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Viorica DESELNICU

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FOREWORD

ICAMS 2016 is offering the framework for presenting the latest results in research, focusing on the field of Materials Science and Innovative Technologies, which records an impressive dynamics and is recognized as a current national and European priority.

The conference provides the opportunity for exchanging ideas and experience with researchers, scientists and experts at international level, and for developing new scientific contributions.

The ICAMS 2016 international event, jointly organised by the National Institute for Research and Development for Textiles and Leather - Division Leather and Footwear Institute (INCDTP-ICPI) and National Authority for Scientific Research and Innovation (ANCSI), took place in Bucharest on 20-22 October 2016. ICAMS 2016 brought together different stakeholders and provided a platform for a better understanding of the European innovation ecosystem while raising awareness of the actions needed to enable synergies and drawing lessons for future actions. Around 130 participants joined the event from several academic and research institutions, public and private sectors, as well as Managing Authorities. Participants presented their experience on research, innovation, policies and the creation of synergies. All these inputs offered insightful elements for discussion in the different participatory sessions throughout the event.

The conference topics included, but were not limited to:

1. **Advanced Materials**
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    - *Materials and innovative products*
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    - *New technologies and methods for managing complexity*
    - *Innovative technologies*
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    - *National / International Standards*
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    - *Policies and Procedures (on a global, national, regional scale)*

We would like to thank all the participants, the International Scientific Committee and all the sponsors that made this scientific event possible. ICAMS Conference has already become a tradition, contributing to the advancement of Materials Science in academic, social and business environments worldwide.

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I.
ADVANCED MATERIALS

INVESTIGATION OF HOT STAMPING PROCESS PARAMETERS IN UP&DOWN MACHINING ON ABS MATERIALS UNDER QUALITY PURPOSES OF DIFFERENT STAMPING PROCESSES

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Hot stamping foil manufacturers have been introducing only an average setting parameters gap. They are requesting that the end users find their most suitable and specific settings by changing parameters with numerous trial processes. Key parameters in machining for a hot stamping process are: die & sample temperature, dwell time and pressure; and these three variables should be proportional with each other. ABS (Acrylonitrile Butadiene Styrene) is one of the most common thermoplastic polymers in the hot stamping industry. A large quantity of identical ABS material, which is used as a water dispenser part by an international brand- Arçelik A.Ş., was ordered. In addition, this was accompanied by multiple types of hot stamping foils which are suitable for use on ABS materials. These foils have different structures and release layers and were chosen and supplied for the experiments. More than one thousand experiments were performed. The most correct parametric values were determined for each targeted pattern foils. Additional tests were performed to determine feasible end-users' harms; moreover, various failures also occurred and these failures were examined. Every result was analysed and compared with their detailed technical photos and graphs. A guide for the industry was established which would establish a new course to help machining operators reach the solution more quickly and easily, in case they have a process mistake.

Keywords: Hot Stamping Foil, Silicone Die, ABS Material.

INTRODUCTION

In the 19th century, hot stamping became a popular method of applying gold tooling or embossing in book printing (Cambras, 2004). The first patent for hot stamping was recorded in Germany by Ernst Oeser in 19th Century (Benedelk, 2010; Oeser, 1901). At the present day, hot stamping foils have been using in many industries, including (but not limited with) packaging, cosmetics, household appliances and automotive, to be applied on a material for value adding purposes. Hot stamping foil printing involves the transfer of a coating from a carrier film to the substrate to be printed by means of heated male die (Brewis and Briggs, 1985). The manufacturing of a typical foil is figured as below:

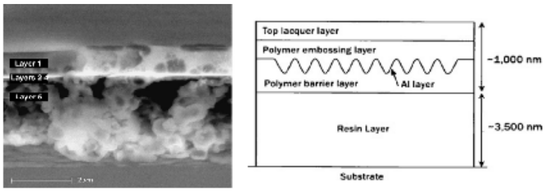

Figure 1. (a) Microtomed cross-section of hot stamping foil showing multilayer structure and (b) a schematic illustration of the layers (Leech, 2009)

Nowadays, hot stamping foil industry optionally has many different structure alternatives to be manufactured in consideration of the customer's needs and this may provide wide selection of different structures & designs for application on different type of materials. Today, it may be defined hot stamping manufacturing process in two different types (Crown Roll Leaf, Inc). In the structure, role of the layers is defined as below in Table 1. Hot stamping foil industry has a big potential in consideration of its further global market position, because it has a very wide application area for value adding by its creative design solutions and additionally allows very high durability in case the choice of foil correctly matches with type of the applied material. The most important key in this industry is to choose the correct material with the correct type of the hot stamping foil and training that matches with the correct process parameters. The present research work has been undertaken with an objective to explore correct parameters although they have been presented by their makers, with an aim of creating a guide for the operators to reach the best stamping results in machining process in case they have failure in the hot stamping process.

Table 1. Layers for Pigment Foils and Metallic Foils (Crown Roll Leaf, Inc)

Base film	Carrier film, normally PET and between 8 µm - 50 µm.
Release layer	Release the structure from the carrier film.
Protection (Colour) Layer	Protection of metallic layer. Pigment colour options.
Metallic Layer*	Brightness layer, usually Aluminium based; but may also be different such as Sn(Tin) for touch display screens or Chromium (Cr) for exterior use (e.g vehicle logos) due to its high durability.
Adhesion Layer	Adhesion on moulding, eligible by reference of the materials stamping on.

**There is no Metallic Layer for Pigment Foils.*

EXPERIMENTAL PROCEDURES

Experiments were occurred in 25 different process parameters. Matrix of experimental chart in different process parameters were formed are given in Table 2. A pneumatic Up&Down Hot Stamping Machine has been built up by a local machine maker. A large quantity of identical ABS material, which is used as a water dispenser part by an international brand-Arçelik A.Ş., two silicone dies and a workpiece sample holding fixture have been supplied by local makers of Arçelik A.Ş. A number of hot stamping foils are supplied for the experiments from an American Company - Crown Roll Leaf Inc. and from a Japanese Company-Katani Sangyo Co. Ltd. Numerous trials for each hot stamping foil have been made separately in consideration of a matrix at 2.75 bar (40 psi) including the dwell time of 0.3 sec, 0.7 sec, 1.1 sec, 1.5 sec and 2.3 sec; also including the temperature of 130°C, 150°C, 170°C, 210°C and 250°C.

Table 2. Matrix of experimental chart in different process parameters for each foil

	130°C	150°C	170°C	210°C	250°C
0.3 sec.	Trial No: 01	Trial No: 06	Trial No: 11	Trial No: 16	Trial No: 21
0.7 sec.	Trial No: 02	Trial No: 07	Trial No: 12	Trial No: 17	Trial No: 22
1.1 sec.	Trial No: 03	Trial No: 08	Trial No: 13	Trial No: 18	Trial No: 23
1.5 sec.	Trial No: 04	Trial No: 09	Trial No: 14	Trial No: 19	Trial No: 24
2.3 sec.	Trial No: 05	Trial No: 10	Trial No: 15	Trial No: 20	Trial No: 25

Figure 2. Silicone die

Figure 3. Infrared heat measurement device

Sample Preparation

Test samples were manufactured by injection moulding. The ABS samples were manufactured by using 100% ABS material. These samples are exactly the same products that has been in use in the refrigerator industry as a water dispenser part by an international brand- Arçelik A.Ş.. Silicone dies and a workpiece sample holding fixture was supplied. Silicone dies were assembled by a combination of 10mm aluminium and 2.5mm silicone; and a workpiece sample holding fixture made by aluminium only. 5-different hot stamping foil samples from an American Company - Crown Roll Leaf Inc. and 5-different hot stamping foils samples from a Japanese Company – Katani Sangyo Co. Ltd. were supplied (Table 3). The samples have technically different structures, such as thickness of the base (carrier) film (between 12 µm and 25 µm), level of the release layer (soft release and hard release), structure of protection (colour) layer and structure of metallic layer (e.g. chromium or aluminium).

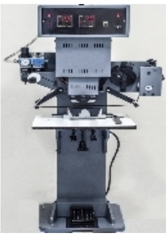

Figure 4. Hot stamping foil machine

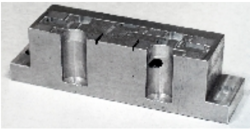

Figure 5. Workpiece sample holding fixture

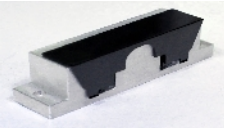

Figure 6. Workpiece sample holding fixture with the ABS sample

In the beginning, pressure of the machine's pneumatic system was set up at 2.75 bar (40psi) and the machine's heat parameter was set up at 130°C and first trial group was made in 5-steps of dwell time parameters between 0.3 secs and 2.3 secs. This changing dwell time method was repeated separately for 5-steps of heating parameters between 130° and 250°C.

Mechanical Characterization

Silicone dies and ABS samples were measured before the stamping process by using a 4-points infrared heat measurement device (Figure 3), according to compare the risk of heat change probabilities due to process speed of serial working conditions and the room temperature conditions. A decision making was done after numerous trials on different hot stamping foils and 10-hot stamping foils were chosen to include this research work. At the room temperature (for ABS samples), 25 trials were done by using each defined hot stamping foil by changing parameters on same reference values (for heating temperatures and dwell times).

Testing Procedure

Results were examined and an analysing was made after trials. The best results were chosen from the trial groups; then scratch and tape test were occurred on to examine the durability and quality of the processes.

Figure 7. One of the best stamped samples, HSF04 - Trial No: 25

Table 3. Definition of Hot Stamping Foils which had been used in the research

Foil Maker	Hot Stamping Foil Sample Number	Product Code	Carrier film thickness	Metal & Colour
Crown Roll Leaf, Inc (USA)	HSF01	BW88/260E	12 µm	Metallic Gold
	HSF02	YEA3200		Pigment Blue
	HSF03	YEA4200		Pigment Yellow
	HSF04	YEA5600		Pigment Green
	HSF05	YED6250		Pigment Pink
Katani Sangyo Co. Ltd. (JAPAN)	HSF06	CX-1001	16 µm	Metallic Silver
	HSF07	TA		Pigment White
	HSF08	HR-5431-F	25 µm	Aluminium
	HSF09	329KH718 ST NC		Tin(Sn)
	HSF10	EM-2009 Exterior		Chromium

RESULTS AND DISCUSSION

Number of hot stamping foil processes were tested (Figure 7 and Figure 8). To get more realistic and more specific results, two industrial leader companies' (from different continents) products were examined in this research. This methodology also enables to pursue a more realistic course as these two companies probably use different raw material resources due to their geographical positions. In this experiment, hot stamping foils which have exactly different structures (except YEA series of Crown Roll Leaf, Inc) were used. Pigment foils from two different series' performances proved adhesion and protection layers' importance. For instance, in comparison of YED series against YEA series, YED series have more pigment coating and it enables more covering. Moreover, metallic foils were used to prove stable quality as this is one of the most earliest established hot stamping foil types. In addition to 12 µm foils, 16 µm foils were used to examine the process difference. Although 12 µm products are usually more popular than 16 µm products in the industry, 16 µm products are also considering in case the shape of the product to make hot stamping process has a geometrically hard design with a radius. The key point is, 16 µm products has 4 µm thicker carrier film and this makes the process enable to work with higher temperature or longer dwell time and pressure in comparison to using 12 µm products. Theoretically, 16 µm products should work with higher parametric values, but in this experiment, 12 µm products needs higher temperature and dwell time. This may be explained as 16 µm products' release layer is softer level than release layer of 12 µm products.

YED series proved better condition in higher temperature than YEA series due to its higher pigment level requires higher process conditions. An interesting point occurred in comparison of YEA4200 against other two products of YEA series. YEA4200 provides its best result in lower stamping temperature and dwell time although there is no technical difference. There should be a momentary issue during the experiment while process of this product was being occurred. The measured and tested results are shown in Table 4. This is very clear that 130°C is very low for a good condition process. When the temperature reaches till 150°C, then the looking gets sharp but the quality of the stamping is not stable. Commonly, their resistance levels are low and this means the process quality still needs higher parametric values for machining settings. 170°C and 210°C are mainly preferred temperature levels for a better hot stamping process.

Table 4. Experiments' detailed test results

TRIAL NUMBER	HOT STAMPING FOIL SAMPLES									
	S 01	S 02	S 03	S 04	S 05	S 06	S 07	S 08	S 09	S 10
Trial No: 01 (130 ^{o}C vs. 0.3 sec.)	1	1	1	1	1	1	2	1	1	1
Trial No: 02 (130 ^{o}C vs. 0.7 sec.)	1	1	1	1	1	1	2	1	1	1
Trial No: 03 (130 ^{o}C vs. 1.1 sec.)	2	1	1	1	1	1	2	1	1	1
Trial No: 04 (130 ^{o}C vs. 1.5 sec.)	2	1	1	1	2	2	2	1	1	1
Trial No: 05 (130 ^{o}C vs. 2.3 sec.)	2	1	2	1	2	2	2	1	1	1
Trial No: 06 (150 ^{o}C vs. 0.3 sec.)	1	2	2	1	1	1	2	1	1	1
Trial No: 07 (150 ^{o}C vs. 0.7 sec.)	2	2	3	1	1	3	3	2	2	1
Trial No: 08 (150 ^{o}C vs. 1.1 sec.)	2	2	3	2	1	3	3	2	2	1
Trial No: 09 (150 ^{o}C vs. 1.5 sec.)	2	2	3	2	2	3	3	2	2	1
Trial No: 10 (150 ^{o}C vs. 2.3 sec.)	5	2	3	2	2	3	3	2	2	1
Trial No: 11 (170 ^{o}C vs. 0.3 sec.)	1	2	3	1	2	2	2	2	2	1
Trial No: 12 (170 ^{o}C vs. 0.7 sec.)	2	3	3	2	2	2	3	5	5	1
Trial No: 13 (170 ^{o}C vs. 1.1 sec.)	2	3	4	2	2	3	3	5	6	2
Trial No: 14 (170 ^{o}C vs. 1.5 sec.)	2	3	4	2	2	4	3	5	6	5
Trial No: 15 (170 ^{o}C vs. 2.3 sec.)	5	3	5	2	2	4	4	5	6	5
Trial No: 16 (210 ^{o}C vs. 0.3 sec.)	1	3	5	1	1	2	4	6	5	5
Trial No: 17 (210 ^{o}C vs. 0.7 sec.)	2	3	5	3	3	2	4	6	6	5
Trial No: 18 (210 ^{o}C vs. 1.1 sec.)	4	4	5	3	5	3	5	6	6	6
Trial No: 19 (210 ^{o}C vs. 1.5 sec.)	4	5	5	4	5	4	5	6	6	6
Trial No: 20 (210 ^{o}C vs. 2.3 sec.)	5	5	5	4	5	4	5	6	6	6
Trial No: 21 (250 ^{o}C vs. 0.3 sec.)	6	3	5	5	4	4	5	6	6	5
Trial No: 22 (250 ^{o}C vs. 0.7 sec.)	6	5	6	6	5	5	5	6	6	5
Trial No: 23 (250 ^{o}C vs. 1.1 sec.)	6	6	6	6	6	6	6	6	6	6
Trial No: 24 (250 ^{o}C vs. 1.5 sec.)	6	6	6	6	6	6	6	6	6	6
Trial No: 25 (250 ^{o}C vs. 2.3 sec.)	6	6	6	6	6	6	6	6	6	6

E.G. S01 -> HSF01 (BW88/260E METALLIC GOLD BY CROWN ROLL LEAF, INC)

Point 1 - not stamped; point 2 - stamped poor; point 3- stamped good; point 4 - stamped very good; point 5 - stamped more than enough (just letter dispersing); point 6 - stamped much more than enough (dispersing)

Figure 8. One of the best stamped samples, HSF07 - Trial No: 20

Figure 9. Background of HSF01, high temp. against low temp.

Due to its carrier film, 25 μm products are designed to cover surfaces by a Roll-On type of machine, rather than Up&Down. These products may also be used under higher temperatures and longer dwell times. In this research, three different types of 25 μm products were used. Their stamping conditions were as expected, did not work well with letter stamping; but also shown that these three foils may easily work with higher temperature and dwell times and this makes the quality of stamping higher. HR-5431-F is a product with a structure of aluminum for its metallic layer. As it makes its durability higher in comparison with non-aluminum products, this is not enough strong against corrosion. EM-2009 Exterior is a product with a structure of Chromium. This product has a better durability than HR-5431-F and mainly preferred in automobile industry due to its stronger structure. 329KH718 is a semi-transparent (approx. 30% transparency) and non-conductive product, which has been used in touch screen panels due to its conductivity blocking structure, because it has Tin(Sn) on its metallic layer that makes this product non-conductive and this makes its characteristics such as a TV controller. This product is mainly used in electronics industry.

The best results of each product has been tested by using scratch and tape methods. To compare the results, 25 µm products provides the best resistances. There was no big differences between 12 µm and 16 µm products. In case of comparison between 12 µm products, YED series has better durability than YEA series.

Figure 10. Poor stamping sample, HSF03 - Trial No: 07

Figure 11. Poor stamping sample, HSF05 - Trial No: 25

CONCLUSIONS

In the present work, a number of testing was occurred and results were tested under durability issues and detailed analysing was reported. The aim of this study was to investigate the effect of parametric settings on hot stamping process and creating a guide for the industry to establish a new course to help machining operators reach the solution more quickly and easily, in case they have a process mistake. The tools used in this research work were almost the same and the results indicate the inverse relation between the stamping temperature and dwell time. With this research work, the process failures would be analysed clearly to reach the solution. This is proved that in case the pressure is stable, the temperature should be increased while the dwell time is set to be decreased for trials to gain quality. Higher temperature & increased dwell time is necessarily if the stamping is in a poor condition; and lower temperature & decreased dwell time is necessarily if the stamping quality is dispersing. The best hot stamping process must be ended with a very sharp looking, high durability against attacks and minimum touch feelings.

Acknowledgement

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REFERENCES

Benedek, I. (2010), *Developments in Pressure-Sensitive Products*, 2nd Edition, Taylor & Francis, p. 514.
Brewis, D.M. and Briggs, D. (1985), *Industrial Adhesion Problems*, New York: Wiley, p. 201.
Cambras, J. (2004), *The Complete Book of Bookbinding*, Lark Books. p. 115. ISBN 978-1-57990-646-7.
Leech, P.W. (2009), “Laser ablation of multi-layered hot stamping foil”, *Journal of Materials Processing Technology*, 209(9), 4281-4285.
Oeser, E. (1901), “Process of manufacturing colored or similar films”, US Patent 665458 A, available at http://www.google.com/patents/US665458.

POLYMER NANOCOMPOSITES PE / PE-G-MA / EPDM / NANO ZnO DYNAMICALLY VULCANIZED WITH PEROXIDE

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Nanocrosslinked elastic-plastic nanocomposites are blends that economically combine, in a highly advanced way, the properties of at least two polymers (elastomer and plastomer) and nanoparticles in a single product. In these mixtures the elastomeric phase is cross-linked in the thermoplastic melt under conditions of thermodynamic technology. This paper presents the development of multiphase elastic-plastic polymeric nanocomposites, dynamically vulcanized, reinforced with nanoparticles for products used in food and pharmaceutical domain, a new variety of composite material based on elastomer (ethylenepropylene-ter-polymer rubber EPDM) and nanofiller (ZnO) dispersed in the plastomer matrix (high density polyethylene-HDPE). By nanometric dispersion in plastomer matrix, elastomers and nanofiller have led to obtaining a high-performance polymer material with multifunctional characteristics (rubber and thermoplastic) and processing properties specific to plastics. Also, the elastomer was dynamically cross-linked in the plastomer matrix, during processing, by classic vulcanization system with peroxide. The crosslinking method is ecological, without release of aromatic nitrosamines, which are carcinogenic in the crosslinking process. Experimented composites were physico-mechanically characterized by standard methods specific to plastics.

Keywords: nanocomposite, dynamical cross-link, peroxide.

INTRODUCTION

Thermoplastic elastomers (TPEs) are a class of copolymers or polymer blends that have the thermoplastic processing and functional characteristics of a conventional crosslinked elastomer. TPEs typically have a structure of two phases, a thermoplastic phase and the elastomeric phase. The thermoplastic phase melts at high temperatures at which the elastomeric phase disperses and thus the TPE becomes processable (Borcea, 2008). The advantages of TPEs compared to plastomers are: possibility of adapting the properties from rigid to low hardness, by varying the amount of plasticizer and/or reinforcing agents, processing by injection with low consumption of time and energy, waste recycling capacity etc. The disadvantages include: high temperature processing, physical and mechanical properties that not suitable for all applications and the material loses its properties after recycling (Lebel, 1994). The thermoplastic compounds - TPE based on rubber/polyolefin - are classified in two distinct classes: one class consists of simple mixing and is referred to as olefin thermoplastic elastomers (TEO) (ASTM D 5593 – 99) and the other class, the rubber phase is dynamically crosslinked obtaining a dynamically crosslinked thermoplastic (TPV) in compliance with (ASTM D 5046 – 98). Dynamically crosslinked thermoplastic materials (TPV) have taken a large marketshare in use as substitutes for polymers, plastics, wood and even metal, due to thermoplastic processability combined with elastomer behavior. TPVs contain elastomeric crosslinked particles into a thermoplastic matrix. The rubber particle size dispersed in thermoplastic and interfacial adhesion with plastomer is the key of these materials, because they have a major influence on the quality and processability of this new material. Dynamic crosslinking process was tested by Gessler (Gessler and Haslett, 1962). The first dynamically crosslinked thermoplastic composite was introduced in 1972 (Fisher, 1972), which partially crosslinked elastomeric phase - EPDM of PP / EPDM compound with peroxide. Significant improvements of these compound properties were made (Coran *et al.*, 1978) by completely crosslinking the elastomer phase dynamically, while maintaining the compound thermoplasticity. TPVs represent the second group of elastic thermoplastic elastomers, the first being styrene block copolymers. These systems have properties similar to crosslinked rubber hardness in the range of 40-90 °Shore A (Harrats *et al.*, 2006; Rane and Abitha, 2015; Ito *et al.*, 2003). TPVs are obtained by melt compounding techniques in extrusion granulators with co-rotating twin screws and a high L (length) / D (diameter) ratio, continuous flow processing technology (Ellul *et al.*, 2004; Harrats *et al.*, 2006). The properties of the composites depend on the polymer, reinforcing agents, the type and amount of compatibilizer and their proportion in compounding and processing conditions. Therefore, the reinforcing agents properties must be known, they influence compound properties, and therefore the domain of interest (Thostenson and Chou, 2005; Koo, 2006). There are different types of nanoparticles that can be embedded into the polymer matrix which are selected depending on their properties and applications. ZnO nanoparticles show antimicrobial activity including for temperature and high pressure resistant spores (Emamifar, 2010). Besides the role of active filler or curing initiator, treating elastomers and plastomers (polyester, polyamide) with ZnO nanoparticles leads to an important reduction of bacterial cells by 78 and 62% respectively, and for fungus by 80.7 and 32% respectively (Kim, 2013).

MATERIALS AND METHODS

Materials

All composites contain the same two polymers: the EPDM elastomer (Nordell IP 3745P-DuPont) and the high density polyethylene (HDPE-SIDPEC, Egyptene), in the ratio 60:40.

In addition to the polymers, other additives were used, as follows:

- compatibilizer: polyethylene graft maleic anhydride (PE-g-MA, Sigma Aldrich);
- crosslinking agent: di (tert-butylperoxiisopropil) benzene, powder 40% with calcium carbonate and silica – Perkadox 14-40B-PD, AkzoNobel, Germany;
- activators: zinc oxide and stearic acid – Bayer, Germany;
- antioxidants: di-lauril-orto-dipropionate (Irganox 1010) and N-isopropyl-N-phenyl-p-phenylendiamine (Irganox 4010), Bayer, Germany.

Preparation of Various Types of Thermoplastic Elastomers

HDPE and Irganox 1010 were mechanically mixed in Brabender Plasti-Corder PLE 360 at 160°C and 80 rot/min for 3 minutes, to melt the plastomer and mix it with the antioxidant. The temperature was decreased to 140°C and was added EPDM, PE-g-MA and Irganox 4010, continuing mixing at a speed of 100 rot/min for 4 minutes. Next, were added the crosslinking activators, ZnO (nano and micro) and stearic acid, at the same temperature and mixing speed, continuing mixing for 2 minutes. For the last added constituent - peroxide, the rotation speed was increased (120 rot/min) and the temperature was decreased to 100°C. The total mixing time was 11-12 minutes. In the table 1 are shown the tested receipts. From the Brabender mixing diagrams, figures 1 and 2, it is seen that the temperature from mixing chamber does not change when adding compatibilizer and nanoZnO (starts from 90°C decrease to 72°C with a maximum of 233°C at the end), while for the mixing forces small changes are observed, the maximum force is when EPDM is added. The values are 144Nm for the control sample and 162Nm for N26 (nanocomposite with 75% ZnO nanopowder).

Table 1. Polymer nanocomposite recipes PE / PE-g-MA / EPDM / nanoZnO dynamically crosslinked with peroxide

Materials/Symbol	N20	N21	N22	N23	N24	N25	N26
HDPE	135	135	135	135	135	135	135
Irganox 1010	2,7	2,7	2,7	2,7	2,7	2,7	2,7
PE-g-MA	-	1,35	4,05	6,75	4,05	4,05	4,05
EPDM	90	90	90	90	90	90	90
Irganox 4010	1,8	1,8	1,8	1,8	1,8	1,8	1,8
ZnO	9	9	9	9	8,1	4,5	2,7
ZnO nano	-	-	-	-	0,9	4,5	6,3
Stearic acid	0,75	0,75	0,75	0,75	0,75	0,75	0,75
Perkadox	11,25	11,25	11,25	11,25	11,25	11,25	11,25

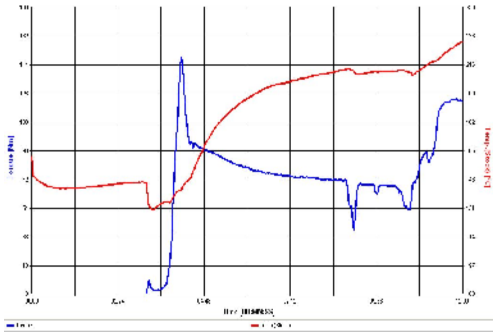

Figure 1. Brabender mixing diagram for the N20 composite control sample

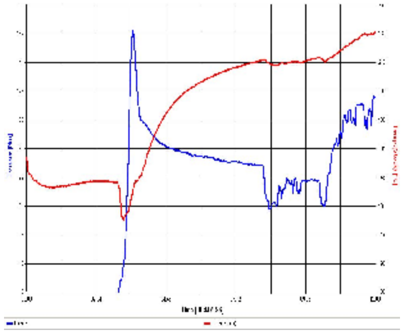

Figure 2. Brabender mixing diagram for nanocomposite with 75% ZnO nanopowders, N26

In order to establish the curing times, the samples were rheologically tested using Monsanto Rheometer with oscillating disc. From the data it is observed that ZnO nanoparticles do not influence the crosslinking time, diagrams are identical for the tests conducted, as demonstrated in figures 3 and 4.

The compound was then compression-molded (using an electrically heated hydraulic press at 170°C under 200 MPa pressure) into a sheet of about 2 mm thick. The sheet was then cooled down to room temperature under same pressure. The specimens were die-cut from the compression molded sheet and used for testing after 24 hours of storage at room temperature.

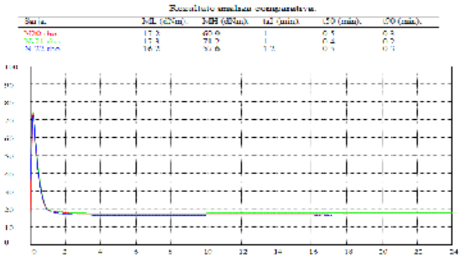

Figure 3. Overlapping rheogram
for samples N20-N22

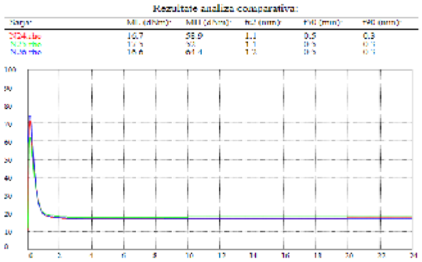

Figure 4. Overlapping rheogram
for samples N24-N26

Apparatus for Physical-Mechanical Tests

Tensile tests of the samples were carried out in compliance to SR ISO 37:2012 using a Schopper Tensile Testing machine 1445, at a constant crosshead speed of 500 ± 5 mm/min.

Hardness of the samples was determined with Shore "A" Durometer, in compliance with SR ISO 7619-1:2011.

Attrition test is carried out with the *roll abrader*, in compliance with SR ISO 4649/2010, and the melt flow index with – Haake Meltflow MT.

RESULTS AND DISCUSSION

Physical-mechanical tests were carried out in the Investigation laboratory from INCDTP - Division ICPI, accredited by RENAR, and materialized in the determination of hardness, elasticity, tensile and tear strength, attrition, residual elongation and elongation at break for thermo-oxidative aging (168h x 100°C) and normal state, and melt flow index.

The hardness, for normal state, is 93°Sh A and increase to 94°Sh with the addition of ZnO nanoparticles. Thermo-oxidative aging increase the hardness value to 98°Sh A, which demonstrates that the curing process continues during processing. Peroxide crosslinking system creates more stable cross bridges between the polymer chains, which are harder to break under temperature action;

The tensile strength falls in the range from 9.6 to 10.5 N / mm^2, for normal state and 8.9 to 9.4 N/mm ^{2} after aging. The high values of this parameter are to be noted, values that are not found in classic rubber blends with the same hardness;

Nanostructuring of EPDM rubber in the polyolefin matrix and PE-g-MA compatibilization requires *elasticity* with increased values at high hardness, performance characteristic of these materials (Tables 2 and 3), maintaining its high values even after accelerated aging;

Tear strength is an index with performance values, maintaining the same values (102-105N/mm) due to tested elastic-plastic nanocomposites morphology (Tables 2, 3).

Table 2. Physical-mechanical characterization, normal state

Symbol	N20	N21	N22	N23	N24	N25	N26
Hardness °Sh A SR ISO 7619-1:2011	93	93	93	93	94	94	94
Elasticity % ISO 4662:2009	22	21	22	22	22	21	20
Tensile strength, N /mm^2 SR ISO 37:2012	10.5	9.7	9.3	9.1	9.0	8.9	8.6
Elongation at break, % SR ISO 37:2012	340	340	360	400	280	240	220
Residual elongation, % SR ISO 37:2012	212	193	200	180	140	180	220
Tear strength, N /mm SR EN 12771:2003	102	118	112.5	104.5	106	105	105
Density, g/cm^3, SR ISO 2781:2010	0.98	0.97	0.96	0.96	0.93	0.93	0.93
Attrition, mm^3, SR ISO 4649/2010	50	41	41	42	43	42	41

Table 3. Physical-mechanical characterization after thermo-oxidative aging for 168h at 70°C

Symbol	N20	N21	N22	N23	N24	N25	N26
Hardness °Sh A SR ISO 7619-1:2011	98	98	98	98	98	98	98
Elasticity % ISO 4662:2009	22	22	24	24	24	24	28
Tensile strength, N /mm^2 SR ISO 37:2012	9,4	9,1	8,3	9,6	9,1	8,9	9,0
Elongation at break, % SR ISO 37:2012	453	345	380	460	420	460	540
Residual elongation, % SR ISO 37:2012	227	210	220	240	140	214	340
Tear strength, N /mm SR EN 12771:2003	127,5	127	118	111	107	105	116

CONCLUSIONS

The experiments pursued the obtaining and characterization of PE/PE-g-MA/EPDM /nanoZnO based polymeric nanocomposites, dynamically crosslinked with peroxide.

The technology for obtaining the elastic-plastic polymer nanocomposites with variable content of ZnO nanoparticles has the advantage of simultaneously crosslinking and processing the elastomer in the plastic melt, HDPE respectively. The nanostructured polymeric materials obtained are blends with the structure of plastic in which crosslinked EPDM elastomer and ZnO nanoparticles are dispersed, and can find the appropriate applications for their advanced properties, substituting existing products made from different rubbers and plastics, offering significant economic benefits to companies. In addition to high temperatures resistance, these materials are anti-microbial, characteristic to be tested biologically.

Acknowledgements

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REFERENCES

ASTM D 5046 – 98: “Standard specification for Fully Crosslinked Elastomeric Alloys”.

ASTM D 5593 – 99: “Standard Classification for Thermoplastic Elastomers-Olefinic (TEO)”.

Borcea, V. (2008), “Radioactive ion implantation of thermoplastic elastomers”, Presses univ. de Louvain.

Coran, Y., Das, B. and Patel, R.P. (to Monsanto Co.) (1978), U.S. Patent 3,130,535.

Ellul, M.D., Tsou, A.H. and Hu, W. (2004), “Crosslink densities and phase morphologies in thermoplastic vulcanizates”, *Polymer*, 45(10), 3351-3358.

Emamifar, A., Kadivar, M., Shahedi, M. and Soleimanian-Zad, S. (2010), “Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice”, *Innovative Food Science & Emerging Technologies*, 11(4),742-748;

Fisher, W.K. (to Uniroyal, Inc.) (1973) U.S. Patent 3,758,643.

Gessler, M. and Haslett, W.H. (to Esso Research and Enginering Co.) (1962), U.S. Patent 3,037,954.

Harrats, C., Thomas, S. and Groeninck, G. (2006), *Micro- and Nanostructured Multiohase Polymer Blend Systems*, Taylor and Francis Group, LLC;

Ito, E.N., Hage, E., Jr., Mantovani, G.L. *et al.* (2003), “Development of phase morphology for extruded and moulded PBT/ABS blends”, *Proceedings of the 19th Annual Meeting of the Polymer Processing Society* PPs-19, Melbourne, CD, S2, 259, 1-5.

Kim, H.J., Pant, H.R., Park, C.H., Tijing, L.D., Hwang, B.S., Choi, N.J. *et al.* (2013), “Electrical Properties of ZnO/NYLON-6 Spider-Wave-Like Nanonets Prepared via Electrospinning”. *Digest Journal of Nanomaterials and Biostructures*, 8(1), 385 – 393.

Koo, J. (2006), *Polymer Nanocomposites. Processing, characterization and applications*, McGraw-Hill Nanoscience and Technology Series, Companies, Inc.

Lebel, M.A. (1994), “Factors fueling the growth of thermoplastic elastomers”, *Plastics Engineering*, 50(1), 23-26.

Rane, A.V. and Abitha, V.K. (2015), “Study of Mechanical, Thermal and Micro structural Properties of EPDM/Polypropylene/Nano clay Composites with Variable Compatibilizer Dosage”, *Journal of Materials and Environmental Science*, 6(1), 60-69.

Thostenson, E., Li, C. and Chou, T. (2005), “Review Nanocomposites in Context”, *Journal of Composites Science & Technology*, 65, 491–516. (Reproduced in part with permission from Elsevier, 2006).

OFF-AXIS TENSILE BEHAVIOR OF MULTISTITCHED COMPOSITES

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The off-axis tensile behavior of multi-stitched composites was investigated. The off-axis tensile strength, tensile modulus and tensile strain values were determined. Angular deformation, lateral shrinkage and displacement measurements were performed on the composites after the tensile tests. Because of the filament breakages caused by multi-stitching, the tensile strengths and modulus of the multi-stitched composites slightly decreased. According to measurements, normal deformation, or angular deformation or lateral shrinkage were occurred on all tested composites. However, a new failure mode was observed on four directional densely stitched composite. This structure had only lateral shrinkage following the angular deformation.

Keywords: Off-axis tensile test, multi-stitched composite, lateral shrinkage.

INTRODUCTION

Textile structural composites can serve as advanced engineering materials owing to their exceptional properties such as high delamination resistance and damage tolerance (Brandt *et al*., 1996; Dexter and Hasko, 1996). A wide range of techniques can be used to fabricate textile preforms for composites including weaving, knitting, braiding, stitching, and various nonwoven production methods (Bilisik and Yilmaz, 2012). Stitching has been shown to increase the fracture resistance of the composite by changing the crack propagation pattern, that is, the crack is forced to follow a more tortuous route between stitches, absorbing a large amount of energy in the process (Mouritz, 2004). It was reported that the through-the-thickness stitching of the preform by using only a few per cent of stitching yarn greatly enhances the out-of-plane tensile modulus of the resulting composite at the cost of some reduction in in-plane tensile and shear moduli (Dickinson *et al*., 1999). It was shown that the positive effect of stitching on the delamination resistance increases as the stitch density is increased. This was attributed to the fact that more closely positioned stitches can be more effective at keeping the individual cracks within a small area without further propagation (Tan *et al*., 2012). In this study, the off-axis tensile behavior of multi-stitched composites was experimentally investigated.

MATERIALS AND METHODS

Multistitched Preform and Composite

E-glass woven fabrics (Cam Elyaf A.S., Turkey) were used for producing multi-stitched preform and composites. Table 1 shows the specifications of E-glass woven fabric.

Table 1. The specifications of E-glass woven fabric

Weave type	Yarn linear density (tex)		Density (per 10 cm)		Weight (g/m^2)	Crimp (%)		Thickness (mm)
	warp	weft	warp	weft		warp	weft	
Plain	2400	2400	16	18	800	1.24	1.20	1.01

E-glass woven fabric layers were stacked one on top of the other according to the sequence ($[(0°/90°)]_4$). The multi-stitched preforms were produced by stitching these layers in through-the-thickness direction according to various stitching patterns such as one-directional stitching in the warp (0°) direction; two-directional stitching in the warp (0°) and weft (90°) directions; four-directional stitching in the warp (0°), weft (90°) and ±bias directions. A multilayer non-stitched preform was produced as a reference sample. Two different stitching densities (2 and 6 step/cm) were used with 1 cm distance between the neighboring stitching lines. Brother DB2-B736-3TR (Brother Industries Ltd, Japan) stitching machine was used with lock stitching type. Kevlar® 129 and nylon 6.6 were used as the bobbin and needle stitching yarns respectively. Table 2 shows the properties of stitching yarns.

Table 2. Properties of stitching yarns

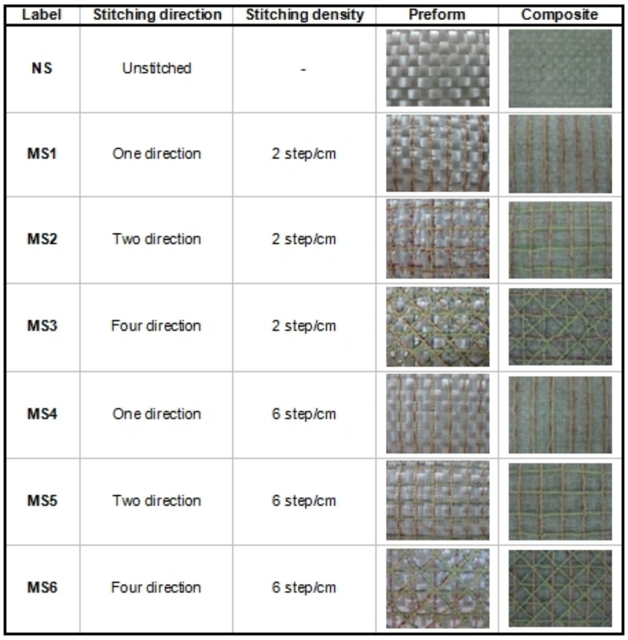
Fiber type	Fiber diameter (µm)	Fiber density (g/cm^3)	Tensile strength (GPa)	Tensile modulus (GPa)	Elongation at break (%)	Yarn linear density (tex)
Kevlar 129	12	1.45	3.4	99	3.3	110
Nylon 6.6	14	1.14	0.6	2.46	41	44

VARTM method was used for composite fabrication. Unsaturated polyester resin (Crystic 703PA, Scott Bader, UK) and hardener (methyl ethyl ketone peroxide, 2 wt. %) were mixed homogenously before applying to the preforms at a temperature of 20°C under vacuum. Figure 1 shows some of the produced preforms and composites.

Composite Tests

The volume fraction, density and void content of the composite samples were determined in accordance with ASTM D3171-99, ASTM D792-9 and ASTM D2734-91 standards respectively. Off-axis tensile tests of the samples were conducted according to ASTM D3039-76 using Shimadzu AG-XD 50 (Japan) testing machine equipped with Trapezium® data acquisition software.

Some measurements were performed on the composites after the application of off-axis tensile load. Image Pro-Plus image analyzing software (MediaCybernetics, USA) was used to measure the angular deformation (θ_s), lateral shrinkage (b_s) and displacement (w) normal to the loading direction. Figure 2 shows the measurements performed on the samples.



Label	Stitching direction	Stitching density	Preform	Composite
NS	Unstitched	-		
MS1	One direction	2 step/cm		
MS2	Two direction	2 step/cm		
MS3	Four direction	2 step/cm		
MS4	One direction	6 step/cm		
MS5	Two direction	6 step/cm		
MS6	Four direction	6 step/cm		

Figure 1. Multi-stitched preforms and composites

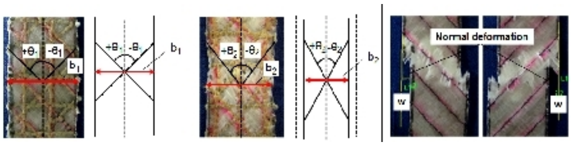


Figure 2. The measurements of the composites after off-axis tensile load was applied

Angular deformation (θ_s) was defined as the change in the angle between the warp and weft yarn sets after the application of off-axis tensile load (Equation 1); lateral shrinkage (b_s) was defined as the change in the sample width after the application of off-axis tensile load (Equation 2); displacement (w) was defined as the recorded displacement normal to the loading direction in off-axis tensile test.

$$\theta s = |\theta 2 - \theta 1| \quad [\text{where, } +\theta 2 < +\theta 1,\ -\theta 2 < -\theta 1] \qquad (1)$$

$$bs = |b2 - b1| \quad [\text{where, } b2 < b1] \qquad (2)$$

Where, θ_s is the angular deformation angle (°); θ_1 is the angle between the warp and weft yarns before the application of off-axis load (°); θ_2 is the angle between the warp and weft yarns after the application of off-axis load (°); b_s is the lateral shrinkage of the sample (mm); b_1 is the specimen width before the application of off-axis load (mm) and b_2 is the specimen width after the application of off-axis load (mm).

RESULTS AND DISCUSSION

Fiber Volume Fraction and Density Results

Figure 3 shows the fiber volume fraction, density, and void content of the samples. The results suggested that stitching impaired the fiber alignment especially at the needle penetration sites. The volume fraction of the stitching yarn increased when the number of stitching directions in the sample is increased depending on the stitching density.

Off-Axis Tensile Results

Figure 3 shows the off-axis tensile strength and modulus of multistitched composites. The off-axis tensile strength and modulus of non-stitched reference sample were slightly higher compared with those of the multi-stitched samples because of the filament breakages that caused by stitching process. The off-axis tensile strength and modulus of 2 step/cm stitched samples were higher than those of 6 step/cm stitched sample because denser stitching caused more filament breakages. A slight difference was observed between +45° and -45° directional off-axis tensile strengths.

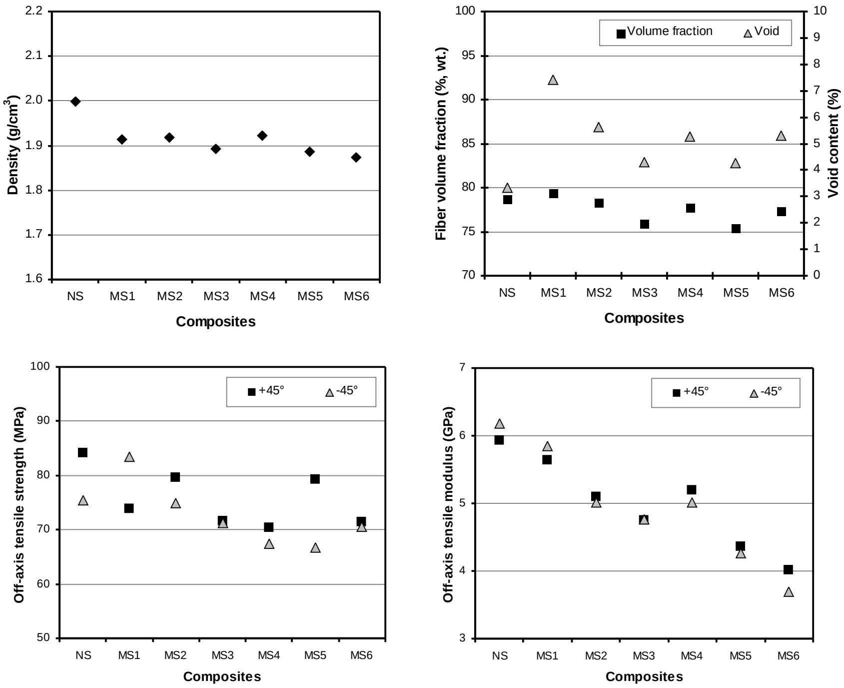


Figure 3. Density, fiber volume fraction, void content, off-axis tensile strength and modulus results of multi-stitched composites

The off-axis tensile strains of the multi-stitched composite samples are depicted in Figure 4. The non-stitched structure yielded slightly higher off-axis tensile strain when compared to the multi-stitched samples except for samples MS5 and MS6 owing to the fact that the displacement of the yarns was restricted by stitching.

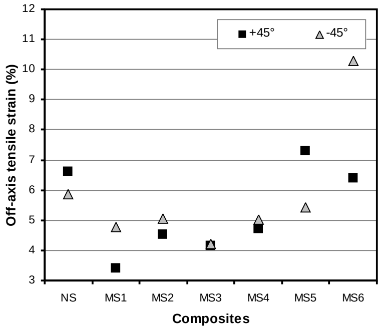


Figure 4. Off-axis tensile strain of multi-stitched composites

Failure Results

Figure 5 and Table 3 show the damaged multi-stitched composites after off-axis tensile test. The failure of multi-stitched composites took place as a combination of matrix, and partial fiber breakage at the surface. The angular deformation of multi-stitched composites increased as the number of stitching directions and the stitching density are increased. The normal deformation was observed generally for all structures except MS6, MS2, MS3 and MS5 which experienced lateral shrinkage in addition to the normal deformation. However, the MS6 sample underwent only lateral shrinkage. The reason is that the off-axis load was confined to a narrow region which, as a consequence, failed abruptly leading to an extensive damage in a larger area involving matrix fracture, fiber pull-out and local multiple fiber breakages.

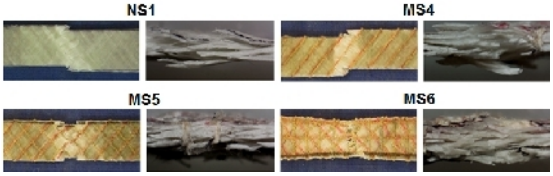


Figure 6. Front face (digital photos, left) and cross sectional (microscopic photos at ×6.7 magnification, right) views of multistitched composites +45°

Table 3. The failure results of multistitched composites after off-axis tensile test

Label	Angular deformation (°)				Normal deformation (mm)		Shrinkage in width (mm)	
	+45°		-45°		+45°	-45°	+45°	-45°
	+θ	-θ	+θ	-θ				
NS	0	1.45	0.94	0	2.78	4.34	0	0
MS1	0	0	0	1.93	4.67	2.79	0	0
MS2	0	0	0.90	0	2.51	2.62	4.57	3.06
MS3	0	0	9.69	3.58	1.43	1.64	1.84	2.72
MS4	0	0	0	0	4.06	4.57	0	0
MS5	5.62	6.75	4.94	6.00	2.14	2.23	4.80	2.23
MS6	3.81	6.84	11.77	9.64	0	0	2.21	2.66

CONCLUSION

The off-axis tensile strengths and moduli of the multi-stitched composites slightly reduced because of filament breakages that resulted from stitching process. The off-axis tensile strain of densely stitched composites was slightly higher compared with that of the non-stitched composite due to restricted movement of yarns. Post-failure examination indicated that four-directional densely stitched composite sample showed (MS6) a new failure mode i.e., it only experienced lateral shrinkage following the angular deformation. These findings suggest that stitching direction and density significantly affect the off-axis tensile behavior of multi-stitched composite materials.

REFERENCES

Bilisik, K. and Yilmaz, B. (2012), “Multiaxis multilayered non-interlaced/non-Z E-glass/polyester preform and analysis of tensile properties of composite structures by statistical model”, *Textile Research Journal*, 82(4), 336-351.

Brandt, J., Drechsler, K. and Arendts, F.J. (1996), “Mechanical performance of composites based on various three–dimensional woven fibre performs”, *Composites Science and Technology*, 56, 381-386.

Dexter, H.B. and Hasko, G.H. (1996), “Mechanical properties and damage tolerance of multiaxial warp-knit composites”, *Composites Science and Technology,* 51, 367-380.

Dickinson, L.C., Farley, G.L. and Hinders, M.K. (1999), “Prediction of effective three-dimensional elastic constants of translaminar reinforced composites”, *Journal of Composite Materials*, 33(11), 1002-1029.

Mouritz, A.P. (2004), “Fracture and tensile fatigue properties of stitched fibre glass composites”, *Journal of Materials: Design and Applications*, 218, 87-93.

Tan, K.T., Watanabe, N. and Iwahori, Y. (2012), “Impact damage resistance, response, and mechanisms of laminated composites reinforced by through-thickness stitching”, *International Journal of Damage Mechanics*, 21(1), 51-80.

ADAPTIVE TEXTILES - BASIC ELEMENT OF DAILY LIFE

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In society there is a high interest for materials that provide a good answer to many contemporary requirements materials that “stress” the environment as little as possible. In recent years, the international scientific world focused its efforts on developing new ways for obtaining polymers, their diversification, in particular by “copying what exists in nature” and the translation of the design center of gravity to the nano – micro level. Currently, new materials are those that respond to the use requirements that are fundamentally designed to meet the demands of smart devices and are environmentally friendly. Adaptive polymers are a high interest area in terms of innovative design, structure, properties and potential application areas.

Keywords: textile, functionalities, adaptive.

OVERVIEW

In society there is a high interest for materials that provide a good answer to many contemporary requirements materials that “stress” the environment as little as possible. In recent years, the international scientific world focused its efforts on developing new ways for obtaining polymers, their diversification, in particular by “copying what exists in nature” and the translation of the design center of gravity to the nano – micro level.

Thus, the convergence in action and the multi-disciplinarity in diversity of design-research-innovation teams defined a new generation of products and related technologies, developed and / or hybrid, in all economic, social and cultural domains. All these actions enabled the development of products with functionalities that scan from passive to active and even bio-intelligent. Textile elements are worldwide recognized as a binder element and a support for nano-functionalities, arising from various fields of science such as chemistry, biology, materials science, information technology and communications etc.

Both in terms of textile components (polymers, fiber, web, strip, yarn) and also the main textile products (woven fabric, knitted fabric and clothing), these functionalities (nonspecific textile field) were attached by various methods (non-textile technologies, hybrid technologies and even textile technologies) on the fabric surface at any level, nano, micro, meso and macro.

Currently, new materials are those that respond to the use requirements that are fundamentally designed to meet the demands of smart devices and are environmentally friendly. Adaptive polymers are a high interest area in terms of innovative design, structure, properties and potential application areas.

ADAPTIVE TEXTILES IN THE CONTEXT OF ADAPTIVE POLYMERS

As smart materials, the adaptable polymers can rapidly, controllable and predictable change their own shape, under the influence of external stimuli. The amplitude, intensity or response frequency of the adaptable polymer are significantly higher compared to the energy developed / expressed by external stimuli (Galaev and Mattiasson, 1999; Hoffman and Stayton, 2004).

The adaptive textile elements/products represent the smart textile category that, after sensing for a short time - via a sensitive element - an external stimulus (default textiles), will compare / analyze information and for a preset threshold, will initiate an answer back to the emitted stimulus environment. The active element of these textiles is an adaptive polymer that is embedded into textile structure at a certain level (nano, micro, meso, and macro).

The adaptive textile elements/products are part of the generation of functionalized textiles.

ADAPTIVE POLYMER TYPES

Adaptable polymers are also called polymers that respond to stimuli by changing. The changes refer to the following levels: size, surface appearance and some features. Examples of stimulants are (Galaev and Mattiasson, 1999; Hu and Chen, 2010): pH, temperature, electric and magnetic fields, mechanical stress, enzyme substrate, agents and chemical solvents, electromagnetic radiation, sonic, biochemical agents.

A number of adaptive materials as: hydrogels-type, particles-type, surface appearance memory polymers-type, smart fiber /films-type, smart textiles-type are known. Some cyclodextrin and chitosan derivatives and copolymers are also part of adaptive materials category.

Energy that mediates the answer received by adaptive material may be: non-covalent interactions type, hydrophobic-type, coulomb forces-type, hydrogen bonding-type, van de Waals-type.

Change Surface Appearance Polymers

- If a material is under the influence of a stimulus like: heat, light, pH, it deformes, and remain in that form for a limited period of time, then returns to its original shape. The applications are numerous in terms of smart textiles, some adaptive facilities, sensors and actuators;

- If a material is subjected to a thermal stimulus, mechanical, electrical, magnetic, it will record a variation in technical parameters such as surface appearance, position, strength, friction etc.

In general, the architecture of such a polymer comprises two basic components, namely: “switch” and “network node”.

The “switch” can be represented by: various limit states, such as crystallization, amorphous, liquid-crystalline phase, supramolecular hydrogen bonds, reversible networks, nano-composite filtration networks;

Network node can be defined by: physical bonds, chemical bonds, and other polymeric bonds (Hu and Chen, 2010).

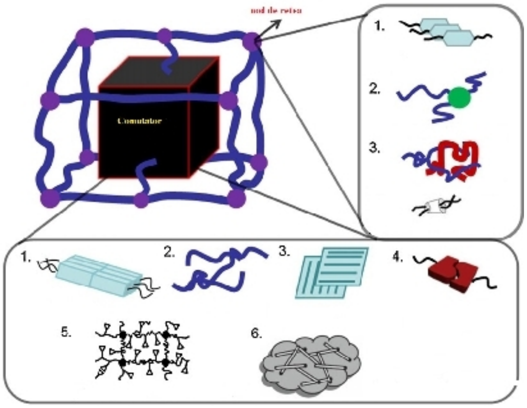

Figure 1. The drawing of the molecular structure of such polymers

Adaptive Hydrogels

- Adaptive hydrogels are polymers that swell in the presence of water and are characterized by 3D shape. The most common applications are related to the temperature and pH stimuli adaptive hydrogels.

Adaptive Particles

- Adaptive particles are those polymers with a spherical shape that under the action of stimuli like: light, heat, temperature, pH, chemical, occurs specific changes. They can be found in the form of powder, suspension, etc. The changes that are frequently targeted are lateral surface and the size of these nanoparticles.

ADAPTIVE TEXTILE TYPES

Adaptable polymers for textiles define groups with specific functionalities, namely: adaptive textiles for thermoregulation with memory shape change, color changing capability, luminescent, conductive.

Phase Change Materials – Materials that Change its State

Use of phase change materials for thermal energy storage has been practiced for several years. Setting the temperature according to body and environment temperature is achieved using phase change materials (PCM - bifunctional microcapsules).

There are two major application categories of phase change materials, namely, temperature control applications (consisting in maintaining an environment temperature at a constant value) and of heat or cold storage applications. Heat can be delivered or extracted from a phase change material without a major change in temperature. The materials capable to alter the state are substances that absorb or release significant amounts of energy during melting process, solidification or sublimation. These materials absorb energy during the heating process when the phase change takes place and in case of cooling process energy can be transferred to the environment while returning to the initial phase (Bajaj, 2001).

Figure 2. Drawing of the operating principle of phase change materials (Phase Change Materials Panels and Balls, 2016)

As materials for storing and reusing the latent heat, inorganic salts and mixtures of salts and paraffin can be used. The effect of thermal barrier for a fabric depends on caloric storage capacity of PCM microcapsules, their quantity and structure of the textile support. When using paraffin, the energy type is the phase change latent heat, stored heat is 200 kJ / kg and the temperature may vary in the range 5°C - 30°C.

The phase change fibers or yarns are used in manufacturing of products for sporting activities, protective equipment for home textile or medical fields.

Shape Memory Materials - Material that Store Shape

These materials are defined as materials that are able to “remember” its original shape and can return to it. The effect is due to thermal or magnetic changes (Smart Technologies for stress free AiR Travel - AST5-CT-2006-030958, 2006). The term for materials that store shape include four classes, namely: alloys (SMA), ceramics (SMC), polymers (SMP) and ferromagnetic alloys (FSMA) differentiated by material type / nature and reaction stimulus type. Those that are used in textile field are the SMP class where everything is based on two polymer properties - elasticity and plasticity. The memory effect consist of the ability of the material to resume form, before the plastic deformation, by simply changing the temperature.

The polymer matrix contains two components, one with higher melting temperature (Cook *et al*., 2005) that can turn into fiber by wet, dry or melting spinning process, or by electrospinning. The fibers can be processed in order to obtain woven, knitted, nonwoven or braided structures (Gök *et al*., 2015), with a broad potential application in the field of protective clothing and medicine (implants, sutures).

Materials Capable of Changing Color - Chameleon

The ability to reversible change the color (due to change optical properties) in according with external environment in response to application of an electric field or electromagnetic, is characteristic of these materials. In accordance with scientific literature, the electro-chromic and electro-luminescence are two directions that can cause color change. Some organic, inorganic or polymeric materials present excellent electrochromic properties. Depending on the energy of external stimulus under the chromic materials react, they are classified as is Table 1:

Table 1. Chromic materials

Framing group	External stimuli
Photochromics	light
Thermochromics	heat
Electrochromics	electricity
Piezochromics	pressure
Solvatochromic	lichid
Carsochromic	electron flux

Chromatic materials are mainly a source of inspiration for designers of clothing that can change color depending on the amount of incident light.

Luminescent Materials

Luminescence is the emission of photons (light) generated when electrons back from the state of excitation to a lower energy state.

The difference between chromic and luminescent materials is that while the first group changes color, the second group emits light (Lakowicz, 2006).

Depending on the nature of the stimulus the following luminescent materials categories can be found:

- *Photo luminescence*: external stimulus is light, we have photo luminescent organic or mineral materials; they are used in the textile industry clothing (clothing destined for activities in nightclubs), or take the safety labels used to detect imitations/fakes; Phosphorescent materials (light emission continues after the period of existence of fluorescence) can be used for protective equipment in case of poor visibility conditions.
- *Electro luminescence*: include inorganic semiconductors, polymers, and small organic molecules; electricity is external stimulus; appropriate LEDs.
- *Chemical luminescence*: chemical reaction (oxidation reaction) is external stimulus
- *Optical luminescence:* it is common in optical fibers widely used in textile sensor role in monitoring the body or environment conditions.

Materials with Conductive Properties

Electrical conductivity of textiles is a field-leading research. Clothing electrical conductive properties - “smart clothing” -uses special textiles or electronics integrated into the textile structure. For producing structures for conductive textile fibers and yarns, metal wires that contain certain levels of inorganic conductive material or conductive films can be used. As fields of use we can mention: professional health monitoring, sports and leisure activities, everyday life.

CONCLUSIONS

Currently new generation materials functionalized materials for passive, active, bio-intelligent are those that respond to the scope of use that are fundamentally designed to meet the demands of smart devices and are environmentally friendly. Adaptable polymers show an area of particular interest in terms of innovative design, the structures and the properties and potential application areas. Textiles are a viable way to introduce reliable in everyday life and in technical areas, these new generations of special polymers.

Acknowledgement

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REFERENCES

Bajaj, P. (2001), Thermally sensitive materials, in X. Tao (ed.), *Smart Fibres, Fabrics & Clothing*, 58.

Cook, F.L., Jacob, K.I., Polk, M. and Pourdeyhimi, B. (2005), “Shape Memory Polymer Fibers for Comfort Wear”, *National Textile Center Annual Report*.

Galaev, I.Y. and Mattiasson, B. (1999), “Smart Polymers and That They Could Do in Biotechnology and Medicine”, *Trend in Biotechnology*, 178(8), 335-340.

Gök, M.O., Bilir, M.Z. and Gürcüm, B.H. (2015), “Shape-Memory Applications in Textile Design”, *Procedia - Social and Behavioral Sciences*, 195, 2160 – 2169.

Hoffman, A.S. and Stayton, P.S. (2004), “Bioconjugate of Smart Polymers and Proteins”, *Brazillian Polymers Conference*, 207/1, 139-152, doi:10.1002/masy.200450314.

Hu, G.L. and Chen, S.J. (2010), “A review of actively moving polymers in textile applications”, *Journal of Materials Chemistry*, 20, 3346-3355.

Lakowicz, J.R. (2006), *Principles of Fluorescence Spectroscopy - Third Edition*, Springer Science+Business Media, LLC.

*** (2016), “Phase Change Materials Panels and Balls”, available from Lizoo: http://www.lizoo.cn/?p=5537.

*** (2006), Smart Technologies for stress free AiR Travel - AST5-CT-2006-030958, available from European Commission - Research & Innovation - Transport: http://ec.europa.eu/research/transport/projects/items/seat_en.htm.

VALIDATION OF METHOD FOR 4-CHLOROANILINE AND 4,4'-BI-O-TOLUIDINE FROM DYED LEATHER

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Toxicity assessment of leather products designed to obtain leather goods must be in accordance with legislation and environmental standards which require the use of chemicals from semi-finished leather processing. Azo dyes will be checked for two banned aromatic amines (4-chloroaniline and 4,4'-bi-o-toluidine) which can be found as a result of splitting azo dyes used to dye the leather. The method will be validated by gas chromatography (GC-MS) and the following parameters will be tested: accuracy (trueness and reliability), precision, sensitivity, detection limit, limit of quantification, working range, linearity.

Keywords: Validation method, azo dyes, leather, 4-chloroaniline, 4,4'-bi-o-toluidine

INTRODUCTION

The most toxic products, mutagens, carcinogens (Eurachem Guide, 1998) were the first to have alarmed European Union and imposed publication of several directives that prohibit or limit the use of these chemicals (Directive 99/51/EC on pentachlorophenol, Directive 2002/61/EC banning azo dyes, Directive 2009/563/EC on eco-labelling footwear, Directive 2010/75/EU on industrial emissions in tanneries, REACH).

Azo dyes account for about 90% of all dyes (Eurachem Guide, 1998) used for finishing leather and textiles. Azo dyes decompose under reductive conditions to form aromatic amines, derivatives of benzidine. 22 aromatic amines are banned in the EU Regulation 552/2009, and 2 more were added in 2015.

EU Regulation provides test method for each type of substrate and defines the detection limit of 30 mg/kg for each amine that may be found in the leather and therefore this is the limit. Other aromatic amines from leather were investigated (www.cdc.gov; Huber, 2010; Chelaru *et al.*, 2016). The objective of this study is to identify the presence of 4-chloroaniline, 4,4'-bi-o-toluidine used in dyed leather designated for clothing manufacture.

MATERIALS AND METHOD

Materials

Methanol; T-butyl methyl ether; Sodium dithionite, minimum purity 87%; Aqueous solution of sodium dithionite, 200 mg/ml, prepared daily; N-hexane; Amines; Methanolic sodium hydroxide 20% (w/v), 20 g of NaOH dissolved in 100 ml of methanol; Distilled water - grade 3 according to SR EN ISO 3696: 2002 - Water used for analytical laboratories. Specifications and methods of analysis.

Method

Testing method for leather is established by EN ISO 17234-1:2015: Chemical tests for the determination of certain azo colorants in dyed leathers - Part 1 - Determination of certain aromatic amines derived from azo colorants.

RESULTS

The studies were performed on bovine hides experimentally dyed in the laboratory obtained with known concentrations of amine in the study (4-chloroaniline and 4,4'-bi-o-toluidine).

4-chloroaniline

4-chloroaniline (CAS 106-47-8) is part of chlorinated organic compounds. The chemical formula (linear form) is $ClC_6H_4NH_2$ and structural form is displayed in Figure 1.

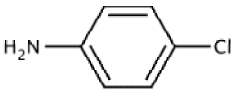


Figure 1. 4-Chloroaniline

4-Chloroaniline is an important compound used in the chemical industry for the production of leather dyestuffs, pesticides and drugs. 4,4' bi-o-toluidine is very toxic, possibly carcinogenic, absorbed through skin (www.cdc.gov). There was a total of 10 specimens of each individual skin sample treated with one of three amines. For each specimen the steps in SR EN ISO 17234-1: 2015 were followed (Table 1).

Table 1. Information about tested component

Component	Concentration	No. of analyses
4-chloroaniline	200 µg/ml	10

Table 2. GC-MS and concentration integrated values

Integrated mass units	Det. (µg/ml)	Integrated mass units	Det. (µg /ml)
23159019	205,64	19992053	203,13
22909319	205,44	19847365	203,01
23659174	206,04	20579603	203,59
19778586	202,96	20077222	203,19
21984956	204,71	22441986	205,07

Table 3. Values for accuracy formula

Accuracy	Domain	Value
Accuracy % = $\frac{X_{average}}{\mu}100$	90-110%	102.14
Bias % = $\frac{X_{average}-\mu}{\mu}100$	-	2.14 %

Table 4. Values for fidelity formula

Fidelity	Domain	Value
CV (RSD) % = $\frac{s}{X_{average}}\times 100$	2-20%	0,58%

Table 5. Values for repeatability formula

Repeatability	Domain	Value
r = 2.8 x s_r	-	3.42 µg/ml

Linearity Domain

Table 6. GC-MS and concentration integrated values for linearity domain

Integrated mass units	(det., µg/ml)
21236364	50
55503678	100
204380880	200

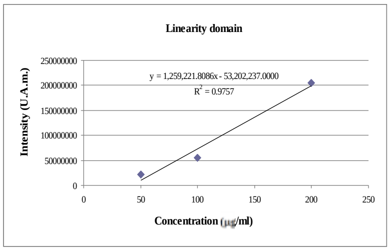


Figure 2. 4-chloroaniline linearity domain

The form of linear regression equation function:

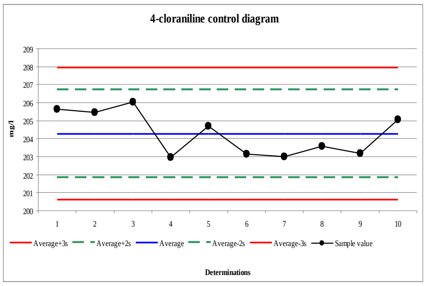
$y = 1259221.88x – 53505237$
b= slope calibration

b= 1259221,88 areas units x μg^{-1}

$R^2 = 0.9757$

Table 7. Values for detection, quantification limit and work domain formula

	Formula	Value
Detection limit	LoD = 3s	3.67 µg /ml
Quantification limit	LoQ = 10 s	10.22 µg/ml
Work domain		
Lower limit	LI = 50 µg/ml	
Upper limit	LS = 200 µg/ml	



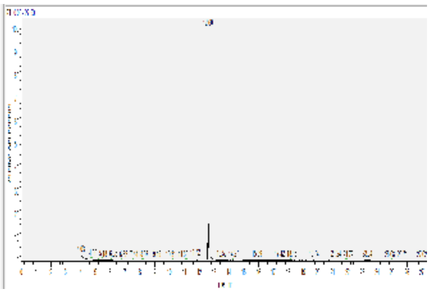


Figure 3. 4-chloroaniline control diagram

Figure 4. 4-chloroaniline GC-MS chromatogram

4,4'-bi-o-toluidine

4,4'-bi-o-toluidine (CAS 119-93-7) is part of organic compound. The chemical formula (linear form) is $(C_6H_4(CH_3)NH_2)_2$ and structural form is displayed in Figure 5.

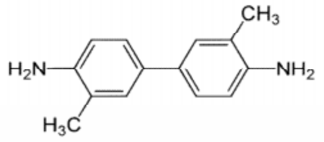


Figure 5. 4,4'-bi-o-toluidine

4,4'-bi-o-toluidine is an intermediate for the production of soluble azo dyes and insoluble pigments used particularly in the textile, leather and paper industries. It is toxic and possibly carcinogenic. It is listed as an IARC Group 2B carcinogen, meaning it is "possibly carcinogenic to humans". Animal studies have shown that animals exposed to 4,4'-bi-o-toluidine developed tumors in the liver, kidney, and mammary glands (www.cdc.gov).

Table 8. Information about tested component

Component	Concentration	No. of analyses
4,4'-bi-o-toluidine	200 µg/ml	10

Table 9. GC-MS and concentration integrated values

Integrated mass units	(det., µg/ml)	Integrated mass units	(det., µg /ml)
1572098500	200,84	1392618607	178,51
1497543548	191,56	1377947260	176,56
1569498199	200,51	1285159747	165,15
1492700267	190,96	1302565826	167,31
1466693430	187,72	1258529985	161,83

Table 10. Values for accuracy formula

Accuracy	Domain	Value
Accuracy % = $\frac{X_{mediu}}{\mu} 100$	90-110%	91.05
Bias % = $\frac{X_{mediu} - \mu}{\mu} 100$	-	-8,95 %

Table 11. Values for fidelity formula

Fidelity	Domain	Value
CV (RSD) % = $\frac{s}{X_{mediu}} \times 100$	2-20%	7.87%

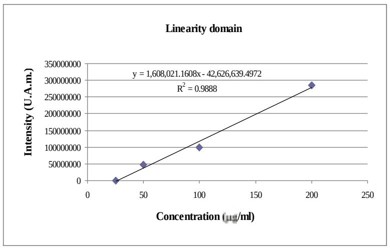
Table 12. Values for repeatability formula

Repeatability	Domain	Value
r = 2.8 x s_r	-	40.15 µg/ml

Linearity Domain

Table 13. GC-MS and concentration integrated values for linearity domain

Integrated mass units	det., (µg/ml)
12882.326	25
47825938	50
98829960	100
285832597	200



The form of linear regression equation function:

$$y = 1608021.1608x – 42626639.4972$$

b= slope calibration

b= 1608021.1608 areas units x μg^{-1}

$R^2 = 0.9888$

Figure 6. 4,4'-bi-o-toluidine linearity domain

Table 14. Values for detection, quantification limit and work domain formula

	Formula	Value
Detection limit	LoD = 3s	4.45 µg /ml
Quantification limit	LoQ = 10 s	14.83 µg/ml
Work domain		
Lower limit	LI = 50 µg/ml	
Upper limit	LS = 200 µg/ml	

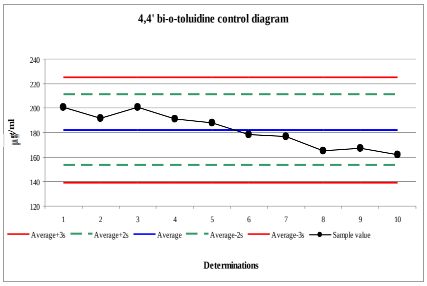


Figure 6. 4,4'-bi-o-toluidine control diagram

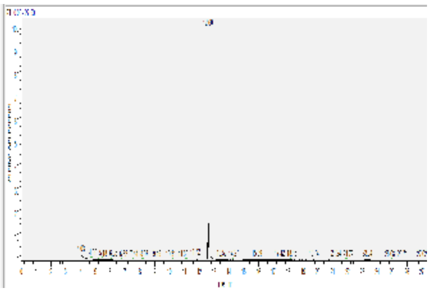


Figure 7. 4,4'-bi-o-toluidine GC-MS chromatogram

CONCLUSION

This paper presents a study for the implementation of gas chromatographic instrumental method for determination of aromatic amines derived from azo colorants used for dying leather and its validation. Official testing method is EN ISO 17234-1:2015: Chemical analysis for the determination of certain azo colorants in dyed leathers - Part 1 - Determination of certain aromatic amines derived from azo colorations.

From the calculation method performance parameters we can see the following results:

- Linearity range of the method is between 25-200 µg/ml range in which the correlation coefficient was in the range 0.9757 to 0.9888. For a better linearity, correlation coefficient must be between 0.9800 and 1.0000.
- Detection limit of the amines ranges from 3.67 µg/ml and 4.45 µg/ml;
- The limit of quantification of the amines ranges from 10.22 µg/ml and 14.83 µg/ml;
- The accuracy is 91.05% and 102.14% and is approximation of the actual value and the value found in the sample. For the gas chromatograph method for the performance must be within 85-110%;

Validated analytical method for the determination of aromatic amines in leather meets all the conditions required to be used and applied.

Acknowledgment

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REFERENCES

Chelaru, C., Macovescu, G., Crudu, M., Gurău, D. (2016), "Validation of Method For Identifying Aromatic Amines From Dyed Leather", *Leather and Footwear Journal*, 16(3), 185-199.

Eurachem Guide (1998), The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics, LGC, Teddington, Middlesex, UK.

Huber, L. (2010), *Validation of Analytical Methods*, Agilent Technologies, Germany.

ISO 11843-6:2013, Capability of detection -- Part 6: Methodology for the determination of the critical value and the minimum detectable value in Poisson distributed measurements by normal approximations.

*** www.cdc.gov/niosh/ipcsneng/neng0026.html, CDC - NIOSH Pocket Guide to Chemical Hazards.

*** www.cdc.gov/niosh/npg/npgd0618.html, CDC - NIOSH Pocket Guide to Chemical Hazards.

STATISTICAL METHOD FOR DIMENSIONAL ANALYSIS OF MICRO/NANOPARTICLES DEPOSITED ONTO TEXTILE SUBSTRATES

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In this work it was developed a morphological analysis method of nano- and microparticles deposited on the textile substrate. This method is based on the statistical analysis of their dimensions measured by SEM (scanning electron microscopy). Towards this development, it was used a knit surface treated with TiO_2 – N – Fe (treatment that gives photocatalytic, antibacterial and antifungal skills). The method consists in the processing of the SEM images (previously obtained) by the insertion of labels with dimension of nano/micro- particles and the calculation of statistical parameters of values obtained. It was processed 10 SEM images of the same material from different places investigated at same magnification (in this case 8000 X), each of these images being processed in software Scandium 5.0. In each image it was measured the dimension of 10 micro/nano- particles. The requirement of a large number of dimensional measurements is because the size of the constituent particles in surface treatments, generally, has some variability. The major advantage of using Scandium 5.0 software is that it calculates automatically the statistical parameters of the set of measured values for all 10 images (average, minimum, maximum, number of values, standard deviation).

Keywords: SEM, statistic, dimension.

INTRODUCTION

Nanoparticles are particles with at least one dimension smaller than 1 micron and potentially as small as atomic and molecular length scales (~0.2 nm). Nanoparticles can have amorphous or crystalline form and their surfaces can act as carriers for liquid droplets or gases. Examples of materials in crystalline nanoparticle form are metal-oxide like TiO_2, CuO, ZnO, AgCl, CuO. Nanoparticulate matter should be considered a distinct form of matter organisation, due to its distinct properties (large reactive surface area and quantum size effects). Many authors limit the size of nanomaterials to 50 nm or 100 nm, the choice of this upper limit being justified by the fact that some physical properties of nanoparticles approach those of bulk when their size reaches these values. However, this size threshold varies with material type and cannot be the basis for such a classification. A legitimate definition extends this upper size limit to 1 micron (micrometer), the sub-micron range being classified as nano. Consequently, this mean the particles with dimensions higher or equal than 1 micrometer are named “microparticles”.

We have developed a morphological analysis method of nano- and microparticles deposited on the textile substrate. This method is based on the statistical analysis of their dimensions measured by SEM (scanning electron microscopy).

In order to improve the dimensional accuracy of these measurements, simultaneously, the calibration of electron microscope was performed with a standard multiscale purchased. This calibration was necessary to guarantee the quality of dimensional measurements (lengths) at several orders of magnification. This standard, produced by Christine Gröpl, is intended for use in calibration of dimensional measurements for a wide range of magnification orders, being a silicon wafer lithographed with a network of lines located from each other at distances in range of 100÷2500 nm.

In this work it was used a knit surface treated with TiO_2-N-Fe (treatment that gives photocatalytic, antibacterial and antifungal skills).

This method consists in the processing of the SEM images (previously obtained) by the insertion of labels with dimension of nano/micro-particles and the calculation of statistical parameters of values obtained. This processing of SEM images was performed with a specialized software called Scandium 5.0.

STATISTICAL METHOD

Next, it will be described the statistical analysis method developed in this work, using as example the material marked 'T4 (a II-a tratare)', which is a fabric treated with micro/nano-particles of TiO_2-N-Fe.

In figures 1÷10 are shown the SEM images obtained at same magnification (8000X), which were obtained using scanning electron microscope FEI Quanta 200, before processing them:

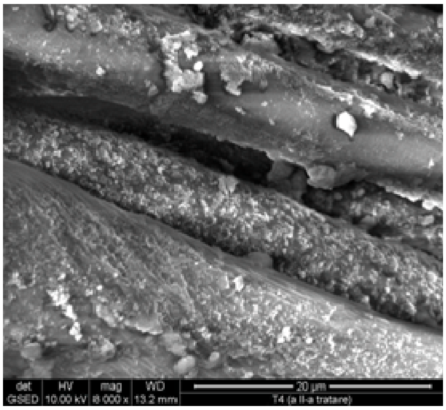

Figure 1. Unprocessed SEM image (no.1)

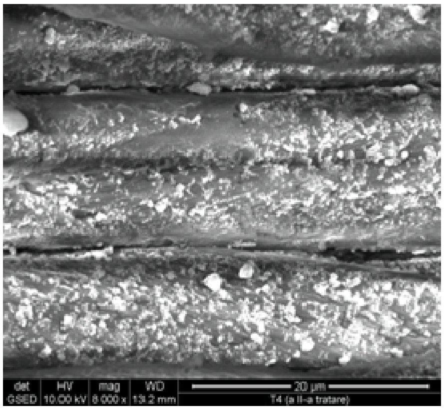

Figure 2. Unprocessed SEM image (no.2)

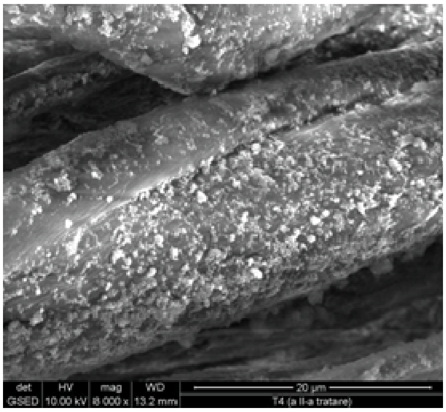

Figure 3. Unprocessed SEM image (no.3)

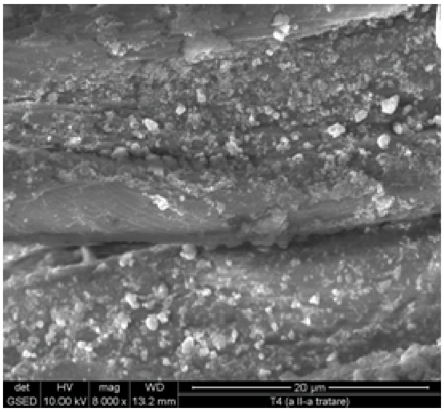

Figure 4. Unprocessed SEM image (no.4)

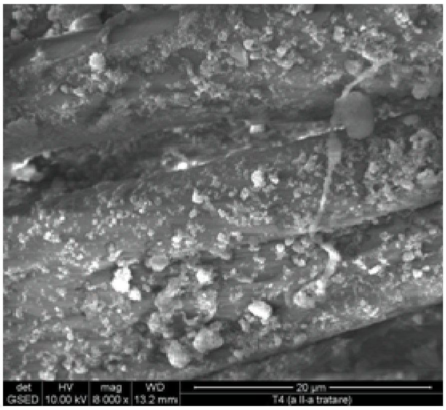

Figure 5. Unprocessed SEM image (no.5)

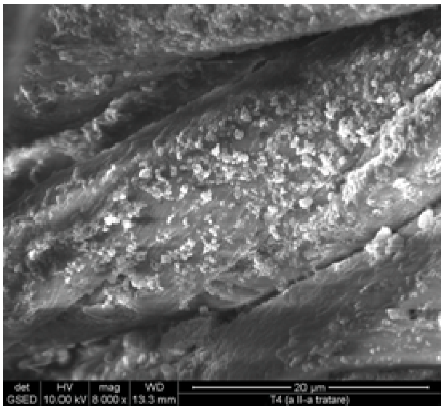

Figure 6. Unprocessed SEM image (no.6)

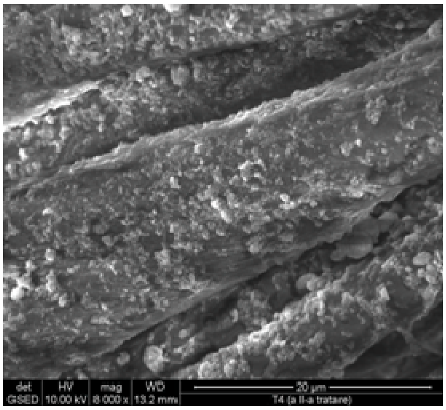

Figure 7. Unprocessed SEM image (no.7)

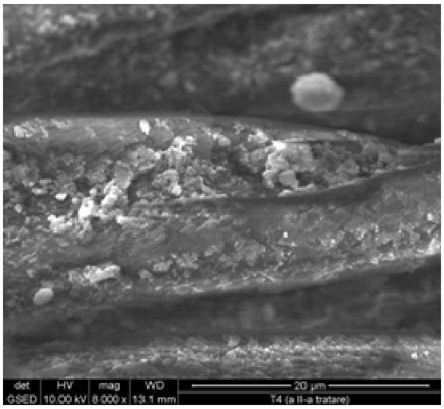

Figure 8. Unprocessed SEM image (no.8)

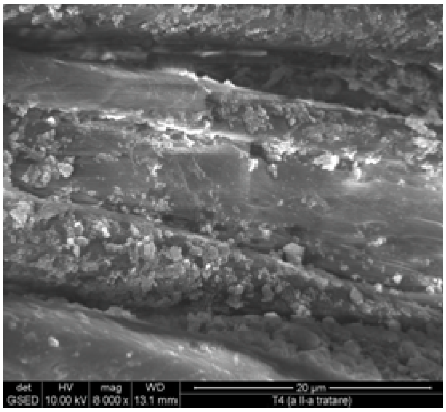

Figure 9. Unprocessed SEM image (no.9)

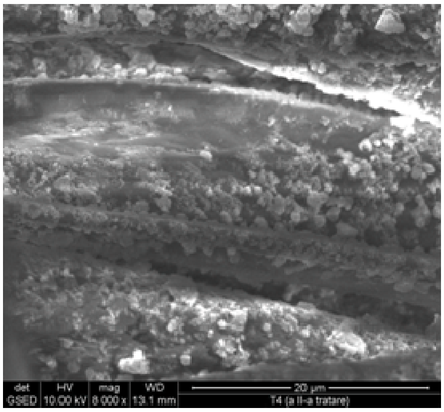

Figure 10. Unprocessed SEM image (no.10)

After the purchase of 10 SEM images of the same material from different places investigated at same magnification (in this case 8000X), each of these images is processed in software Scandium 5.0, measuring within each image the dimension of 10 micro/nano-particles.

The measure unit of particle size must be the same for all measurements of each image (in this case was chosen micrometer – μm).

The requirement of a large number of dimensional measurements is because the size of the constituent particles in surface treatments, generally, have some variability. In this work were made 100 measurements (10 images x 10 measurements) onto the same textile material.

In figures 11÷20 are shown the SEM images after processing in the software Scandium 5.0 (the screenshots of application window used are shown):

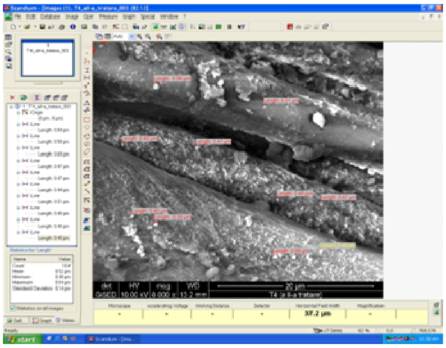

Figure 11. Processed SEM image (no.1)

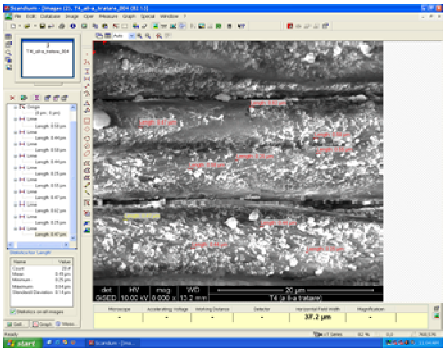

Figure 12. Processed SEM image (no.2)

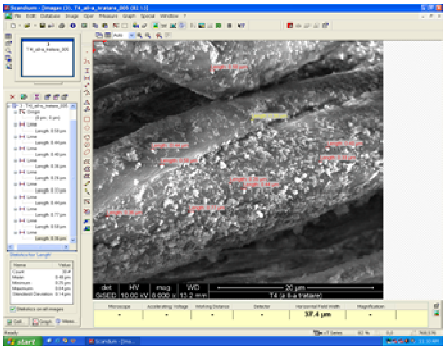

Figure 13. Processed SEM image (no.3)

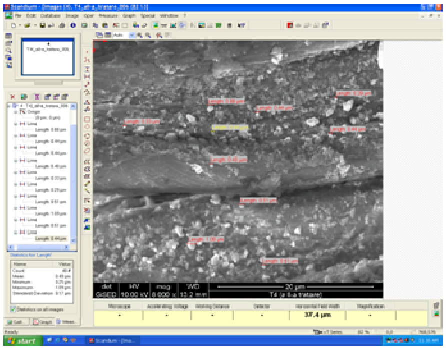

Figure 14. Processed SEM image (no.4)

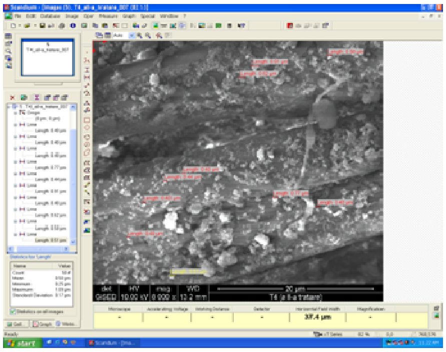
Figure 15. Processed SEM image (no.5)

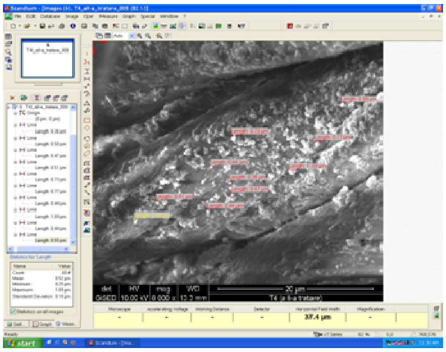
Figure 16. Processed SEM image (no.6)

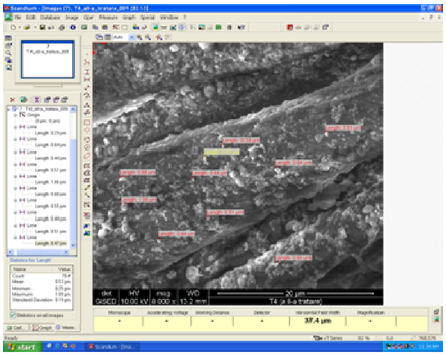
Figure 17. Processed SEM image (no.7)

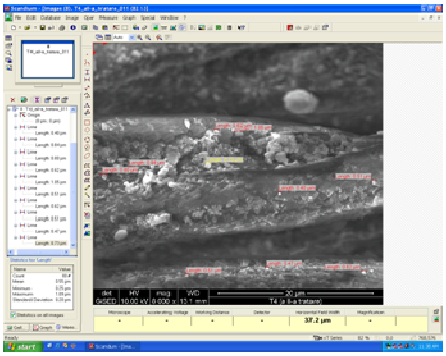
Figure 18. Processed SEM image (no.8)

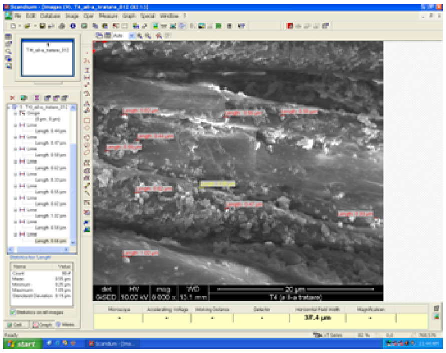
Figure 19. Processed SEM image (no.9)

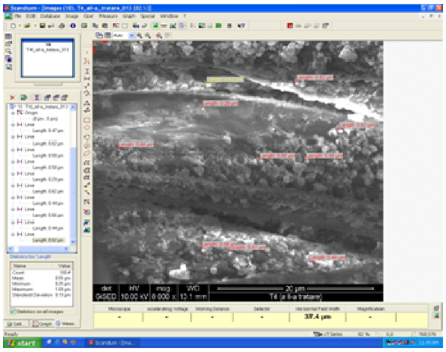
Figure 20. Processed SEM image (no.10)

The major advantage of using Scandium 5.0 software is that it calculates automatically the statistical parameters of the set of measured values for all 10 images (average, minimum, maximum, number of values, standard deviation). The statistical parameters determined by the software are displayed in the bottom-left corner of the application window.

In this work, the software calculated for all 100 values of the particle size (dimension), the following values of the statistical parameters (Table 1):

Table 1. Statistical parameters

Statistical parameter	Value of statistical parameter
1. Total number of measurement values	100
2. Average of measurement values	0.55 μm
3. Minimum of measurement values	0.25 μm
4. Maximum of measurement values	1.09 μm
5. Standard deviation of measurement values	0.19 μm

These values of statistical parameters listed in Table 1 (for the 100 measurement values) are displayed in the window which corresponds with tenth processed image (in the screenshot from Figure 20). The reason is that the software updates the values of statistical parameters at each measurement added in the current image imported into Scandium 5.0. For this reason, the final result of the statistical calculation for the whole set of measurements is displayed in the window that contains the latest SEM image imported into software.

As shown in Figures 11÷20, dimensional measurement values are inserted in the SEM images imported. The operator is who chooses formations (particles) to which measured size. The formations (particles), which it is measured size, are chosen by the operator.

CONCLUSIONS

This statistical analysis of the dimension of particles from textile surface treatments, is useful to evaluate the grade of variability of particle sizes. Also, this method serves for categorizing the type of particles (nanoparticles or microparticles) from treatments investigated. In case of this work, by statistical point of view, the particles analysed may be considered nanoparticles (average and standard deviation of sizes is lower than 1μm).

Acknowledgements

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REFERENCES

FEI Company (2005), *Quanta Training Course,* FEI Company, Eindhoven (Netherlands).

Buzea, C., Pacheco, I.I., and Robbie, K. (2007), “Nanomaterials and nanoparticles: Sources and toxicity”, *Biointerphases*, 2(4), MR17 - MR172.

INFLUENCE OF THE HEAT BONDING ON AIR PERMEABILITY

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Usually, in footwear technology the upper materials are backed with heat bonding textile materials in order to increase the tensile and tear strength and the thermal resistance of the entire structure due to higher thickness. The paper studies the effects of the backing with heat bonding textiles on air permeability, an important comfort characteristic. The influence of the heat bonding with lining on the air permeability was studied using 5 types of bovine leather for upper materials and 4 types of knitted and woven fabrics for lining. The method to determine the air permeability is relatively simple and known. The air flow passing through the sample fixed on the device with the face upward is measured for certain values for the pressure difference. The conclusion drawn from the experiment is somewhat surprising: the air permeability increases with the thickness. This contradicts the general behaviour of textile structures, for which the air permeability decreases with thickness.

Keywords: footwear, air permeability, thermo-adhesive lining.

INTRODUCTION

The use of heat bonding is extending, due to the development of new materials with different physical, chemical and mechanical properties, as well as a large range of synthetic adhesives. The welding gives the structure an increased resistance to weather conditions and different chemical agents. This thermal joining was first called laminating.

Regardless of the position on the human body, the process requires a ground material and a material with thermal adhesive characteristics that is called reinforced lining. The upper material is bonded with the reinforced lining in order to obtain improved tensile and tear strength and, according to experimental data, also air permeability.

MATERIALS AND METHODS

The air permeability of a material or a system is defined as the amount of air passed over a surface under a certain pressure difference in a unit time. Generally, the pressure difference between layers is 0.2 to 20 mm water column.

The air permeability is calculated with the following relation:

$$P_a = \frac{V}{t \cdot A}\text{, (m}^3\text{/m}^2\text{.min;l/m}^2\text{.s)} \qquad (1)$$

Where:
V – volume of air passing through the sample under a preset difference of pressure (m^3; mm water column; l);
t – duration of air passing, (min, s);
A – sample surface (m^2).

The ratio between the volume of air V (m^3 or litres) and the time when the air flows through the material t (minutes or seconds) defines the air flow q (l/h).

The influence of the heat bonding with lining on the air permeability was studied using 3 types of bovine leather for upper materials and 4 types of knitted and woven fabrics for lining, presented in Table 1.

Table 1. Materials

	Material	Code
Upper materials	Embossed leather	A3
	Box with corrected grain	A4
	Buff leather	A5
	Thermo-adhesive knitted fabric	C3
Linings	Thermo-adhesive knitted fabric	C5
	Thermo-adhesive woven fabric	C6
	Thermo-adhesive woven fabric	C9

The two materials, the upper and the lining were cut and then welded using a heat bonding press and then cut to sample size.

The parameters of bonding proccess are the temperature, between 140-160°C, 4 atm pressure and the time of pressing around 6 – 8 seconds. Those parameters are setted in according with the adhesive caracteristics and the finishing of leather surface. The adjustment of bonding parameters must be set in optimum limits, because otherwise can damage the color and even the structure of the leather.

It is important to have a perefect jointting structure between leather and reinforcement lining, flexible and also resistant. The unsoldering resistance should be between 0,3 – 0,5 N/mm^2.

The method to determine the air permeability is relatively simple and known. The air flow passing through the sample fixed on the device with the face upward is measured for certain values for the pressure difference. In this case, the air flow was measured for a pressure difference of 20 mm water column. For each material, the air permeability was measured three times.

RESULTS AND DISCUSSIONS

Table 2 presents the average values for the experimental data.

Table 2. Air permeability

Code	q (l/h)	A (cm^2)	P_{ai}=q/(6*A) (m^3/min.m^2)
A3	10	20	0.083333333
A3+C3	34	20	0.283333333
A3+C5	18	20	0.15
A3+C6	21	20	0.175
A3+C9	15	20	0.125
A4	3	20	0.025
A4+C3	28	20	0.233333333
A4+C5	11	20	0.091666667
A4+C6	15	20	0.125
A4+C9	8	20	0.066666667
A5	6	20	0.05
A5+C3	38	20	0.316666667
A5+C5	24	20	0.2
A5+C6	24	20	0,2
A5+C9	20	20	0,166666667

Generally, the materials used for leather products have low air permeability, but the values can be improved through heat bonding. Figures 1 to 3 present the variation of the air flow for different upper leather bonded or not with reinforced linings.

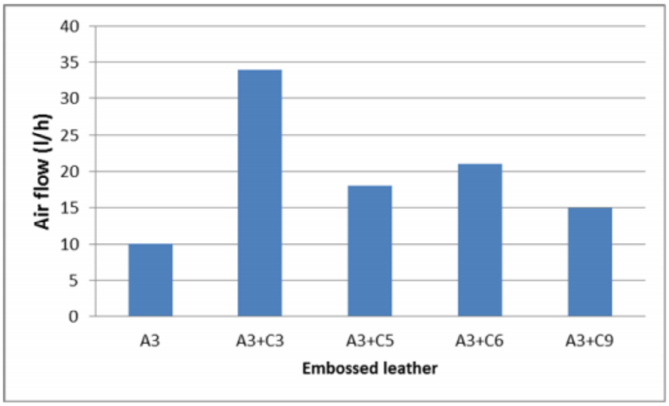

Figure 1. The air flow for embossed leather

The structures resulting from the embossed leather and different types of the reinforced lining present a major increased air flow. For structure A3+C9 the air flow was improve with 50% and in case of structure A3+C3 up to 240% more than A3.

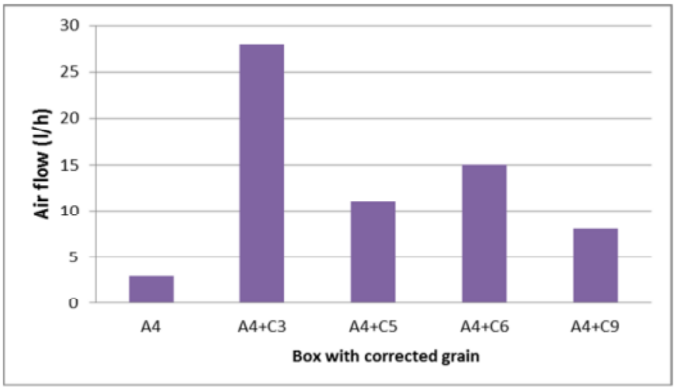

Figure 2. The air flow for box with corrected grain

Those structures obtain from box with corrected grain and reinforsed lining present an increase of the air flow with over 800% for A4+C3 and around 160% for A4+C9. For this upper structure was obtain the most important increase of the air permeability for all type of used linings.

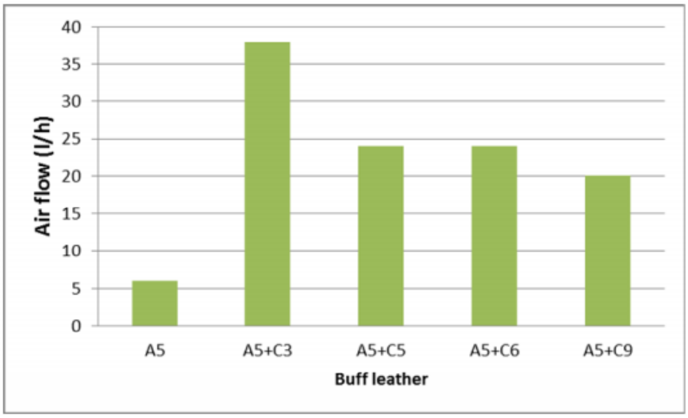

Figure 3. The air flow for buff leather

Like in previous two cases, the air flow is remarkable improved by the heat bonding. In this case, the structure A5+C3 has an air flow with over 500% more than A5 and with more than 200% for A5+C9.

The discussion was making only for the structures with biggest or the lowest increase of the air flow after heat bonded with reinforced lining: C3 has the best results and C9 has the worst results. This mean, the air permeability depends of type of lining (knitted or woven fabric), thickness and design of fabric.

In conclusion, the permeability of the leather variants is considerably improved by heat bonding with a jersey knitted fabric, thickness 0.735 mm.

The graphics presented in Figs. 4 to 6 and the regression equations in Table 3 show relation between air permeability and thickness of the structures.

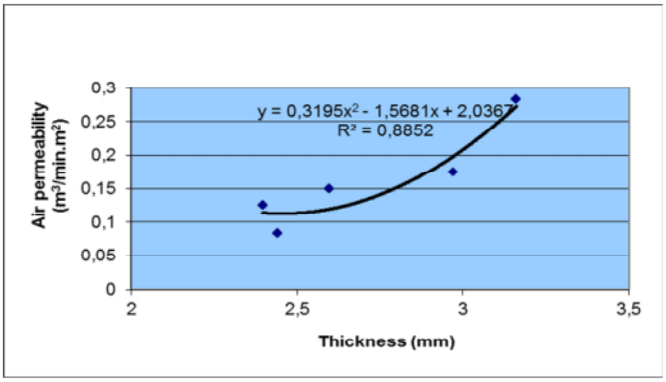

Figure 4. The air permeability for embossed leather

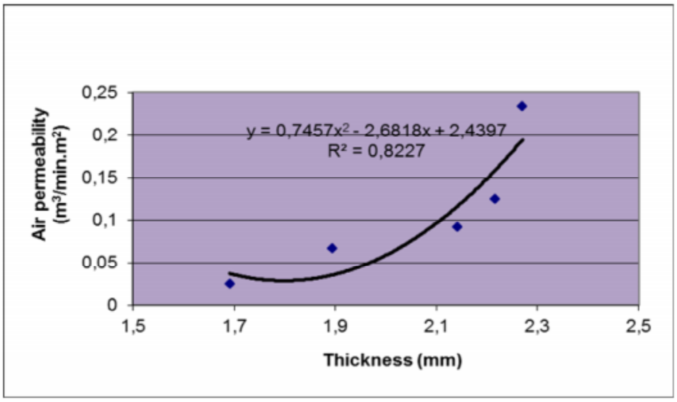

Figure 5. The air permeability for box with corrected grain

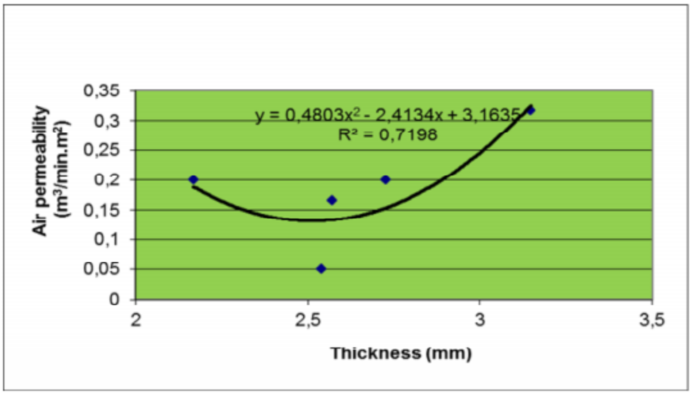

Figure 6. The air permeability for buff leather

The regression equations presented in Table 3 are polynomial functions and the values of the correlation coefficient are close to 1, indicating a strong correlation between the two parameters.

Table 3. Regression equation and correlation coefficient

Regression equation	Correlation
$y = 0.3195x^2 - 1.5681x + 2.0367$	$R^2 = 0.8852$
$y = 0.7457x^2 - 2.6818x + 2.4397$	$R^2 = 0.8227$
$y = 0.4803x^2 - 2.4134x + 3.1635$	$R^2 = 0.7198$

The conclusion is important and surprising: the air permeability increase with thickness and heat bonding linings. It is surprising because it is against of the low of the textile structures and against of the general opinion that the heat bonding afect in a negative way the permeability of the upper.

CONCLUSIONS

This paper investigates the effects of the backing with heat bonding textiles on air permeability and the results can be concluded there by:

1. When analysing the effects of the thermal treatment and pressure on the air permeability it can be concluded that the heat bonding of upper leather parts increase it.
2. A strong correlation was determined for all experimental variants between air permeability and the overall thickness.
3. The conclusion drawn from the experiment is somewhat surprising: the air permeability increases with the thickness. This contradicts the general behaviour of textile structures, for which the air permeability decreases with thickness. In this case, the thermal process associated with heat bonding causes the contraction of the entire structure and therefore increases porosity.

REFERENCES

Dragomir, A. (2012), *Foot comfort*, Performantica Publishing, Iaşi, Romania.

Dragomir, A., Mitu, S. and Conachi, A. (2004), "Estimation of covering surface with thermoadesives of the interlinings", *1st International Student Conference*, *LEATHER, FOOT & SHOES*, Zlim, The Czech Republic.

Mitu, S. and Dragomir, A. (2002), "Method of calculating the thermoadhesive covering surface for materials", *The XII Romanian Textiles and Leather Conference*, Structures and Technologies in Leather Goods, Performantica Publishing, Iaşi.

SIMSANO, (2007), *Structural-functional analysis of footwear that compensates for locomotor deficiencies and prevents foot diseases*, PARTNERSHIPS program, Contract no. 41064/2007 – stage I.

*** (2007), *Theoretical and experimental research on factors influencing comfort of products and systems made of leather and substitutes*, stage I, Project PN II-RU-TD 476/2007.

PHOTOCATALYTIC PERFORMANCES OF TEXTILES COATED WITH GRAPHENE OXIDE/TIO$_2$ NANOCOMPOSITES – PART 1

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The objective of this research work was dedicated to the preparation of photocatalytic textiles based on graphene (0.5%wt)/TiO$_2$ composite. Four different methods were tested to coat the cotton knit: dipping twice the knit in composite (a); dipping twice the knit in composite and finally in a polyacrylate binder (b); dipping the knit in a mixture of composite/polyacrylic binder (c), and treatment of the materials in adhesive polymer followed by immersion in composite dispersion (d). The surface, morphology and distribution of nanoparticles covering the textile materials and also the elemental composition were investigated by scanning electron microscopy coupled with X-ray energy dispersive spectroscopy (EDAX). The modifications of chemical and physical properties (surface morphology, hidrophily, electrical resistivity, and elemental composition) have been analysed. The obtained values after determination of electrical resistivity demonstrate that method of treating cotton knits with graphene/TiO$_2$ cause a very slight decrease of surface resistivity and a 10 times increase of volume resistivity compared to the blank sample. The results have demonstrated that the applied coatings induce minor modifications of the initial untreated materials.

Keywords: cotton, graphene/ TiO$_2$, photocatalytic.

INTRODUCTION

TiO$_2$ is recognized as one of the best photocatalytic material, intensively investigated for water purification and environment decontamination. The main disadvantage of TiO$_2$ is its low efficiency on visible range. To overcome this limitation it was doped with different metals and non-metals, and more recently by preparing TiO$_2$/graphene nanocomposite (Xuan *et al*., 2013). The studies (Tolasz *et al*., 2015) have shown that graphene could acts as a sensitizer, and TiO$_2$ as a substrate in the heterojunction system, promoting the separation of photo-induced electron-hole pairs, the electrons being transferred from TiO$_2$ to graphene, while the holes remaining in TiO$_2$ drive the oxidation process (Pan *et al*., 2012). An intimate interfacial contact between graphene and TiO$_2$ is necessary to increase the electron-hole pair production and avoid their recombination. Unfortunately, TiO$_2$ nanoparticles are highly agglomerated onto graphene nanosheets, diminishing the ability to create holes and oxidize the contaminants. More than that, when deposited on irregular textile surfaces, the nanoparticles are not uniformly distributed and the photocatalytic efficiency is much lowered (Karimi *et al*., 2015). New methods to prepare better graphene/TiO$_2$ composites and to more uniformly coat the textiles are required for developing enhanced applications. Our work was directed to the methods development to coat cotton knit with large amount of graphene/TiO$_2$ and to the evaluation of the textiles properties (surface morphology, hydrophilicity, electrical resistivity, elemental composition) modifications. Four different methods were tested to coat the cotton knit: dipping twice the knit in composite (a); dipping twice the knit in composite and finally in a polyacrylate binder (b); dipping the knit in a mixture composite/polyacrylic binder (c) and treatment of the materials in adhesive polymer followed by immersion in composite dispersion (d).

EXPERIMENTAL

Materials

Graphene (0.5% wt)-TiO$_2$ prepared by sonication process was provided by NanoXplore (Canada). Itobinder AG, polyacrylic binder was purchased from LJ

Specialities, UK. Ethanol pro-analysis and distilled water were used to prepare Graphene (0.5% wt)-TiO_2 (GT) dispersions.

Textile fabric: 100% cotton knit, 213 g/m^2, 1.08 mm thick.

Methods

Preparation of Graphene-TiO_2 Composites Dispersion

0.05g graphene (0.5% wt) -TiO_2 were introduced in a mixture of distilled water / ethanol and placed in an ultrasonic bath for one hour at 30°C. A milky unstable dispersion was obtained (experiment 1). To stabilize the dispersion an acrylic polymer, Itobinder AG, is added dropwise over 30 minutes under ultrasonic stirring (exp. 2-6). The parameters of the prepared solutions are presented in Table 1.

Table 1. The parameters of the graphene (0.5% wt)-TiO_2 solutions

No.	Graph. - TiO_2, [g/L]	Graph.- TiO_2, g	Dist. water mL	EtOH mL	Itobinder AG		Sol. vol. mL	Stirring time, min	Aspect
					mL	%			
1	0.5	0.05	67.7	32.3	-	-	100	60	Milky White dispersion
1*	0.23	0.05	117.7	32.3	60	28.57	210	30	Homogeneous solution
2	0.5	0.05	47	23	30	30	100	60	Milky White dispersion
3	0.26	0.05	94	46	50	26.31	190	60	Homogeneous solution
4	0.5	0.1	140	60	10	5	200	60	Milky White dispersion
5	0.5	0.1	140	60	6	3	200	10	Milky White dispersion
6	0.5	0.1	140	60	4	2	200	10	Milky White dispersion

Treatment Methods of Textile Materials

Method a: cotton knit is immersed into the graphene/TiO_2 dispersion (experiment 1) and maintained 10 minutes in an ultrasonic bath at 30°C and then for another 20 minutes without ultrasound at 20°C, with occasionally stirring. The knit was removed from the bath and dried at 100°C. The dried fabric was re-immersed in the dispersion prepared according to experiment 1 and maintained 10 minutes at 30°C on ultrasonic bath, then squeezed and dried in an oven at 100°C. Knit notation: T_1S_2-2.

Method b: in the solution prepared according to experiment 1, remaining from the 2nd treatment of cotton fabric, 82.3mL of water and 60 mL Itobinder AG are added dropwise for 30 minutes and ultrasonicated. The cotton knit is immersed in the resulted milky homogeneous solution and maintained for 10 minutes in the ultrasonic bath at 30°C. Then, the cotton fabric was removed, squeezed and dried in an oven at 100°C. Knit notation: T_1S_2-2ITO.

Method c: cotton knits were immersed into the graphene/TiO_2 solutions prepared according to experiments 2 and 3 and maintained for 10 minutes in an ultrasonic bath at 30°C and then, another 20 minutes without ultrasound at 20°C, stirring occasionally.

The cotton knits were removed from the bath and dried at 100°C.

Knits notations: T_2S_2: knit treated with the solution prepared according to experiment 2; T_3S_2: knit treated with the solution prepared according to experiment 3.

Method d: cotton knits were introduced in 200mL Itobinder AG solution and stirred mechanically for 10 minutes; after that the fabrics are removed from the bath, squeezed and immersed in 200mL solution containing 0.1g graphene (0.5% wt)/TiO_2, 140mL distilled water and 60mL ethanol (experiments 4-6); the fabrics are maintained in the ultrasonic bath for 10 minutes at 30°C and, then removed from the bath, squeezed and dried in an oven at 90-100°C.

Knits notations: T_4S_2: knit treated with the solution prepared according to experiment 4; T_5S_2: knit treated with the solution prepared according to experiment 5; T_6S_2: knit treated with the solution prepared according to experiment 6.

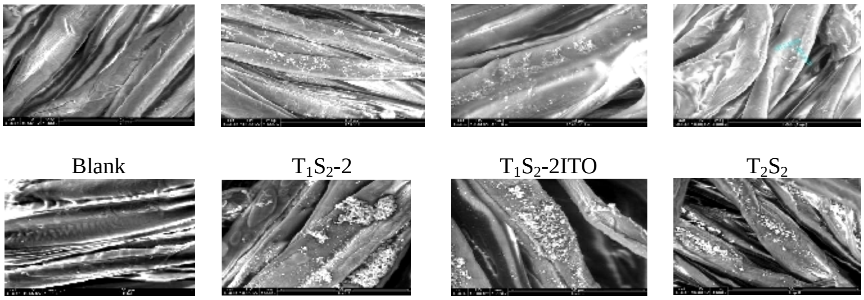
Characterization of Coated Textiles

The morphology and chemical composition of treated and untreated fabrics were investigated by scanning electron microscopy (SEM, Quanta 200, FEI, Holland) equipped with energy dispersive X-ray (EDX) microanalyzer. The hydrophilicity was investigated by measuring the contact angle on VCA Optima equipment, and electrical resistivity with concentric rings (PRS-801) device according to SR EN 1149: 2006.

RESULTS

Surface Morphology of Cotton Knits Treated with Graphene (0.5%)/TiO_2

SEM images of the cotton knits surfaces treated with graphene (0.5%)/TiO_2 are shown in Figure 1.



Blank　T_1S_2-2　T_1S_2-2ITO　T_2S_2

T_3S_2　T_4S_2　T_5S_2　T_6S_2

Figure 1. SEM Images of cotton knits treated with graphene/0.5% TiO_2

SEM images show particle deposition on the surface of cotton fibers. The addition of polyacrylic binder (method b, the sample T_1S_2-ITO) leads to the deposition of relatively large quantities of particles on the fibers surface. This method has beneficial effects on reducing both environmental pollution due to the high degree of treatment bath exhaustion and on process total cost reduction because is no longer necessary to change the treatment bath, the consumption of water, energy and labor being highly reduced. T_2S_2 and T_3S_2 materials, treated by method c are covered with thick layers of polymer that includes most of the particles graphene/TiO_2. Pretreatment of knitted fabric with polyacrylic binder and subsequently with graphene/TiO_2 (d method, samples

T_4S_2, T_5S_2, T_6S_2) favors the deposition of large quantities of particles compared to using a binder/composite mixture (method c). Apparently, the binder forms a uniform film on the fibers surface, increasing the adherence of particles onto the substrate.

Elements Quantification by SEM/EDAX on the Cotton Knit Surface Treated with Graphene (0.5%)/TiO_2

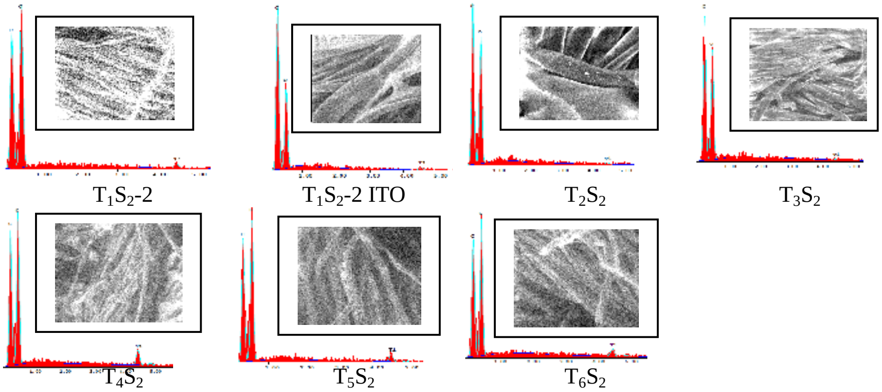

T_1S_2-2 T_1S_2-2 ITO T_2S_2 T_3S_2 T_4S_2 T_5S_2 T_6S_2

Figure 2. EDAX spectra of cotton knits treated with graphene (0.5%)/TiO_2

Table 2. Elements quantification by EDX on the knit treated with graphene (0.5%)/TiO_2

Element	Knit blank	T_1S_2-2	T_1S_2-2 ITO	T_1S_2-2 ITO – sample 2	T_2S_2	T_3S_2	T_4S_2	T_5S_2	T_6S_2
C K	45.99	40.76	57.23	58.07	51.46	51.69	34.4	38.1	37.53
O K	54.01	51.88	38.29	36.58	47.52	47.74	45.9	48.9	49.34
TiK	0	7.36	4.48	5.35	1.02	0.57	19.6	12.9	13.12
Total	100	100	100	100	100	100	100	100	100

The amount of particles deposited on the fabrics depends on the concentration of composite graphene/TiO_2 and the acrylic binder, the number of immersions, and the method of material treatment. Thus, lowering the concentration of graphene/TiO_2 from 0.5 to 0.25g/L (experiments 1-1 and 3-4 *) lowers to about half the amount of TiO_2 deposited on the material. Doubling the number of immersion increases the amount of TiO_2 deposited on the material. On textiles treated once (sample T_1S_2-ITO, 5.35% Ti K) a small amount of TiO_2 (5.35% Ti K) is found compared to those treated by double impregnation (sample T_1S_2-2; 7.36% TIK). This decrease is even more dramatic if the cotton fabric is treated once with preformed mixtures of graphene-TiO_2-polyacrylic binder. Thus, in the case of knitted fabrics treated with the same concentration of TiO_2, TiK concentration decreases from 7.36% (sample T_1S_2-2) if treated with 0.5g/mL graphene/TiO_2 to 1.02% (sample T_2S_2) if treated with binder/graphene-TiO_2 mixture.

In the case of textiles treated initially with polyacrylic binder (method d, samples T_4S_2, T_5S_2, T_6S_2) significant increases of the quantities of TiO_2 deposited is observed. Increasing the concentration of binder from 2-3% to 5% lead to increase by 33.2 - 33.8% of the amount of TiO_2 deposited. But if the concentration of binder greatly increase (exp. 2, 3) it was observed a massive decline in the number of particles deposited on the surface of knitted fabrics due to the formation of clusters of large dimensions remaining in the fleet of treatment, no longer having the ability to migrate to the surface of the fibers.

Determination of the Contact Angle

T_1S_2-2 T_1S_2-2 ITO T_2S_2

Figure 3. Contact angle of cotton knit treated with graphene (0.5% wt)/TiO_2

Table 3. Mean values of knits contact angle treated with graphene (0.5%wt)/TiO_2

Sample	Left angle	Right angle	Wetting time, s
T_1S_2-2	0	0	< 2
T_1S_2-2 ITO	131.16	131.44	> 300
T_2S_2	126.762	127.04	> 300
T_3S_2	133.76	131.30	300
T_4S_2	135.32	136.06	56
T_5S_2	136.72	137.11	27
T_6S_2	132.27	132.33	16.6

Contact angle values and short wetting time demonstrates that treatment with 0.5g/L graphene-TiO_2 (T_1S_2-2) does not modify the hydrophilic properties of cotton. Adding of acrylic binder in high concentrations induces a pronounced hydrophobic character of the materials (T_1S_2-2 ITO, T_2S_2) and a harsh handle due to thick polymer layer formed on the surface.

Determination of Electrical Resistivity

Electrical resistivity was calculated according to the equation:

Surface resistivity: $\rho = Rs \times [2\pi / \ln (R2 / R1)] = R \times k\ [\Omega]$ (1)

where: ρ - surface resistivity; R_S - resistance surface; R_1: the outer electrode radius; R_2: inner electrode radius; k: geometric coefficient.

Volume resistivity: $\rho_v = (A / L) \times R_s$, μΩ · cm. (2)

where: ρ_v = volume resistivity; A = transversal area; L = length (material thickness)

Table 4. Electrical resistivity of knit cotton treated with graphene (0.5%)/TiO_2

No.	Sample	Surface resistivity, $\Omega x10^{12}$	Vol. resistivity, $\Omega xcm\ x10^{13}$	Thickness, mm
1	Blank Cotton knit	5.03	3.13	1.22
2	T_1S_2-2	4.43	33	1.19
3	T_1S_2-2 ITO	4.86	3.69	1.12
4	T_2S_2	41.8	48.9	1.19
5	T_3S_2	71.8	73.7	1.20

In principle graphene, because of its conjugated structure should determine an increase in conductivity, knowing that it has a mobility of 15 000 $cm^2 \cdot V^{-1} \cdot s^{-1}$ and a resistivity of 10^{-6} $\Omega \cdot cm^{-1}$ (Geim and Novoselov, 2007; Chen *et al.*, 2008). Also, graphene is a zero band gap material and working function of -4.42eV – less than anatase TiO_2 (-4.40 eV) (Czerw *et al.*, 2002; Ocak *et al.*, 2009). This makes graphene to be a good acceptor of TiO_2 donated electrons, thus avoiding recombination with gaps that remain on TiO_2. The obtained values demonstrate that treating cotton knits with graphene/TiO_2 cause a very slight decrease of surface resistivity and a 10 times increase of volume resistivity compared to the blank sample. The polyacrylic acid treatment determines the resistivity of both types increases, in particular when treated with the graphene-TiO_2 and polyacrylate mixtures (method c). Increased resistivity can be attributed to the small amount of graphene, to its covering with layers of polyacrylate and uneven deposition without providing continuous contact between small plates of graphene coating the cotton fabric.

CONCLUSIONS

Four methods to deposit the composite graphene/TiO_2 have been used: double treatment only with composite material (a) to deposit a large quantity of graphene, treatment with graphene/TiO_2 followed by reprocessing in the same bath in which a polyacrylic binder was added so as to provide both an high exhaustion of the bath and a better fixation of the particles (method b), treatment with graphene/TiO_2/polyacrylic binder mixture (method c) to lower the cost of energy, water, labor and initial treatment with polyacrylic binder followed by double immersion in graphene/TiO_2 dispersion to increase particles adhesion to the substrate and to deposit a larger quantity of particles on the material. Both double impregnation (method a) and pre-treatment with polyacrylic binder (method d) facilitates the deposition of greater amounts of composite compared to simple impregnation. Increasing the amount of binder cause, on one hand good hydrophobization of materials, an increase of electrical resistivity, increase of particles adherence and on the other hand decrease of the number of particles deposited on the material because of the formation of large clusters that remain in solution, no longer having the ability to migrate on the material surface and because of the thick polymer layer that coats uniform both cotton fibers and graphene/TiO_2 particles.

Acknowledgements

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REFERENCES

Chen, J.-H., Jang, C., Xiao, S., Ishigami, M., Fuhrer, M.S. (2008), “Intrinsic and extrinsic performance limits of graphene devices on SiO_2”, *Nature Nanotechnology*, 3(4), 206-209.

Czerw, R., Foley, B., Tekleab, D., Rubio, A., Ajayan, P.M., Carroll, D.L. (2002), “Substrate-interface interactions between carbon nanotubes and the supporting substrate”, *Physical Review B*, 66(3), 033408.

Geim, A.K., Novoselov, K.S. (2007), “The rise of graphene”, *Nature Materials*, 6(3), 183-191.

Karimi, L., Yazdanshenas, M.E., Khajavi, R., Rashidi, A. and Mirjalili, M. (2015), “Functional finishing of cotton fabrics using graphene oxide nanosheets decorated with titanium dioxide nanoparticles”, *The Journal of The Textile Institute*, 1122-1134.

Ocak, Y.S., Kulakci, M., Kılıçoğlu, T., Turan, R., Akkılıç, K. (2009), “Current–voltage and capacitance–voltage characteristics of a Sn/Methylene Blue/p-Si Schottky diode”, *Synthetic Metals*, 159(15–16), 1603-1607.

Pan, X., Zhao, Y., Liu, S., Korzeniewski, C.L., Wang, S. and Fan, Z. (2012), “Comparing graphene-TiO_2 nanowire and graphene-TiO_2 nanoparticle composite photocatalysts”, *ACS Applied Materials & Interfaces*, 3944-3950.

Tolasz, J., Vomáčka, P., Štengl, V. and Bludská, J. (2015), “Photocatalytic composite materials based on graphene and titanium oxide prepared by different methods”, in A.L. Araújo, C.A. Mota Soares, et al. (eds.), *7th ECCOMAS Thematic Conference on Smart Structures and Materials, SMART 2015*.

Xuan, P. Zhao, Y., Wang, S. and Fan, Z. (2014), “TiO_2/graphene nanocomposite for photocatalytic application, Materials and processes for energy: communicating current research and technological developments”, in: A. Mendez-Vilas (ed.), *Materials and processes for energy: communicating current research and technological developments*, 913-920.

PHOTOCATALYTIC PERFORMANCES OF TEXTILES COATED WITH GRAPHENE OXIDE/TIO_2 NANOCOMPOSITES – PART 2

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The objective of this work was dedicated to the preparation of photocatalytic textiles based on graphene (0.5%wt)/TiO_2 composite. Four different methods were tested to coat the cotton knit: dipping twice the knit in composite (a); dipping twice the knit in composite and finally in a polyacrylate binder (b); dipping the knit in a mixture composite/polyacrylic binder (c) and treatment of the materials in adhesive polymer followed by immersion in composite dispersion (d). To evaluate the photocatalytic effect of the materials after washing treatment, samples were exposed to ultraviolet and visible light. The trichromatic coordinates of the exposed and non-exposed samples were measured on Hunterlab spectrophotometer. Morphological aspect and TiO_2 existing on the materials surface after washing were evaluated by scanning electron microscopy and energy dispersive analysis. Cotton fabric treated by method b has the most pronounced photocatalytic effect under UV light, probably because of carboxylic groups of polyacrylic binder that facilitates photooxidation.

Keywords: cotton, graphene/ TiO_2, photocatalytic.

INTRODUCTION

TiO_2 is recognized as one of the best photocatalytic material, intensively investigated for water purification and environment decontamination. The main disadvantage of TiO_2 is its low efficiency on visible range. To overcome this limitation it was doped with different metals and non-metals, and more recently by preparing TiO_2/graphene nanocomposite (Xuan *et al*., 2013). The studies (Tolasz *et al*., 2015) have shown that graphene could acts as a sensitizer, and TiO_2 as a substrate in the heterojunction system, promoting the separation of photo-induced electron-hole pairs, the electrons being transferred from TiO_2 to graphene, while the holes remaining in TiO_2 drive the oxidation process (Pan *et al*., 2012).

Our study was focused on the analysis of the photocatalytic effects of Graphene (0.5% wt.)-TiO_2 deposited on textile materials under visible, UV light and their durability at washing.

EXPERIMENTAL

Materials

Graphene (0.5% wt)-TiO_2 prepared by sonication process was provided by NanoXplore (Canada). Itobinder AG, polyacrylic binder was purchased from LJ Specialities, UK. Ethanol pro-analysis and distilled water were used to prepare Graphene (0.5% wt)-TiO_2 (GT) dispersions.

Textile fabric: 100% cotton knit, 213 g/m2, 1.08 mm thick.

Methods

Preparation of Graphene-TiO_2 Composites Dispersion

0.05g graphene (0.5% wt.) -TiO_2 were introduced in a mixture of distilled water / ethanol and placed in an ultrasonic bath for one hour at 30°C. A milky unstable dispersion was obtained (experiment 1). To stabilize the dispersion an acrylic polymer, Itobinder AG, is added dropwise over 30 minutes under ultrasonic stirring.

Treatment Methods of Textile Materials

Method a: cotton knit is immersed into the graphene/TiO_2 dispersion (experiment 1) and maintained 10 minutes in an ultrasonic bath at 30°C and then for another 20 minutes without ultrasound at 20°C, with occasionally stirring. The knit was removed from the bath and dried at 100°C. The dried fabric was re-immersed in the dispersion prepared according to experiment 1 and maintained 10 minutes at 30°C on ultrasonic bath, then squeezed and dried in an oven at 100°C. Knit notation: T_1S_2-2.

Method b: in the solution prepared according to experiment 1, remaining from the 2nd treatment of cotton fabric, 82.3mL of water and 60 mL Itobinder AG are added dropwise for 30 minutes and ultrasonicated. The cotton knit is immersed in the resulted milky homogeneous solution and maintained for 10 minutes in the ultrasonic bath at 30°C. Then, the cotton fabric was removed, squeezed and dried in an oven at 100°C. Knit notation: T_1S_2-2ITO.

Method c: cotton knits were immersed into the graphene/TiO_2 solutions prepared according to experiments 2 and 3 and maintained for 10 minutes in an ultrasonic bath at 30°C and then, another 20 minutes without ultrasound at 20°C, stirring occasionally.

The cotton knits were removed from the bath and dried at 100°C.

Knits notations:

T_2S_2: knit treated with the solution prepared according to experiment 2;

T_3S_2: knit treated with the solution prepared according to experiment 3.

Method d: cotton knits were introduced in 200mL Itobinder AG solution and stirred mechanically for 10 minutes; after that the fabrics are removed from the bath, squeezed and immersed in 200mL solution containing 0.1g graphene (0.5% wt.)/TiO_2, 140mL distilled water and 60mL ethanol (experiments 4-6); the fabrics are maintained in the ultrasonic bath for 10 minutes at 30°C and, then removed from the bath, squeezed and dried in an oven at 90-100°C.

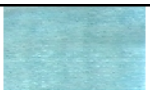
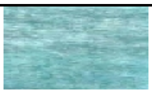
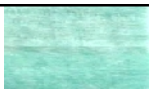
Knits notations: T_4S_2: knit treated with the solution prepared according to experiment 4; T_5S_2: knit treated with the solution prepared according to experiment 5; T_6S_2: knit treated with the solution prepared according to experiment 6.

RESULTS

Photocatalytic Effect Assessment

In the following, we evaluated the photocatalytic effect of graphene (0.5%)/TiO_2, stained with MB and exposed to UV and Vis light. Also, the photocatalytic effect was evaluated in terms of treatment durability to washing. Aspect of the fabrics and color changes of methylene blue (MB), the dye used for staining the treated and untreated fabrics are presented in Tables 1-6.

Table 1. Aspect of cotton knits treated with graphene (0.5%)/TiO_2, stained with MB and exposed to UV light

Time, Sample	**0h**	**11h**	**15h**	**21h**	**28h**
Cotton blank					
T_1S_2-2					

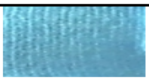
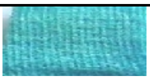
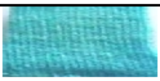
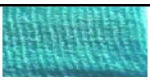
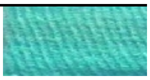
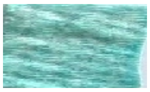
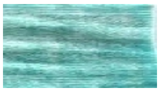
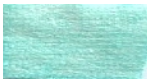
Time, Sample	**0h**	**11h**	**15h**	**21h**	**28h**
T_1S_2-2 ITO					
T_2S_2					
T_3S_2					

Table 2. Trichromatic coordinates of cotton knits treated with graphene (0.5%)/TiO_2, stained with methylene blue and exposed to UV light for 28 hours

Sample	**L***	**a***	**b***	**dL***	**da***	**db***	**dE***	**dC***	**dH***	**dL**	**Note**
Blank	84.55	-11.7	-7.22	0.07	4.01	2.34	4.64	-4.64	0.1	0.08	2.5
T_1S_2-2	83.97	-12.31	-8.03	1.53	3.72	3.34	5.23	-4.96	-0.66	1.83	2.5
T_1 S_2-2 ITO	72.08	-19.79	-18.9	**2.84**	1.66	6	**6.85**	-5.5	-2.92	3.17	2.5
T_2 S_2	82.35	-13.52	-8.94	-0.03	4.1	2.98	5.07	-5.07	-0.2	-0.04	2.5
T_3 S_2	85.43	-12.82	-5.91	0.17	3.99	3.13	5.07	-4.97	-1.01	0.21	2.5

By exposure to UV light, the higher brightness (dL*) and color (dE*) differences are presented by sample T_1S_2-2 ITO followed by the same sample but untreated, with polyacrylic binder (T_1S_2-2). The other samples were similar to the untreated cotton knit. The pronounced photocatalytic effect of sample T_1S_2-2 ITO is due to both relatively large amount of photocatalyst present on the material surface and to the carboxylic groups of polyacrylic binder, which due to attraction of a greater amount of water from the atmosphere facilitates photooxidation.

Table 3. Aspect of cotton knits treated with graphene (0.5%)/TiO_2, stained with 0.0064 g/L MB and exposed 60 hours to UV light

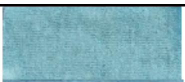
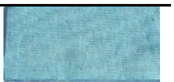
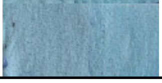
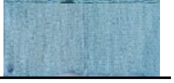
Time (h)	**Blank**	**T_4S_2**	**T_5S_2**	**T_6S_2**
0				
12				
24				
46				
62				

Table 4. Trichromatic coordinates of cotton knits treated with graphene (0.5%)/TiO_2, stained with methylene blue and exposed to UV light for 62 hours

Sample	**L***	**a***	**b***	**dL***	**da***	**db***	**dE***	**dC***	**dH***	**Note**
Blank	83.87	-10.33	-6.45	1.16	5.24	4.53	7.02	-6.88	-0.85	**2**
T_4S_2	82.44	-11.76	-9.12	-0.34	2.21	1.79	2.86	-2.84	-0.06	3.5
T_5S_2	83.71	-11.02	-7.57	0.65	2.74	2.18	3.56	-3.5	-0.22	3
T_6S_2	82.8	-12.12	-9	0.51	1.96	2.02	2.86	-2.78	-0.42	3.5

Even after a long time exposure to UV radiation, the treated materials shows a less intense fading compared to the control sample. It can be noted that the biggest change in color is found on T_5S_2 material, treated with the small amount of graphene/TiO_2.

Table 5. Aspect of cotton knits treated with graphene (0.5%)/TiO_2, stained with 0.0064g / L MB and exposed to visible light 8 hours

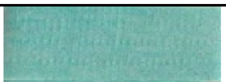
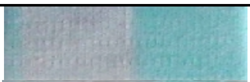
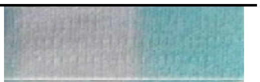
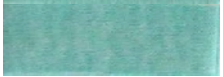
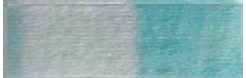
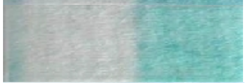
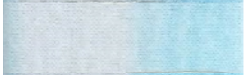
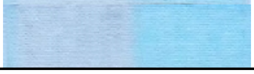
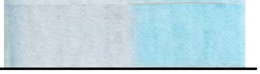
Sample/ Time (h)	**0**	**2 hours**	**8 hours**
Blank			
T_1S_2-2			
T_2S_2			
T_3S_2			
T_4			
T_5			
T_6			

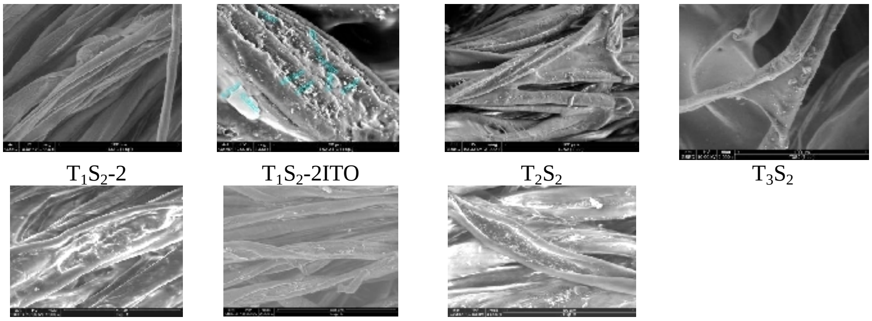
Table 6. Trichromatic coordinates of cotton knits treated with graphene/0.5% TiO_2, stained with MB and exposed to visible light for 8 h

Sample	**L***	**a***	**b***	**dL***	**da***	**db***	**dE***	**dC***	**dH***	**Note**
Blank	87.15	-1.54	-2.12	4.15	13.36	8.7	16.47	-15.79	2.19	1
T_1S_2-2	87.07	-2.22	-2.68	3.92	12.76	7.9	15.51	-14.86	2.1	1
T_2 S_2	87.82	-2.89	-1.94	5.52	12.91	9.79	17.11	-16.19	-0.39	1
T_3 S_2	91.32	-2.42	0.52	**7.08**	14.48	10.46	19.21	-17.13	-5.06	1
T4S2	84.57	-2.07	-3.03	4.05	12.99	10.12	16.96	-16.32	2.17	1
T5S2	87.2	-1.77	-1.89	4.2	11.4	7.95	14.52	-13.85	1.15	1
T6S2	85.75	-2.25	-2.94	2.82	12.99	7.56	15.29	-14.8	2.58	1

Under visible light, the results demonstrate an increase in the brightness (dL*) and color (dE*) difference as the amount of TiO_2 present on the surface of the material decreases. Surprisingly is the more intense discoloration of methylene blue in case of blank sample when compared to samples T_4S_2-T_6S_2. It is assumed that due to the high amount of graphene/TiO_2 these samples absorb a much larger amount of dye compared to untreated fabric cotton. Dye photo-discoloration is more intense under visible light than under UV light. It can be observed a growing prevalence of fading with increasing concentration of acrylic polymer binder. Dye photo-discoloration is more intense than under visible light under UV light.

The treatments Durability to Washing

Morphological aspect and TiO_2 existing on the materials surface after washing were evaluated by scanning electron microscopy and energy dispersive analysis, results being shown in Figures 1 and 2 and Table 7.

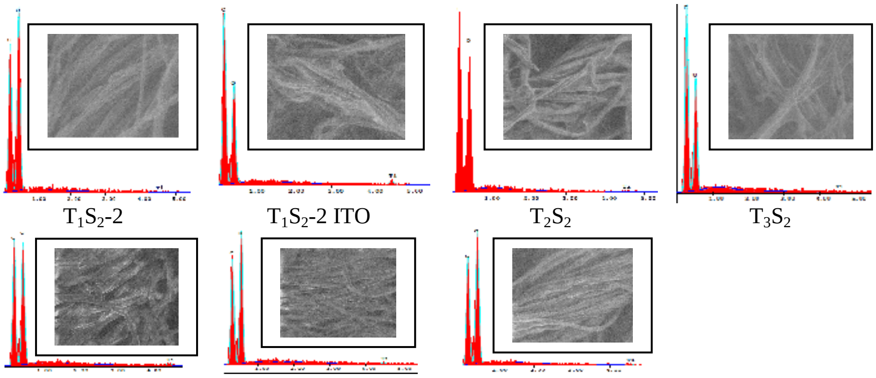


T_1S_2-2 T_1S_2-2ITO T_2S_2 T_3S_2

T_4S_2 T_5S_2 T_6S_2

Figure 1. SEM analysis of cotton fabric treated with graphene/0.5% TiO_2 after washing

A relatively high number of particles are present on the materials surface after washing. Thick layers of acrylic polymer show a high adhesion after washing, the composite particles being firmly fixed on the surface.



T_1S_2-2 T_1S_2-2 ITO T_2S_2 T_3S_2

T_4S_2 T_5S_2 T_6S_2

Figure 2. SEM/EDAX analysis of cotton knit treated with graphene/0.5% TiO_2 after washing

Table 7. EDAX quantification of %wt Ti on the cotton knit surface treated with graphene (0.5%)/TiO_2 after washing

Element,%Wt.	**T_1S_2-2**	**T_1S_2-2 ITO**	**T_2S_2**	**T_3S_2**	**T_4S_2**	**T_5S_2**	**T_6S_2**
TiK_i	7.36	4.48-5.35	1.02	0.57	19.62	12.99	13.12
TiK_s	1.21	6.03	1.81	1.07-1.71	3.81	3.89	5.21
Δ(TiK_s- iK_i)*, %	- 83.56	+ 12.71	+ 77.45	+ 200	- 80.58	-70	- 60.3

* TiK_i – initial; TiKs – after washing

It can be observed an entirely different washing behavior depending on the method of treatment. Thus, after washing, the fabric T_1S_2-2 treated only with graphene/TiO_2 gives approximately 83.56% of the initial amount of TiO_2. Subsequent treatment with polyacrylic binder and usage of mixture binder/composite contributes decisively to fixation of composite particles on the surface of the fabric. The high concentration of TiO_2 found on washed cotton knits T_1S_2-2 ITO, T_2S_2 washed T_3S_2 is due to a slight exfoliation of the polymer layer which allows re-dispersing TiO_2 particles on the surface of the fabric. The largest amount of TiO_2 left on the material after washing is found on the fabric T_1S_2-2 ITO treated twice, followed by T_2S_2 treated with the highest concentration of polyacrylic binder. Instead, knits treated by method d, although initially had high amount of titanium deposited on the surface, after washing the amount of TiO_2 removed from the material has a rate of 60-80%. It is assumed that, using a concentration below 5mL/L polyacrylic binder does not provide a high adhesion of the particles onto the substrate.

CONCLUSIONS

From the four methods used to deposit the composite graphene/TiO2 that have been used, both double impregnation (method a) and pre-treatment with polyacrylic binder (method d) facilitates the deposition of greater amounts of composite compared to simple impregnation. Subsequent treatment with polyacrylic binder and usage of binder/composite mixture increase the treatment durability at washing.

Cotton fabric treated by method b has the most pronounced photocatalytic effect under UV light, probably because of carboxylic groups of polyacrylic binder that facilitates photooxidation. Under visible light photocatalytic efficiency decreases as the quantity of graphene/TiO_2 present on the material surface increases.

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REFERENCES

Pan, X., Zhao, Y., Liu, S., Korzeniewski, C.L., Wang, S. and Fan, Z. (2012), “Comparing graphene-TiO_2 nanowire and graphene-TiO_2 nanoparticle composite photocatalysts”, *ACS Applied Materials & Interfaces*, 3944-3950.

Tolasz, J., Vomáčka, P., Štengl, V. and Bludská, J. (2015), “Photocatalytic composite materials based on graphene and titanium oxide prepared by different methods”, in A.L. Araújo, C.A. Mota Soares *et al*. (eds.), *7th ECCOMAS Thematic Conference on Smart Structures and Materials, SMART 2015*.

Xuan, P. Zhao, Y., Wang, S. and Fan, Z. (2014), “TiO_2/graphene nanocomposite for photocatalytic application, Materials and processes for energy: communicating current research and technological developments”, in: A. Mendez-Vilas (ed.), *Materials and processes for energy: communicating current research and technological developments*, 913-920.

THE STUDY OF Ag/TiO$_2$ NANOMATERIALS USE FOR LEATHER AND FOOTWEAR INDUSTRY

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Ecologic and health effects of applying materials with advanced functions for leather surface finishing are priorities for the European leather industry and contribute to the increase of added value and durability of leather and fur articles. The innovative properties of Ag/TiO$_2$ nanomaterials (NMs) on leather surface are due to their antimicrobial, self-cleaning and flame retardant characteristics. The NANO_SAFE_LEATHER project aims to contribute to the industrial development of safer and advanced leathers that make use of Ag/TiO$_2$NMs, which are ecological alternatives to volatile organic biocides, organic solvents and halogenated flame retardants. The cytotoxicity study of the Ag/TiO$_2$NM's efficiency for leather functionalization related to the dose response on human health is very important for their large scale application in footwear industry. The paper presents the strategy of research regarding the leather surface finishing by spraying and by physical deposition of Ag/TiO$_2$NMs, the impact of NMs use in industrial environment and in connection with wearing behavior. *In vitro* assay to study the cytotoxicity of the Ag/TiO$_2$NMs, leather functionalized with Ag/TiO$_2$NMs and leached Ag/TiO$_2$NMs on different human cells, the cell morphology and cell death mechanism as well as the exposure risk assessment on lung cells will allow to estimate for the first time the impact of NMs industrial application in leather and footwear industry and on consumer health. *In silico* study of human cells in vitro interacted with Ag/TiO$_2$NMs provides information on the potential changes induced by the NPs on cellular morphology and helps build a domain ontology.

Keywords: nanomaterials, Ag/TiO$_2$NMs, human health, leather surface, footwear industry.

INTRODUCTION

Human exposure to nanomaterials (NMs) is inevitable as NMs diversify and become more widely used and, as a result, NMs toxicology research is extremely important for health and the environment. Silver nanoparticles (AgNPs) based materials are already being used in wound dressings, catheters, cosmetics, textiles, plastic materials and household products due to their antimicrobial activity. There were already identified 260 products on the market with AgNPs and with antibacterial, antifungal, antiviral properties and other 400-500 products are estimated to be processed with NMs (www.foodandwatereurope.org).

Recent research demonstrated the efficiency of photocatalytical activity in the UV domain and particularly in the visible domain of nanomaterials based on titanium dioxide doped with nanosilver against a wide spectrum of microorganisms. Other properties of nanomaterials relate to photocatalytical decomposition of dirt on the treated surface or heat/fire resistance. The area of smart properties of nanomaterials includes photocatalytical decomposition of volatile organic compounds, which allows elimination of unpleasant odours or reduction of fogginess inside vehicles. These properties have been exploited to develop multifunctional textiles (https://www.ic.gc.ca/eic/site/textiles-textiles.nsf/eng) and leather with antimicrobial, self-cleaning properties and/or resistance to heat/fire (Lkhagvajav *et al*., 2015; Gaidau *et al*., 2011; Bitlisli and Yumurtas, 2008; Petica *et al*., 2015; Gaidau *et al*., 2014; Gaidau *et al*., 2013; Yang *et al*., 2012).

AgNPs and nano TiO_2, single or doped (Ag/TiO_2NPs), are the most efficient in terms of multifunctional properties: antimicrobial, self cleaning or flame retardant characteristics. The recent progresses (Bitlisli and Yumurtas, 2008; Petica *et al*., 2015; Yang *et al*., 2012) in leather surface functionalization with NPs open a huge area of application in leather industry for innovative footwear, smart upholstery and garment leathers. It is already known that 20–25% of the world's population has skin mycoses, making these one of the most frequent forms of infection. Increasingly frequent *tinea pedis* caused by *T. rubrum* and *T. interdigitale* infection seems to be a characteristic of highly developed countries with booming sport and fitness facilities, increasing prevalence of obesity and diabetes mellitus and an ageing population. The leather surface treated with AgNPs and doped TiO_2 has been proved to protect feet against bacteria and fungi and is an ecological alternative to volatile organic biocides. Leather surface functionalized with doped TiO_2 has flame retardant properties, self cleaning activity under UV-Vis light and allows replacing of organic halogenated materials, while improving leather durability.

Einaga (2006) showed that deposition of silver on the surface of titanium dioxide is effective for improving the activity of titanium dioxide, and the optimum concentration was determined for 2% silver by weight when photocatalytic activity increased four times. Increased activity of titanium dioxide nanoparticles doped with noble metals, particularly silver, was demonstrated for *Escherichia coli* (Jingbo *et al*., 2013).

Silver nanoparticles are predominantly prepared via chemical reduction, an industrially applicable method due to its simplicity and high efficiency (Cao *et al*., 2010; Nainani *et al*., 2012).

The methods of synthesizing NPs include redox, electrochemical, hydrothermal, photochemical, growth method, microemulsion synthesis, synthesis using microwave, methods of evaporation and condensation, etc. Silver nanoparticles of different shapes and sizes can be obtained by chemical methods, depending on the precursors and conditions of synthesis, influencing antimicrobial properties.

Obtaining Ag/TiO_2 doped nanomaterials by chemical methods is a more recent approach and involves modifying the known sol-gel methods by impregnation and chemical reduction of silver ions during the synthesis of titanium dioxide, followed by calcination at 400°C with its transformation from amorphous to anatase crystalline form (Kedziora *et al*., 2012). Another method of obtaining two- and three-component composites is based on the Stöber method (Stöber and Fink, 1968) by means of which core-shell structures of silicon and titanium dioxide-nanosilver are obtained. Electrochemical synthesis of Ag/TiO_2 nanoparticles, the method adopted in this paper, has the advantage of obtaining pure products without reaction intermediaries, compared with the chemical synthesis methods (Anicai *et al*., 2013). NPs deposition by physical methods (vacuum deposition) is another innovative method approached in the research project, intended for the leather substrate with improved environmental effects compared with conventional spraying treatments.

The paper presents the main strategies addressed in the NANO_SAFE_LEATHER project for synthesis and characterization of nanoparticles and leather treated therewith in order to evaluate the cytotoxicological potential of their use in the industry, thus enriching the European database on the toxicological impact of NPs application.

MATERIALS AND METHODS

Processing the Surface of Leather with Ag/TiO_2 Nanoparticles

The research strategy targets deposition of silver-based nanoparticles onto the surface of leather using conventional techniques of spraying film-forming polymers containing Ag/TiO_2NP and Ag/N-TiO_2 NP and physical deposition techniques based on DC Reactive Sputter Deposition.

Obtaining and Characterizing Ag/TiO_2 NPs and Ag/N-TiO_2 NPs and Leather Surfaces Coated therewith Using Conventional Techniques and Physical Deposition

Ag/TiO_2NP and Ag/N-TiO_2NPs were obtained by electrochemical deposition of silver on the surface of TiO_2NPs using the so-called “sacrificial anode method” (Stöber and Fink, 1968). For this purpose, TiO_2NPs powder of 20 nm average size (TitanPE Technologies, Inc., China) well dispersed with Na-PAA in deionized water and electrodes of 99.999% purity Ag plates (155 x 27 x 0.5 mm) were used. The electrochemical process was carried out under current densities between 0.01-0.06 mA.cm^{-2} for 2-8 hours involving a constant current pulse reversed generator with a mechanical stirrer. The resulted dispersion was washed, centrifuged, dried and ground in powder form.

The main assessed characteristics of the NPs were the concentration of silver deposited on TiO_2NP (ICP –OES, Agilent 725), the average particle size of water dispersion in Na-PAA and Zeta potential (Nanosizer, Nano ZS Malvern).

The crust leather surfaces for footwear lining were prepared (SC Taro Commimpex LTD) for finishing by spraying and by physical deposition through DC Reactive Sputtering method.

The classical finishing of leather surface was done by spraying of film forming polymers mixed by mechanical and ultrasound stirring (Petica *et al.*, 2015) of Ag/TiO_2NP and Ag/N-TiO_2NP powders. The leather surfaces were evaluated under UV/visible light irradiation (VL 204 lamp with irradiation at 365 nm and 500W halogen lamp) for model stains of Methylene Blue discoloration (self-cleaning effect) and contact angle modification (VGA Optima XE). The antibacterial sensitivity tests against *Escherichia coli* and *Staphylococcus aureus* were performed according to EN ISO 20645.

The main parameters of DC reactive sputtering method were set for specific characteristics of leather support and by using a pure target of silver and a pure target of titanium to achieve antimicrobial characteristics.

The resistance against *Staphylococcus epidermidis was* tested by agar diffusion method for the leathers treated by DC Reactive Sputtering.

The surface characteristics of leather samples uncoated and coated with Ag/TiO_2 by DC Reactive Sputtering were determined through the sessile drop contact angle technique. The photocatalytic activity of the leather samples covered with Ag/TiO_2NPs by DC Reactive Sputtering were evaluated by the decomposition of an organic dye (Methylene Blue) with combined UV/visible light irradiation.

The leather wearing behavior was preliminary tested by tribological method using 4 different counterpart materials that cover a wide range of frictions, namely PPH (Polypropylene), POM (Polyoxymethylene), PTFE (Polytetrafluoroethylene), PA 6.6 (Polyamide 6.6 or Nylon 6.6), PUR (Polyurethane) and NBR 70 (Nitrile rubber).

Cytotoxicity Test Strategy for Ag/TiO*2* NP Evaluation. *In Silico* Study of Human Cells

In vitro cytotoxicity studies of nanoparticles using different cell lines, incubation times, and colorimetric assays are increasingly being published. *In vitro* model systems provide a rapid and effective means to assess nanoparticles for a number of toxicological endpoints. They also allow development of mechanism-driven evaluations and provide refined information on how nanoparticles interact with human cells in many ways. Such studies can be used to establish concentration–effect relationships and the effect-specific thresholds in cells. The cytotoxicity study of the Ag/TiO_2NP’s efficiency for leather functionalization related to the dose response on human health is very important for their large scale application and has not been done so far. *In vitro* assessment of the impact of Ag/TiO_2NPs and of the leather finished with Ag/TiO_2NPs, on different human cell lines (HaCaT keretinocytes, A549 cell line) using cytotoxicity analysis (such as MTT cell survival assay), cellular morphology, the investigation of cell death mechanisms and of the intracellular signaling pathways activated by manufactured Ag/TiO_2NPs will enrich the knowledge on NPs effects on human health at cellular level.

In silico approach, which tests experimental data by computer simulation analysis, will provide information on the morphology of human cells. Morphological data extracted from the cytotoxicity studies will be processed to obtain virtual representations of human cells.

DISCUSSIONS AND CONCLUSIONS

Ag/TiO_2 NPs and Ag/N-TiO_2 NPs were synthesized by loading concentrations of 0.5 to 1.6% silver on the surface of TiO_2NPs and N-TiO_2 NPs.

The average particle sizes of Ag/TiO_2 NPs and Ag/N-TiO_2 NPs in aqueous dispersions were of 45 nm and of 69 nm, with Zeta potentials of -40mV, and -47 mV, respectively, which means good stability.

The leather surface characterization under UV/Vis light exposure showed photocatalytic properties attributed to active species of O and OH- generation with effect on contact angle decreasing (ten times as compared to the unexposed surface) and hydrophilic characteristics. The treated leather surfaces with Ag-TiO_2NPs were sensitive to tested bacteria (*Escherichia coli* and *Staphylococcus aureus)* as compared to untreated leather surfaces.

Ag/TiO_2 NMs samples were deposited on leather surface by DC Reactive Magnetron Sputtering. Two targets were used (a pure target of silver and a pure target of titanium) and current density was altered in order to change the chemical composition of the films. Other parameters of deposition as work pressure, gas flux were varied in order to optimize the films deposition and to protect the organic support of leather. The method allows depositing alternative layers of silver (with concentration up to 10%) and/or titanium dioxide with antibacterial or photocatalytic properties.

The photocatalytic properties under UV/Vis irradiation were achieved only on titanium dioxide covered leather surfaces against Methylene blue simulated stains while the antibacterial properties were successfully achieved on leather surfaces covered by Ag/TiO_2 NPs.

The primary results of hydrophobic characterization showed that the introduction of silver promotes an increase of contact angle with values round to 110º.

The preliminary agar diffusion test held at some leather functionalized with Ag/TiO_2 NPs against *Staphylococcus epidermidis* showed a clear halo (absence of bacterial growth) around the samples, which may indicate antibacterial activity in the preliminary depositions, and these results will be verified with quantitative tests further on in the project.

The tribological tests intend to estimate the wearing behavior of leather treated with Ag/TiO_2 NPs. In this regard the coefficient of friction as a function of the sliding was recorded for 6 counterpart combinations from which 4 were selected (PTFE, PA 6.6, PUR and NBR 70). The tests were performed at different loads of 1, 5 and 10 N with radii of 5, 7 and 10 mm, respectively. The angular velocity was adjusted for each radius to keep the linear velocity constant at 10 cm/s. The sliding distance was set to 2000 laps. The experiments are in progress and will set the best parameter for leather surface wearing behavior evaluation by tribological tests.

Cytotoxicity studies were performed by exposing human keratinocytes (HaCaT cells) to various concentrations of different Ag/TiO_2 NPs for 48 hours and the cellular viability was determined by MTT assay. The organization of actin cytoskeleton was visualized by fluorescence microscopy after incubation of cells with different concentrations of Ag/TiO_2 NPs, by staining the cells with fluorescently-labeled Phalloidin. The intracellular reactive oxygen species (ROS) levels were quantified using the fluorogenic dye 2',7'-dichlorofluorescein diacetate (DCFH-DA), that measures hydroxyl, peroxyl and other ROS within the cell, using a TECAN spectrophotometer. The research is in progress and will allow establishing the dose response for Ag/TiO_2 NPs use in industrial environment.

In silico approach to study the influence of the MNMs on human cells provides information on the potential changes induced by the NPs on cellular morphology and help build a domain ontology.

The main conclusions of NANO_SAFE_LEATHER project are as follows:

► successful deposition of Ag/TiO_2 NPs and Ag/N-TiO_2 NPs on leather surface was carried out by classical technologies;

► leather surfaces treated with Ag/N-TiO_2 NPs showed photocatalytic properties with improved self-cleaning effect in visible domain and antimicrobial resistance;

► AgNP, TiO_2NPs and Ag/TiO_2NPs were successfully deposited on leather surface by DC Reactive Magnetron Sputtering method;

► the main parameters of DC Reactive Magnetron Sputtering method were set for soft structure of leather surface without damaging the material and using two targets (Ti and Ag);

► self-cleaning properties were developed on leather surface covered with TiO_2NPs by DC Reactive Magnetron Sputtering;

► antimicrobial properties on leather surface were tested successfully for leather surface covered by DC Reactive Magnetron Sputtering with Ag/TiO_2NPs;

► electrochemical NPs deposited on leather surface in film forming polymers by spraying showed photocatalytic properties under UV/Vis light exposure attributed to the active species generation (O and HO^-) with hydrophilic surface effect, proved by contact angle measurements;

► Ag/TiO_2NPs deposited on leather surface by DC Reactive Magnetron Sputtering generated hydrophobic structure of leather surface proved by increased contact angle at the value of 110º. This new characteristic opens the way to developing self-cleaning properties based on hydrophobic effect of NPs;

► the tribological test of leather surface is an innovative approach for wearing behavior evaluation of leather surface treated with NPs and setting the appropriate parameters (loads, angular velocity, sliding distance, counterpart material) was the first progress of the research;

► cytotoxicity studies were performed on human cells exposed to different concentration of Ag/TiO_2NPs according to different protocols for evaluation of cell growth, death mechanism, intracellular reactive oxygen species quantification with the final aim of establishing the dose response for working in industrial environment;

► *in silico* studies of human cells interacted with Ag/TiO_2NPs will allow to predict and to explain the NPs influence on cell life and risks on human health.

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REFERENCES

Anicai, L., Petica, A. and Gavriliu, S. (2013), "Electrochemical procedure of obtaining ecologic disperse nanostructured systems with photocatalytic and antimicrobial activity", Ro.Patent no.125498/30.05.2013.

Bitlisli, B.O. and Yumurtas A. (2008), "Self cleaning leathers-the effect of nano TiO_2", *Journal of the Society of Leather Technologists and Chemists*, 92(5), 183-186.

Cao, X.L., Cheng, C., Ma, Y.L. and Zhao, C.S. (2010), "Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities", *Journal of Materials Science: Materials in Medicine*, 21, 2861–2868.

Einaga, H. (2006), "Effect of silver deposition on TiO_2 for photocatalytic oxidation of benzene in the gas phase", *Reaction Kinetics and Catalysis Letters*, 88(2), 357−362.

Gaidau, C., Petica, A., Dragomir, T., Iovu, H. and Andronescu, C. (2011), "Ag and Ag/TiO_2 nano-dispersed systems for treatment of leathers with strong antifungal properties", *Journal of the American Leather Chemists Association*, 106(3), 102-109.

Gaidau, C., Petica, A., Fleancu, M., Popescu, M. and Piticescu, R.M. (2014), "Innovative materials for smart properties on leather surface", 10th AICLST, November 24th– 26th, Okayama, Japan.

Gaidau, C., Petica, A., Micutz, M., Danciu, M. and Vladkova, T. (2013), "Progresses in Treatment of Collagen and Keratin Based Materials with Silver Nanoparticles", *Central European Journal of Chemistry*, 11(6), 901-911.

Jingbo, L., Zhimin, W., Zhiping, L. and Sajid, B. (2013), "Effective bactericidal performance of silver decorated titania nano-composites", *Dalton Transactions*, 42, 2158-2166.

Kedziora, A., Strek, W., Kepinski, L., Bugla-Ploskonska, G. and Doroszkiewicz W. (2012), "Synthesis and antibacterial activity of novel titanium dioxide doped with silver", *Journal of Sol-Gel Science and Technology*, 62, 79–86.

Lkhagvajav, N., Koizhaiganova, M., Yasa, I., Celik, E. and Sari, O. (2015), "Characterization of antimicrobial performances of nano silver coatings on leather material", *Brazilian Journal of Microbiology*, 46(1), 41-48.

Nainani, R., Thakur, P. and Chaskar, M. (2012), "Synthesis of Silver Doped TiO_2 Nanoparticles for the Improved Photocatalytic Degradation of Methyl Orange, *Journal of Materials Science and Engineering B*, 2(1), 52-58.

Petica, A., Gaidau, C., Ignat, M., Sendrea, C. and Anicai, L. (2015), "Doped TiO_2 Nanophotocatalysts for Leather Surface Finishing with Self-Cleaning Properties, *Journal of Coatings Technology and Research*, 12(6), 1153-1163.

Stöber, W. and Fink, A. (1968), "Monodisperse silica of spheres in the micron size range", *Journal of Colloid and Interface Science*, 26, 62-69.

Yang, W., Wang, X., Gong, Y., Li, H., Chen, W. and Gaidau, C. (2012), "Preparation of Antibacterial Sheepskin With Silver Nanoparticles, Potential for Use as a Mattress for Pressure Ulcer Prevention", *Journal of the American Leather Chemists Association*, 107(3), 85-92.

*** https://www.ic.gc.ca/eic/site/textiles-textiles.nsf/eng

*** www.foodandwatereurope.org

COMPOSITION, PROPERTY AND STRUCTURE OF CHROME COMPLEXES IN NEUTRALIZING EFFLUENT

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In order to interpret the reason of chromium complexes release from the chrome tanned leather during the neutralizing process, the hide powder was tanned by chromium sulfate liquor with 33% basicity (BCS), and then the tanned collagen was treated by general neutralizing process to obtain neutralizing effluent. Ion exchange chromatography (IEC) and gel filtration chromatography (GFC) were used to separate neutralizing effluent; Ultraviolet-visible absorption spectroscopy (UV-Vis) and Fourier Transform Infrared Spectroscopy (FT-IR) were combined to analyze the structure of chrome complexes in the neutralizing effluent. The results indicated that the neutralizing effluent mainly consisted of anionic-zero valent, monovalent and bivalent chrome complexes; the molecular weight of chrome complexes in neutralizing effluent was far less than BCS; releasing chromium complexes were mainly combined with ligands through single point coordination and Cr^{3+} was mainly combined with SO_4^{2-} and $HCOO^-$ by single point coordinate bond. Combined all of the results above, it could deduce that the main constitutes of anionic-zero valent and low electro-positive, smaller molecular, mainly combined with single point coordination are the properties and structural characteristics of the chromium complexes released from chrome tanned leather in neutralizing process.

Keywords: hide powder; neutralizing effluent; chrome complex

INTRODUCTION

Chrome-tanned leather has excellent performance, so chrome tanning is still dominant in the leather industry. But the chromium pollution problem is increasingly brought to the attention, chrome tanning method is facing severe challenges, therefore minimize chromium pollution has significant environmental benefits. After chrome tanning, the chrome release in post-tanning process in the post-tanning process like rewetting, neutralizing, retanning, dyeing and fatliquoring effluents, leading Cr_2O_3 content of post-tanning effluents reached 10~450mg/L that is significantly more than the limit standard (Zhou *et al.,* 2012) are still needed to face. In order to solve chrome pollution problems, chrome release in post-tanning process must be highly valued and adopt corresponding measures.

Organic retanning agents, dyestuffs and fatliquoring agents are mainly anionic materials used in subsequent process of wet blue. The neutralizing process is usually used to raise the pH and adjust the charge to promote penetration of anionic materials. Therefore, there is a certain amount of chrome released in neutralizing process. This article studied the composition, structure and properties of chrome complexes released from the chrome tanned skin collagen in neutralizing effluent.

The hide powder was tanned by chromium sulfate liquor with 33% basicity (BCS), and then the tanned collagen was treated by general neutralizing process to obtain neutralizing effluent. Ion exchange chromatography (IEC) and gel filtration chromatography (GFC) were used to separate neutralizing effluent; Ultraviolet-visible absorption spectroscopy (UV-Vis) and Fourier Transform Infrared Spectroscopy (FT-IR) were used to analyze the structure of chrome complexes.

EXPERIMENTAL

Preparation of Neutralizing Effluent

Hide powder was tanned by 0.2mol/L BCS for 2h at constant temperature bath oscillator (25°C, 200r/min). 1.2% $NaHCO_3$ was added four times to basify, the final pH of tanning liquor was 3.8. The tanned hide powder was dried at 40°C after tanning.

0.2% formic acid and 150% water were added in dried hide powder, and then put in constant temperature bath oscillator for 2h (38°C, 200r/min). Then, the hide powder was washed by running water for 10min. 2% sodium formate and 150% water were added and put in constant temperature bath oscillator for 90min (35°C, 200r/min). After filtered with nylon filter cloth, neutralizing effluent was prepared.

Ion Exchange Chromatography Separation

5ml neutralizing effluent was filtered by 450nm microporous membranes, and then added into SP Sephadex C-25 column (inner diameter 2.0cm, packed 20.0cm). Elution conditions were as following: eluent velocity for 2.5 mL/min, followed by using H_2O, $NaClO_4$ (0.5, 1.0, 2.0 mol/L), HCl (2.0, 3.0 mol/L) to elute. UV-Vis spectrophotometer (Shanghai Jinghua Technology Corporation) was used to detect absorbance at 420nm and compare with BCS separated compositions (Li *et al.*, 1992).

Gel Filtration Chromatography Separation

5ml neutralizing effluent was filtered by 450nm microporous membranes, and then added into SP Sephadex G-25 column (inner diameter 2.0cm, packed 80.0cm). Elution conditions were as following: eluent velocity for 2.5 mL/min and H_2O was used to elute. UV-Vis spectrophotometer was used to detect absorbance at 420nm and compare with BCS separated compositions (Davis and Scroggie, 1973).

UV-Vis Analysis

After IEC separation, collected components were freeze-dried and then dissolved by distilled water. UV-Vis spectrophotometer was used to scan obtained liquor at 300-800 nm. R was calculated by molar absorption coefficient of chrome in 420nm and 580 nm.

FT-IR Analysis

FT-IR spectrum in the 4000–400 cm^{-1} was recorded with a Nicolet 10 FT-IR spectrophotometer (American Thermo Scientific Corporation). Samples were prepared by grinding about 1 mg freeze-dried components with 100 mg KBr, and the mixture was pressed into very thin disks and scanned for 32 times.

RESULTS AND DISCUSSIONS

Charge Characteristics of Chrome Complexes in Neutralizing Effluent

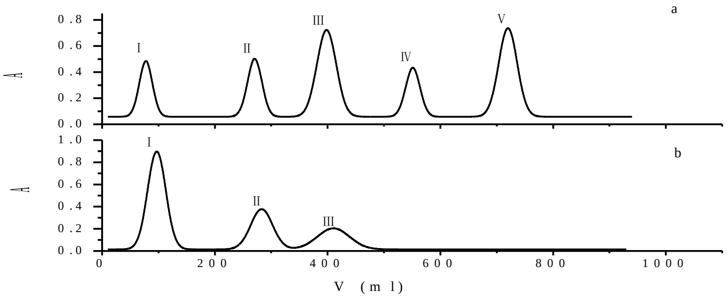

Figure 1. Ion exchange chromatography of BCS (a) and neutralizing effluent (b)

IEC of BCS and neutralizing effluent are shown in Fig.1. As can be seen from Fig.1, at the same elution conditions, BCS is separated into five components (anionic-zero valent, monovalent, bivalent, trivalent and tetravalent chrome complexes), and neutralizing effluent is divided into three components (anionic-zero valent, monovalent and bivalent chrome complexes). Based on IEC peak area, the composition of chrome complexes in BCS and neutralizing effluent can be calculated and listed in Table 1.

Molecular Weight of Chrome Complexes in Neutralizing Effluent

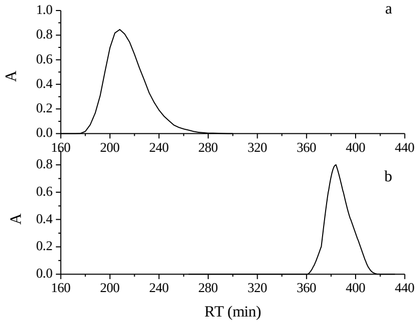

Figure 2. Gel filtration chromatography of BCS (a) and neutralizing effluent (b)

GFC is often used to analyze the molecular weight distribution to study the structure of coordination compounds. The retention time of the large molecular weight complexes is shorter while the small molecular weight is longer during the GFC separation process. GFC results of BCS and neutralizing effluent are shown in Fig.2. As shown in Fig.2, during 172min and 280min, chrome complexes in BCS are washed off completely (Fig.2 a); between 368min and 408min, chrome complexes in neutralizing effluent are washed off completely (Fig.2 b). Accordingly, the molecular weight of chrome complexes in neutralizing effluent is far less than BCS. The small molecular weight chrome complexes cannot combine with skin collagen by stable combination, therefore small chrome complexes are easier to release.

UV-Vis Results

Table 2. R values of each constituent in neutralizing effluent

R values	I	II	III	IV	V
BCS	1.08	1.10	1.09	1.15	1.36
neutralizing effluent	0.95	0.80	0.83	-	-

The combination way between ligands and Cr^{3+} can be determined by R values, R values of each constituent in neutralizing effluent are listed in Table 2. The results in Table 2 showed that, the R value of chrome complex components I, II, III, IV and V in BCS are 1.08, 1.10, 1.09, 1.15 and 1.36 respectively, however, the R value of chrome complex components I, II and III in neutralizing effluent are 0.95, 0.80 and 0.83. The R values of I, II and III are all less than 1.19 indicating that Cr^{3+} mainly combined with ligands through single point coordination and there are less bridge linkages between Cr^{3+} and ligands.

FT-IR Results of Chrome Complex in Neutralizing Effluent

Characteristic absorption peak and coordination mode of I to III components of chrome complex in neutralizing effluent are shown in Table 3. It is seen that 4 absorption peaks are found in 900-1300cm^{-1} for chromium complex in component I, which indicate that interconnection mode for SO_4^{2-} and central ion Cr^{3+} is two-point coordination. In the same way, 2 and 3 absorption peaks in component II and III respectively demonstrate that SO_4^{2-} is free state and coordinate with Cr^{3+} by single point coordination.

One asymmetric and one symmetric stretching vibration absorption peak are respectively found in 1550-1680cm^{-1} and 1370-1465cm^{-1} of chrome complex in component I, II and III, moreover, the interval of these two absorption peaks are small. Therefore single point coordination is speculated between Cr^{3+} and $HCOO^-$ (Chen and Li, 2011).

Table 3. FT-IR absorption peak and coordination mode of chromium complex in neutralizing effluent

Component	Scope (cm^{-1})	Peak (cm^{-1})	Num.	Ligand	Coordination mode
I	900-1300	995,1048,1148,1114	4	SO_4^{2-}	two-point
	1680-1550 1465-1370	1633 1405	2	$HCOO^-$	single point
	3400	3439	1	OH	----
II	900-1300	941,1088	2	SO_4^{2-}	free
	1680-1550 1465-1370	1630 1430	1	$HCOO^-$	single point
	3400	3423	1	OH	----
III	900-1300	941,1087,1141	3	SO_4^{2-}	single point
	1680-1550 1465-1370	1631 1422	1	$HCOO^-$	single point
	3400	3441	1	OH	----

Chromium complex Structure in neutralizing effluent is speculated by valence state of IEC (Tab.1) and R values (Tab.2). Its possible structures are shown in Fig.3.

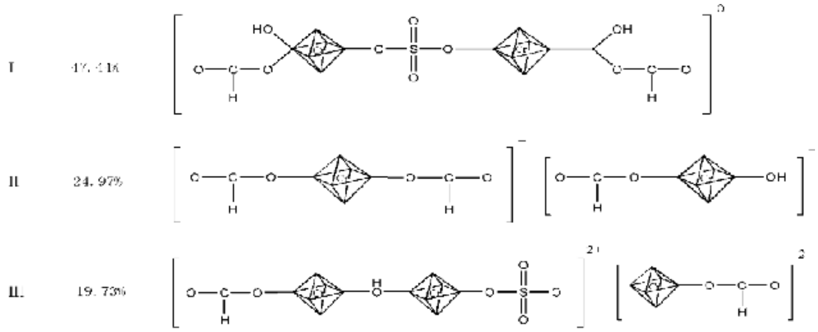

Figure 3. Possible structures of chrome complexes in neutralizing effluent

CONCLUSIONS

The results of IEC and GFC indicate that the neutralizing effluent mainly consisted of anionic-zero valent and low-positive electricity (+1 and +2) chrome complexes; the molecular weight of chrome complexes in neutralizing effluent is far less than the chromium sulfate liquor with 33% basicity. The results of UV-Vis and FT-IR show that releasing chromium complexes is mainly combined with SO_4^{2-} and $HCOO^-$ by single point coordinate bond. Combined all of the results above, the main characteristics of the chromium complexes released from neutralizing process of chrome tanned leather are anionic-zero valent and low electro-positive component, smaller molecular, mainly combined with ligands through single point coordination.

Acknowledgements

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REFERENCES

Chen, W.Y. and Li, G.Y. (2011), *Tanning Chemistry*, China Light Industry Press (the third edition), Beijing.

Davis, M.H. and Scroggie, J.G. (1973), “Investigation of Commercial Chrome-Tanning Systems, Part I”, *Journal of the Society of Leather Technologists and Chemists*, 57(1), 13-18.

Li, G.Y., Cheng, J. and Zhang, M.R. (1992), “The research on the composition of chromium sulfate tanning liquor”, *China Leather*, 21(10), 8-19.

Zhou, J., Hu, S.X., Wang, Y.N., He, Q., Liao, X.P., Zhang, W.H. and Shi, B. (2012), “Release of Chrome Tanning and Post Tanning Process”, *Journal of the Society of Leather Technologists and Chemists*, 96(4), 157-161.

NEW POLYMERIC COMPOUNDS WITH HIGH TEMPERATURE AND IMPACT RESISTANCE

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This paper focuses on development and characterization of new impact and flame resistant advanced materials based on polyamide / compatibilizer / polycarbonate / glass fibers (GF) functionalized with organo silane. Polyamides (PA) are highly crystalline hard materials, that have high impact resistance, burn slowly or are self-extinguishing. Polycarbonates (PC) are thermoplastic polymers intended for oil and high temperatures resistant materials. To increase impact resistance, polymeric compounds were reinforced with short functionalized glass fibers. The thermoplastic polymeric materials are visionary and materials for the future, with special properties, depending on the type of elastomers, the type of reinforcement material, the ratio of reinforcement, the functionalization of the reinforcing material, the type of functionalization agent, the ratio of elastomer-compatibilizing agent-reinforcing agent, etc. Thus by determining the optimum types and ratios of polycarbonate, polyamide, compatibilizer, functionalized glass fibers and optimal processing conditions and parameters, special properties can be achieved: flame retardant and especially self-extinguishing properties, physical-mechanical, chemical and technological properties, superior to the current standards in the field. Due to their characteristics the new materials can be employed in development of finished goods for the automotive and electrical insulation industry, which must meet European legislation (directives) into force.

Keywords: hybrid composites, impact resistance, thermal resistance

INTRODUCTION

Manufacturers requested performant materials for processing and use, which determined development of new polymer structures, with reinforcing agents, having optimized properties compared to traditional materials used in this domain. Automotive and electrical insulation industries are interested in easy to process new polymeric materials, characterized by resistance to shock, high temperature, and direct flame or even self-extinguishing properties. The existence of new reinforcing agents such as glass fiber (Loewenstein, 1993), simple or functionalized, compounded with polymeric materials leads to new composites that provide expected qualitative performance and to greening their processing technology.

The thermoplastic polymers are innovative future materials, with special properties, depending on the type of elastomer, the type and degree of reinforcement material, the functionalization of the reinforcing material, the type of functionalization agent, the elastomer-compatibilizing-reinforcing agent ratio etc. These properties offer high use value in fields which require materials with performance characteristics: dimensional stability, resistance to UV radiation, solvents, aggressive chemical agents, waterproof, electric resistance (Pokharkar and Sivaram, 1995; Park *et al*., 2002; Liu *et al*., 2000; Jones and Ballone, 2010; Liaw and Chang, 1997; Kricheldorf and Luebbers, 1990). In addition to the above, another important property is thermal resistance at a wide range of temperatures, and impact resistance. Properties are the result of a complex of original characteristics which arise from physical, mechanical and chemical interactions that occur during manufacturing processes that result in a polymer nanostructure.

In this paper polyamide (PA) was selected as the continuous phase, due to the fact that it can be processed at high temperatures (200-230°C), giving the finished product thermal resistance properties. Polycarbonate (PC), disperse phase, polymer with wide industrial applications (Sweileh *et al*., 2010; Lee *et al*., 2002), was also selected due to its high impact resistance.

By establishing optimal types and amounts of polycarbonate, polyamide, compatibilizer (ε-caprolactam), functionalized glass fiber and optimum processing technological parameters, high performance properties can be achieved, such as: impact, thermal resistance and especially self-extinguishing properties.

MATERIALS AND METHODS

Materials

In order to achieve the thermal resistance hybrid composites, the following materials were used: (1) Polyamide - standard PA for injection - Sebamid 6 s3c (Basplast), (2) Polycarbonate - CALIBRE^{TM} polycarbonate resin, density 1.2g/cm^3 (Trinseo Gmb), (3) simple Glass Fiber - BMC3 4.5 mm, (4) Organosilane - 3-Aminopropyl)trimethoxysilane, Mw-179.29 g/mol, bp:91-92°C (lit) (Sigma-Aldrich Chemistry, USA).

Method

In order to obtain the impact and heat resistant polymeric composites, the first task was to functionalize the glass fiber. The treatment has an important role on the final properties of the glass fibers and consequently on the final polymer composite that incorporates them. Thus, the treatment method of the glass fiber was designed as follows:

- In a plastic container (to avoid silanization directly on the walls of the glass container) are added weighed glass fibers;
- Ethyl alcohol is added so that they are fully immersed in alcohol;
- They are mechanically mixed for 3-4 hours;
- Add 3-aminopropyltrimethoxysilane(APTMS), functionalization agent - 5% wt, dropwise into the mixture of glass fibers and alcohol. When introducing organosilane, the glass fibers should be completely immersed in alcohol and mixing continuous to ensure homogeneity of the mixture; mixing continues for 3 hours to ensure the binding of functionalization agent to the glass fiber;
- Treated glass fibers are dried for 24 hours at 80°C.

The PA/PC polymeric composites, compatibilized with ε-caprolactam, and reinforced with organosilane treated glass fiber, were made using a Plasti-Corder Brabender mixer, type 350 E (Figure 1). Considering the optimal technological parameters, compounds were processed into finished products (plates) by molding method, using electrically heated press. After stabilization for 24 hours at room temperature, the plates were submitted to physical-mechanical characterizations.

The method for achieving multiphase polymer composites is as follow: PA / ε-caprolactam / PC / GF (simple or treated with APTMS) was added in the mixer, temperature was 200°C (zone 1) / 200°C (zone 2) / 200°C (zone 3). Set mixing speed at 100 rot/min to ensure plasticizing components and uniform dispersion of glass fibers. Simple or treated fiberglass was added to the mixture in minute 8 and 13 respectively, continuing mixing to a maximum of 15 minutes. The total mixing time is 10-15 minutes / formulation.

Figure 1. Plasti-Corder Brabender Mixer 350 E

The obtained polymer composite granules are added in the molds, to process them according to test specimens used for physical-mechanical characterization for finished products, using the electrically heated press, TP 600, by means of compression method, between its platters at temperature of 220°C and 300KN pressure for 2 minutes preheating, 2 minutes actual forming in the press and 10 minutes cooling.

Table 1. PA / ε-caprolactam / PC / GF polymeric composites

Samples	UM	P1	P2	PG1	PG2	P3	P4	P5	P6	P7	P8
Polyamide	%	100	-	90	50	70	70	70	70	70	70
Polycarbonate	%	-	100	10	50	30	30	30	30	30	30
ε-caprolactam	%	-	-	-	-		5	-	-	5	5
Glass fiber (treated with APTMS)	%	-	-	-	-	-	-	-	10	-	10
Glass fiber (simple)	%	-	-	-	-	-	-	10	-	10	-

RESULTS AND DISCUSSIONS

The structural determinations were carried out on an IR molecular absorption spectrometer with double beam, in the range of 4000-600 cm^{-1}, using a 4200 FT-IR equipped with ATR crystal diamond and sapphire head. Samples analyzed are divided into four categories:

- Reference samples: PA, PC, and ε-caprolactam;
- Samples with different PA/PC ratio (90-50%) ,PG$_{1,}$ PG$_2$,P$_3$;
- Samples with ε-caprolactam P$_{4,}$;
- Samples with GF (simple or treated) P$_5$, P$_6$, P$_7$, P$_8$.

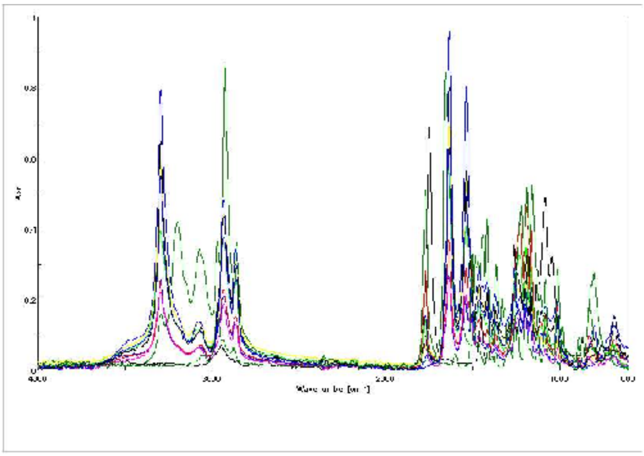

Figure 2. Overlapping IR spectra of PA / ε-caprolactam / PA/GF polymer architectures

From overlapping spectra is noted the presence of PA and PC in the polymeric composites. The presence of PA and PC in variable percentages, by the intensity of characteristic peaks, is observed. Note that the plastomer (PA and PC) is in excess of the other components, being the main ingredient. The compatibilizer, and GF cannot be distinguished very well in this image, due to the small amount (ε-caprolactam max.5% and GF 10% wt plastomer).

The results of the physical-mechanical properties of the samples obtained for the polymer composites based on PA and PC, compatibilized with ε-caprolactam and glass fiber reinforced, compared with the reference samples are shown in Figures 3, 4 and 5.

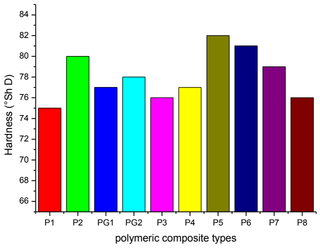

Figure 3. Variation of hardness for composites P1-P8

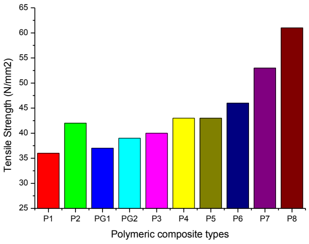

Figure 4. Variation of tensile strength for composites P1-P8

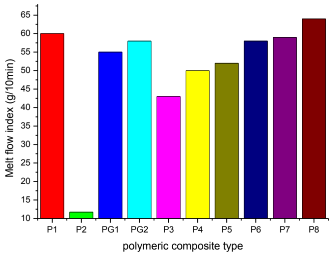

Figure 5. Variation of Melt Flow Index for composites P1-P8

Hardness decreases when adding the ε-caprolactam compatibilizer. When adding simple glass fibers, hardness increases but using the treated glass fibers instead, the hardness decreases by 1-2°Sh D. Thus, for the polymeric composites containing compatibilizer and treated fiberglass this property decreases by 2-3°Sh D.

Tensile strength decreases when mixing the plastomers, PA and PC, based on the ratio used between them. The addition of compatibilizer improves the tensile strength compared to composites P_3. Treated glass fiber without the compatibilizer decreases the tensile strength to the PG_2 (PA / PC 50/50%) level; while the use of compatibilizer between the two polymers (PA/ PC) increases the value of the tensile strength. In that sense P_8 composite presents values of 61 N/mm^2.

Elongation at break in composites PG_1-P_8, with added compatibilizer, simple or treated glass fibers could not be read due to the fact that they demonstrate increased stiffening.

Melt Flow Index - It is noted that there are considerable differences between the viscosities of the two base polymers (PA and PC) which affects the flow of material. Thus, there were obtained values for PA 60g / 10 min and 11.7g / 10 min PC. Melt flow Index ranges, for the obtained composite materials, varies widely between these two extremes, by the base polymer ratio, absence/presence of compatibilizer, absence/ presence and the type of glass fiber. Thus, flow index of composites PG_1-P_3 decreases with increasing percentage of PC. The addition of the compatibilizing agent in a proportion of 5% increases the melt index value. Using 10% simple glass fiber in composite P_3 (70/30), without compatibilizing agent has no major effect. When using compatibilizer and treated glass fiber the melt flow index strongly increases. Thus, composites PA / compatibilizer / PC / with treated GF - P_8 present flow values of 64 g/10 min, at a temperature of 230°C.

CONCLUSIONS

Samples of PA / PC without compatibilizer are not homogeneous, which makes the dispersion of the matrix PC on PA matrix to be achieved at high speed, the mixture is rigid and becomes brittle after cooling.

Introducing compatibilizer (ε-caprolactam) significantly improves the mixture processing, dispersion of discontinuous phase - PC is uniform, the mixture is homogenous and non-brittle after cooling.

Increasing the percentage of compatibilizer does not visibly improve processability.

Functionalization was carried out by immersing the glass fibers in a solution of ethanol and 3-aminopropyl trimethoxysilane (5%) was added dropwise. Then the fibers were dried at 80°C for 24 hours.

Treated glass fiber increases tensile strength and slightly decrease hardness, it disperses evenly, it is not necessary to increase the rate of mixing.

Hardness decreases when adding the ε-caprolactam compatibilizer. For the polymeric composites containing compatibilizer and treated fiberglass, this property decreases by 2-3°Sh D.

Tensile strength decreases when mixing the plastomers, PA and PC, based on the ratio used between them. Treated glass fiber with the compatibilizer between the two polymers (PA/ PC), greatly improve the tensile strength. In that sense P_8 composite presents values of 61 N/mm^2.

Melt Flow Index - It is noted that there are considerable differences between the viscosities of the two base polymers (PA and PC) which affects the flow of material. Using 10% treated glass fiber in composite P_3 (70/30), with compatibilizing agent, increases the melt flow. Composites PA / compatibilizer / PC / with treated GF - P_8 present flow values of 64 g/10 min, at a temperature of 230°C.

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REFERENCES

Jones, R.O. and Ballone, P.A. (2010), “Combined density functional and study of polycarbonate”, *Molecules*, 15, 3680.

Kricheldorf, H. and Luebbers, D. (1990), “Polymers of carbonic acid. 3. Thermotropic polycarbonates derived from 4,4`-dihydroxybiphenyl and various diphenols”, *Macromolecules*, 23, 2656-2662.

Lee, J., Song, C., Kim, J.I. and Kim, J.H. (2002), ”Preparation of aromatic polycarbonates nanoparticles using supercritical carbon dioxide.” *Journal of Nanoparticle Research*, 4, 53-59.

Liaw, D.J. and Chang, P. (1997), “Synthesis and characterization of aromatic and brominated aromatic polycarbonates by two-phase-transfer-catalyzed polycondensation of bisphenols with trichloromethyl chloroformate”, *Journal of Applied Polymer Science*, 63, 195-204.

Liu, Z., Cunha, A.M., Yi, X.S. and Bernardo, A.C. (2000), “Key properties to understand the performance of polycarbonate reprocessed by injection molding”, *Journal of Applied Polymer Science*, 77, 1393-1400.

Loewenstein, K.L. (1993), *The Manufacturing Technology of Continuous Glass Fibers*, 3rd revised ed., Elsevier.

Park, J.H., Hyun, J.C., Kim, W.N., Kim, S.R. and Ryu, S.C. (2002), “Extensional and complex viscosities of linear and branched polycarbonates”, *Macromolecular Research*, 10, 135-139.

Pokharkar, V. and Sivaram, S. (1995), “Poly(alkylene carbonate)s by the carbonate interchange reaction of aliphatic diols with dimethyl carbonate: Synthesis and characterization”, *Polymer*, 36, 4851-4854.

Sweileh, B.A., Al-Hiari, Y.M., Kailani, M.H. and Mohammad, H.A. (2010), “Synthesis and Characterization of Polycarbonates by Melt Phase Interchange Reactions of Alkylene and Arylene Diacetates with Alkylene and Arylene Diphenyl Dicarbonates”, *Molecules*, 15, 3661-3682.

NANOMATERIALS BASED ON TiO_2 FOR EFFLUENT POLLUTANT PHOTODEGRADATION

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One of the most recent nanomaterials that has attracted great attention due to its unique properties is titanium dioxide. TiO_2 powders possess interesting optical, dielectrical and catalytical properties, which results in industrial applications such as pigments, fillers, catalyst supports and photocatalysts. Photocatalysis over titanium dioxide has been heavily investigated as a promising means of treating a wide variety of emerging pollutants and recent research effort has been devoted to improving the photocatalytic efficiency of TiO_2 through a variety of materials engineering approaches, such as enhancing the visible-light activity of TiO_2 through doping. Tannery wastewater represents a serious environmental and technological problem, owing to the large amounts of poorly biodegradable organic chemicals used. The use of photocatalytic TiO_2 nanoparticles to decontaminate industrial tannery wastewater has not been yet investigated. That is why, this paper aims to investigate the synthesis and characterization of TiO_2 and N doped TiO_2 nanoparticles used for the photodegradation of effluent pollutants from the leather industry. The new nanoparticles were synthesized through chemical routes and were characterized by dynamic light scattering and diffuse reflectance spectra measurements. Their photocatalytic activity was tested against an organic pollutant model, Orange II (OII).

Keywords: nanomaterials, TiO_2, photodegradation

INTRODUCTION

Recently, water shortages are becoming an increasing problem due to scientific advances following rapid industrial growth, environmental pollution, depleted water resources, global warming causing abnormal climate changes and uncontrolled groundwater evolution (Fujishima *et al*., 2009).

The rapid development of manufacturing technology after the industrial revolution has significantly improved the standards of living, but it is becoming a threatening factor for human health and the environment. Pollutants are changing both quantitatively and qualitatively and the number of chemicals currently in circulation is 38,000, with more than 300 new materials being synthesized every year due to the diversification of industrial structures and high-tech industry (Schneider *et al*., 2014).

In line with national development, growth and policy, industrial wastewater is becoming more contaminated and difficult to process.

Organic compounds, toxic pesticides and manure emission from each industry are polluting drinking water and rivers, which is becoming a worldwide contamination with increased severity.

The wide area of water pollution, diversification and non-biodegradable compounds has become a problem that cannot be solved by the natural cleansing cycle (Malato *et al*., 2009; Chong *et al*., 2010).

Moreover, in the case of water treatment technology, which includes non-biodegradable organic compounds, it is very difficult to completely remove pollutants with existing treatment technology.

Tannery waste water represents a serious environmental and technological problem, owing to the large amounts of poorly biodegradable organic chemicals used. Nowadays, most tanneries have a wastewater treatment process that usually consists of three stages:

flow homogenisation, dosing coagulants and flocculants; primary decanting to remove suspended solids as well as most of the Chemical Oxygen Demand (COD); biological treatment with subsequent secondary decanting to remove most of the pollutant content (COD and Biological Oxygen Demand (BOD_5)). However, a tertiary treatment is often still required to refine the COD and remove the colour and/or some organic recalcitrant compounds. As a result, other methods are being increasingly explored as alternatives to classical physico-chemical and biological processes.

The search for the development of new and inexpensive methods for the treatment of industrial wastewater is always on progress.

Different methods of separation, degradation and elimination have been used on different polluting chemical agents which are generally present in wastewater coming out from the industrial sector. The treatment of such pollutants can be achieved using an advanced oxidation process (AOP), like the heterogeneous photocatalysis due to its efficiency and low cost, as well as to the fact that it allows the complete degradation of organic pollutants to CO_2 and inorganic acids.

Recently, the application of TiO_2 photocatalysts has mainly been focused on the decomposing toxic and hazardous organic pollutants in contaminated air and water, which is of great importance for the environmental protection (Shon *et al*., 2013; Sharma and Sharma, 2012; Cloete *et al*., 2012).

However, the use of photocatalytic TiO_2 coatings to decontaminate industrial tannery wastewater has not been investigated yet.

Titanium dioxide (TiO_2) is the most widely used semiconductor photocatalyst due to its stability, non-toxicity and relatively low cost (Savage and Diallo, 2013).

However, color removal from wastewater is the most complex and difficult task.

Dyes are usually the first contaminant to be recognized in industrial wastewater due to their high visibility even in minute concentrations (<1 ppm). These colored wastewaters are a considerable source of eutrophication as well as non-aesthetic pollution that can produce dangerous byproducts by further oxidation, hydrolysis, or other chemical reactions taking place in the wastewater phase. Beyond the toxic effects of dyes in wastewater streams, the presence of dyes can cause reduced light penetration resulting in reduced photosynthetic activity thus making oxygen unavailable for biodegradation of microorganisms in the water.

Textile industry, leather tanning industry, paper industry, food industry, hair colorings, photoelectrochemical cells and light-harvesting arrays also contribute to the presence of dyes in wastewater. Majority of dyes used in various industries are toxic and carcinogenic thus posing a serious hazard to humans as well as to marine ecosystem. Therefore, the impact of dyes released into the environment has been extensively studied in the last few years (Herrmann, 2011).

Among major dye categories, azo dyes are the largest group of colorants and over 50% of all the dyes used in industries are azoic dyes.

Apart from their physically unpleasant nature and toxicity, the ever increasing massive production rate of dyes due to increasing industrialization has led to the necessity of an effective treatment. Therefore, in order to treat such obvious and challenging effluents, a wide range of technologies have been tested to reduce their potential magnified impacts on the environment (Ohno *et al*., 2012).

Traditional physical techniques such as activated carbon, adsorption, reverse osmosis, ultrafiltration can be used for dye removal. However, these processes simply transfer the pollutants from one medium to another causing secondary pollution. This generally requires further treatment of solid-wastes and regeneration of the adsorbent, which adds more cost to the process. Chemical processes such as chlorination, ozonation, adsorption on organic or inorganic matrices, precipitation, chemical oxidation processes, advanced oxidation processes and photodegradation through photocatalysis are also commonly being used for synthetic dye removal.

However, toxic unstable metabolites as a result of most of these processes impart adverse effects on animal and human health.

Biological processes involving microbiological or enzymatic decomposition and biodegradation have also been used for dye removal from wastewaters. However, it has been found that these conventional biological treatment processes are ineffective for synthetic dyes having recalcitrant nature.

In recent years, a broad range of synthetic dyes have been extensively studied to develop a more promising technology based on AOP that has the ability to oxidize contaminants quickly and non-selectively. AOP rely on in situ production of highly reactive hydroxyl radicals ($OH^{\cdot}$) which can virtually oxidize any compound present in the water matrix, often at a diffusion controlled reaction speed. These radicals are produced with the help of one or more primary oxidants (e.g. ozone, hydrogen peroxide, oxygen) and/or energy sources (e.g. ultraviolet light) or catalysts (e.g. titanium dioxide).

Heterogeneous photocatalysis has proved to be as an efficient tool for degrading both atmospheric and aquatic organic contaminants. It uses the sunlight in the presence of a semiconductor photocatalyst to accelerate the remediation of environmental contaminants and destruction of highly toxic molecules. The type of the radiation used depends on the type of catalyst i.e. pure TiO_2 works under UV light (370–415 nm).

In order to assess the degree of dye photodegradation achieved during the treatment, general formation of CO_2 and inorganic ions is determined. However, it is impossible to measure the exact concentration of these ions in case of real wastewaters. In such cases the determination of total organic carbon (TOC) or the measurement of the chemical oxygen demand (COD) or the biological oxygen demand (BOD) is used to monitor extent of dye mineralization.

Among various types of photocatalysts, TiO_2 assisted photocatalytic oxidation has received much attention in the last few years due to its non-toxicity, strong oxidizing power and long-term photostability (Akpan and Hameed, 2010). TiO_2 is a white powder semiconductor having a wide band gap of 3.0–3.2 eV. In general, there are three types of titanium dioxide i.e. anatase, rutile and brookite. Most of the studies have been carried out with anatase phase due to its high photocatalytic efficiency and adsorption affinity for the organic compounds as compared to the rutile phase.

There are various research studies on photocatalytic degradation of dyes using TiO_2 in several modified forms for performance enhancement under visible light. These include adsorption and surface complexation on TiO_2, non-metal doping, lanthanide ion doping, transition metal doping, noble metal doping and multi-atom doping. The main purpose of doping is to decrease the band gap of pure TiO_2 (3.2 eV for anatase phase) to bring the absorption band from UV to visible region.

There are various reports available on non-metal doping of TiO_2, especially with boron, carbon, sulfur, nitrogen and fluorine.

The main objective of non-metal doping is to bring the absorption band of TiO_2 to visible region.

The nitrogen-doped TiO_2 photocatalysts have been tested for the decomposition of aqueous solutions of organic compounds and dyes under UV and visible light illumination (Sun *et al.*, 2014).

Parida and Naik (2012) reported the degradation of methylene blue and methyl orange using N-doped TiO_2 showing 67% and 59% of degradation after 4 hours irradiation under visible light source.

Selvaraj *et al*. (2013) measured the photocatalytic degradation of the reactive triazine dyes including reactive yellow 84, reactive red 120 and reactive blue 160 on N-doped TiO_2 anatase and P25 in the presence of natural sunlight. It was reported that reactive yellow 84 indicated a faster degradation on N-doped TiO_2 in sunlight than the commercial Aeroxide P25.

EXPERIMENTAL

In this study, simple (TiO_2NPs) and N doped TiO_2 nanoparticles (N-TiO_2NPs) were synthesized via chemical routes. In order to prepare simple TiO_2 nanoparticles, titanium butoxide $Ti(OC_4H_9)_4$ (97% Sigma Aldrich) was used as starting material. N doped TiO_2 nanoparticles were obtained through the calcination of a mixture formed from TiO_2 nanoparticles and urea. The obtained nanoparticles are presented in Figure 1.

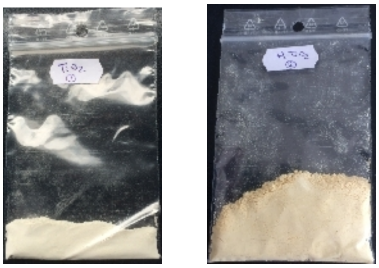

Figure 1. TiO_2NPs (left) and N-TiO_2NPs (right)

RESULTS AND DISCUSSION

The new obtained nanoparticles were characterized by dynamic light scattering technique using Zetasizer Nano ZS, Malvern. The results are presented below.

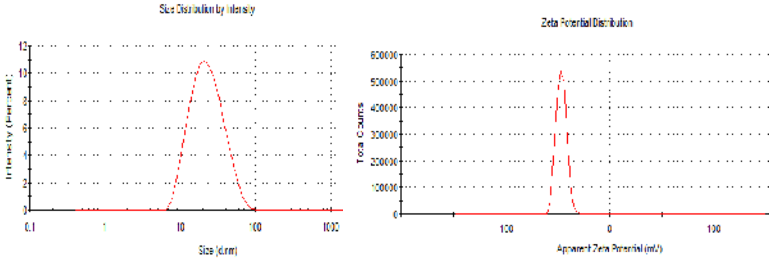

Figure 2. Size distribution (left) and Zeta potential (right) for TiO_2NPs

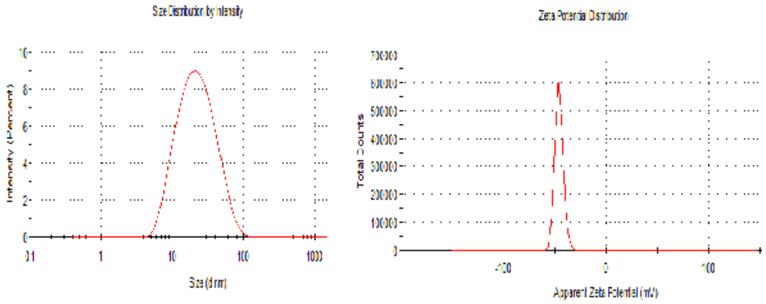

Figure 3. Size distribution (left) and Zeta potential (right) for N-TiO_2NPs

TiO_2 nanoparticles have sizes of about 25 nm, the N-doped around 30 nm and Zeta potential is -45 mV for the simple and -47 mV for the doped ones, suggesting high stability of samples.

In order to have more information about the reactions of the photocatalyst materials with photon energies, UV-Vis diffuse reflectance spectra were recorded.

In Figure 4 are presented diffuse reflectance spectra of N-TiO_2NPs comparative with undoped TiO_2.

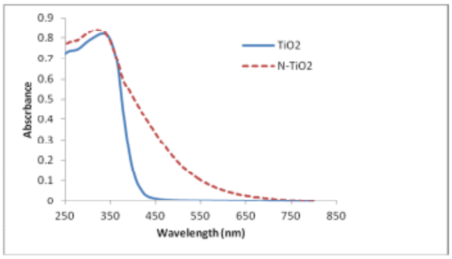

Figure 4. UV-Vis diffuse reflectance spectra for simple and N doped TiO_2 nanopowders

Figure 4 shows a significant absorption shift in the visible domain for N-TiO_2 powder.

To evidence photocatalytic degradation for a 20 ppm OII solution, in the presence of 0.1 g/L TiO_2 and N doped TiO_2 nanoparticles, absorbance spectra under visible light irradiation (150 W halogen lamp) were recorded (Table 1).

Table 1. UV-Vis absorbance of OII solutions with TiO_2 based nanoparticles after 1 hour of irradiation

Nanoparticles	Absorbance
TiO_2	0.28
N-TiO_2	0.05

Knowing that the initial value for the absorbance of OII was around 1, one can observe that the best value was obtained for N doped TiO_2 nanoparticles.

N-TiO_2NPs exhibited a significant increase in OII discoloring efficiency as compared with TiO_2 ones.

CONCLUSIONS

New doped nanoparticles with improved photocatalytic properties for effluent pollutant photodegradation were developed. TiO_2 dispersions characterized by dynamic light scattering technique indicate that these are very stable and well dispersed. The shifted photocatalytic activity in visible domain, depending on the type of doping element, was evidenced by diffuse reflectance spectra.

Owing to its many advantages mainly involving most stable and active naturally occurring photocatalyst, TiO_2 is, so far, seen as the best catalytic material for degradation of various contaminants and sustainable environmental remediation technology.

Photodegradation of industrial dyes using improved TiO_2 has presented a somewhat promising and effective treatment technology.

The results demonstrate the potential use of new synthesized photocatalytic TiO_2 nanoparticles in tannery waste water treatment.

Acknowledgement

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REFERENCES

Akpan, U.G. and Hameed, B.H. (2010), "Parameters affecting the photocatalytic degradation of dyes using TiO_2-based photocatalysts: a review", *Journal of Hazardous Materials*, 170, 520–529.

Chong, M., Jin, J., Chow, C.W. and Saint, C. (2010), "Recent developments in photocatalytic water treatment technology: a review", *Water Research*, 44, 2997–3027.

Cloete, S., Eugene, T., Kwaadsteniet, M., Botes, M. and López-Romero, M. (2012), Nanotechnology in Water Treatment Applications, Caister Academic Press.

Fujishima, A., Zhang, X. and Tryk, D.A. (2009), "Heterogeneous photocatalysis: from water photolysis to applications in environmental cleanup", *International Journal of Hydrogen Energy*, 32, 2664–2672.

Herrmann, J.M. (2011), "Heterogeneous photocatalysis: fundamentals and applications to the removal of various types of aqueous pollutants", *Catalysis Today*, 53, 115–129.

Malato, S., Fernández-Ibáñez, P., Maldonado, M.I., Blanco, J. and Gernjak, W. (2009), "Decontamination and disinfection of water by solar photocatalysis: recent overview and trends", *Catalysis Today*, 14, 59-71.

Ohno, T., Sarukawa, K., Tokieda, K. and Matsumura, M. (2012), "Morphology of a TiO_2 photocatalyst (Degussa P-25) consisting of anatase and rutile crystalline phases", *Journal of Catalysis*, 203, 82–86.

Parida, K. and Naik, B. (2012), "Synthesis of mesoporous TiO2− x N x spheres by template free homogeneous co-precipitation method and their photo-catalytic activity under visible light illumination", *Journal of Colloid and Interface Science*, 333, 269–276.

Savage, N. and Diallo, M.S. (2013), Nanomaterials and water purification: Opportunities and challenges, Springer.

Schneider, J., Matsuoka, M., Takeuchi, M. and Zhang, J. (2014), "Understanding TiO_2 photocatalysis: mechanisms and materials", *Chemical Reviews*, 114, 9919–9986.

Selvaraj, A., Sivakumar, S. and Balasubramanian, V. (2013), "Photocatalytic degradation of triazine dyes over N-doped TiO2 in solar radiation", *Research on Chemical Intermediates*, 39, 2287–2302.

Sharma, V. and Sharma, A. (2012), "Nanotechnology: An Emerging Future Trend in Wastewater Treatment with its Innovative Products and Processes", *International Journal of Enhanced Research In Science Technology & Engineering*, 15, 238-252.

Shon, H.K., Phuntsho, S., Chaudhary, D.S., Vigneswaran, S. and Cho, J. (2013), "Nanofiltration for water and wastewater treatment –a review", *Drinking Water Engineering and Science*, 6, 47–53.

Sun, J., Wang, X., Sun, J., Sun, R. and Qiao, L. (2014), "Photocatalytic degradation and kinetics of Orange Gusing nano-sized Sn(IV)/TiO2/AC photocatalyst", *Journal of Molecular Catalysis A: Chemical*, 260, 241–246.

COMFORT AND ANTIFUNGAL PROPERTIES OF ORTHOTIC MATERIALS USED IN FOOTWEAR

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An orthotic material could be defined as an externally applied device, which modifies the function by supporting or controlling a body part. Shoe orthoses are devices placed inside a shoe to accommodate anatomical abnormalities, to relieve pressure at a specific site or to alter the transmission of forces during gait. They work by applying force in a controlled manner and maybe accommodative or corrective devices. Besides, previous reviews have revealed that foot orthotics can prevent some lower limb overuse injuries, particularly femoral stress fractures and shin splints. These reviews highlight that further research in this area is still required, particularly in the form of wear comfort and antimicrobial properties of orthoses. Up to now, according to our knowledge there has been no reported research on these characteristic properties of orthoses materials in footwear. For this purpose, antifungal activity with *Aspergillus niger*, air permeability and water vapour permeability properties of five different orthotic materials were investigated in the present study. The results demonstrated that foot orthotic samples fulfilled the wear hygiene properties and could be used as safe antifungal materials for foot orthoses.

Keywords: orthoses, footwear, wear hygiene, antifungal

INTRODUCTION

Shoe inserts and/or orthoses are generally used to improve comfort, daily performance and/or to prevent the feet from the injuries (Nigg *et al.*, 1997). Shoe orthoses are also described as the devices placed inside the wear material to accommodate anatomical abnormalities, to relieve pressure or to alter the transmission of forces (Kurup *et al.*, 2011). Besides, foot inserts and orthoses have a great potential to prevent lower extremity ailments, including ankle or knee pain or, more specifically, plantar fasciitis, posterior tibial syndrome, Achilles tendonitis, patellar femoral pain syndrome, or osteoarthritis (Mündermann *et al*. 2001; Collins *et al*. 2007; Landorf and Keenan 2007; Murley *et al*. 2009).

In addition, requirement of comfort for shoe inserts and orthotics is crucial, however, limited information is available in the literatures about the effect of orthotic materials on footwear comfort (Mündermann *et al.*, 2001), particularly in the form of antimicrobial properties and comfort behaviors of orthoses. According to our knowledge, there has been no reported research on the antifungal and comfort properties in terms of air and water vapor permeability of orthotic materials in footwear.

In this study, antifungal activity with *Aspergillus niger* and comfort properties in terms of air permeability and water vapour permeability of orthotic materials such as dyatec, plastazote, thermoformable cork and 2 types of pedilin (2mm and 10mm) were investigated whether the materials could be used as an antifungal shoe device by providing a footwear comfort during gait.

MATERIAL AND METHOD

Material

In the present study, dyatec, plastazote, thermoformable cork, and 2 types of pedilin (2mm and 10mm) were investigated as orthotic materials. All chemicals used in the study were of analytical grade and purchased from Merck, Germany.

Methods

Antifungal Activity

The antifungal activity was performed in accordance with ASTM D 4576-86: 1996 Standard Test Method. The orthoses samples were cut into pieces having the surface of 1 inch^2 and the assays were done in duplicates with *Aspergillus niger* test organism. The test samples were placed in the center of Petri vessels and later the growth medium (*potato dextrose agar*-PDA), was filled up to the upper level of the samples. The Petri vessels were incubated for two weeks at the temperature of 26-30°C. Later, at 3, 7 and 14 days the Petri vessels were checked and evaluated visually according to the assessment as given below. In addition, the samples in Petri dishes were also photographed.

Table 1. Assessment of antifungal activity

0	mould absent on the surface of sample
0.5	less than 12% of sample surface is covered with micelle
1	25% of sample surface is covered with micelle
2	50% of sample surface is covered with micelle
3	75% of sample surface is covered with micelle
4	100% of sample surface is covered with micelle

Air Permeability

Orthotic samples were classified and conditioned for minimum 48 hours in an acclimatized room having a temperature of 20 ± 2°C and a relative humidity of 65% ± 5. The air permeability values of the samples were determined by means of a Devotrans branded air permeability measuring device. Measurements were performed by determining in m^3 the amount of air that flows for 5 minutes under 200 Pa and 500 Pa pressure with a ring of 100 cm^2; the results were expressed in cm^3/cm^2.s. The test was repeated at 3 different points of the samples and the mean values were calculated (Kanli *et al*., 2010).

Water Vapor Permeability

The water vapor permeability was performed in accordance with TS EN ISO 14268 (2004) standard.

RESULTS AND DISCUSSION

High air permeability and water vapor permeability properties are known as the most important requirements for foot comfort and hygiene of the materials for the medical devices and shoes.

Table 2. Air and water vapor permeability results of the orthotic materials

Materials	Air Permeability ($cm^3/cm^2.s$)	Water Vapor Permeability ($mg/cm^2.h$)
Cork	0.007	228.53
Plastazote	0.017	753.05
Pedilin (2 mm)	0.027	984.34
Pedilin (10 mm)	0.013	313.87
Dyatec	8.600	35135.98

The air and water vapor permeability test results of orthotic materials are shown in Table 2. Dyatec provided the highest water vapor and air permeability values such as 35135.98 $mg/cm^2.h$ and 8.600 $cm^3/cm^2.s$ respectively. Permeability values of the other orthoses were found quite lower than the Dyatec. However, comparable air permeability results were obtained from the cork, plastazote and pedilin materials. In contrast to air permeability results, water vapor permeability of the orthotic materials was determined quite satisfactory. Although low air permeability lead to a negative impact in terms of the air transfer in the footwear and resulted an uncomfortable feeling, pedilin (2mm) and plastazote could be differed due to their high water vapor permeability values. Milasiene (2003) indicated that the decrease in the water vapor permeability depends on the reduced porosity of the materials and the air permeability values of the materials could be directly affected from the same factors of water vapor permeability (Milasiene *et al*., 2003).

In addition to orthotic materials, the same samples were covered with goat lining leather and also analyzed for the determination of their antifungal activity. The antifungal activity test results and their photos were given in Table 3 and 4 respectively.

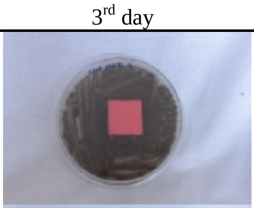
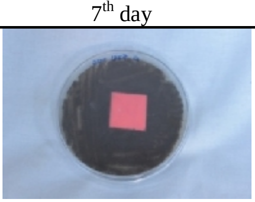
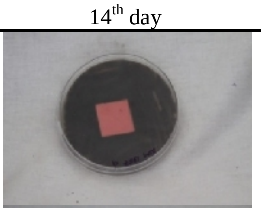
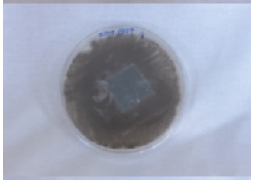
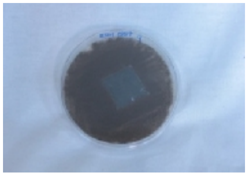
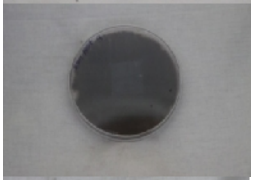
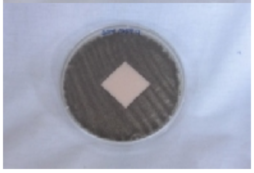
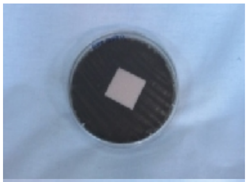
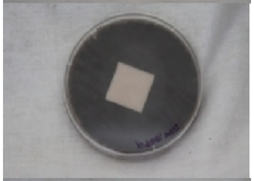
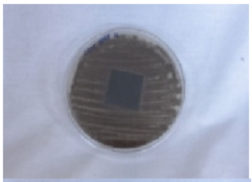
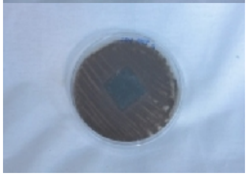
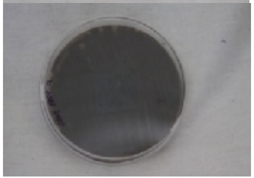
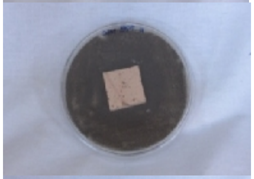
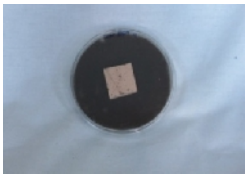
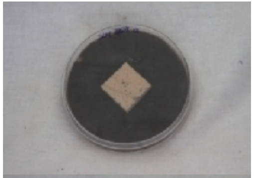
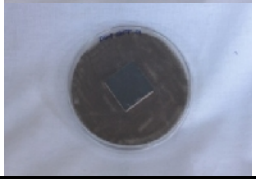
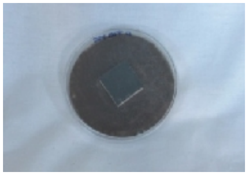
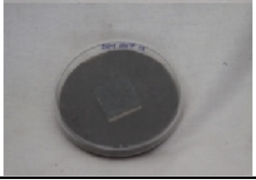
Table 3. Antifungal activity test results of the orthoses materials

Materials	3rd day	7th day	14th day
Dyatec	0	0	0
Dyatec with Leather	0	0.5	1
Plastazote	0	0	0
Plastazote with Leather	0-0.5	0.5-1	2
Cork	0	0	0
Cork with Leather	0	0.5	1
Pedilin (10 mm)	0	0	0
Pedilin with Leather (10 mm)	0	0.5	1
Pedilin (2 mm)	0	0	0-0.5
Pedilin with Leather (2 mm)	0	0.5	1
Leather	0-0.5	1	2-3

The 14th day results of antifungal activity with *Aspergillus niger* showed that there was no fungal growth on Dyatec, Plastazote, Cork, and Pedilin (10 mm) samples (Table

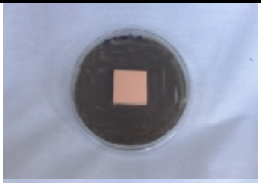
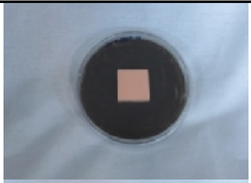
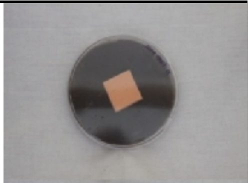
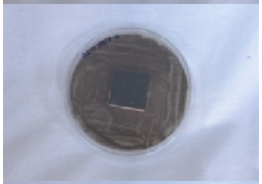
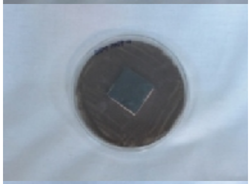
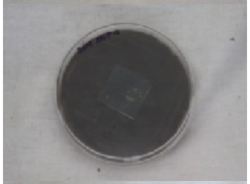
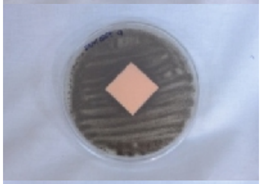
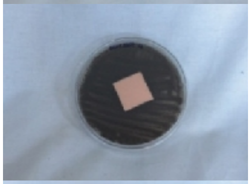
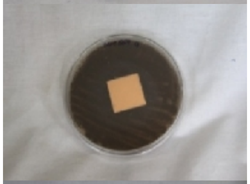
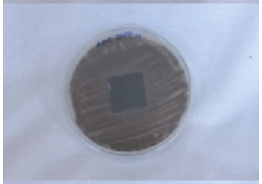
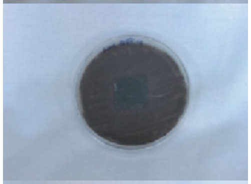
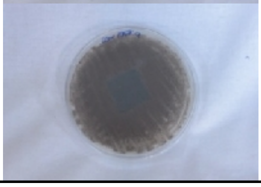
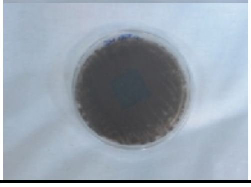
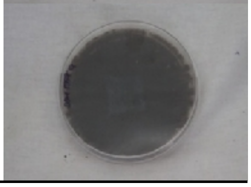
4), although the surface of Pedilin (2 mm) samples were covered with the organism micelles nearly in proportion of 12%.

Table 4. Final appearance of orthoses for the antifungal activity

		Aspergillus niger	
	3rd day	7th day	14th day
Dyatec			
Dyatec with leather			
Plastazote			
Plastazote with leather			
Cork			
Cork with Leather			

The results of orthotic samples covered with goat lining leather were found worse than the orthoses without lining leather. The lining leather was covered with *Aspergillus niger* nearly 65% of its area at the last assessment day. The reason of lower antifungal properties of the samples covered with leather was the lower antifungal property of the leather. The lowest antifungal effect was determined from Plastazote with leather samples.

Table 5. Final appearance of materials for the antifungal activity

	Aspergillus niger		
	3rd day	7th day	14th day
Pedilin (10 mm)			
Pedilin with leather (10 mm)			
Pedilin (2 mm)			
Pedilin with leather (2 mm)			
Leather			

CONCLUSION

In this study, antifungal activity with *Aspergillus niger*, air permeability and water vapor permeability properties of five different orthotic materials were investigated and following conclusions have been drawn; a) Orthoses could be characterized as antifungal materials; b) Only pedilin (2 mm) samples were covered with micelles of the organism in low proportions; c) Dyatec, Plastazote, Cork, and Pedilin (10 mm) demonstrated their safely usage as antifungal orthoses due to their effective antifungal properties against *Aspergillus niger*; d) Dyatec had the highest air and water vapor permeability results among the orthoses.

REFERENCES

ASTM D 4576-08. (2008), "Standard Test Method for Mold Growth Resistance of Wet-Blue (Leather)".

Collins, N., Bisset, L., McPoil, T. and Vicenzino, B. (2007), “Foot orthoses in lower limb overuse conditions: a systematic review and meta-analysis”, *Foot Ankle International*, 28, 396–412.

Kanli, N., Adiguzel Zengin, A.C. and Bitlisli, B.O. (2010), “The effects of different finishing types on water vapour and air permeability properties of shoe upper leathers”, *ICAMS 2010 – 3rd International Conference on Advanced Materials and Systems*, 16-18 September 2010, Bucharest, Romania, 63-66.

Kurup, H.V., Clark, C.I.M. and Dega, R.K. (2012), “Footwear and orthopaedics”, *Foot and Ankle Surgery*, 18, 79–83.

Landorf, K.B. and Keenan, A.M. (2007), *Do foot orthoses prevent injury? In evidence-based sports medicine*, Malden, MA: Blackwell, 73–92.

Milasiene, D., Jankauskaite, V. and Arcisauskaite, R. (2003), “Prediction of Stress Relaxation in Laminated Leather Layers”, *Materials Science (Medžiagotyra)*, 9(1),73-79.

Mündermann, A., Stefanyshyn, D.J. and Nigg, B.M. (2001), “Relationship between footwear comfort of shoe inserts and anthropometric and sensory factors”, *Medicine & Science in Sports & Exercise*, 1939-1945.

Murley, G.S., Landorf, K.B., Menz, H.B. and Bird, A.R. (2009), “Effect of foot posture, foot orthoses and footwear on lower limb muscle activity during walking and running: A systematic review”, *Gait & Posture* 29, 172–187.

Nigg, B.M., Khan, A., Fisher, V. and Stefanyshyn D. (1998), “Effect of shoe insert construction on foot and leg movement”, *Medicine & Science in Sports & Exercise*, 550-555.

TS EN ISO 14268. (2014), “Leather - Physical and mechanical tests - Determination of water vapour permeability”, Turkish Standard Institute, Ankara, Turkey.

MEDICAL BIOPRODUCTS COLLAGEN QUANTIFICATION BY HYDROXYPROLINE DETERMINATION

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The paper presents a method of quantifying collagen from medical collagen-based bioproducts obtained in the Collagen Research Department of INCDTP – Division ICPI by determining hydroxyproline. Collagen differs from usual proteins in that it contains a higher concentration of certain amino acids. Almost a third of collagen is made up of glycine, the smallest amino acid, and another third is made up of proline and hydroxyproline, the active form of proline. As hydroxyproline has been found in very few proteins other than collagen, hydroxyproline determination is used as a marker to quantify collagen levels from various medical products. Collagen is quantified by multiplying hydroxyproline content by the 6.6 factor. The proposed method was validated to establish performance parameters and to check accordance with the set goal, by determining: limit of detection, limit of quantification, selectivity, sensitivity, robustness, accuracy and reliability of the method.

Keywords: collagen, hydroxyproline, medical bioproducts

INTRODUCTION

Collagen analysis helps to characterize and ensure the safety of a multitude of healthcare products that incorporate this extracellular protein matrix. These applications include wound care, burn care, orthopedic graft products, tissue engineering, hemostatic sponges, injectables for soft tissue augmentation, as a vehicle for drug delivery and as an ingredient in skin and hair care products. Collagen is a highly versatile material and there is a growing interest in the processing and characterization of many types of collagen as developers find new applications. Due to its excellent biocompatibility and biodegradability, well-defined structure, biological characteristics and method of interaction with the body, collagen is one of the most frequently used biomaterials for medical treatment. Extracted in the form of aqueous solution or gel, type I fibrillary collagen may be modelled into various products: medical devices, artificial implants, drug release systems, creams and scaffolds for tissue regeneration, with important role in medicine (Albu *et al.*, 2011; Albu and Titorencu, 2011; Albu *et al.*, 2012a; Vranceanu *et al*., 2012; Albu *et al.*, 2012b; Albu *et al.*, 2015).

Collagen itself is considered an active drug/principle, used – in various forms – as hemostatic and dressing in the treatment of various types of wounds. Collagen is the basis of intercellular matter of conjunctive tissue found in bones, teeth, cartilage, tendons, ligaments, skin, blood vessels and has an important role in a series of physiological processes, provides resistance and structural integrity to the body. An increase in catabolism and collagen regeneration are important information in the pathogenesis of many diseases.

Collagen, a natural protein, cannot heal infected tissue by itself, as bacteria may use it as a substrate. In severe wound infections, systemic drug administration may lead to insufficient drug concentration at the infected site or to side effects associated to the drug and/or systemic toxicity. This deficiency found its successful resolution in local drug applications, by developing drug release systems using collagen as substrate and an antibiotic/antiseptic as drug for infection control (Albu and Titorencu, 2011; Vranceanu *et al*., 2012; Albu *et al.*, 2007; Albu *et al.*, 2010).

The presence of collagen in the body is essential for healing minor skin injuries, as well as wounds in different tissues, for repairing cartilage, ligaments and bones, including even dental degeneration. A natural polymer, collagen is made up of 20 amino acids, arranged in characteristic sequences that form a highly complex conformational structure, organized into four levels, called primary, secondary, tertiary and quaternary structures.

Collagen differs from regular proteins by the fact the it includes a higher concentration of certain amino acids. Almost a third of collagen composition is glycine, the smallest amino acid, and another third is proline and hydroxyproline, the active form of proline, an amino acid specific to collagen. Hydroxyproline is an amino acid that is synthesized from the irreversible post-translational hydroxylation of proline by prolyl hydroxylase. Hydroxyproline is found almost exclusively in the protein collagen, in the Y position of the repeating tripeptide Gly-X-Y. By allowing sharp twisting of the collagen helix, hydroxyproline helps to stabilize the structure of collagen. Since hydroxyproline has been found on so few proteins other than collagen, measurement of hydroxyproline has been used as a marker to quantify levels of collagen and/or gelatin (partial hydrolysis of collagen resulting in a mixture of protein and peptides). In addition, hydroxyproline measurement has been used to identify certain diseases that involve breakdown of collagen.

Quantification of collagen proteins in biomedical products is performed using a determination hydroxyproline method, in which the amount of hydroxyproline is measured and converted to the amount of collagen. However, the conversion factor can be inaccurate because of variation in the content of hydroxyproline. However, in converting hydroxyproline content to collagen type II content, there is little consensus on the appropriate factor. The hydroxyproline conversion factor (CF) for cartilage collagen content is highly variable according to different laboratories: CF = 6.94; 7.1; 7.6; 8.3; or 10. Experimentally CF = 6.6 for bovine collagen type I (De Ceuninck *et al*., 2004).

The total collagen content can then be extrapolated by multiplying amount of total hydroxyproline content in each sample by a factor of 6.6, based on the fact that hydroxyproline represents 14.4% of the amino acid composition of collagen in most mammalian tissues. (De Ceuninck et al., 2004).

Several experimental hydroxyproline determination approaches were found in the literature differing depending the nature of the material to be tested (Macovescu *et al*., 2016).

Samples subjected to analyses are first hydrolysed with acid to release hydroxyproline. This is generally performed using hydrochloric acid solution 6M or sulphuric acid 6M at temperatures from 110 to 130°C for 10 to 24 hours, either in sealed tubes or in reflux condensers. Free hydroxyproline is most conveniently quantified colorimetrically after oxidation to pyrrole, which is then reacted specifically with p-dimethylaminobenzaldehyde (Ehrlich's reagent) to produce an intense red-brown compound. Chloramine-T is now generally preferred as oxidant in pyrrole formation.

MATERIALS AND METHOD

Method Principle

Hydroxyproline determination is performed taking into account the following three basic steps:

a) hydroxyproline is derivatized from collagen by hydrolysis with sulphuric acid, at high temperature, 105°C;

b) hydroxyproline is oxidized by adding Chloramine-T, and the oxidation product is subjected to decarboxylation to pyrrole, in an acid medium at high temperature;

c) pyrrole combines, in an acid medium, with p-dimethylaminobenzaldehyde (DMAB) and the resulting addition product is determined by measuring the solution absorbance at 558 nm.

Hydroxyproline content is calculated and expressed as mass percentage.

Reagents

Only known analytical grade reagents (SIGMA ALDRICH) and distilled water, demineralized water or water equivalent in purity are used.

1. **Sulphuric acid solution**, 3 mol/L.
2. **Buffer solution**, pH = 6.8, consisting of:

- 26.0 g citric acid monohydrate;
- 14.0 g sodium hydroxide;
- 78.0 g sodium acetate anhydrous

Reagents are dissolved in 500 mL water and quantitatively transferred in a 1 litre volumetric flask. 250 mL N-propanol are added and water is filled up to the mark. When stored at the temperature of 4°C in the dark, this solution is stable up to a few weeks.

3. **Chloramine-T**

1.41 g N-chloro-p-toluenesulfonamide sodium salt trihydrate (Chloramine-T) are dissolved in 100 mL buffer solution. This solution is prepared immediately before use.

4. **Colour reagent**

10.0 g p-dimethylaminobenzaldehyde are dissolved in 35 mL perchloric acid solution [60% (m/m)] and then 65 mL isopropanol are slowly added. This solution is prepared on the day it is used.

5. **Hydroxyproline**, **standard solutions**

A stock solution is prepared by dissolving 50 mg hydroxyproline in water in a 100 mL volumetric flask. 1 drop sulphuric acid solution is added and filled up to the mark with water. This solution is stable for at least 1 month, stored at 4°C.

On the day of use, 5 mL stock solution is transferred into a 500 mL volumetric flask and filled up to the mark with water. Four standard solutions are then prepared by diluting 10 mL, 20 mL, 30 mL and 40 mL of this solution with water up to 100 ml to obtain hydroxyproline concentrations of 0.5 μg/mL, 1 μg/mL, 1.5 μg/mL, and 2 μg/mL, respectively.

Equipment

- Spectrometer, suitable for use at a wavelength of 558 nm ± 2 nm, or a photoelectric colorimeter with an interference filter with maximum absorption at 558 nm ± 2 nm. Glass cells with optical path length of 10 mm are used.
- Adjustable oven at 105°C ± 2°C;
- Analytical scales with accuracy of 0.0001 g;
- Adjustable water bath at 60°C.

Work Method

Sample Preparation

a. Approximately 0.5-1 g sample are weighed with an accuracy of 0.0001 g in hydrolysis tubes so that the sample does not adhere to the walls.

b. 10 mL sulphuric acid solution (1) are added, the tube is covered and placed in the oven at 105°C ± 2°C for 16 hours for hydrolysis.

c. The resulting hydrolysate is transferred into a 250 mL volumetric flask and filled with water up to the mark.

d. Using a pipette, a volume V is added into a 250 mL volumetric flask and filled with water up to the mark. Volume V will be taken so that hydroxyproline content would range between 0.5 µg/mL and 2 µg/mL.

e. 4.00 mL of this solution (d) is transferred into a test tube and 2.00 mL Chloramine-T reagent is added (3). The solution is stirred and left at room temperature for 20 min ± 1 min.

f. 2.00 mL colour reagent (4) is added, mixed thoroughly and the lid of the tube is covered with aluminium or plastic foil (5.6).

g. The tube is rapidly transferred into the water bath (5.7), set at 60°C and heated for 20 minutes precisely.

h. The tube is cooled under tap water stream for at least 3 minutes and left at room temperature for 30 min.

i. Absorbance is measured at 558 nm ± 2 nm in a glass cell compared to a blank of reagents.

j. To convert HYP to collagen, we can multiply the result by factor of 6.6

Calibration Curve

The procedure described from item **e.** to **i.** included is performed on standard hydroxyproline using 4.00 ml of the four diluted solutions. The calibration curve is plotted. Values for analyzed samples are read and concentration is calculated depending on the mass of the sample, dilutions and the sample volume **V** taken in item **d.**

RESULTS AND DISCUSSIONS

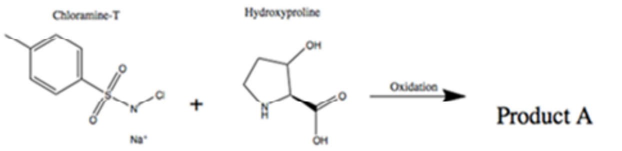
Collagen biomaterials for medical use from the Collagen Department of INCDTP - Division ICPI, namely collagen hydrolysates, gels and matrices used in wound treatment (Pancol, Gevicol), were studied to determine hydroxyproline (Albu *et al.*, 2012b; Albu *et al.*, 2015; Albu *et al.*, 2007; Albu *et al.*, 2010). Samples were physically-chemically characterised and the results are presented in Table 1:

Table 1. Characterization of collagen biomaterials for medical use

Characteristics Biomaterial	Dry matter, %	Ash*, %	Total nitrogen*, %	Appearance
Pancol batch 4	85.69	2.37	15.68	Spongious white foil
Pancol batch 5	84.40	2.93	16.29	Spongious white foil
Gevicol batch 4	87.47	2.62	16.35	Spongious violet foil
Gevicol batch 5	86.72	2.46	15.90	Spongious violet foil
Collagen gel batch 4	2.98	0.87	16.28	Transparent gel
Collagen gel batch 5	3.45	0.43	16.08	Transparent gel
Hydrolysate COL 24	84.95	1.99	16.97	Yellowish powder
Hydrolysate COL 25	85.55	1.76	17.15	Yellowish powder

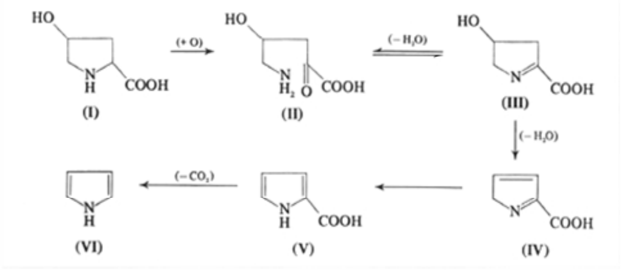
*values are recalculated without volatile matter

Chloramine-T (N-chloro-4-toluenesulfonamide sodium salt) was used as oxidation agent, as its indisputable advantages include easy decomposition of its excess and absence of coloured reduction products. The oxidation reaction is performed in a buffer solution with pH ~ 6.8. Hydroxyproline oxidation is illustrated by the following reactions:



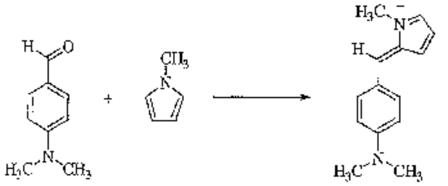
(1)

The postulated mechanism for the oxidation of hydroxyproline to pyrrole is as follows (2): first hydroxyproline (I) is oxidized to a linear compound, α-keto-γ-hydroxy-δ-aminovaleric acid (II), which is in equilibrium with the pyrroline-4-hydroxy -2-carboxylic acid with cyclic structure (III). The loss of water gives an unstable structure (IV), which spontaneously rearranges to pyrrole-2-carboxylic acid (V). The final step of decarboxylation to pyrrole (VI) takes place during the heating after the addition of the chromogenic reagent for pyrrole, p-dimethylaminobenzaldehyde (Etherington and Sims, 1981).



(2)

Chromophore formation is illustrated in the following reaction:



(3)

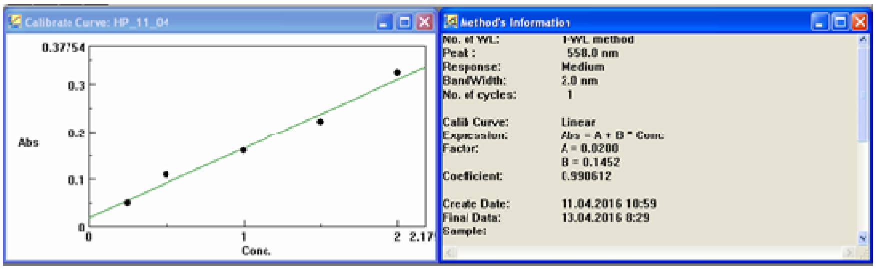
As the products tested have a much higher collagen content, the amount of sample tested was modified. Also, as the product in question is collagen hydrolysate, we considered it necessary to use more diluted mineral acids for hydrolysis, 3 molar.

Values for hydroxyproline and collagen in bioproducts for medical use determined using the adapted method are in accordance with literature data. Each value is the average of 10 replicated determinations, presented in Table 2.

Table 2. Hydroxyproline and collagen content of biomaterials for medical use

Biomaterial	Pancol batch 4	Pancol batch 5	Gevicol batch 4	Gevicol batch 5	Coll. gel batch 4	Coll. gel batch 5	Hydrolysate COL 24	Hydrolysate COL 25
Hydroxyproline,%	14.01*	14.22*	14.53*	14.65*	15.02*	15.12*	13.53*	13.77*
Collagen, %	92.46*	93.85*	95.89*	96.69*	99.13*	99.66*	89.30*	90.88*

* values are recalculated without volatile matter



No. of WL: 1-WL method
Peak : 558.0 nm
Response: Medium
BandWidth: 2.0 nm
No. of cycles: 1

Calib Curve: Linear
Expression: Abs = A + B * Conc
Factor: A = 0.0200
B = 0.1452
Coefficient: 0.990612

Create Date: 11.04.2016 10:59
Final Data: 13.04.2016 8:29
Sample:

Figure 2. Linearity range for concentrations ranging between 0.5 µg/mL and 2 µg/mL

Ten replicated samples of 1.5 µg/mL hydroxyproline concentration were prepared and Yi values were measured (integrated units) for signal intensity at 558 nm. Based on the equation of the calibration curve, Xi (µg/L) values of concentration obtained experimentally were calculated.

From the calculation of performance parameters of the studied method, the following are noted:

- The linearity range of the method was between 0.5-2 µg/mL, interval where the value of the correlation coefficient was 0.9906;
- If one takes into account smaller concentrations, the value of the correlation coefficient is 0.9903;
- Limit of detection was set for a concentration of 0.1088 µg/mL;
- Limit of quantification was set for a concentration of 0.1295 µg/mL;
- Accuracy is 99.36% and represents closeness between the real value and the determined value in the analysed sample;
- Standard deviation value is 0.00285 µg/mL (Macovescu *et al*., 2016).

REFERENCES

Albu, M.G. and Titorencu, I. (2011), "Biocompatibility study of collagen nerve conductors", *Leather and Footwear Journal*, 11(4), 329-340.

Albu, M.G., Deselnicu, V., Ioannidis, I., Deselnicu, D.C. and Chelaru, C. (2015), "Chemical functionalization and stabilization of type I collagen with organic tanning agents", *Korean Journal of Chemical Engineering*, 32(2), 354-361, DOI: 10.1007/s11814-014-0197-x.

Albu, M.G., Ficai, A. and Lungu, A. (2010), "Preparation and characterization of collagen matrices obtained at different freezing temperatures", *Leather and Footwear Journal*, 10(3), 39-50.

Albu, M.G., Ghica, M.V., Tang, K., Liu, J. and Coara, Gh. (2012a), "Rheological behavior of some collagen extracts", *Leather and Footwear Journal*, 12(3), 193-200.

Albu, M.G., Leca, M. and Trandafir, V. (2012b), "Rheological behaviour of some collagen creams", *Leather and Footwear Journal*, 12(4), 257-270.

Albu, M.G., Titorencu, I. and Chelaru, C. (2011), "The stability of some collagen hydrogels", *Leather and Footwear Journal*, 11(1), 11-20.

Albu, M.G., Trandafir, V., Leca, M. and Carsote, C. (2007), "Characterization of collagen-gentamicin systems used in controlled release of drugs", *Leather and Footwear Journal*, 7(4), 3-9.

De Ceuninck, F., Sabatini, M. and Pastoureau, P. (eds.) (2004), *Methods in Molecular Medicine*, Vol. 101: Cartilage and Osteoarthritis, Volume 2: Structure and In Vivo Analysis, © Humana Press Inc., Totowa, NJ.

Etherington, D.J. and Sims, T.J. (1981), "Detection and Estimation of Collagen", *Journal of the Science of Food and Agriculture*, 32, 539-546.

Macovescu, G., Chelaru, C., Kaya Albu, M.G. and Albu, L. (2016), "Determination of hydroxyproline in collagen biomaterials for medical use and validation of method", *Leather and Footwear Journal*, 16(2), 147-162.

Vranceanu, M.D., Saban, R., Albu, M.G. and Antoniac, I. (2012), "Preparation and characterization of collagen: amorphous calcium phosphate composites", *Leather and Footwear Journal*, 12(3), 215-222.

RADIATION SYNTHESIS AND CHARACTERIZATION OF POLY(ACRYLAMIDE-CO-ACRYLIC ACID) HYDROGELS USED FOR THE ABSORPTION OF HEAVY METALS

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The purpose of the paper is to present the synthesis and characterization of hydrogels prepared by free-radical copolymerization of acrylamide and acrylic acid in aqueous solutions using potassium persulfate as initiator and trimethylolpropane-trimethacrylate as crosslinker, via radiation technique. The influence of the absorbed dose on the swelling properties, diffusion coefficient and network parameters of hydrogels was investigated. The swelling of hydrogels loaded with metal cations (copper, chromium) was investigated.

Keywords: hydrogels, heavy metals, electron beam

INTRODUCTION

Hydrogels are three-dimensionally cross-linked hydrophilic polymers capable of swelling and retaining huge volume in the swollen state, even under pressure. These macromolecule networks can absorb water, many hundreds of times than their dried weight. Due to their unique characteristics like hydrophilicity, swelling in aqueous media, non-soluble nature in aqueous fluids and ionic aspects, they are applied in biomedicine, bioengineering, pharmaceutical, food industry or agriculture (Karadag *et al*., 2000; Bardajee *et al*., 2008; Saraydin *et al*., 2000). The various techniques adopted for hydrogels preparation are the physical and chemical cross-linking, the grafting polymerization and the radiation cross-linking (Said *et al*., 2004; Fei *et al*., 2000; Liu *et al*., 2002). The initiation of chemical reactions by using radiation is increasingly used for novel hydrogels obtaining. The radiation technique is more preferable than the chemical one, because of the advantages offered by the gently control of cross-linking level through the variation of the absorbed dose. It is a simple additive-free process which is happening at any temperature, the reactions such as polymerization, cross-linking and grafting can be easily controlled and the treatment can be limited to a specific area (Karadag *et al*., 2004). The processing of materials by irradiation with accelerated electrons removes many drawbacks of the conventional technologies, because ionizing radiation initiates polymerization without thermal input from the outside, due to free radicals that are formed at the interaction with the monomers and especially with the solvent (water in this case). So, a good solution to produce polymeric materials is to use ionizing radiation and especially electron beams, which direct their energy in the entire volume of the monomeric solutions to be irradiated.

The purpose of this study is to present the synthesis and characterization of some hydrogels prepared by free-radical copolymerization of acrylamide and acrylic acid in aqueous solutions using potassium persulfate as initiator and trimethylolpropane trimethacrylate as cross-linker. The free-radical copolymerization was realized by electron beam irradiation in the dose range of 2.4 to 7.2 kGy in atmospheric conditions and at room temperature. The cross-linker was used in conjunction with the radical cure systems in order to improve physical properties of the final product. The obtained hydrogels were investigated through swelling and diffusion experiments. The heavy metals (Cu^{2+} and Cr^{6+}) removal was evaluated at room temperature.

EXPERIMENTAL

Materials

The materials used for the obtaining of hydrogels are shown in Table 1: acrylamide (AMD) and acrylic acid (AA) as co-monomers, potassium persulfate (PP) as initiator and trimethylolpropane trimethacrylate (TMPT) as cross-linker. All reagents were obtained from LACHEMA, Germany and were used directly, without purification.

Preparation and Irradiation of the Samples

Two different types of aqueous solutions based on acrylamide and acrylic acid for the irradiation experiments were prepared: (a) the first type based on AMD (5 mol/L), AA (0.5 mol/L), PP ($3.7\text{x}10^{-3}$ mol/L) and TMPT ($2.95\text{x}10^{-3}$ mol/L) - noted H-TMPT-1 and (b) the second type on AMD (5 mol/L), AA (0.5 mol/L), PP ($3.7\text{x}10^{-3}$ mol/L) and TMPT ($5.90\text{x}10^{-3}$ mol/L) - noted H-TMPT-2. The solutions were placed in polyvinylchloride (PVC) containers of 3 cm diameter and irradiated in atmospheric conditions and at room temperature of 25°C with 2.4 kGy, 4.8 kGy and 7.2 kGy. After irradiation, the obtained hydrogels were cut into pieces of 3±4 mm length, dried in air for 3 days and in a laboratory oven at 50°C for 12 hours to constant weight and then stored in desiccators. The dried hydrogels were used to determine the swelling, diffusion and network parameters.

Experimental Installation and Sample Irradiation

Experiments were carried out with an experimental installation consisting mainly of the following units: an electron linear accelerator (ALIN-10) of 6.23 MeV energy and 75 mA peak current of the electron beam and an irradiation chamber containing the samples of monomer solution. The optimum values of the EB peak current I_{EB} and EB energy E_{EB} to produce maximum output power P_{EB} for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: $E_{EB} = 6.23$ MeV, $I_{EB} = 75$ mA, $P_{EB} = 164$ W ($f_{EB} = 100$ Hz, $\tau_{EB} = 3.5$ μs). The EB effects are related to the absorbed dose (D) expressed in Gray or J kg^{-1} and absorbed dose rate (D*) expressed in Gy s^{-1} or J kg^{-1} s^{-1}.

RESULTS AND DISCUSSION

Network Studies

One of the most important structural parameters for the characterization of the cross-linked polymers is M_c, the average molar mass between cross-links, which is directly related to the cross-link density. The swelling equilibrium is widely used to determine M_c. According to the theory of Flory and Rehner for a perfect network, M_c is calculated using the following relation (Karadag *et al.*, 2001):

$$M_c = -V_1 d_p \frac{\nu_S^{1/3} - \nu_S / 2}{\ln(1-\nu_S) + \nu_S + \chi \nu_S^2} \tag{1}$$

where V_1 is the molar volume of the solvent (in this case water: 18 cm^3 mol^{-1}), d_P is the polymer density (1.106 g cm^{-3}), υ_S is the volume fraction of the polymer in the swollen gel (cm^3) and is equal to 1/S, χ is the Flory–Huggins interaction parameter between the solvent and polymer.

The value of χ is calculated as follows (Yiamsawas *et al*., 2007; Ding *et al*., 1991; Karadag *et al*., 1997):

$$\chi = 0.431 - 0.311\nu_S - 0.036\nu_S^2 \tag{2}$$

The cross-link density, *q*, is defined as being the mole fraction of the cross-linked units.

$$q = \frac{M_0}{M_C} \tag{3}$$

where M_0 is the molecular weight of the repeating units from polymer and is calculated using the following relation (Yiamsawas *et al*., 2007; Karadag *et al*., 1997).

$$M_0 = \frac{(m_{AMD} \times M_{AMD}) + (m_{AA} \times M_{AA}) + (m_{TMPT} \times M_{TMPT})}{m_{AMD} + m_{AA} + m_{TMPT}} \tag{4}$$

where m_{AMD}, m_{AA} and m_{TMPT} are the masses of acrylamide, acrylic acid and cross-linker (TMPT) expressed in grams and M_{AMD}, M_{AA} and M_{TMPT} are the molar masses of acrylamide, acrylic acid and TMPT expressed in g mol^{-1}.

Another parameter used for cross-link density characterization is υ_e which represents the number of elastically effective chains totally included in a network per volume unit and it is calculated with the following relation:

$$\upsilon_e = \frac{d_p N_A q}{M_0} \tag{5}$$

where N_A is the Avogadro number.

The values of parameters M_c, *q* and υ_e were calculated as above and are listed in Table 1.

Table 1. The variation of the number-average molar mass between cross-links (M_c/g mol^{-1}), cross-link density (*q*) and number of elastically effective chains (υ_e) with the amount of TMPT and absorbed dose

TMPT (mol/l)		Absorbed dose (kGy)	
	2.4	4.8	7.2
		M_c x 10^{-3}	
2.95 x 10^{-3}	449 717	309 883	184 895
5.90 x 10^{-3}	994 785	498 836	235 255
		q x 10^{7}	
2.95 x 10^{-3}	1.597	2.318	3.885
5.90 x 10^{-3}	0.729	1.453	3.082
		υ_e x 10^{14}	
2.95 x 10^{-3}	2.459	3.569	5.982
5.90 x 10^{-3}	1.112	2.217	4.701

From the Table 1 it is observed that the number-average molar mass between cross-links of hydrogels has increased with the increasing of the amount of cross-linker (TMPT) but has decreased with the increasing of absorbed dose. The values of the cross-link density and number of elastically effective chains are inversed due to the value of number-average molar mass between cross-links. The increasing of the amount of cross-linker decreases the cross-linking density, because the hydrogels obtained are not stiff. Increasing the cross-linker concentration increases the M_c between the two main backbones. A large value of M_c indicates long chains between the two backbones. The obtained results show that the M_c values are affected by the absorbed dose. The increasing of absorbed dose leads to the decreasing of M_c, since the hydrogel becomes more and more dense.

Other important parameters used for the assessment of networks are gel pore size or mesh size (ξ) and porosity (P %). Using the calculated values of number average molecular mass between cross-links, *Mc*, the mesh size was determined using the following equation (Thakur *et al*., 2011):

$$\xi = \upsilon_S^{-1/3} \sqrt{\frac{2C_n M_C}{M_r}} \tag{6}$$

where υ_S is the volume fraction of the polymer in the swollen gel, *l* is the length of the C–C bond along the polymer backbone (0.154 nm), C_n is the Flory characteristic ratio of the polymer and M_r is the molecular mass of the repeated unit.

The characteristic ratio, C_n, for poly(AMD-co-AA) hydrogels was taken as the weighted average of C_n values for poly(AMD) and poly(AA) chains, according to their molar ratio in the hydrogel (C_n was taken 8.8 and 6.7 for poly(AMD) and poly(AA), respectively).

The porosity P(%) of the obtained hydrogels was determined using the following equation (Karadag *et al*., 2001):

$$P(\%) = \frac{V_d}{1 - V_d} \times 100 \tag{7}$$

where V_d is the volume ratio of water at equilibrium. The values of the mesh size, ξ (nm) and porosity (P) are shown in Table 2.

Table 2. The variation of mesh size (ξ) and porosity (P) with the amount of TMPT and absorbed dose

TMPT (mol/l)	Absorbed dose (kGy) 2.4	4.8	7.2
		ξ/nm	
2.95×10^{-3}	133.70	103.21	72.14
5.90×10^{-3}	232.38	143.70	85.25
		P(%)	
2.95×10^{-3}	98.05	97.56	96.68
5.90×10^{-3}	98.78	98.76	97.12

As it is presented in Table 2, the mesh size and porosity have increased with the increasing of the amount of cross-linker (TMPT) and have decreased with the increasing of the absorbed dose. The mesh size is related with the space available for transport of a solute or of a solvent in a network. The increasing of the mesh size and porosity has as a result the increasing of water content in the hydrogel. More than that, from the results it is observed that the degree of cross-linking had a significant influence on the mesh size. Hydrogels having higher degrees of cross-linking have a relatively shorter distance between two cross-linking points and as a result, the mesh sizes and porosity of these hydrogels are lower.

Adsorption of Ions from Aqueous Solutions

The obtained hydrogels have been tested to remove toxic heavy metals from aqueous media, even if their application on large scale may not be a practical solution due to exorbitant costs. The heavy metals (Cu^{2+} and Cr^{6+}) removal was evaluated at room temperature on water samples synthesized from $CuSO_4*5H_2O$ (0.5 wt/vol%) and $K_2Cr_2O_7$ (0.5 wt/vol %).

The adsorption of ions was studied by the following procedure: 0.01 g of dry hydrogel was introduced in 5 mL of aqueous solution containing Cu^{2+} or Cr^{6+} ions for 72 h. Then, the gel was removed from the solution and the ions retention was monitored by UV-VIS absorption spectrophotometry. The heavy metals adsorption performance is presented in terms of “adsorption efficiency” and was calculated as follows:

$$E(\%) = \frac{C_0 - C_1}{C_0} \times 100 \tag{8}$$

where C_0 is the initial heavy metals concentration (Cu^{2+} and Cr^{6+}), C_1 is the heavy metals concentration after adsorption.

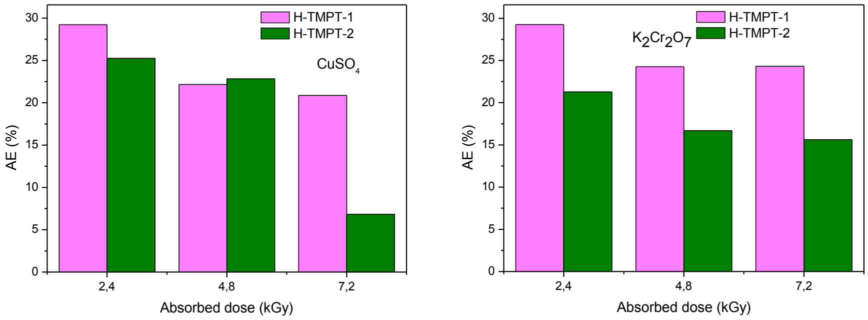

Figure 1. Heavy metals removal efficiency (%) as a function of electron beam dose (kGy)

In Figure 1 the heavy metals removal is represented depending on the absorbed dose used for the hydrogels obtaining. It is observed that the heavy metals adsorption performance decreases with the increasing of the absorbed dose. Thus, low radiation doses leads to the obtaining of efficacious hydrogels for heavy metals removal. This is related with the decrease of mesh size (ξ) and porosity (P) due to the increase of degree of cross-linking when the radiation dose has increased. The mesh size and porosity are related with the space available for transport of the liquid in the polymeric network, so their decreases indicate that the hydrogels present modest possibilities of water adsorption.

CONCLUSIONS

This study was carried out to illustrate the synthesis of poly[acrylamide-co-acrylic acid]-hydrogels using potassium persulfate as initiator and trimethylolpropane-trimethacrylate as cross-linker, via the radiation technique. The obtained hydrogels were investigated through swelling analysis. The number-average molar mass between cross-links of hydrogels increased with the increasing of the amount of cross-linker (TMPT) but decreased with the increasing of absorbed dose. Also, the mesh size and porosity have increased with the increasing of the amount of cross-linker (TMPT) and have decreased with the increasing of the absorbed dose. Hydrogels having higher degree of cross-linking have a relatively shorter distance between two cross-linking points and for this reason, the mesh sizes and porosity of these hydrogels have decreased. Also, the swelling of hydrogels loaded with metal cations (copper, chromium) was investigated. The efficiency in heavy metals adsorption decreases with the increasing of the absorbed dose, because the mesh size (ξ) and porosity (P) are connected in the same way with the absorbed dose.

REFERENCES

Bardajee, G.R., Pourjavadi, A., Sheikh, N. and Amini-Fazl, M.S. (2008), “Grafting of acrylamide onto kappa-carrageenan via g-irradiation: Optimization and swelling behavior”, *Radiation Physics and Chemistry*, 77(2), 131-137.

Ding, Y.Z., Aklonis, J.J. and Salovey, R. (1991), “Model filled polymers. VI. Determination of the crosslink density of polymeric beads by swelling”, *Journal of Polymer Science Part B: Polymer Physics*, 29(8), 1035-1038.

Fei, B., Wach, R.A. Mitomo, H., Yoshii, F. and Kume, T. (2000), “Hydrogel of biodegradable cellulose derivatives. I. Radiation-induced crosslinking of CMC”, *Journal of Applied Polymer Science*, 78(2), 278-283.

Karadag, E., Saraydin, D. and Güven, O. (1997), “Influence of some crosslinkers on the swelling of acrylamide-crotonic acid hydrogels”, *Turkish Journal of Chemistry*, 21, 151-161.

Karadag, E., Saraydin, D. and Guven, O. (2004), “Water absorbency studies of c-radiation crosslinked poly(acrylamide-co-2,3-dihydroxybutanedioic acid) hydrogels”, *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 225(4), 489-496.

Karadag, E., Saraydin, D., Caldiran, Y. and Guven, O. (2000), “Swelling studies of copolymeric acrylamide/crotonic acid hydrogels as carriers for agricultural uses”, *Polymers for Advanced Technologies*, 11, 59-68.

Karadag, E., Saraydin, D., Sahiner, N. and Guven, O. (2001), “Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors”, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 38(11), 1105-1121.

Liu, P., Zhai, M., Li, J., Peng, J. and Wu, J. (2002), “Radiation preparation and swelling behavior of sodium carboxymethyl cellulose hydrogels”, *Radiation Physics and Chemistry*, 63(3-6), 525-528.

Said, H.M., Abd Alla, S.G. and El-Naggar, A.W.M. (2004), “Synthesis and characterization of novel gels based on carboxymethyl cellulose/acrylic acid prepared by electron beam irradiation”, *Reactive and Functional Polymers*, 61(3), 397-404.

Saraydin, D., Karadag, E. and Guven, O. (2000), “Relationship between the swelling process and the releases of water soluble agrochemicals from radiation crosslinked acrylamide/itaconic acid copolymers”, *Polymer Bulletin*, 45, 287-294.

Thakur, A., Wanchoo, R.K. and Singh, P. (2011), “Structural Parameters and Swelling Behavior of pH Sensitive Poly(acrylamide-co-acrylic acid) Hydrogels”, *Chemical and Biochemical Engineering Quarterly*, 25(2), 181–194.

Yiamsawas, D., Kangwansupamonkon, W., Chailapakul, O. and Kiatkamjornwong, S. (2007), “Synthesis and swelling properties of poly[acrylamide-co-(crotonic acid)] superabsorbents”, *Reactive and Functional Polymers*, 67(10), 865–882.

DETERMINATION AND MAPPING OF TEAR LOAD, TENSILE STRENGTH AND EXTENSION VALUES ON OVERALL AREA OF GOATSKIN LEATHERS

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Leather can be defined as a protein based fibrillary network, which consists mainly from collagen. The look, length and thickness of these fiber bundles are different in various organs of the body. Thus, leather is not a uniform material from a structural perspective and the properties of a leather piece depend on the position and direction over its area. The aim of this study was to measure some physical properties of goatskin leathers such as tear load, tensile strength and extension over the whole area and to draw maps showing regional variations. For this aim, whole the surface areas of 12 goatskin leathers have been sampled. Thickness, tear load, tensile strength and extension at break values of each sample have been measured and recorded. Then maps were drawn by using MATLAB (Matrix Laboratory) software that allows matrix manipulations, plotting of functions and data. The findings showed that the tear load, tensile strength and extension properties change directionally and locational over the area of the leather. These maps will provide information for usage area of the leathers especially for cutting in footwear production.

Keywords: leather, goatskin, physical properties

INTRODUCTION

Depending on the fields of use, specific performance characteristics are expected from the leathers (Urbanija *et al.*, 2004). Especially special mechanical performance properties are expected from shoes, which carries the body weight and related with comfort and health.

When choosing a good leather product and evaluating its quality, people always pay close attention to its handle character, although its physical-mechanical properties contribute to capability of withstanding wear (Xiao-Lei *et al.*, 2006). Also the shoe upper leathers are affected by foot movements and must protect foot from outer impacts. Physical resistance of shoe upper leathers should be high if the quality is desired.

Leather processing can simply be defined as, modification of hides/skins by a sequence of chemical and physical treatments. Various physical, chemical and fastness properties are required from leather products depending on their field of use (Ork *et al.*, 2014). Leather is an intermediate industrial product with numerous applications in downstream sectors. It can be cut and assembled into shoes, clothing, leather goods, furniture and many other items of daily use (Joseph *et al.*, 2009). However leather is not a uniform material from a structural perspective. The physical properties of leather change depending on the animal type and the animal individually. Furthermore these properties exhibit variations in different parts over the leather area (Mutlu *et al.*, 2014).

The aim of this study is to measure physical properties such as tear load, tensile strength and % extension of goat shoe upper leathers over the whole areas in both directions: perpendicular and parallel to the backbone and to compare the results and to draw maps showing distributions of physical properties that can give information on cutting of leathers for shoe production with the aim of achieving high quality.

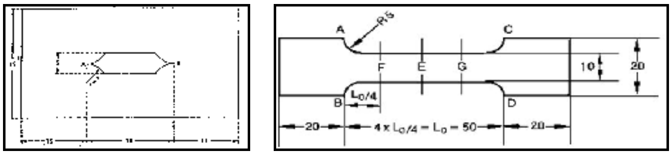
MATERIAL AND METHOD

Material

- 12 chromium tanned goatskin leathers obtained from a shoe upper leather manufacture company,
- Hydraulic press and press knives for cutting leather specimens,
- Satra-Thickness gauge for thickness measurement of leather specimens,
- Shimadzu AG-IS Tensile Tester and Trapezium-2 software for testing physical properties,
- MATLAB R2012b software for drawing maps of strengths and extensions.

Method

The test samples were conditioned according to TS EN ISO 2419, at 23 ± 2°C and 50% ± 5 RH (Anonymous 2015). The thicknesses of test samples were measured according to TS 4117 EN ISO 2589 standard (Anonymous 2016a) by using Satra-Thickness gauge. Physical properties of leather samples were determined by using Shimadzu AG-IS Tensile Tester and Trapezium-2 software following standard test methods: TS EN ISO 3377-2 - Determination of tear load - Part 2: Double edge tear (Anonymous 2016b) and TS EN ISO 3376 - Determination of tensile strength and percentage extension (Anonymous 2012).



(a) (b)

Figure 1. Dimensions of press knives used for tear load (a) and tensile strength/extension (b)

The samples were taken perpendicular to the backbone line from 3 leathers and parallel to the backbone line from the other 3 leathers for both tests. Numbers of samples are given in Table 1.

Each sample was coded regarding to its position over the leather area. Tear load, tensile strength and % extension data were entered into MATLAB spreadsheet cell according to its original position. Then the maps of strengths and percentage of extensions were plotted by using the “Contour Plots-contourf” function in MATLAB.

Table 1. Sampling directions and number of samples for tear load (a), tensile strength and percentage of extension (b)

Sampling Direction to Backbone	Number of Samples (a)	Number of Samples (b)
Perpendicular 1	500	387
Perpendicular 2	577	390
Perpendicular 3	567	400
Parallel 1	390	332
Parallel 2	492	220
Parallel3	565	217
Total	3091	1946

RESULTS AND DISCUSSION

The minimum, maximum and mean values of thickness and tear load measurements for the samples are given in Table 2. The mean thickness values are between 1.06-1.10mm. When the data are compared, it is seen that tear load results of perpendicular samples are higher than parallel samples. UNIDO recommends a minimum of 30 N of tear load for shoe upper leathers. The tear load results of goat shoe upper leathers are compatible with the acceptable quality standards advised by UNIDO (1996).

Table 2. Thickness and tear load of perpendicular and parallel samples

		Perpendicular		Parallel	
		Thickness (mm)	Tear load (N)	Thickness (mm)	Tear load (N)
1	Mean	1.16	76.77	0.94	58.96
	Min	0.90	33.27	0.77	25.00
	Max	1.60	120.13	1.30	87.23
2	Mean	1.02	77.73	1.19	74.84
	Min	0.82	36.45	0.98	43.01
	Max	1.35	109.89	1.80	102.27
3	Mean	1.00	75.19	1.17	64.77
	Min	0.73	25.63	0.92	30.03
	Max	1.35	107.27	1.45	114.90
Mean	Mean	1.06	76.56	1.10	66.19
	Min	0.82	31.78	0.89	32.68
	Max	1.43	112.43	1.52	101.47

The minimum, maximum and mean values of thickness, tensile strength and percentage extension measurements for the samples are given in Table 3. The mean thickness of leather samples are between 0.92-1.03mm. When the data are compared, the tensile strength results of perpendicular samples are higher than parallel samples. UNIDO recommends a minimum 20 N/mm^2 of tensile strength of shoe upper leathers. The tensile strength results of goat shoe upper leathers are compatible with the acceptable quality standards advised by UNIDO (1996).

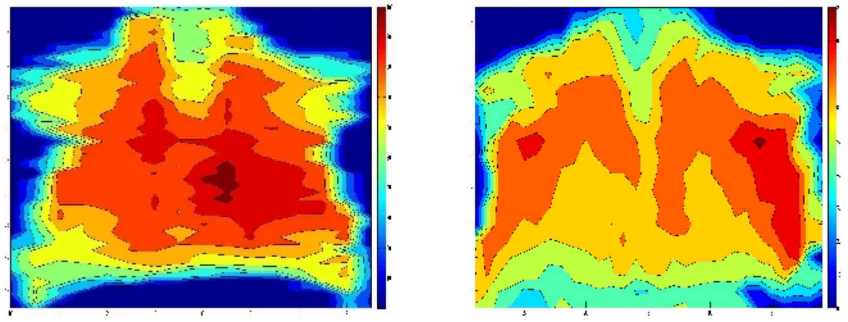
Tensile-elastic properties or elasticity is defined as a property where a body tends to recover to its original state after being deformed. If a body is affected by a force this can lead to certain stresses in the body causing its deformation, i.e. extension (Urbanija *et al.*, 2004). The elastic properties of shoe materials are important in two ways. The first one is the extension of material with the feet movement which provides comfort to the user. The second one is the extension needed in production of shoes in lasting process. These extensions should be in limits so that no damage would occur in the shoe material.

It is observed that there is a significant difference in percentage of extension depending on the sampling direction (Table 3). The mean extension percent of parallel samples are % 39.41 and higher than the mean extension percent of perpendicular samples, which is % 28.68. Thanikaivelan *et al.* (2006) have investigated that maximum breaking load and percentage extension at break values of the shoe upper leather tested at various gauge lengths for the dumbbell specimens in both parallel and perpendicular directions to the backbone and the percentage extension of perpendicular and parallel to the backbone found as 44 % and 45 % at 9.53 cm gauge length respectively.

Table 3. Thickness, tensile strength and percentage of extension of perpendicular and parallel samples

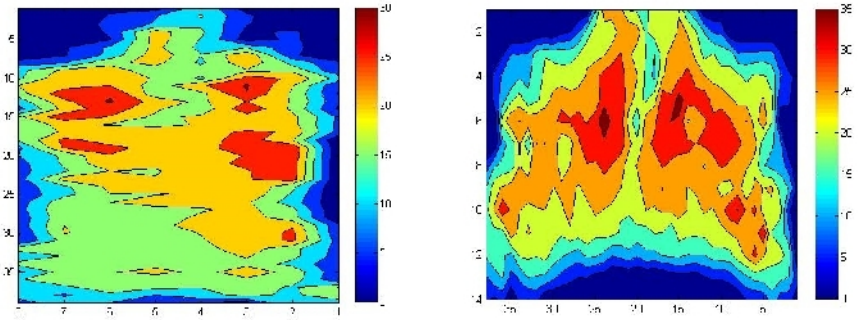
			Perpendicular			Parallel	
		Thickness (mm)	Tensile strength (N/mm^2)	Extension (%)	Thickness (mm)	Tensile strength (N/mm^2)	Extension (%)
1	Mean	0.87	24.812	33.08	1.12	18.09	37.93
	Min	0.70	6.24	11.28	0.93	6.28	10.25
	Max	1.24	42.65	107.96	2.21	32.29	86.53
2	Mean	0.92	23.86	27.71	1.00	19.96	38.04
	Min	0.69	5.59	10.01	0.72	4.70	11.93
	Max	1.89	38.69	78.85	1.41	34.44	102.08
3	Mean	0.98	23.42	25.26	0.99	22.35	42.26
	Min	0.80	4.59	8.37	0.71	6.40	15.35
	Max	1.35	39.09	79.15	1.27	37.22	125.36
Mean	Mean	0.923	24.03	28.68	1.03	20.13	39.41
	Min	0.73	5.47	9.89	0.79	5.79	12.51
	Max	1.49	40.14	88.65	1.63	34.65	104.66

The directions of maximum and minimum stretch in the area now recognized as the “Official Sampling Position” (OSP) run respectively parallel and perpendicular to the backbone. However across the rest of the hide the direction of minimum and maximum values varies. The maximum values run more or less in the direction of the hair follicle, as this roughly follows the direction of the underlying fiber structures (Daniels, 2007; Mutlu *et al.*, 2014). The mean tensile strength values vary in the range of 5.47- 40.14 N/mm^2 and 5.79-34.65 N/mm^2 for perpendicular and parallel samples respectively. This means 7 times strength variations over the area for the same leather. The tear load values vary in the range of 31.78-112.43N and 32.68-101.47N for perpendicular and parallel samples respectively. UNIDO standards are 20N/mm^2 for tensile strength and for tear load 30N. So, the same leather can meet or fail the quality standards depending on the region of sampling.. Fig.2-4 shows the maps of the means of tear load, tensile strength and percentage of extension values over the area of whole goat skin leathers.



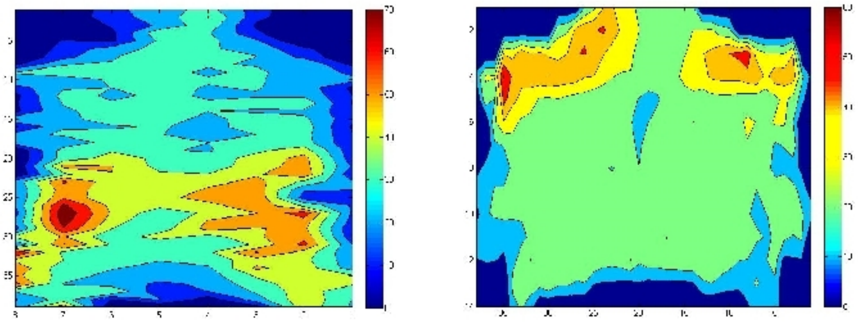
(a) (b)

Figure 2. Perpendicular dimension (a), parallel dimension (b) of tear load



(a) (b)

Figure 3. Perpendicular dimension (a), parallel dimension (b) of tensile strength



(a) (b)

Figure 4. Perpendicular dimension (a), parallel dimension (b) of percentage of extension

CONCLUSIONS

From the results of this study following conclusions were concluded:

- Shoe upper goat leathers have different physical properties depending on the direction of sampling. Tear load and tensile strength are higher when the samples are perpendicular to the backbone line. However percentages of extension at break values are higher when the samples are parallel to the backbone.

- Shoe upper goat leathers have different physical properties depending on the location of sampling. Tear load, tensile strength and percentage of extension values can vary up to three, seven and ten times respectively depending on the sampling location.
- When the maps are observed, the tear load and tensile strength are higher at the sides of backbone line of leathers and decrease gradually moving to the edges. The backline, neck and tail areas are also weaker.
- The physical tests of leathers are done with the samples taken from standard sampling location (TS EN ISO 2418, 2006) but, even for a leather which can pass the quality tests, its weak areas can fail the quality.
- The pattern cutter should be aware of the change of leather properties depending on the cutting location and direction and should place the patterns considering the forces they would subject to during the usage. These maps of physical properties can be guiding for the shoe pattern cutters and thus provide information to make higher quality shoes.

REFERENCES

Anonymous (2006), TS EN ISO 2418 Leather - Chemical, physical and mechanical and fastness tests - Sampling location, Turkish Standard (TSE), Ankara.

Anonymous (2012), TS EN ISO 3376 Leather - Physical and mechanical tests - Determination of tensile strength and percentage extension, Turkish Standard (TSE), Ankara.

Anonymous (2015), TS EN ISO 2419 Leather - Physical and mechanical tests - Sample preparation and conditioning (ISO 2419:2012), Turkish Standard (TSE), Ankara.

Anonymous (2016a), TS EN ISO 2589 Leather - Physical and mechanical tests - Determination of thickness, Turkish Standard (TSE), Ankara.

Anonymous (2016b), TS EN ISO 3377-2 Leather - Physical and mechanical tests - Determination of tear load - Part 2: Double edge tear, Turkish Standard (TSE), Ankara.

Daniels, R. (2007), “The Scientific and Commercial Significance of Variations in Strength and Stretch in Leather”, *World Leather*, November, 20-25.

Joseph, K. and Nithya, N. (2009), “Material flows in the life cycle of leather”, *Journal of Cleaner Production*, 17, 676–682.

Mutlu, M.M., Ork, N., Yegin, O. and Bas, S. (2014), “Mapping the Variations of Tensile Strength over the Area of Sheepskin Leather”, *Annals of The Universıty of Oradea*, 15, 157-162.

Ork, N., Ozgunay, H., Mutlu, M.M., Ondogan, Z. (2014), “Comparative Determination of Physical and Fastness Properties of Garment Leathers Tanned With Various Tanning Materials for Leather Skirt Production”, *Tekstil ve Konfeksiyon*, 24(4), 413-418.

Thanikaivelan, P., Shelly, D.C. and Ramkumar, S.S. (2006), “Gauge Length Effect on the Tensile Properties of Leather”, *Journal of Applied Polymer Science*, 101, 1202–1209.

UNIDO (1996), *Acceptable Quality Standards in the Leather and Footwear Industry*, United Nations Industrial Development Organization, Vienna.

Urbanija, V. and Gersak, J. (2004), “Impact of the Mechanical Properties of Nappa Clothing Leather on the Characteristics of Its Use”, *Journal of the Society of Leather Technologists and Chemists*, 88, 181-190.

Xiao-Lei, Z., Qing-Lan, L. and Wei-Ping, Z. (2006), “Evaluation Of Leather Handle Character By Discriminatory Two Class Analysis”, *Journal of the Society of Leather Technologists and Chemists*, 91, 201-207.

DEVELOPMENT OF INCLUSION COMPLEXES BASED ON ESSENTIAL OILS AND CYCLODEXTRIN FOR NATURAL FUR TREATMENT

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Essential oils are liquid substances, with oily appearance, insoluble in water, soluble in alcohol and organic solvents, with specific odour and are known for their remarkable perfume and their therapeutic qualities. Inclusion complexes of any plant extract with cyclodextrins may be used in several areas: medicine, pharmacy, perfumery, textiles, the food industry, as well as in the leather and fur industry. The paper presents the spectrometric method (FT-IR) of characterizing inclusion complexes based on essential oils and β-cyclodextrin, to highlight the obtained inclusion complexes that will be used for finishing woolen sheepskin, in order to prolong the perfuming effect of volatile oils.

Key words: woolen sheepskin, finishing, essential oils, cyclodextrin

INTRODUCTION

Improvement of fur processing technologies is closely related to chemical auxiliaries used in various operations, in order to increase processing yield and to delay or prevent unwanted phenomena that affect the quality of furs and fur items (Chirita *et al*.,1999; Maier *et al*., 2010; Bălău Mândru *et al*., 2011; Niculescu *et al*., 2014; Niculescu *et al*., 2015; Niculescu *et al*., 2015)

Essential oils are liquid substances, with oily appearance, insoluble in water, soluble in alcohol and organic solvents, with specific odour and are known for their remarkable perfume and their therapeutic qualities (Ciulei *et al*., 1993; Constantinescu *et al*., 2004; European Pharmacopeia, 2005).

Cyclodextrins (CD) are unstable compounds and usually combine with other chemicals to form a stable aqueous compound. Inclusion complexes of any plant extract with cyclodextrins may be used in several areas: medicine, pharmacy, perfumery, textiles, the food industry, as well as in the leather and fur industry.

There is particular interest in the use of cyclodextrins to prepare bioproducts with long-term effect. Some researchers have used β-CD and encapsulated complex materials, such as oleoresins, essential oils (sage, jasmine, rose, etc.) and fatty acid compounds (linoleic acid) with cyclodextrin (Chen *et al*., 2009; Cabrales *et al*., 2012; Chen *et al*., 2011).

The paper presents the spectrometric method (FT-IR) of characterizing inclusion complexes based on essential oils and β-cyclodextrin, to highlight the obtained inclusion complexes that will be used for finishing woolen sheepskin, in order to prolong the perfuming effect of volatile oils.

EXPERIMENTAL

Materials

Lavender oil (Solaris, Romania), containing 33% linalyl acetate, 29% linalool, 4% lavandulyl acetate, terpinene, ocimene, caryophyllene etc.; Orange oil (Solaris, Romania), containing 94% limonene, pinene, myrcene, octanal, linalool, etc.; Ethanol (Chemical Company, Germany), colorless liquid, boiling point 78.37°C, density - 0.79g/cm^3; β-cyclodextrin (Redox, Romania), white powder, purity > 99,0%, melting point 290-300°C.

Methods

Synthesis of materials based on plant extracts for biological protection and fragrance of fur was conducted in a glass flask using a heating and homogenization installation (Velp) and an ultrasonic bath (Elmasonic S 15 H).

Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) measurements were run with a Jasco instrument (model 4200), in the following conditions: wavenumber range – 600-4000 cm^{-1}; data pitch – 0.964233 cm^{-1}; data points – 3610: aperture setting – 7.1 mm; scanning speed – 2 mm/s; number of scans – 30; resolution – 4 cm^{-1}; filter – 30 kHz; angle of incident radiation – 45° (Coates *et al*., 2000; Moldovan, 2001).

Obtaining the Inclusion Complexes Based on Essential Oils and β-cyclodextrin

The receipts and methodology for obtaining the inclusion complexes based on essential oils and β-cyclodextrin the inclusion complexes based on essential oils and β-cyclodextrin are described in (Arama *et al*., 2012).

Inclusion complexes were prepared by co-precipitation method, using lavender essential oil (labelled LAV) and β-CD, orange essential oil (labelled POR) and β-CD, and a mechanical mixture from the same components, namely LAV and β-CD, and POR and β-CD was prepared as control.

A. Mechanical Mixture

A mechanical mixture was prepared as control (labelled AM-LAV-CD) from lavender oil: β-CD by mixing in a glass flask for 5 minutes to get a homogeneous mixture. It was labelled LAV-CD. A mechanical mixture was prepared as control (labelled AM-POR-CD) orange oil: β-CD by mixing in a glass flask for 5 minutes to get a homogeneous mixture. It was labelled POR-CD.

B. Inclusion Complex

The inclusion complex of the lavender oil: β-CD was prepared by co-precipitation. The same method was used to prepare the inclusion complex of orange oil: β-CD.

For good homogenization of mixture component and precipitates, a heating and homogenization equipment and an ultrasonication device were used. Flasks were kept at 40°C, for 48 hours in the water bath. Filtration led to the microcrystalline precipitate, that was dried in the oven at 40°C for 48 hours. The same method was used to prepare products based on orange oil.

Lavender oil was added to screw cap vials containing β-CD in 5 ml ethanol:water solution (25:75 v/v). The ratio was 1-5 parts lavender oil (or orange oil): 1 part β-CD in 5 ml ethanol:water solution (25:75 v/v), according to data and labels presented in Table 1.

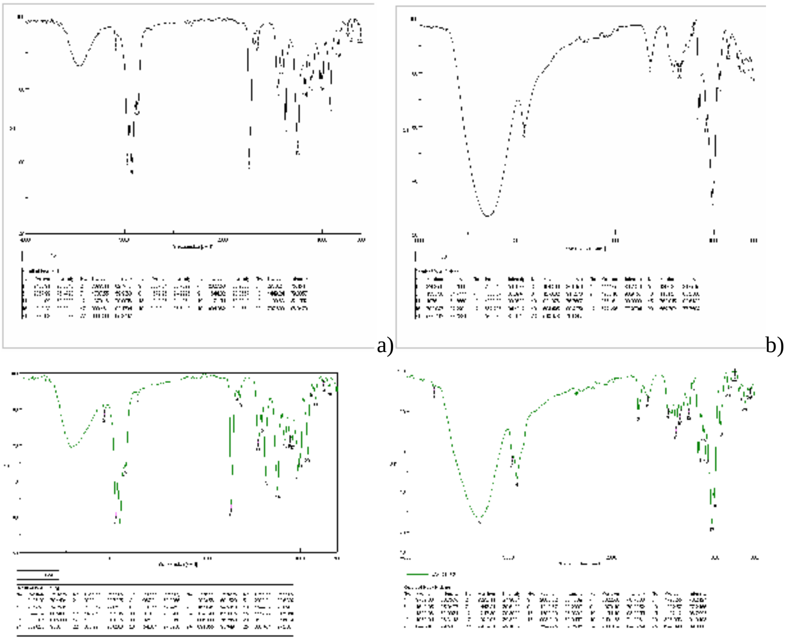
Table 1. Amounts of essential oils and β–CD used to obtain precipitates

Precipitate label	LAV or POR ratio	β–CD ratio
LAV-CD-1	LAV -1	1
LAV-CD-2	LAV- 2	1
LAV-CD-3	LAV-3	1
LAV-CD-4	LAV-4	1
LAV-CD-5	LAV-5	1
POR-CD-1	POR-1	1
POR-CD-2	POR-2	1
POR-CD-3	POR-3	1
POR-CD-4	POR-4	1
POR-CD-5	POR-5	1

RESULTS AND DISCUSSION

Characterization of Inclusion Complexes Based on Essential Oils and β-cyclodextrin Pigment Pastes by Spectroscopy (FT-IR)

FT-IR spectra of samples are presented in Figure 1 for: a-lavender oil (LAV), b-β-cyclodextrin (CD), c-mechanical mixture of lavender oil-β-cyclodextrin (AM), d- co-precipitate obtained from lavender oil-β-cyclodextrin, in a ratio of 1:5, by co-precipitation (CO). Figure 2 presents FT-IR spectra for: a-orange oil (POR), b-mechanical mixture of orange oil-β-cyclodextrin (AM), c-co-precipitate obtained from orange oil-β-ciclodextrin, in a ratio of 1:5, by co-precipitation (CO).



a) b)

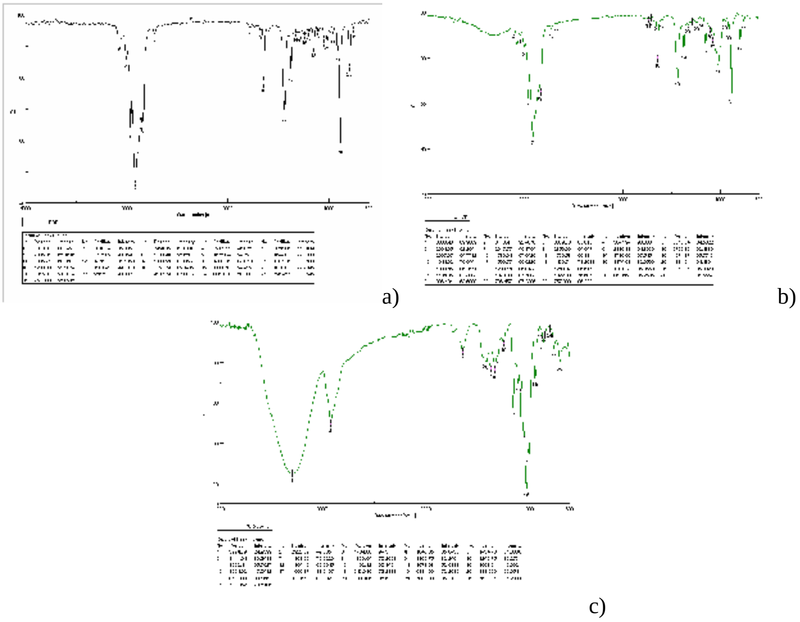
c) d)

Figure 1. a) FT-IR spectrum for LAV; b) FT-IR spectrum for CD; c) FT-IR spectrum for AM (LAV-β-CD); d) FT-IR spectrum for CO (LAV-β-CD, 5:1)

Prominent spectra for lavender oil are: 3455, 2967, 2923, 1737, 1449, 1370, 1240 and 918 cm^{-1}. In general, the stretching region of the hydroxyl group, O-H, was observed in the 3200-3600 cm^{-1} range. The band at 3455 cm^{-1} indicates the presence of hydroxyl group in lavender. The band of alkanes (C-H) is noticed at 1350-1512 cm^{-1}. The band of lavender oil at 1370 cm^{-1} indicates the presence of C-H group. Stretched bands at 1000-1260 cm^{-1} indicate the presence of the ether group (C-O). The band of lavender oil at 1240 cm^{-1} indicates the presence of C-O group. The peaks of carbonyl group band (C=O) appeared in the range of 1700 to 1750 cm^{-1}. The band of lavender oil at 1737 cm^{-1} indicates the presence of C=O group (Fig. 1.a.). Prominent spectra of β-CD are: 3298, 1643 and 1020 cm^{-1}. The band at 3298 cm^{-1} indicates the presence of the hydroxyl group in β-CD, and the band at 1643 cm^{-1} indicates the presence of the CD ring (Fig. 1.b.).

FT-IR spectra of the mechanical mixture consisting of lavender oil and β-CD overlap and intensities of characteristic peaks of lavender oil are higher (Fig. 1.c.). FT-IR spectra of inclusion complexes differ from that of the mechanical mixture consisting of lavender oil and β-CD (Fig. 1.d.).

The characteristic peaks of lavender oil, C=O stretching vibrations at 1737 cm^{-1} shifted to 1736 cm^{-1} and are of much lower intensity. The characteristic peaks of lavender oil, C-O stretching vibrations at 1240 cm^{-1} disappeared and can no longer be seen. Water in β-CD led to the broad peak of O-H masking the presence of O-H in lavender oil. The absorption band at 1412 cm^{-1} due to deformation vibration of O-H bond appeared as a result of the band at 1449 cm^{-1} shifting, due to the increasing number of O-H groups, with the introduction of β-CD into the system. The wavelength of the vibration of CD ring from 1643 cm^{-1} was shifted to 1645 cm^{-1}, which shows that the lavender molecule was incorporated into the hydrophobic cavity of the CD (Fig. 1.d.).


a) b)

c)

Figure 2. a) FT-IR spectrum for POR; b) FT-IR spectrum for AM (POR-β-CD); c) FT-IR spectrum for CO (POR-β-CD, 5:1)

Prominent spectra of orange oil are: 2964, 2917, 1644, 1436, 1375 and 886 cm^{-1}. The band of orange oil at 1644 cm^{-1} indicates the presence C=O group. The band of alkanes (C-H) is noticed at 1350-1512 cm^{-1}. The band of orange oil at 1375 cm^{-1} indicates the presence of alkane C-H group (Fig. 2.a.). FT-IR spectra of the mechanical mixture consisting of orange oil and β-CD overlap and intensities of peaks characteristic to orange oil are lower (Fig. 2.b).

FT-IR spectra of inclusion complexes differ from that of the mechanical mixture consisting of orange oil and β-CD (Fig. 2.c.). The transition of C-H band in the orange oil from 1375 cm^{-1} to higher wave numbers, 1410 cm^{-1}, indicates that C-H strengthened and participated in complexation (Fig. 2.c.). The presence of water in β-CD led to the broad peak of O-H, masking the presence of O-H in the orange oil. The wavelength of the CD ring vibration from 1643 cm^{-1} shifted to 1644 cm^{-1}, which shows that the orange oil molecule was incorporated into the hydrophobic cavity of the CD (Fig. 2.c.).

Treatment of natural fur (sheep suede) with fragrance materials was carried out by wetting the surface of fur.

CONCLUSIONS

▪ Inclusion complexes of essential oils and β-cyclodextrin were prepared: inclusion complex of lavender oil and β-cyclodextrin, and orange oil and β-cyclodextrin, both prepared using the co-precipitation method.

▪ Methods of treating natural furs with new fragrant materials and with essential oils as such (in the float or by wetting the surface of fur) were established.

▪ The fragrance effect on fur treated with inclusion complexes based on lavender oil and β-CD and orange oil and β-CD is better and lasts much longer compared to fur treated with essential oils as such.

▪ Depending on the amount of product applied to the surface of fur, the fragrance effect is different, the higher the amount of product applied on the fur, the stronger the scent.

Acknowledgements

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REFERENCES

Arama, C., Monciu, C.M. and Nicolescu, C. (2012), "Preparation and characterization of inclusion complexes between repaglinide and beta-cyclodextrin, 2-hydroxypropyl-beta-cyclodextrin and randomly methylated beta-cyclodextrin", *Farmacia*, 58(1).

Bălău Mândru, T., Deselnicu, V., Niculescu, O. and Pruneanu, M. (2011), "New antifelting-antistatic treatments of keratinous substrates", *Leather and Footwear Journal*, 11(1), 21-32.

Cabrales, L., Abidi, N. and Hamood, A. (2012), "Cotton Fabric Functionalization with Cyclodextrins", *Journal of Materials and Environmental Science*, 3(3), 561.

Chen, L. and Wang, C. (2009), "Application of Cyclodextrins in Textile Processing", *Textile Auxiliaries*, 26(3).

Chen, W., Liu, X., Dai, P., Chen, Y. and Jiang, Z. (2011), "Preparation and characterization of β-cyclodextrin-based microcapsules for sustaining release of Jasmine oil", Advanced Materials Research, 239-242(5), 624.

Chiriţă, Gh. and Chiriţă, M. (1999), *Treatise on leather and fur chemistry and technology*, vol. I and II, Gh. Asachi, Iaşi.

Ciulei, I., Grigorescu, E. and Stănescu, U. (1993), *Medicinal Herbs, Phytochemistry and Phytotherapy*, Medical Press, Bucharest, vol. 2.

Coates, J. (2000), "Interpretation of Infrared Spectra. A Practical Approach", in R.A. Meyers (ed.), *Encyclopedia of Analytical Chemistry*, Wiley, 10815.

Constantinescu, G., Haţieganu, E. and Buşuricu, F. (2004), *Medicinal Herbs Used in Therapeutics*, Medical Press, Bucharest.

Council of Europe (2005), *European Pharmacopeia*, vol. II, ESCOP Strasbourg.

Maier, S.S., Deselnicu, V. Maier, V. and Niculescu, O. (2010), "The influence of some technological parameters on tanning sheep furs pickled using dicarboxylic acids", *Leather and Footwear Journal*, 10(4), 33-44.

Moldovan, Z. (2001), Instrumental Analysis Methods, University of Bucharest Press.

Niculescu, O., Leca, M., Moldovan, Z. and Deselnicu, D.C. (2015), "Obtaining and characterizing a product with antifungal properties based on essential oils and natural waxes for finishing natural leathers", *Revista de Chimie* (Bucharest), 66(11), 1733-1736.

Niculescu, O., Leca, M., Moldovan, Z. and Deselnicu, D.C. (2015), "Research on obtaining products for fragrance and biological protection of natural leathers and furs", *Revista de Chimie* (Bucharest), 66(12), 1956-1959.

Niculescu, O., Tonea, R.A. and Tonea, S. (2014), "Product for biological protection and fragrance of fur and fur items", patent application, OSIM A/00395/2014.

COLORIMETRIC CHARACTERIZATION OF LEATHER ASSORTMENTS FINISHED WITH ECOLOGIC MATERIALS

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Leather is finished by coating the dermal substrate with disperse finishing systems containing: pigments, binders, natural and synthetic waxes, preservatives, plasticizers, thickeners, fillers, odorizers, penetrating agents, solvents. To prepare the pigment pastes, black iron oxide pigments were used – admitted by the legislation in force, Bindex BRILLANT acrylic binder as carrier resin – also having protective colloid properties, lauryl alcohol ethoxylated with 7 moles of ethylene oxide – fully biodegradable – as a dispersing agent and stabilizer and natural oils as plasticizers: castor oil, flax oil or poppy seed oil. The pigment pastes to be used in the final dressing for the dry finishing of natural leather. In this paper, a finishing composition, technology for leather assortments and characterization by CIE L*a*b* colorimetric method are presented. After thermal and artificial light aging, the colorimetric characteristics (CIE L*a*b*) change, depending on the temperature of the heat treatment and UV light, leather assortment, and type of final dressing. The highest resistance to light after aging under the influence of artificial light has the leathers finished with polyurethane or acrylic dressing in comparison with those finished with nitrocellulose dressing.

Keywords: leather, finishing, pigment pastes, colorimetric method

INTRODUCTION

Leather is finished by coating the dermal substrate with disperse finishing systems containing: pigments, binders, natural and synthetic waxes, preservatives, plasticizers, thickeners, fillers, odorizers, penetrating agents, solvents (Lange, 1982; Heidemann, 1994; Chirita and Chirita, 1999).

Environmental and toxicity concerns have led to new alternatives for the industry of ancillary finishing materials (Niculescu *et al.*, 2013; Niculescu *et al.*, 2015; Florescu *et al.*, 2009).

Flax and poppy seed oils, used as plasticizers, increase the films' resistance to aging over time (Lăzărescu, 2009; Istudor, 2011).

The paper presents the physical-chemical characterisation of newly obtained pigment pastes, developing a finishing composition using the pigment pastes for the basecoat and final dressing (polyurethane, acrylic or nitrocellulose) and CIE L*a*b* colorimetric method characterisation of cattle leather finished and aged using various methods (Tarlea and Ilie, 2006; Tarlea and Ilie, 2008; Niculescu and Leca, 2005).

EXPERIMENTAL

Materials

Bovine Leather chrome tanned, retanned, fatliquored and dyed (black), 1.2-1.4 mm thick from INCDTP-ICPI, Romania.

Finishing auxiliaries from (Triderma, Germany, 2012; Florescu *et al.*, 2009).

Black pigment paste -PN - obtained by a process described in (Niculescu and Manta, 2013; Niculescu *et al.*, 2015).

The product was used as handle modifier containing black iron oxide based on iron tetroxide (Fe_3O_4 – 94%), acrylic binder, castor, flax or poppy seed oils, stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide with the following characteristics: dry substance – 30.22-31.34, pH (10% solution) – 6.8-7.1, ash – 23.90-25.04%;

Feeling agent -Wax-AGE 7- obtained by o process described in (Niculescu *et al*., 2013). The product was used as handle modifier containing beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide with the following characteristics: dry substance – 18.96, pH (10% solution) – 7.3, Ford cup viscosity Φ4 – 27 s, kinematic viscosity, cSt – 10.48, density – 0.975 g/cm^3.

Methods

Colorimetric measurements were performed using a MINOLTA spectrophotometer (CM 2002), with light impulses from a xenon lamp with 0.8 cm aperture. Light reflection is focused on a silicon photo diode with wavelengths between 400 and 700 nm (10 nm steps) and L*a*b* values (chromatic coordinates: brightness, red/green and yellow/blue). In the CIE L*a*b* space used in colorimetric analysis by reflection, the tint (represented by fundamental colours – red, green, yellow and blue), brightness or clarity (represented by the chromatic stimulus varying from black to white) and saturation (purity of colour) are expressed according to three coordinates: L*, a* and b*. On the X axis, a* parameter has values between red ($a^* > 0$) and green ($a^* < 0$) stimuli, on the Y axis, b* parameter varies between yellow ($b^* > 0$) and blue ($b^* < 0$) stimuli, and L* parameter, on polar Z axis, represents brightness (grey axis), varying between white and black. (DIN 6174, 1976; Jawahar *et al*., 2013; X-Rite, 2007).

The colour difference parameters of the analyzed (P) and reference (R) samples are expressed by the following equations:

$$\Delta a^* = a^*_P - a^*_R \tag{1}$$

$$\Delta b^* = b^*_P - b^*_R \tag{2}$$

$$\Delta L^* = L^*_P - L^*_R \tag{3}$$

Based on these equations, the following parameters can be calculated:

- chromatic shifting, through the relation:

$$\Delta E^* = [\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}]^{1/2} \tag{4}$$

- saturation or purity difference, using the relation:

$$\Delta C^* = [a^*_P{}^2 + b^*_P{}^2]^{1/2} - [b^*_R{}^2 + L^*_R{}^2]^{1/2} \tag{5}$$

- tint difference, through the relation:

$$\Delta H^* = [\Delta E^{*2} - \Delta L^{*2} - \Delta C^{*2}]^{1/2} \tag{6}$$

Obtaining New Pigment Pastes

The receipts and methodology for obtaining the pigment pastes are described in (Niculescu and Manta, 2013; Niculescu *et al*., 2015) and composition is presented in Table 1.

Materials used for obtaining of new black pigment paste are: black iron oxide pigment, acrylic polymer, non-ionic tensioactive agent, wax emulsion made from beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide, castor oil, flax and poppy seed oils, used as plasticizers.

Table 1. The composition of new pigment pastes

Quantities	New pigment paste - PN 1	New pigment paste - PN 2	New pigment paste - PN 3
Black iron oxide, (%)	30	30	30
Polyacrylic binder, %	40	40	40
Ethoxylated lauric alcohol, %	9	9	9
Castor oil, %	9	-	-
Flax oil, %	-	9	-
Poppy seed oil, %	-	-	9
Wax emulsion, %	3	3	3
Water, %	9	9	9

The framework technology for dry finishing of bovine leather into natural grain box assortments, black, is presented in Table 2.

Table 2. Technology for dry finishing of bovine leather into black natural grain box assortments

Operation	Dispersion composition/application method
Application of dispersion I (basecoat)	100 g/L pigment paste (PN 1, PN 2 or PN 3) 30 g/L aqueous wax emulsion 300 g/L aqueous acrylic dispersion, or 150 g/L aqueous acrylic dispersion and 150 g/L aqueous polyurethane dispersion, or 200 g/L aqueous acrylic dispersion and 100 g/L aqueous polyurethane dispersion 70 g/L water Application by spraying (2 passes dispersion I)
Intermediate pressing	In hydraulic press with the mirror or fog plate, parameters: - temperature – 50-60°C; pressure – 50-100 atm
Application of dispersion I	By spraying (2-3 passes dispersion I)
Application of final dressing (fixing)	Emulsion/dispersion with the following composition: 700 g/L aqueous nitrocellulose, acrylic or polyurethane emulsion 20 g/L aqueous wax emulsion for handle 280 g/L water Application by spraying (2 passes final dressing)
Final pressing	In hydraulic press with the mirror plate, parameters: - temperature – 70-80°C; pressure – 50-100 atm.

Application of the final dressing (fixing films deposited onto the dermis) was performed in three variants: FN – nitrocellulose, FA – acrylic and FP – polyurethane, presented in Table 3.

Table 3. Technological variants for fixing bovine leather into natural grain box assortments

Components of final dressing (g/L)/Variant	FN	FA	FP
Roda lac 93	700	-	-
Medacril EFP34	-	700	-
Roda pur 5011	-	-	700
Wax emulsion AGE 7	20	20	20
Water	280	280	280

Testing Artificially Aged Finished Leather

Finished leathers were artificially aged and tested according to ISO 17228/2006 standard.

Mechanical characteristics of finished natural grain box assortments in the same variants but artificially aged were determined. The following abbreviations were used:

- IT1 – leather aged at 50°C for 7 days;
- IT2 – leather aged at for 7 days;
- IL – leather aged with artificial light (Xenotest) for 7 days.

RESULTS AND DISCUSSION

Characterization of Pigment Pastes

New pigment pastes were characterized by physical-chemical analyses. Physical-chemical characteristics are presented in Table 4.

Table 4. Physical-chemical characteristics of pigment pastes

Characteristics/samples	New pigment paste - PN 1	New pigment paste - PN 2	New pigment paste - PN 3
Dry substance, %	31.34	30.84	30.22
pH 10% solution	6.8	7.0	7.1
Ash, %	25.04	24.65	23.90

The new pigment paste are viscous and homogeneous fluids and dry substance indicate that they are more concentrated pastes. They are stable over time, without sediments of phase separation and have the characteristics of concentrated pastes.

Characterization of Finishing Leathers by Colorimetric Method

Finished leathers were artificially aged and tested according to the CIE LAB system.

Chromatic characteristics of natural grain Box assortments (variants PN1-PN3 for finishing with using the pigment pastes for base coat and variants FN, FA and FP for final dressing) non-aged and aged using the methods IT1, IT2, IL, plus those aged in artificial light (UV) for 7 days–IUV obtained using the prepared pigment pastes are given in Table 5.

Table 5. Values of colorimetric parameters for finished unaged black natural grain box leather samples

Sample	Technological variant	CIE L*	CIE *	CIE b*	CIE C*	CIE H*
P 1	PN1, FN	25.05	0.23	-0.60	0.65	290.55
P 2	PN2, FN	24.65	0.20	-0.69	0.71	286.47
P 3	PN3, FN	25.47	0.23	-0.51	0.55	294.24
P 4	PN1, FA	25.14	0.16	-0.65	0.67	283.97
P 5	PN2, FA	24.48	0.17	-0.69	0.71	284.08
P 6	PN3, FA	25.16	0.17	-0.64	0.66	285.2
P 7	PN1, FP	24.94	0.21	-0.57	0.61	290.67
P 8	PN2, FP	24.70	0.21	-0.70	0.73	286.32
P 9	PN3, FP	25.25	0.13	-0.54	0.56	284.01

Variation of colorimetric parameters for finished natural grain box leather samples P1-P9 aged using IT1, IT2, IL and IUV methods is shown in Table 6.

Table 6. Variation of colorimetric parameters for finished aged black natural grain box leather samples using IT1, IT2, IL and IUV methods

Sample	Method	ΔL*	Δa*	Δb*	ΔE*
P 1	IT1	0.54	0.29	0.05	0.61
	IT2	0.83	-0.1	0.26	0.88
	IL	0.89	-0.09	0.02	0.40
	IUV	0.87	-0.09	0.1	0.88
P 2	IT1	0.48	-0.05	-0.02	0.48
	IT2	0.61	-0.1	-0.23	0.66
	IL	0.86	-0.09	0.13	0.87
	IUV	0.53	-0.03	-0.16	1.24
P 3	IT1	0.24	0.1	-0.11	0.28
	IT2	0.29	-0.1	0.1	0.32
	IL	0.69	-0.15	0.09	0.72
	IUV	0.52	-0.07	0,05	0.09
P 4	IT1	0.28	-0.04	-0.06	0.19
	IT2	0.5	-0.09	0.09	0.52
	IL	0.44	-0.04	0.09	0.45
	IUV	0.43	0.12	0.23	0.77
P 5	IT1	0.18	-0.04	0	0.09
	IT2	0.37	-0.18	0.21	0.32
	IL	0.33	0	0.01	0.33
	IUV	0.21	-0,07	0.12	0.17
P 6	IT1	0.14	-0.07	0.8	0.87
	IT2	0.16	-0.19	0.32	0.52
	IL	0.29	-0.04	0.02	0.39
	IUV	0.13	-0.04	0.06	0.15
P 7	IT1	0.1	-0.04	-0.05	0.12
	IT2	0.18	-0.19	0.35	0.44
	IL	0.2	0.03	0	0.40
	IUV	0.11	-0.11	0.01	0.22
P 8	IT1	0.1	-0.13	0.02	0.17
	IT2	0.14	-0.14	0.08	0.21
	IL	-0.01	0.01	0.04	0.04
	IUV	-0.01	-0.17	0.4	0.43
P 9	IT1	0.02	0.01	-0.05	0.05
	IT2	0.05	0.01	0.02	0.05
	IL	-0.38	-0.05	0.19	0.43
	IUV	-1.02	0.04	-0.16	1.03

The samples finished using black pigment pastes (P1-P9) containing the plasticizers: castor oil (P1, P4 and P7), flax oil (P2, P5 and P8) or poppy seed oil (P3, P6 and P9) and nitrocellulose (P1-P3), acrylic (P4-P6) or polyurethane (P7-P9) dressing. Samples P2 and P3 aged using IT1, IT2, IL and IUV methods have lower values of ΔL* parameter compared to P1 and are darker, which indicates a positive influence of the acrylic and polyurethane dressing on fastness to light of leathers finished compared to nitrocellulose dressing. Samples P5 and P6 aged using IT1, IT2, IL and IUV methods have lower values of ΔL* parameter compared to P4 and are darker, which indicates a positive influence of the acrylic and polyurethane dressing on fastness to light of leathers finished. Samples P8 and P9 aged using IT1 and IT2 methods have lower values of ΔL* parameter compared to P7 and are darker, which indicates a positive influence of the acrylic and polyurethane dressing on fastness to light of leathers finished compared to nitrocellulose dressing.

Samples P8 and P9 aged using IL and IUV methods have negative values for brightness ($\Delta L^*<0$), i.e. are darker compared to sample P7. Samples P4-P9 aged using IL and IUV methods have ΔL^* lower than P1-P3, and therefore are darker. Leather samples finished with acrylic and polyurethane dressing and aged using the specified methods have lower ΔL^* values and change their colour less compared to those finished with nitrocellulose dressing. The lower values of ΔL^* also indicate the positive influence of plasticizer poppy seed oil used on resistance to ageing.

CONCLUSIONS

- Thermal and artificial light ageing change colorimetric characteristics (CIE L*a*b*) compared to those of unaged samples, depending on the ageing method, leather assortment and type of final dressing.
- The highest values for fastness to light after artificial light ageing were those of leathers finished with polyurethane or acrylic dressing and the lowest, those finished with nitrocellulose dressing.
- Flax and poppy seed oils, used as plasticizers, improve resistance to ageing of coating.

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REFERENCES

Chiriţă, Gh. and Chiriţă, M. (1999), *Treatise on leather and fur chemistry and technology*, vol. I and II, Gh. Asachi, Iaşi.

Florescu, M., Marcu, C., Coară, G., Albu, L. and Acsinte, D. (2009), “Polymer binder and processing thereof”, Patent RO 122205/2009.

Heidemann, E. (1994), *Fundamentals of Leather Manufacturing*, Eduard Roether KG Druckerei und Verlag, Darmstadt.

Istudor, I. (2011), *Concepts on the chemistry of painting*, ACS, Bucharest, 98, 176, 212.

Jawahar, M., Kanth, S.V., Rajangam, V. and Kannan, C.N. (2013), “Mathematical regression technique to correlate visual and instrumental colour analysis for leather industry”, *XXXII Congress of IULTCS*, May 29-31, 2013, Istanbul, Turkey, 247.

Lange, J. (1982), *Leather Finishing*, Leather library vol. V, Umschau, Frankfurt.

Lăzărescu, L. (2009), *Oil Painting Technique*, Polirom, Bucharest, 204.

Niculescu, O. and Leca, M. (2005), “Rheological behaviour of some aqueous pigment pastes used in finishing natural leather”, *Leather and Footwear Journal*, 5(4).

Niculescu, O. and Manta, A. (2013), “Inorganic pigment pastes for natural leather finishing”, Patent Application RO A/00533/2013.

Niculescu, O. *et al*. (2013), “Waxy product for finishing natural leather and leather items”, Patent Application RO A/00532/2013.

Niculescu, O., Leca, M., Staicu, T., Micutz, M. and Moldovan, Z. (2015), “Rheological behavior of ecological pigment pastes for natural leather and fur finishing”, *Revista de Chimie* (Bucharest), 66(10), 1549-1553.

Tarlea, M. and Ilie, Gh. (2006), “Assessing the Color Levelness and Similitude in Finished Bovine Leather by Means of COLORMASTER Software”, *Leather and Footwear Journal*, 6(4).

Tarlea, M. and Ilie, Gh. (2008), “Analysis of Leathers’ Colour by COLORMASTER Programme”, Leather and Footwear Journal, 8(3).

Triderma (2012), *Leather finishing auxiliaries*, Germany.

X-Rite Inc. (2007), *A Guide to Understanding Color Communication*, available at https://www.xrite.com/documents/literature/en/L10-001_Understand_Color_en.pdf.

*** (1976), DIN 6174, Determination Colorimetric of Color Distances, CIE LAB of 1976.

*** (2006), SR EN ISO 17228-2006, Determination of ageing resistance of leathers.

INVESTIGATION OF THE PROPERTIES OF POLYMER-TITANIUM TANNED LEATHER

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In the present time, despite all the advantages, the use of chrome-based tanning components are reduced or excluded in leather production, primarily, because of its toxicity and ineffective use of the agents. The new chrome-free tanning method has been offered to replace ship skin pickling with polymeric treatment of rawhide and subsequent titanium tanning. Ammonium titanyl sulfate is used as a tanning agent while polymaleate – as a polymeric material. It has been established that the replacement of pickling with polymeric treatment improves diffusion and exhaustion of titanium tannins from the solution, which, in turn, ensures the formation of the Wet-White leather structure and its thermostability. Polymer-titanium tanning system not only increases the consumption of tanning agents, reduces treatment duration, but also improves physical and mechanical properties of finished leather: strength, elongation, volume yield, thickness yield, porosity, etc. This was confirmed by the results of scanning electronic microscopy, which prove that a tanning method affects morphological changes in leather structure and uniform distribution of tannin into the derma.

Keywords: polymer-titanium tanning, leather, structure, properties

INTRODUCTION

The need for more efficient use of raw materials and energy resources as well as greening of leather processing industry impose strict requirements to manufacturing processes and chemical materials used. In the tanning process it refers, first of all, to reducing treatment duration, restricting or even complete exclusion chromium compounds in the production cycle. The last requirement is explained by instability in time and oxidation potential of these compounds to bio-toxic chromium (IV) (Environmental Problems, 2003; Klimova, 1990).

Titanium tanning is one of the alternatives to chrome-based tanning and makes it possible to produce leather with performance properties close to chrome tanned leather, which are long-time resistant to environmental factors (Peng *et al*., 2007; Metelkin and Rusakova, 1980; Cherkashin and Chursin, 2012; Adiguzel Zengin *et al*., 202; Seggiani *et al*., 2014). The main drawbacks of titanium-tanned leather are stiffness and insufficient strength of the front layer due to uneven distribution of tanning compounds into the derma, which significantly reduces operational properties of leather goods. The existing methods of improving titanium tanning, which are based on a combination of titanium compounds with synthetic, organic (tannin, aldehydes) and mineral (zirconium, aluminum, silicon) tanning agents, improve leather structure and strength, but do not facilitate to strengthen certain important organoleptic, hygienic, elastic and dactylic properties of leather (Covington, 1988; Bandino and Plains, 1995; Halmetova *et al*., 2005; Madiev *et al*., 2009).

Therefore, it is important to further improve titanium-based tanning system using modern, non-toxic chemical materials that can ensure diffusion, uniform distribution and maximum fixation of tanning agents into the derma to make the leather structure and properties, as well as reduce the environmental impact (Nikonova and Andreyeva, 2015).

We have previously studied the process of tanning sheepskins using ammonium titanylsulfate in the presence of unsaturated polymaleate and polyacrylate polymeric compounds. The results of testing showed that this tanning system reduces processing time while ensuring relatively high hydrothermal stability and appropriate formation of Wet-White leather structure (Nikonova *et al.*, 2015; Nikonova *et al.*, 2016).

The objective of this study is to determine the effects of polymer-titanium tanning on the finished leather structure and its properties.

MATERIALS AND METHODS

Raw and Chemical Materials

Bated pelt of sheep skin obtained during leather garment manufacturing technology was used for the study (Balberova *et al.*, 1986).

Polymaleate (product Kro) was applied as a polymeric material. It is a commercial technical material for leather production by Codyeco S.p.A. (Italy), which is non-toxic, water-soluble, and electrolyte resistant reagent of 21.5% active agent content (on dry residue).

Sodium chloride (95.0%), sulfuric acid (96.0%), and sodium hydrocarbonate (96.0%) were also applied in the study. The tanning was complete with ammonium titanyl sulfate of 40.4% basicity and 22.4% active agent content (on TiO_2).

All indicated materials for leather processing are technical products.

Experimental Conditions

The bated pelt from sheep skin was divided in two samples by the method of asymmetric trimming. One sample of the sheepskin (Experiment) was treated using polymer-titanium tanning, the replacement of traditional ship skin pickling with polymeric treatment. After polymeric treatment, the used solution was exhausted and the next processing was carried in the new solution with 5.0% sodium chloride pretreatment for 15 minutes to prevent acid swelling. Consumption of Kro was in amount 1.1%, temperature 36-38°C, process time − 90 minutes, and consumption of water − 1.0. The pH values after polymeric treatment was 6.5±0.1. The consumption of tanning agent in salt solution was 3.8% (expressed as TiO_2) at 36-38 °C. The pH values at the beginning of the tanning process range 1.1±0.1. Sodium hydrocarbonate was added in amount of 3.5% (1:20 w/v) to get pH values 3.9±0.2, which provided to improve the fixation of tanning agents during diffusion of titanium compounds in semi-finished cross-section.

Another part of the sheepskin (Control) was tanned with 5.0% titanium subsequent pickling with sodium chloride (5.0% in consumption) and sulfuric acid (0.6% in consumption) at 20-24°C (Metelkin and Rusakova, 1980; Balberova *et al.*, 1986).

In all cases, the finish of the tanning process was determined by the stable boiling temperature. The duration of titanium-pickling tanning process was 9.0 hours, while polymer-titanium tanning lasted for 5.0 hours. Consumption of chemical materials was calculated based on limed pelt mass of samples.

All subsequent processes and operations were carried out using the known technology (Balberova *et al.*, 1986).

The study was carried out in a laboratory setting with 10-liter shaking containers being used, thus ensuring the required temperature and constant mixing (frequency of shaking 8-12 min^{-1}). Physical and mechanical testing and chemical analysis was provided to determine how polymer-titanium tanning affects the finished leather properties.

Research Methods

Both traditional physical and chemical testing and modern methods of analysis were applied to ensure unbiased results of the study.

The pH control of solutions was determined by a pH meter PATECH PH-013M.

Tanned solutions were analyzed using a photocolorimeter AE-30F (ERMA Inc., Japan) for determination of TiO_2 content as per official method (Golovteeva *et al.*, 1971).

Physical and chemical testing and modern methods of leather analysis were conducted using official methods: moisture content ISO 4684:2005 (IULTCS/IUC 5), shrinkage temperature ISO 3380:2015 (IULTCS/IUP 16), hide substance ISO5397:1984 (IULTCS/IUC 10), strength of surface ISO 3379:2015 (IULTCS/IUP 9), strength and percentage extension ISO 3376:2011 (IULTCS/IUP 6), apparent density ISO 2420:2002 (IULTCS/IUP 5), мeasurement of thickness ISO 2589:2016 (IULTCS/IUP 4), мeasurement of area ISO 11646:2014 (IULTCS/IUP 32), content of substances extracted with organic solvents ISO 4048:2008 (IULTCS/IUC 4), water vapour absorption ISO 17229:2016 (IULTCS/IUP 42), water vapour permeability ISO 14268:2012 (IULTCS/IUP 15). Volume yield and porosity was calculated using the official method (Danilkovich, 2006).

The impact of leather processing on the formation of the porous structure of semi-finished Wet-White leather and uniformity of distribution of tanning agents was measured by electron microscopy. The testing was performed using a scanning electronic microscope (SEM JSM-6490-LV, JEOL, Japan) with integrated electron microprobe analyzer INCA Enerdy and energy dispersive spectroscopy (EDS+WDS, OXFORD, UK) with 50-20000 times magnification.

The diffusion of tanning agents into derma was measured (yellow colored sections after hydrogen peroxide treatment) every 30 minutes using an optical microscope Bresser Researcher Bino (Bresser, Germany) with 40-50 times magnification as per official testing method (Metelkin and Rusakova, 1980; Danilkovich, 2006).

RESULTS AND DISCUSSION

No complications were noticed during processing of leather samples; both tanned Wet-White samples and finished leather have a clean and silky grain smoothness and nice feel, were more filled and soft compared to control leather samples as per both sensory and visual assessment. Furthermore, it was discovered that the polymeric treatment prior to titanium tanning provide to reduce this process in 1.8 times and increase the exhaustion of titanium tanning agent from a solution in 1.6 times (table 1).

Table 1. Description of waste tanning waters

Index	Exhaustion of tanning agent (%)	Content of TiO_2 in waste solution (g/l)
Experiment	95.7	1.60
Control	94.8	2.56

The impact of tanning method on the morphological changes in collagen structure shown in SEM-micrographs of cross-sections of the tanned leather as presented in Figures 1 and 2.

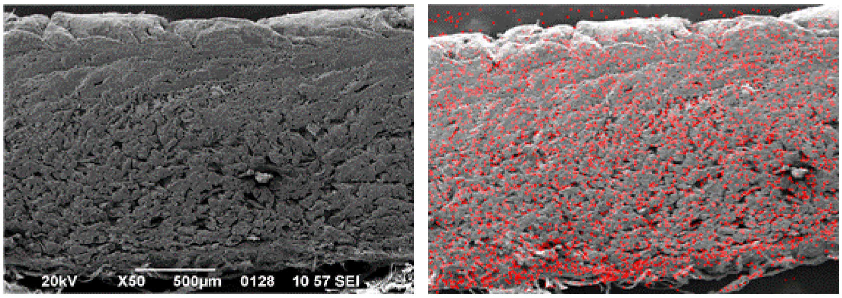

Figure 1. SEM-micrographs and EDS analysis of titanium-pickled tanned leather

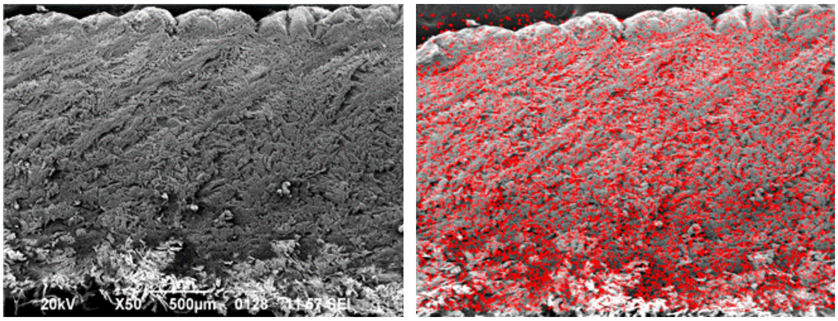

Figure 2. SEM-micrographs and EDS analysis of polymer-titanium tanned leather

As shown in figure 1, in cross-sections of titanium-pickling tanned leather samples the interlacing bundles of collagen are traced poorly, the spread of voids on the section area is unequal with a substantial increase in density in the direction of the grain surface. Since, of the insufficient structure loosening, the titanium-tanning agents are distributed unevenly into the derma.

SEM-micrographs of polymer-titanium semi-finished lather cross-sections (figure 2) show better interweaving of second-level fibers, uniform structure density throughout the cut area, increased distance both between individual fibers and their clusters, which will ensure proper diffusion and uniform distribution of titanium tanning agents into the derma and positively affect leather properties.

The results of physical and mechanical testing and chemical analysis (table 2) confirm that several leather parameters have been improved after applying a new tanning method, which involves the replacement of pickling with polymeric treatment (product Kro) and subsequent titanium tanning. Compared to the control leather sample the following indicators have been improved: strength in 1.3 times; strength of surface in 1.4 times; eextension at 10 MPa in 1.2 times; titanium oxide content in 1.2 times;

thickness yield by 8.8%; area yield by 10.1%; volume yield by 14.7%; shrinkage temperature by 12 °C, porosity by 10.4%.

Table 2. Influence of tanning method on the properties of leather

Index	Experi-ment	Control
Moisture content (%)	12.0	12.2
Content of TiO_2 (%)	6.4	5.2
Content of substances extracted with organic solvents (%)	9.5	11.0
Tensile strength (MPa)	12.4	10.0
Tear strength (N/mm)	28.0	20.0
Strength of surface (MPa)	12.4	8.7
Extension at tensile strength (%)	35.5	30.0
Volume yield (cm^3/100g)	139.6	119.1
Yield of thickness relative to bated pelt (%)	111.4	101.5
Yield of area relative to bated pelt (%)	89.0	80.0
Shrinkage temperature (°C)	92.0	80.0
Apparent density (g/cm^3)	0.38	0.42
Water vapour permeability (mg/$cm^2 \cdot h$)	2.48	2.47
Porosity, %	66.7	59.7

CONCLUSIONS

The study has investigated the impact of polymer-titanium tanning on garment sheepskin leather properties.

It was revealed that polymeric treatment before titanium tanning will improve performance and consumer leather properties. Polymer-titanium tanning system can increase the strength, elongation, volume yield, thickness and area yield, and porosity. These were also confirmed by the results of microscopic testing of semi-finished Wet-White leather, which show that titanium tanning agents are distributed more uniformity into the derma if a polymaleate (product Kro) is applied before tanning. The advantages of polymer-titanium tanning include the reduced processing cycle, chrome-free tanning system, better exploitation of titanium tanning agents and leather materials that will positively affect on the environment, energy and resources.

REFERENCES

Adiguzel Zengin, A.C., Crudu, M., Maier, S.S., Deselnicu, V., Albu, L., Gulumser, G., Bitlisli, B.O., Basaran, B. and Mutlu, M.M. (2012), “Eco-leather: chromium-free leather production using titanium, oligomeric melamine-formaldehyde resin, and resorcinol tanning agents and the properties of the resulting leathers”, *Ekoloji: International Journal of Environment*, 82. http://dx.doi.org/10.5053/ekoloji.2011.823.

Balberova, N.A., Mikhailov, A.N., Shulenkova E.I. and Kut’in, V.A. (1986), *Directory for tanner (Technology)*, Legprombytizdat, Moskow, 256 (in Russian).

Bandino, G. and Plains, M. (1995), “Process titanium-based mixed tannins”, US Patent 5500020.

Cherkashin, I.V. and Chursin, V.I. (2012), “Properties and application of the new combined tanning in leather production”, *Leather and shoes industry*, 4, 23-26 (in Russian).

Covington, A.D. (1988), “Leather Tanning Process Using Aluminum (III) and Titanium (IV) Complexes”, US Patent 4731089.

Danilkovich, A.G. (2006), *Laboratory practice at the rate of chemistry and technology of leather and fur,* Phoenix, Kyiv, 340 (in Ukrainian).

Environmental problems (2003), *Leather and footwear industry*, 2, 14.

Golovteeva, A.A., Kutsidi, D.A. and Sankin, L.B. (1971), *Laboratory practical work course of chemistry and technology of leather and fur*, Legkayaindustriya, Moskow, 288 (in Russian).

Halmetova, Sh.T., Shirt, M.S. and Madiev, W.K. (2005), “Development tanning compounds of aluminum, titanium and zirconium in the double-bath method in the production of leather tanning”, *International collection of scientific papers* "Technical regulation the basic component of the quality of service management products and services", SHAKHTY, 45-47 (in Russian)

Klimova, D.M. (1990), “Clinical safety and hygiene aspects of the impact on an organism the chromium and other chemicals”, in: A. A. Smagulov et al. (Ed.), Alma-At., Aktyubinsk, 148.

Madiev, W.K., Evtyushkin, M.I. and Kudabayev, A.K. (2009), “The process of tanning glove-haberdashery leather”, KZ Patent 21584 (in Russian).

Metelkin, A.I. and Rusakova N.T. (1980), *Titanium tanning*, Legkayaindustriya, Moskow, 152. (in Russian).

Nikonova, A.V., Andreyeva, A.O. and Maistrenko, L.A. (2015), “Research of tanning process using titanium compounds and polymeric materials”, *Bulletin of KhNU*, 4(227), 93-97 (in Ukrainian).

Nikonova, A.V., Andreyeva, A.O. and Maistrenko, L.A. (2016), “Determination of the optimal parameters of titanium tanning process ussing polymeric compound based on a maleic acid”, *Bulletin of KhNU*, 1 (233), 196-201 (in Ukrainian).

Nikonova, A. and Andreyeva, O. (2015), “The perspective of using titanium compounds in leather and fur production”, *VI Ukrainian-Polish Scientific dialogues*/ Khmelnytsky National University, Khmelnytsky-Yaremche.

Peng, B., Shi, B., Ding, K., Fan, H. and Shelly, D.C. (2007), “Novel titanium (IV) tanning for leathers with superior hydrothermal stability”, *Journal of the American Leather Chemists’ Association*, 102(10), 297-305.

Seggiani, M., Puccini, M., Vitolo S., Chiappe, C., Pomelli, C.S. and Castiello D. (2014), “Eco-friendly titanium tanning for the manufacture of bovine upper leathers: pilot-scale studies”, *Clean Technologies and Environmental Policy*, 16, 1795-1803.

CURED ANTIBACTERIAL COMPOUND BASED ON SILICONE RUBBER AND TiO_2 AND ZnO NANOPARTICLES

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Vulcanizing elastomers is a main step, with a major impact on the final product properties. The amount and type of curing agent, curing time, temperature and pressure are important factors that control the degree of crosslinking and the final properties of the product. The vulcanization system used and required quantities of curing agents are selected based on the elastomer used and the envisaged characteristics. The uses of silicone rubber vulcanizates are vast, but they are preferred in medicine and food because relative to rubber mixtures based on other elastomers, they do not contain substances like antioxidants, accelerators, sulfur and other ingredients that are not permissible in terms of toxicology. The purpose of this paper is to develop a compound reinforced with TiO2 and ZnO nanoparticles based on silicone rubber having antibacterial properties determined by the nature and chemical structure of the matrix and the dispersed phase, processing conditions and curing, using innovative technologies for the development of competitive products for the food and medical industries. These advanced materials and technologies will help improve product quality, environmental protection, human health, antibacterial and antifungal sterilization of products, increased turnover for companies and technological competitiveness at international scale.

Keywords: siliconic rubber, antibacterial compound, nanoparticles.

INTRODUCTION

Lately, the field of polymer compounds has featured new improvements in synthesis of elastomers reinforced with nanopowders with antibacterial properties, offering the possibility of obtaining new advanced polymer structures, and the possibility of extending their area of application (Barton, 1990).

Reinforcement promotes physical, chemical and radicalic bonds between ingredient particles (nanoparticles, antioxidants, accelerators, stabilizers, plasticizers, active and inactive fillers, etc.) and macromolecules of silicone elastomer (silicone rubber) as a result of which a rubber-ingredient complex with special properties is formed (Zhou *et al*., 2012).

Due to the wide range of temperatures at which silicone elastomers may be used, from -100°C to +315°C, they can be applied in various fields (Petreus, 1999; Dobrinescu, 1971; Horn *et al*., 1997). They are used mainly in the development of products for the food and medical industry due to their high temperature resistance specific to sterilization, including the fact that they do not contain substances such as antioxidants, curing accelerators, etc. (Stelescu, 2010; Malcolm *et al*., 2003; Mashak, 2008; Mashak *et al*., 2006). They can also be used as insulating material in electrical industry, aircraft industry, textile industry, and machine building industry (Stelescu, 2010).

The antibacterial elastomeric compounds based on silicone elastomer, silicone rubber in this paper, nanometric reinforcing agents - ZnO (zinc oxide) and TiO_2 (titanium dioxide), crosslinking agents - PD (dicumyl peroxide) and fillers (chalk) were characterized physico-mechanically (Fallahi *et al*., 2003) for hardness, elasticity and tensile strength. The performance of antibacterial elastomeric structures depends on the development technology, processing parameters, the vulcanization system used and the curing agents used in compounding, etc. (Taghizadeh *et al*., 2004; Kajihara *et al*., 2003).

EXPERIMENTAL PROCEDURE

Materials

The following materials were used to develop the antibacterial compound based on silicone elastomer: (1) silicone rubber (Elastosil R701/70-OH: polydimethylsiloxane with vinyl groups, (dynamic) viscosity of over 9,000,000 mPa·s, in the form of paste, density - 1.32 g/cm^2, color - opaque); (2) stearin (white flakes, max 0.5% moisture, max 0.025% ash); (3) active zinc oxide (ZnO - 93-95% precipitate in the form of white powder, density - 5.5 g/cm, surface area - between 45 and 55 m^2/g); (4) zinc oxide nanoparticles (ZnO - white powder, 99.99% trace metals basis); (5) chalk (precipitated $CaCO_3$ - white powder, molecular weight 100.09); (6) di(tert-butylperoxyisopropyl) benzene, powder 40% with calcium carbonate and silica (PD) - Perkadox 14-40B (1.65 g/cm^3 density, 3.8% active oxygen content, pH 7, assay: 39.0-41.0%).

Procedure

Polymer nanocomposites based on silicone rubber, reinforced with zinc oxide nanoparticles and crosslinked with dicumyl peroxide were obtained by mixing on a laboratory roll of 1 Kg without heating, yielding formulations in the form of 3-4 mm thick sheets. Basic materials were added to the mixture in different proportions according to Table 1, respecting the order of mixing.

Table 1. Formualtions of polymer nanocomposites based on rubber, reinforced and crosslinked

Sample code	UM	CS_1	CS_2	CSZ_1	CSZ_2	CSZ_3	CSZT
Silicone Rubber	g	200	200	150	150	150	150
Stearin	g	10	10	7,5	7,5	7,5	7,5
ZnO	g	8	8	4,5	3	1,5	1,5
ZnO (nanoparticles)	g	-	-	1,5	3	4,5	4,5
TiO_2 (nanoparticles)	g	-	-	-	-	-	1,5
Creta	g	20	20	15	15	15	15
PD (Pekcadox)	g	15	30	11,25	11,25	11,25	11,25

For physical-mechanical characterization, plates were made by molding method using an electrically heated press. Antibacterial polymer nanocomposites based on silicone elastomer, reinforced with ZnO and TiO_2 nanoparticles and crosslinked with PD are placed in a mold specific to specimens used for finished products and physico-mechanical characterization with the following dimensions: l50mm x 2mm x l50mm; 70mm x 70mm x 6mm, forming in the press according to the established optimal parameters. Optimum curing time was determined using the Monsanto rheometer.

Figure 1. Electric press used to process specimens (for physical-mechanical testing)

RESULTS AND DISCUSSIONS

The resulting polymer composites were tested according to the physical-mechanical standards in force - normal state: hardness, °Sh A - SR ISO 7619-1:2011; elasticity, % - ISO 4662:2009; tensile strength, N/mm^2 - SR ISO 37:2012; elongation at break, % - SR ISO 37:2012. After conditioning for 24 hours at room temperature, samples were subjected to physico-mechanical measurements, in accordance with the above standards.

Physico-mechanical characterizations are shown in Table 2.

Table 2. Physical-mechanical characterisation

Symbol	CS_1	CS_2	CSZ_1	CSZ_2	CSZ_3	CSZT
Hardness ^{0}Sh D SR ISO 7619-1:2011	65	64	65	66	65	65
Elasticity, %, ISO 4662:2009	12	14	12	12	12	12
Tensile strength, N/mm^2, SR ISO 37:2012	3,4	3,8	3,4	3,8	3,8	3,8
Residual elongation, %, SR ISO 37:2012	36	36	37	39	42	43

The physical-mechanical analysis performed for these mixtures shows the following:

- *Hardness*. Antibacterial polymer nanocomposites based on silicone rubber, reinforced and cured with PD by adding ZnO and TiO2 nanoparticles, show no significant changes compared to control samples, CS_1 and CS_2.

- *Elasticity*. Similar to hardness, adding nanoparticles and crosslinking agent in different proportions keeps elasticity constant. Elasticity increases in the case of samples with a higher percentage of crosslinking agent, due to silicone rubber vulcanization (sample CS_2).

- *Tensile strength*. By introducing reinforcing agent (ZnO and TiO_2 nanoparticles) and crosslinking agent (dicumyl peroxide) into the formulations, tensile strength reaches values from 3.4 N/mm^2 to 3.8 N/mm^2. Therefore tensile strength increases compared to CS_1 and CS_2 control samples depending on the percentage of reinforcing agent introduced in mixtures.

- *Residual elongation* increases with the addition of reinforcing agent. The higher the proportion of reinforcing agent in the form of nanoparticles, the higher the elongation at break.

CONCLUSIONS

The aim of the work was the development of polymer nanocomposites based on silicone rubber, reinforced with ZnO and TiO_2, in the presence of curing agents - dicumyl peroxide (Perkadox - powder 40% with calcium carbonate and silica) and their physical-mechanical characterization determining hardness, elasticity, tensile strength, and elongation at break.

Polymer nanocomposites based on silicone elastomer, reinforced and crosslinked were processed using elastomer processing machinery and physical-mechanical characterization was performed according to the standards in force.

Adding ZnO and TiO_2 nanoparticles does not influence the hardness of antibacterial polymer nanocomposites. Also elasticity is not influenced by adding nanoparticles in the formulations.

Tensile strength increases compared to control samples CS_1 and CS_2 by adding reinforcing agent, and residual elongation increases as the percentage of reinforcing agent is higher.

From experimental data, we can say that antibacterial polymer nanocomposites based on silicone rubber, reinforced with ZnO and TiO_2 nanoparticles and crosslinked with dicumyl peroxide show feasibility of application in pharmaceutical and food industry.

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REFERENCES

Barton, F.M. (1990), *Handbook of Polymer–Liquid Interaction Parameters and Solubility Parameters*, CRC Press, Inc., Boca Raton, Florida.

Dobrinescu, A. (1971), *New types of elastomers for special purposes*, Ministry of Light Industry, Centre for Documentation and Technical Publications, Bucharest.

Fallahi, D., Mirzadeh, H. and Khorasani, M.T. (2003), “Physical, mechanical, and biocompatibility evaluation of three different types of silicone rubber”, *Journal of Applied Polymer Science*, 8, 2522–2529.

Hron, P., Slechtova, J., Smetana, K., Dvorankova, B. and Lopour, P. (1997), “Silicone rubber–hydrogel composites as polymeric biomaterials. IX. Composites containing powdery polyacrylamide hydrogel”, *Biomaterials*, 18(15), 1069–1073.

Kajihara, M., Sugie, T., Sano, A., Fujioka, K., Urabe, Y., Tanihara, M. and Imanishi, Y. (2003), “Novel method to control release of lipophilic drugs with high potency from silicone”, *Chemical and Pharmaceutical Bulletin*, 51(1), 11–14.

Malcolm, K., Woolfson, D., Russell, J., Tallon, P., Mc Auley, L. and Craig, D. (2003), “Influence of silicone elastomer solubility and diffusivity on the in vitro release of drug from intravaginal rings”, *Journal of Controlled Release*, 90(2), 217–225.

Mashak, A. (2008), “In vitro drug release from silicone rubber–polyacrylamide composite”, *Silicon Chemistry*, 3(6), 295-301.

Mashak, A. and Taghizadeh, S.M. (2006), “In vitro progesterone release from γ-irradiated cross-linked polydimethylsiloxane”, *Radiation Physics and Chemistry*, 75(2), 229–235.

Petreus, O. (1999), *Polymer materials*, Cermi, Iasi.

Stelescu, M.D. (2010), “Characteristics of silicone rubber blends”, *Leather and Footwear Journal*, 10(3), 51-58.

Taghizadeh, S.M., Mashak, A., Jamshidi, A. and Imani, M. (2004), “Study of progesterone release mechanisms from a silicone matrix by a new analytical method”, *Journal of Applied Polymer Science*, 91, 3040–3044.

Zhou, H., Wang, H., Niu, H., Gestos, A., Wang, X. and Lin, T. (2012), “Fluoroalkyl silane modified silicone rubber/nanoparticle composite: a super durable, robust superhydrophobic fabric coating”, *Advanced Materials*, 24, 2409-2412.

MORPHOLOGICAL AND STRUCTURAL CHARACTERISATION OF A DYNAMICALLY CURED MMT-REINFORCED ANTIMICROBIAL POLYMER COMPOSITE

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Compounding elastomers and plastics by reactive melt processing in the presence of correct curing systems has led to elastic-plastic alloys with advanced properties. At global scale, however, the new polymeric architectures obtained by dynamic curing, made compatible and reinforced with nanometric particles expand their area of application. Dynamic curing yields elastic-plastic alloys that combine features of both components (elastomer and plastomer) such as chemical resistance, low permeability to water, resistance to extreme temperatures, ozone and UV, low temperature flexibility, resistance to aggressive chemical environments, etc. Through dynamic curing, elastomer particles (EPDM rubber) are more easily dispersed in the polymer matrix (plastomer - PP). This paper aims to morpho-structurally characterize (DSC, SEM, FT-IR) a dynamically cured polymer composite based on polypropylene and ethylene propylene diene terpolymer rubber, made compatible with polypropylene-graft-maleic anhydride reinforced with nanometric particles with antimicrobial properties (MMT), and addition of crosslinking agents. The dynamically cured polymer-based composite based on PP/EPDM/PP-g-MA/MMT/curing agents was obtained using technologies specific to elastomers and plastics and characterized according to current standards.

Keywords: polymeric composite, dynamic vulcanization, morphostructural characterization

INTRODUCTION

In recent years, renowned companies worldwide have changed their production by focusing on special polymers with particular properties. The trend of developing new advanced hybrid polymeric composites, from mixtures of polymers, mixtures of elastomers with olefin plastomers and nanometric reinforcing agents, leads to the development of a new field of application (Stelescu *et al.*, 2011; Vîlsan *et al.*, 2009).

Compounding elastomers and plastics by reactive melt processing in the presence of correct curing systems has led to elastic-plastic alloys with superior properties and special qualities that continue to broaden their area of use in the footwear industry and other branches of economy (Anandhan and Bandyopadhya, 2011; Coran and Patel, 1980; Fisher, 1975; Ionescu *et al.*, 2008). Their vulcanization is a milestone, with a major impact on the properties of the final product (Stelescu *et al.*, 2013; Stelescu *et al.*, 2011). The amount and type of curing agent, time, curing temperature and pressure are important factors that control the degree of crosslinking and the properties of the final product (Volintiru and Ivan, 1974). The resulting advanced materials can be used as thermoplastic elastomers because they have rubber-like properties, but are processed as thermoplastics and do not require vulcanization to manufacture the finished product (Sönmez *et al*., 2014a; Sönmez *et al*., 2014b; Alexandrescu *et al*., 2014).

The new materials and advanced technologies improve the quality of products, environmental protection through recycling, protection of human health by removing pollutants during production, increase turnover of businesses and provide technological competitiveness worldwide. (Vîlsan *et al*., 2009).

The performance of polymer alloys, based on plastomer - PP, elastomer - EPDM, made compatible with PP-g-MA and reinforced with MMT nanoparticles, depends on their concentration and morphology, the processing parameters, the type of auxiliary materials used in compounding, and the equipment used to obtain polymer alloys, etc. (Niţuică *et al*., 2014).

EXPERIMENTAL PROCEDURE

Materials

The following materials were used: (1) polypropylene (PP), impact copolymer Tipplen K 948; (2) ethylene-propylene-diene (EPDM) terpolymer rubber, NORDEL IP 4760, specific gravity – 0.872, Mooney viscosity – 60 MU, ethylene content – 67.5 wt%, ethylidene norbornene (EBN) contents – 5.0 wt%, molecular weight distribution – medium, propylene content – 27.5 wt% (3) polypropylene-graft-maleic anhydride (PP-g-AM), average Mw~9.100 by GPC, average Mn~3,900 by GPC, maleic anhydride 8-10 Wt.%, manufactured; (4) montmorillonite (MMT), Nanoclay, surface modified I.31.PS, contains 0.5-5wt% aminopropyltriethoxysilane, 15-35wt% octadecylamine; (5) di(tert-butylperoxyisopropyl) benzene, powder 40% with calcium carbonate and silica (PD) - Perkadox 14-40B (1.65 g/cm^3 density, 3.8% active oxygen content, pH 7, assay: 39.0-41.0%).

Procedure

Polymer composites based on PP/EPDM, compatibilized with PP-g-MA, reinforced with MMT and crosslinked with dicumyl peroxide were obtained in a Plasti-Corder Brabender Mixer and then plates were moulded for morpho-structural characterization and other purposes by molding method using an electrically heated press, considering the optimal technological processing parameters. The basic materials were added to the mixture in different proportions, as follows: PP - 90 and 50%, EPDM rubber - 10 and 50% PP-g-MA - 5%, PD - 3%, and the percentage of MMT nanoparticles varied from 1 to 3 and 7%. Formulas for $M_{115}M_1$, $M_{115}M_2$, $M_{115}M_3$ samples contained: PP - 90%; EPDM - 10%; PP-g-MA - 5%; PD - 3% and MMT - 1/3/7% and for $M_{315}M_1$, $M_{315}M_2$, $M_{315}M_3$ samples: PP - 50%; EPDM - 50%; PP-g-MA - 5%; PD - 3%; MMT - 1/3/7%.

Polymer composites based on plastomer - PP, elastomer - EPDM, compatibilized with PP-g-MA, reinforced with nanometric particles (MMT) and crosslinked with dicumyl peroxide (Perkadox, PD) were obtained by mixing at the speed of 280 rpm, the temperatures in the three zones are 165/175/175°C, air cooled, stirring for 3-5 minutes after adding the crosslinking and reinforcing agent.

For the characterization, the obtained polymer composite is added in the molds, using the electrically heated press, TP 600, by means of compression method, between its platters at temperature of 165°C and 150 KN pressure for 2 minutes preheating, 10 minutes actual forming in the press and 10 minutes cooling with water.

Characterization of Composites

Composites were characterized in terms of morphological and structural properties using adequate techniques. The specific methods for morphological and structural characterization of dynamically cured polymer nanocomposites are as follows: in terms of morphology, measurements were made using scanning electron microscopy - SEM and thermal behaviour was determined by differential scanning calorimetry - DSC, while FT-IR spectroscopy was carried out to determine the structure.

SEM analysis was performed with scanning electron microscope QUANTA INSPECT F - Netherlands, provided with field emission gun (FEG) with a resolution of 1.2 nm and energy dispersive X-ray spectrometer (EDS) with MnK resolution of 133 eV.

The thermal behavior of the sample was monitored by TG-DSC using a Netzsch 449C STA Jupiter device, in a closed aluminum crucible and heated with 10 K min^{-1} from room temperature to 200°C at the rate of 20 mL min^{-1} dry air. Spectral FT-IR measurements were carried out using a molecular absorption FT-IR ATR spectrometer, Able Jasco 4200, with a double beam in the 4000-600 cm^{-1} range, equipped with ATR diamond crystal and sapphire head.

RESULTS AND DISCUSSIONS

FT-IR Spectrometry

For polymer composites based on PP/EPDM, reinforced compatibilized and cured with PD, IR sequences and vibration assignments of samples $M_{115}M_1$, $M_{115}M_2$, $M_{115}M_3$, $M_{315}M_1$, $M_{315}M_2$, $M_{315}M_3$, are shown in Figures 2 and 3. Vibration assignments are similar to those obtained for polypropylene and EPDM elastomer, Table 1.

Table 1. Vibration assignments and IR frequencies of samples based on PP/EPDM/PPg-MA/MMT/PD

Sample code	Frequency	Intensity	Vibration assignment
PP	1455,74	0,178386	(CH_2) and (CH_3)
	1375,83	0,248779	(CH_2) (CH)O
	1255,9	0,021153	(CH_2) (CH)
	1166,83	0,0409114	Presence of isopropyl group
EPDM	1463,71	0,1755108	(CH_2) CH_3 asymmetric
	1375, 96	0,0827913	CH_3 symmetric
	721,247	0,0512599	(CH_2) crystallinity

It is noticed that the amount of PP is higher than that of the other ingredients, due to the fact that it represents the disperse phase and is the main material. The main absorption bands of PP and EPDM as well as their assignments are: 1375 – (CH_2) (CH)O, 1455 – (CH_2) and (CH_3) and at 1166,83 - presence of isopropyl group. The overlapping spectra show the presence of EPDM in variable percentages, and the absorption bands of EPDM are the following: 728 – CH_2 crystallinity, 1375 –symmetric CH_3 deformation, 1464 – CH_2 scissor vibration and CH_3 asymmetric.

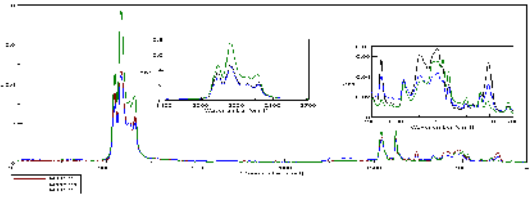


Figure 2. FT-IR spectrum for samples with 90% PP, 10% EPDM, 5% PP-g-MA, 1/3/7% MMT, 3% PD ($M_{115}M_1$ – 1%MMT, $M_{115}M_2$ – 3%MMT, $M_{115}M_3$ – 7%MMT)

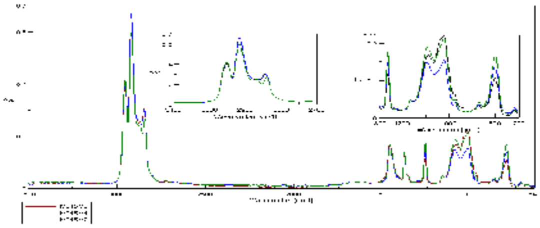
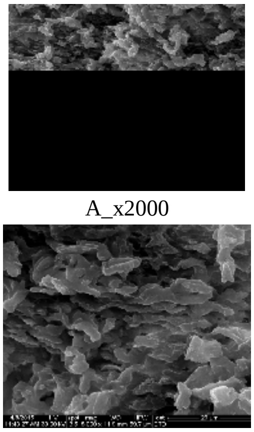


Figure 3. FT-IR spectrum for samples with 50% PP, 50% EPDM, 5% PP-g-MA, 1/3/7% MMT, 3% PD ($M_{315}M_1$ – 1%MMT, $M_{315}M_2$ – 3%MMT, $M_{315}M_3$ – 7%MMT)

Scanning Electron Microscopy (SEM)

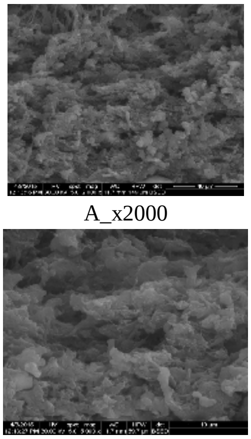
SEM micrographs (Figures 4 and 5) of samples based on PP/EPDM/PP-g-MA/MMT/crosslinkers - PD show a homogeneous dispersion of the elastomer (EPDM) and MMT nanoparticles, as well as crosslinking agents (PD) in the mass of dynamically cured polymer composites, which proves that dynamically vulcanized polymeric nanoalloys are properly obtained at effective temperatures and mixing times depending on the characteristics of plastomers, elastomers, nanopowders and crosslinking agents used in the compounds.



A_x2000

B_ x5000

Figure 4. SEM images, sample $M_{315}M_1$



A_x2000

B_x5000

Figure 5. SEM images, sample $M_{315}M_3$

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was carried out in a temperature range from 120°C-190°C, and the area was measured between 130°C-180°C, Figures 6-8.

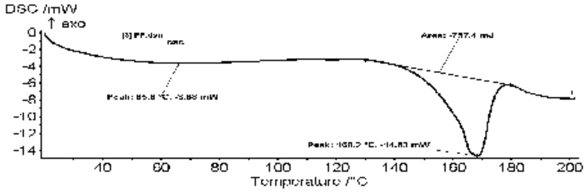


Figure 6. DSC analysis of PP sample (as such)

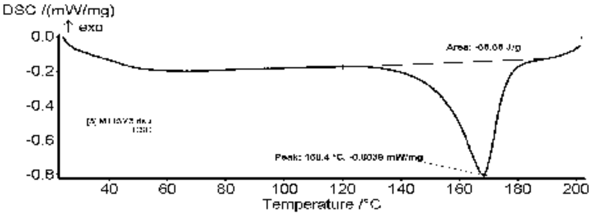


Figure 7. DSC analysis of $M_{115}M_3$ sample (90% PP; 10% EPDM; 5% PP-g-MA; 3% D; 7% MMT

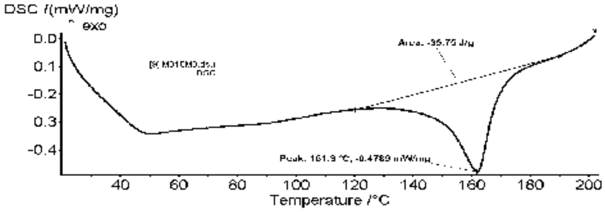


Figure 8. DSC analysis of $M_{315}M_3$ sample (50% PP; 50% EPDM; 5% PP-g-MA; 3% PD; 7% MMT)

The thermograms recorded for the samples M115M3 (90% PP, 10% EPDM, 7% MMT) and M315M3 (50% PP, 50% EPDM, 7% MMT), with PP as the basic material in which the other components disperse, show that the amount of EPDM is in greater proportion in the M315M3 sample. A decrease in temperature, is also noticed, due to changes in viscosity of samples by adding different proportions of EPDM and MMT.

CONCLUSIONS

Scanning electron microscopy was performed in the region of the fracture on the gold film coated samples. SEM analysis of the fracture was conducted in order to highlight the dispersion of the EPDM elastomer in the plastomer matrix, of montmorillonite nanoparticles, and also the homogeneity of the compounded after the dynamic vulcanization and pressing. SEM micrographs of dynamically vulcanized polymer composite and nanocomposite samples indicate a homogenous dispersion of EPDM rubber in the plastomeric matrix, MMT nanoparticles and curing agents used in compounding vulcanized polymer nanoalloys. DSC measurements in a temperature range from 120°C to 190°C and area measurement of samples subjected to the determination were carried out in a temperature range of 130°C-180°C. The values of the melting temperature of the thermograms reflect small changes in the samples, compared to the thermogram recorded for polypropylene, which is the basic material in which the other components are distributed.

The FT-IR spectra show the main absorption bands of polypropylene and EPDM, and their vibration assignments: 1375 - (CH_2) (CH)O, 1455 - (CH_2) and (CH_3), at 1167 - the presence of isopropyl group, and for EPDM absorption bands are the same as the dynamically vulcanized polymer composites (728 - CH_2 crystallinity, 1375 - CH_3 symmetric deformation, 1465 - CH_2 and CH_3 asymmetric shear vibration).

The experimental data of dynamically vulcanized polymer nanocomposites based on PP/EPDM/PP-g-MA/MMT/PD demonstrate the possibility of their application in the footwear industry and for consumer goods.

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REFERENCES

Alexandrescu, L., Sönmez, M., Juganaru, M. and Juganaru, B. (2014), “Polymer structures based on chloroprene rubber and butadiene-co-acrylonitrile for vulcanizates soles intended for usage in high-risk heat environment”, patent no. RO 129558 A2.

Anandhan, S. and Bandyopadhya, S. (2011), “Polymer Nanocomposites: From Synthesis to Applications”, *Nanocomposites and Polymers with Analytical Method*, ISBN: 978-953-307-352-1.

Coran, A.Y. and Patel, R.P. (1980), “Rubber-Thermoplastic Compositions. Part I. EPDM-Polypropylene Thermoplastic Vulcanizates”, *Rubber Chemistry and Technology*, 53, 141-148.

Fisher, W.K. (1975), “Thermoplastic blends of partially cured monoolefinic copolymer rubber and polyolefin plastic”, US Patent 3, 862, 106

Ionescu, F., Alexandrescu, L., Vîlsan (Niţuică), M., Georgescu, M., Ficai, M., Vîlsan, S., Ciobotaru, V., Moldovan, Z., Teişanu, F., and Grigorescu, V. (2008), “Ageing resistance under dynamic conditions – a quality performance of the structured elastoplastic materials”, *Proceedings of the 2nd International Conference on Advanced Materials and Systems*, CERTEX, ISBN 978-973-1716-39-8, 52-56.

Niţuică (Vîlsan), M., Meghea, A., Sönmez, M., Georgescu, M., Alexandrescu, L., Gurău, D., Stelescu, M.D., and Vasilescu, I.O. (2014), “Polymeric nanostructures based on polyolefins and rubber for the footwear industry”, *Proceedings of the 5th International Conference on Advanced Materials and Systems*, CERTEX, Bucharest, ISSN: 2068-0783, 103-108.

Sönmez, M., Alexandrescu, L., Georgescu, M., Niţuică (Vîlsan), M., Gurău, D., Ficai, A., and Ficai, D. (2014a), “Processing and morphological and structural characterization of polypropylene / silicon carbide nanocomposites”, *27th International Symposium on Polymer Analysis and Characterization*, ISPAC, Les Diablerets, Switzerland, June 16-18, 111.

Sönmez, M., Niţuică (Vîlsan), M., Alexandrescu, L., Georgescu, M., Gurău, D., Vasilescu, I.O., Ficai, D., and Ficai, A., (2014b), “Influence of coupling agents on the polymeric material/disperse material interface”, *Proceedings of the 5th International Conference on Advanced Materials and Systems*, CERTEX, Bucharest, ISSN: 2068-0783, 121-126.

Stelescu, M.D. (2011), *Thermoplastic elastomers based on ethylene-propylene rubber (EPDM), which can be used in the footwear industry*, Performantica Press, Iasi, ISBN: 978-973-730-809-2.

Stelescu, M.D., Mănăilă, E., and Crăciun, G. (2013), “Vulcanization of ethylene-propylene–terpolymer-based rubber mixtures by radiation processing”, *Journal of Applied Polymer Science*, 128(4), 2325-2336.

Vîlsan (Niţuică), M., Ficai, M., Georgescu, M, Panţuru, L., Chelaru, C., and Dragomir, T. (2009), “Application of advances polymers in the footwear industry”, *Leather and Footwear Journal*, 9(1), 33.

Volintiru, T. and Ivan, Gh. (1974), *Technological fundamentals of elastomer processing*, Technical Press, Bucharest.

THE EFFECT OF THE FUNCTIONALIZING AGENT TYPE ON PROCESSABILITY, MECHANICAL AND THERMAL PROPERTIES OF POLYPROPYLENE-BASED COMPOSITES

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The aim of this study is to monitor the influence of the addition of glass fibers (GF_s) treated with polydimethylsiloxane (PDMS) and aminopropyltrimethoxysilane (APTMS) on processability and mechanical and thermal properties of polypropylene. The composites based on PP/GFs were processed on a twin screw extruder-granulator, using 20% glass fibers, relative to the polymer mixture (PP/PP-g-MA). Composite granules obtained by extrusion were used in order to determine flow indices and the values obtained demonstrate that the addition of 20% GF_s does not raise serious processability problems (viscosity of the mixture does not vary very widely). Thermal stability tests under load - HDT demonstrate that the best thermal stability is that of the composite reinforced with GF_s-PDMS. Mechanical tests also demonstrate that tensile strength, resistance to bending, modulus and elongation at break are superior for the composite reinforced with PP/GF_s-PDMS compared with the one reinforced with GF_s-APTMS. This can be attributed to the fact that there is a better compatibility between polydimethylsiloxane-treated fibers and the polypropylene matrix compared with those functionalized with aminopropyltrimethoxysilane. Moreover, glass fibers treated with polydimethylsiloxane are more stable to changes in temperature and pressure that the composite is subjected to during processing. TG-DSC results demonstrate a higher thermal stability of the composite with the addition of functionalized fibers.

Keywords: polypropylene, melt flow index, thermal behavior.

INTRODUCTION

Short glass fibers (GFs) are the most commonly used reinforcing agents in composites based on polypropylene (PP), mainly due to their physical and mechanical properties and the cost price (Biswas *et al.,* 2014; Aguilar *et al.,* 2014). Final properties of composite materials are strongly influenced by the strength and stability of the interphase that develops at the fiber/polymer matrix boundary and by mass ratio between components (Etcheverry and Barbosa, 2012). Adhesion between phases is also affected by aggressive environmental conditions, temperature, humidity as well as final tests that the material will be subjected to during the exploitation process (Ota *et al.,* 2005). Due to the non-polar nature of polypropylene (PP), interaction with inorganic disperse phase is low. Both chemical compounds (functionalization agents) applied to the fiber surface, and coupling agents may be used to improve compatibility. Functionalization agents improve adherence to interface through physical and chemical bonds that develop between components in the system (Zaretsky *et al.,* 2004). Moreover, functionalization agents protect glass fiber surface from aggressive environmental conditions such as humidity and reactive fluids (Sockalingam and Nilakantan, 2012).

Organosilanes are considered the most widely used functionalization agents that improve interfacial bonding of glass fibers reinforced composites. Organosilanes' ability to improve adherence depends on several factors such as the nature, type, silane layer thickness, and surface treatment method used for fibers (Broughton *et al.*, 2010; Rausch *et al.*, 2010).

Another commonly used method to improve the glass fiber/polymer interphase is the introduction of a low amount of a polymer modified with specific groups in the thermoplastic matrix (Lin *et al.*, 2015).

Polar groups may be introduced in the polypropylene chains by reactions with species that contain functional groups in their structure, such as ester, carboxylic or anhydride groups (Mäder *et al.*, 1996). This methodology is used in composites based on PP/GF_s, where small amounts of polypropylene-graft-maleic anhydride (PP-g-MA) is introduced into the mixture, in order to react with the amino group on the surface of the silanized glass fibers (Szentes *et al.*, 2012).

MATERIALS AND METODS

Materials

The materials used in this study were the following: polypropylene co-polimer TIPPLEN K 948; polypropylene-graft-maleic anhydride (PP-g-AM), average Mw~9.200 by GPC, average Mn~3,900 by GPC, maleic anhydride 8-10 wt.%; poly (dimethylsiloxane) (PDMS), grade: analytical standard, vapor pressure: 153 mmHg (20^0C), density: 0.82 g/mL, molecular weight: 236.53 wt.%; (3-aminopropyl)trimethoxysilane (APTMS): assay: 97%, refractive index n20/D0: 1.424, density: 1.027 g/mL, molecular weight: 179.29 wt.%; borosilicatic fiber type E, length=4.5 mm, diameter=13 µm, alkaline oxide content>1.

Method

Composite materials based on polypropylene reinforced with glass fibres are obtained in two stages:

- In the first stage – 100g of glass fibers were added in 1 litre of solution containing ethyl alcohol, distilled water and a percentage of 0.5% of functionalizing agent (PDMS or APTMS), relative to the amount of glass fibers. The mixture was maintained under continuous stirring for 24 hours, followed by drying in a hot air oven at a temperature of 80°C and subsequently at 130°C to complete the silanization reaction and evaporation of alcohol.
- The second stage consists in developing the composite on a counter-rotating twin screw extruder granulator as follows: polypropylene powder is introduced in the extruder along with maleic anhydride grafted polypropylene, and glass fibers functionalized with PDMS or APTMS, according to the following 9 zones temperature profile of the extruder: 130-138-161-174-193-189-167- 156-146°C, with screw rotation speed of 100-300 rpm. From composite granules, specimens with the size of 100x10x4 mm were obtained in a laboratory electric press, with the following processing parameters: Temperature = 175°C; Pressure = approx. 150 kN; Preheating time = 15 min; pressing time = 15 min; Cooling time (at 300 kN pressing) = 12 min.

After conditioning at room temperature the specimens were subjected to mechanical and thermal determinations.

Tested formulas based on composites reinforced with glass fiber are presented in Table 1.

Table 1. Formulas of composites based on polypropylene reinforced with treated or untreated glass fibers

Raw materials / Symbol	MU	PP	P0	P01	P1	P2	P3
PP K948	%	100	97	90	77	77	77
PP-g-AM	%	-	3	-	3	3	3
PP/GF_S untreated	%			20	20	-	-
GF_S - PDMS	%	-	-	-	-	20	-
GF_S - APTES	%	-	-	-	-	-	20

Characterization

Counter-rotating twin screw extruder granulator, TSE 35 type; Electrically heated press, TP 600 with the following characteristics: pump pressure max. 300 bar, pressing surface 400 x 400 mm, work temperature 150-300°C adjustable; HDT thermal stability under load was evaluated using Qualitest HDT1, according to SR EN ISO 75, using 2°C/min heating rate, 0.34 mm standard deflection at 1.8 MPa flexural stress, in siliconic oil environment; Tensile and flexural (3-point bending) tests were performed using INSTRON 5982 machine, equipped with 10 and 100 kN load cells, on a minimum of 6 specimen per test; Thermal behaviour (DSC-TG) of the samples was determined with the Netzsch 449C STA Jupiter device. Samples were introduced in a closed aluminium crucible and heated at 10 K min^{-1} from room temperature to 200°C, with dry air flow of 20 mL min^{-1}.

RESULTS AND DISCUSSION

Mechanical Tests

Table 2 shows the physical-mechanical values obtained for the control sample (PP) and those of composites based on polypropylene reinforced with 20% glass fibers treated with PDMS or APTMS and untreated.

The hardness of composites does not vary significantly, relative to the reference value (PP) of approximately 6°Sh D. Increasing mixture hardness within these (acceptable) limits does not raise serious technological problems during processing.

Flexural strength, Young's modulus and breaking load of the mixtures are significantly improved when using GF_s functionalized with PDMS (P2) and APTMS (P3), due to bonds that form at the interphase, compared with values obtained for the control sample (PP). This can be attributed to the interactions occurring between amino groups present on the surface of treated GF_s and anhydride groups of PP-g-AM. In composites reinforced with untreated GF_s (P01 and P1) values obtained from mechanical determinations are much lower. This is due to reinforcing phase crowding and pores generating in the material, due to evaporation of water molecules on the surface of glass fibers.

Elongation% is maximum in the case of mixture (P0) due to the addition of 3% PP-g-MA. It acts as a plasticizer and reduces the viscosity of the mixture. In the case of mixture (P01), elongation value decreases to 1.95% due to the introduction of 20%, untreated glass fibers which leads to increased viscosity of the mixture. As shown in the case of composites containing both PP-g-AM and GF$_s$-PDMS or GF$_s$-APTMS (P2 and P3), elongation at break values increase due to a better adhesion between phases. Moreover, depending on the type of functionalizing agent applied to the surface of the fibers, elongation shows maximum values comparable to those of the mixture (P0). This allows the use of high amounts of reinforcing agents without affecting processability.

The density of the tested mixtures increases with the addition of glass fibers.

Table 2. Values of physical-mechanical determinations obtained for control samples and composite materials

Determinations/Symbol	PP	P0	P01	P1	P2	P3
Hardness °Sh D	66	65	69	70	72	70
Tear load, N	50.48	51.02	53	54.71	67.42	60.94
Resistance to bending, MPa	33.38	33.81	33.73	34.19	40.98	38.62
Young's modulus (GPa)	1.63	1.73	2.34	2.45	2.75	2.55
Elongation (%)	3	3.28	1.95	2.3	3.13	2.9
Density, g/cm^3	0.694	0.682	1.093	1.190	1.190	1.190

Melt Flow Index (MFI)

Tests for determining the melt index were carried out at a temperature of 180°C using a pressing force of 5 kg.

The MFI results of the six samples are shown in Figure 1 and indicate that with the addition of treated or untreated glass fibers, the MFI value decreases. This can be attributed to the presence of fibers in the melt and their partial alignment, which affect the viscoelastic dynamic of the melt and thus decrease the mobility of molecular chains. However, the flow properties of blends that contain glass fibers are improved significantly by the introduction of coupling agents such as PP-g-MA, and depending on the type of organosilane applied to the surface of the fibers. The best yield, relative to the control sample (PP) is that of mixture (P0), because the PP-g-MA reduces the degree of crystallinity (molecular weight) of polypropylene. In the case of the mixture (P2, and P3, respectively) containing PDMS or APTMS treated glass fiber and PP-g-MA, the flow properties are far superior (71-69.6 g/10min) compared to the mixtures (P01 and P1). This demonstrates that by the appropriate treatment of the glass fibers surface and by introducing PP-g-AM, the viscosity of the mixture is significantly reduced and can be processed easily by injection with high amounts of reinforcing agent without raising serious processability problems and excessive wear of equipment.

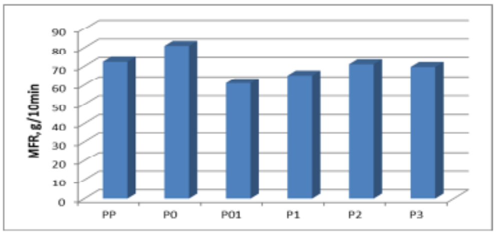

Figure 1. Values of flow index obtained on control samples and PP/ GF$_s$ treated and untreated composite samples

Thermal Stability under Mechanical Loading - HDT

Softening or deflection temperature determinations (HDT) of blends based on polypropylene reinforced with treated and untreated glass fibers were performed in compliance with SR EN ISO 75, and the resulting values are presented in table 3.

The analysis shows that the thermal stability of the blends reinforced with untreated glass fibers (P01), GF_s-PDMS (P2) or GF_s-APTMS (P3) improve the thermal stability of the composites compared with the control sample (PP) by about 60°C. This can be attributed to the fact that by adding glass fibers thermal stability improves and flammability of the products reduces. However, the best thermal stability is that of P2 blend, due to the presence of Si-O-Si group in the PDMS main chain, which gives an excellent temperature stability.

Table 3. Values of HDT deflection temperature obtained on control samples and PP/GF_s composite samples

Symbol	Temp [°C]
PP (K 948) (175 °C)	64,9
P0 (175 °C)	71,1
P01 (175 °C)	80
P1 (170 °C)	95,3
P2 (170 °C)	125,5
P3 (170 °C)	109,6

Complex Thermal Analyses

The thermal analyses of the four samples reveal important differences from both thermal behavior and mass changes in the range of 30 to 900°C. From the point of view of the thermal behavior we can observe that the melting point of the four samples is in the 170 and 180°C.

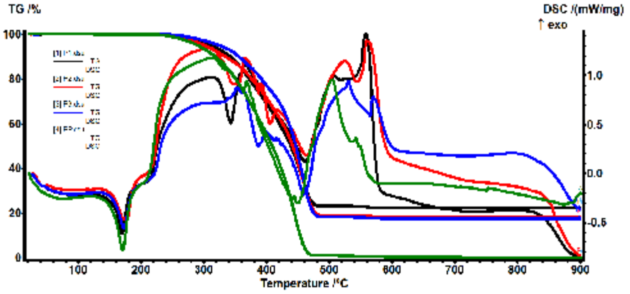

Figure 2. DSC-TG curves recorded on control samples and PP/PP-g-AM/GFs composite samples

The use of the functionalized glass fibers lead to an increasing stability of the samples, the melting point increasing while, the use of bare glass fibers lead to a decrease of the stability proved by the decreasing of the melting point. The very good compatibility between PP and GF_s-PDMS can be highlighted by the splitting of this peak into two peaks (centered at 172.0 and 178.0°C). All the three composite samples are completely burned at ~600^0C, the last strong burning effect being centered at 558.4;

560.2 and 570.3°C while the bare PP exhibit this effect at 550°C. The mass loss was analyzed and based on these data it can conclude that P2 and P3 lost 4.19 and respectively 4.90% comparing to P1 which means that the degree of functionalization of GF_s was ~22% in both cases.

CONCLUSIONS

The results obtained from mechanical determinations show that the addition of small amounts of coupling agent (PP-g-MA) and surface treatment of fibers with organo-silanes, especially PDMS, considerably improve adherence to interphase through amino and anhydride groups. The flow properties of blends are strongly influenced by the type of treatment applied to the surface of the fibers and the presence of a coupling agent because they influence the viscosity of the mixture, in a positive sense. Thermal stability, HDT, shows significantly higher values compared with the control sample (PP), in composites containing glass fibers, especially in the case of blends containing PDMS modified glass fibers.

Acknowledgements

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REFERENCES

Aguilar, H., Yazdani-Pedram, M., Toro, P., Guijada, R., Angel, M. and Manchado, L. (2014), “Synergic effect of two inorganic fillers on the mechanical and thermal properties of hybrid polypropylene composites”, *Journal of the Chilean Chemical Society*, 59(2), 2468-2473.

Biswas, A.K., Cherif, C., Hund, R.D., Shayed, M.A. and Hossain, M. (2014), “Influence of coatings on tensile properties of glass fiber”, *Materials Science*, 20(1), 50-54.

Broughton, W.R., Lodeiro, M.J. and Pilkington, G.D. (2010), “Influence of coupling agents on material behaviour of glass flake reinforced polypropylene”, *Composites Part A Applied Science and Manufacturing*, 41, 506–514.

Etcheverry, M. and Barbosa, E.S. (2012), “Glass fiber reinforced polypropylene mechanical properties enhancement by adhesion improvement”, *Materials*, 5, 1084-1113.

Lin, J.H., Huang, C., Liu, C.F., Chen, C.K., Lin, Z.I. and Lou, C.W. (2015), “Polypropylene/short glass fibers composites: Effects of coupling agents on mechanical properties, thermal behaviors, and morphology”, *Materials*, 8, 8279–8291.

Mäder, E., Jacobasch, H.-J., Grundke, K. and Gietzelt, T. (1996), “Influence of an optimized interphase on the properties of polypropylene/glass fibre composites”, *Composites Part A Applied Science and Manufacturing*, 27, 907–912.

Ota, W.N.; Amico, S.C.; Satyanarayana, K.G. (2005), “Studies on the combined effect of injection temperature and fiber content on the properties of polypropylene-glass fiber composites”, *Composites Science and Technology*, 65, 873–881.

Rausch, J., Zhuang, R.C. and Mäder, E. (2010), “Systematically varied interfaces of continuously reinforced glass fibre/polypropylene composites: Comparative evaluation of relevant interfacial aspects”, *eXPRESS Polymer Letters*, 4(9), 576–588.

Sockalingam, S. and Nilakantan, G. (2012), “Fiber-matrix interface characterization through the microbond test”, *International Journal of Aeronautical and Space Sciences*, 13(3), 282-295.

Szentes, A., Varga, Cs., Horváth, G., Bartha, L., Kónya, Z., Haspel, H., Szél, J. and Kukovecz, Á. (2012), “Electrical resistivity and thermal properties of compatibilized multi-walled carbon nanotube/polypropylene composites”, *eXPRESS Polymer Letters*, 6(6), 494–502.

Zaretsky, E., Botton, G.D. and Perl, M. (2004), “The response of glass fibers reinforced epoxy composite to an impact loading”, *International Journal of Solids and Structures*, 41, 569 – 584.

COMPARISON OF CHARACTERISTICS OF NATURAL RUBBER COMPOUNDS WITH VARIOUS FILLERS

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Natural rubber is the most frequently used elastomer worldwide due to its low prices, renewability, non-toxicity and excellent physical properties. Natural rubber is not used as such but in the form of mixtures which generally contain: fillers for reinforcement, plasticizers, crosslinking agents and other ingredients that confer different characteristics to the final product. Reinforcement of elastomers by incorporating fillers is a process of great practical and technological importance that greatly enhances the physical-mechanical properties of the composite material and reduces the cost price. The origin of such mechanical reinforcement has been linked to the interaction between the rubber and filler as well as the homogeneous dispersion of the filler within the polymer matrix. In natural rubber technology, knowledge of the conditions necessary to achieve more efficient natural rubber–filler interactions is improving continuously. The purpose of this article is to determine the influence of different types of mineral or organic fillers on the properties of natural rubber mixtures. The following were determined for the mixtures obtained: curing characteristics, hardness, elasticity, tensile strength, elongation at break and tear strength. The results indicate that both organic and inorganic fillers have led to improved physical and mechanical characteristics.

Keywords: natural rubber, filler, curing characteristics, physical-mechanical characteristics

INTRODUCTION

Reinforcement of a polymer matrix through the incorporation of fillers is a common industrial practice that greatly enhances the physic-mechanical properties of the composite material. The origin of such mechanical reinforcement has been linked to the interaction between the polymer and filler as well as the homogeneous dispersion of the filler within the polymer matrix. In natural rubber (NR) technology, knowledge of the conditions necessary to achieve more efficient NR–filler interactions is improving continuously. Reinforcement of elastomers with certain ingredients is a process of great practical and technological importance. The purpose of this article is to investigate the influence of different types of mineral or organic fillers on the characteristics of natural rubber compounds. Usage of natural rubber only to produce rubber products is disadvantageous in economical and environmental aspect. It will be more efficient if the rubber is reinforced with filler as its additive without affecting its properties. Moreover, the increasing interest in using natural fillers as reinforcement in natural rubber to substitute the conventional fillers has become one of the main concerns nowadays.

In this research work, the influence of the type of filler on the characteristics of some composites based on natural rubber was studied. Five types of filler used in the study were: precipitated silica, precipitated calcium carbonate, sawdust, hemp and starch. It can be seen that there are two mineral fillers, of which an active one (precipitated silica) and a semi-reinforcing one (precipitated calcium carbonate) (Evans, 2001; Franta, 1989), and three organic fillers, made of natural fibre, namely: sawdust, hemp and starch. Samples were crosslinked using peroxide and a polyfunctional monomer. Research papers have been published suggesting that appropriate polyfunctional monomers (PFMs), also called coagents, added in polymer matrix could be used to obtain desired physical properties of the blend. Coagents are multifunctional organic molecules which are highly reactive towards free radicals. Previous studies (Stelescu *et al*., 2014; Mănăilă *et al*., 2014) show that the most efficient PFM in the case of natural rubber was polyfunctional monomer trimethylolpropane trimethacrylate (TMPT).

EXPERIMENTAL

Materials

To obtain rubber mixtures the following materials were used:

- Natural rubber (NR) for pharmaceutical use, Crep from Sangtvon Rubber Ltd, in the form of white rubber sheets, Mooney viscosity 67.64 ML (1 '+ 4') 100°C, volatile matter content of 0.5%, nitrogen content 0.45%, ash content of 0.25%, impurity content of 0.026%;
- Richon IPPD antioxidant (4010 NA) N-isopropyl - N-phenyl - phenylene diamine, 98% purity, molecular mass 493.6374;
- For crosslinking blends, the following were used: Perkadox 40 benzoyl peroxide (density 160 g/cm^3, 3.8% active oxygen content, 40% peroxide content, pH 7) and polyfunctional monomer trimethylolpropane trimethacrylate Luvomaxx TMPT DL 75 (22 % ash, pH 9.2, density 1.36 g/cm^3, 75 ± 3 % active ingredient);
- The compatibilizing agent - maleated natural rubber (NR-g-AM) was obtained by roll mixing NR with 5 phr (parts per 100 parts of rubber) of maleic anhydride for synthesis, S6855208, produced by Merck KGaA, Germany (melting point 52°C) and 0.75 phr of Perkadox 40; the resulting mixture was kept at a temperature of 160°C for 30 minutes and then used as such.
- Five different types of fillers were tested, of which 2 mineral fillers and 3 types of natural fibers:
    - Precipitated silica Ultrasil VN$_3$, Evonik Industries (pH=6.2, SiO$_2$ content 97%);
    - Chalk, Omyacarb 2T-TN, Tetravion Ltd., Thessaloniki, Greece, surface treated, very fine calcium carbonate powder with good dispersion properties (98.6% CaCO3, 1.1% MgCO3, pH=9);
    - Ground hemp (thread length of max 3 mm);
    - Sawdust;
    - Starch - produced by Lach-Ner - soluble potato starch (water insoluble substances 0.28%; loss on drying 16.9%, easily biodegradable: BOD$_5$ – 0.6 g/g – and COD – 1.2 mg/g) plasticized with glycerin: produced by SC CHIMREACTIV SRL (free acidity 0.02%, density 1.26 g/cm^3, purity 99.5%); the starch was dried at 80°C for 24 h. To obtain the plasticized starch, the starch (50%), water (20%) and then glycerol (30%) were mixed at 70°C for 15 minutes at 50-100 rpm until obtaining a homogeneous mixture. It was left for 1 hour at room temperature, then in an oven at 80°C for 22 h and then for 2 hours at 110°C.

Rubber Compounding

Compounding process was carried out by using a laboratory two-roll mill machine. Formulations are presented in Table 1. First, the NR and NR-g-AM was added into two roll mill. The temperature of this machine was 30-50°C. Afterwards, antioxidant, filler and lastly the vulcanizing agent were added into the compounding. The control sample, NR was also prepared by using two roll mill machine. No filler was added in the control sample (M). This sample was used to compare the properties between NR composites with different filler. The time taken to complete all process was about 18 to 20 minutes. Nip gap, the speed roll mill, time of mixing and the sequence of adding ingredients were kept constant for all formulations.

Table 1. Formulations

Mixture symbol	M (phr)	R (phr)	A (phr)	U (phr)	C (phr)	I (phr)
Natural rubber	95	95	95	95	95	95
NR-g-AM	5	5	5	5	5	5
Glycerine-plasticized starch	-	-	64	-	-	-
Sawdust	-	50	-	-	-	-
Ultrasil VN3	-	-	-	50	-	-
Chalk	-	-	-	-	50	-
Hemp	-	-	-	-	-	50
Peroxide	8	8	8	8	8	8
TMPT	3	3	3	3	3	3
Antioxidant 4010	1	1	1	1	1	1

Preparation of Tensile Test Sample

The test specimen sheets of all compounds were produced using compression moulding. The temperature of the compression moulding machine was kept constant at 165°C. The time taken to produce the specimen sheets was based on the curing time (t_{90}) from the curing testing.

Laboratory Tests

Curing Characteristics

Curing characteristics were determined by an oscillating disk rheometer (Monsanto), at 165°C for 24 min, according to the SR ISO 3417/1997. Delta torque or extent of crosslinking is the maximum torque (MH) minus the minimum torque (ML). Optimum cure time (t_{90}) is the time to reach 90 % of the delta torque above minimum. Scorch time (ts_2) is taken as the time to reach 2 % of the delta torque above minimum. Optimum cure time (t_{90}) is the time to reach 90 % of the delta torque above minimum. The cure rate index (CRI) of the recipe was calculated according to the following formula:

$$CRI = 100 : (t_{90} - ts_2) \quad (1)$$

The CRI is a measure of the rate of vulcanization based on the difference between optimum vulcanization time, t_{90}, and incipient scorch time, ts_2.

Physical-Mechanical Characteristics

Tensile strength and tearing strength tests were carried out with a Schopper strength tester with testing speed 460 mm/min, using dumb-bell shaped specimens according to ISO 37/2012, respectively, angular test pieces (Type II) according to SR EN 12771/2003. Hardness was measured by using a hardness tester according to ISO 7619-1/2011 using 6-mm thick samples. Elasticity (rebound resilience) was evaluated with a Schob test machine using 6-mm thick samples, according to ISO 4662/2009. Residual elongation is the elongation of a specimen measured 1 min after rupture in a tensile test. It was calculated using the formula:

$$\text{Residual elongation}(\ ) = \frac{L - L_0}{L_0} \times 100 \tag{2}$$

where: L_0 is the initial length between two marks and L is the length between the marks 1 min after the sample broke in a tensile test.

RESULTS AND DISCUSSIONS

Cure Characteristics

The curing characteristics, expressed in terms of the optimum curing time, t_{90}, scorch time (ts_2), CRI minimum torque ML, maximum torque MH and torque value DM, (dNm) for the NR samples with different types of fillers, are reported in Table 2. A minimum torque, ML, is a measure of stiffness of the unvulcanized test specimen taken at the lowest point of the cure curve. A maximum torque, MH, is a measure of stiffness or shear modulus of the fully vulcanized test specimen at vulcanization temperature. In other words, it is also a measure of crosslink density (Lopez-Manchado *et al*., 2003; Arroyo *et al*., 2003). It is noticed that the mixture filled with starch had a different behavior than the other mixtures, that can be determined from the existence of plasticizer - glycerine, which led to a plasticized mixture, obtaining smaller values of MH and ML. For other mixtures, it was noticed that introducing the filler leads to an increase in CRI and a decrease in optimum vulcanization time (highest/lowest values were seen when using precipitated silica, followed by sawdust and hemp).

Table 2. Rheological characteristics of blends

Characteristic/ Mixture symbol	M	C	U	A	R	I
The minimum torque M_{min} (dNm)	22.5	21.3	25.4	15.3	21.1	24
The maximum torque M_{max} (dNm)	74.2	74.2	74.2	35.6	74.2	74.3
Torque value ΔM (dNm)	51.7	52.9	48.8	20.3	53.1	50.3
Scorch time,T_{s2} (minutes)	1.52	1.52	1.33	1.9	1.2	1.31
T_{50} (minutes)	5.77	5.1	3.68	5.26	3.74	3.9
Optimal curing time,T_{90} (minutes)	12.47	9.34	5.45	14.6	6.22	6.81
Cure Rate Index (CRI), min^{-1}	9.13	12.79	24.27	7.87	19.92	18.18

Physical-Mechanical Characteristics of Samples

Physical–mechanical characteristics of samples are presented in Figure 1. Hardness increases when adding the filler, thus indicating that all the filler types had a reinforcing effect on mixtures. 100% elongation modulus, tensile strength and the tear strength increased in mixtures containing filler. Elasticity and elongation at break show an uneven variation and residual elongation shows low values, indicating a good return to its original state after applying a force, and therefore, an efficient crosslinking.

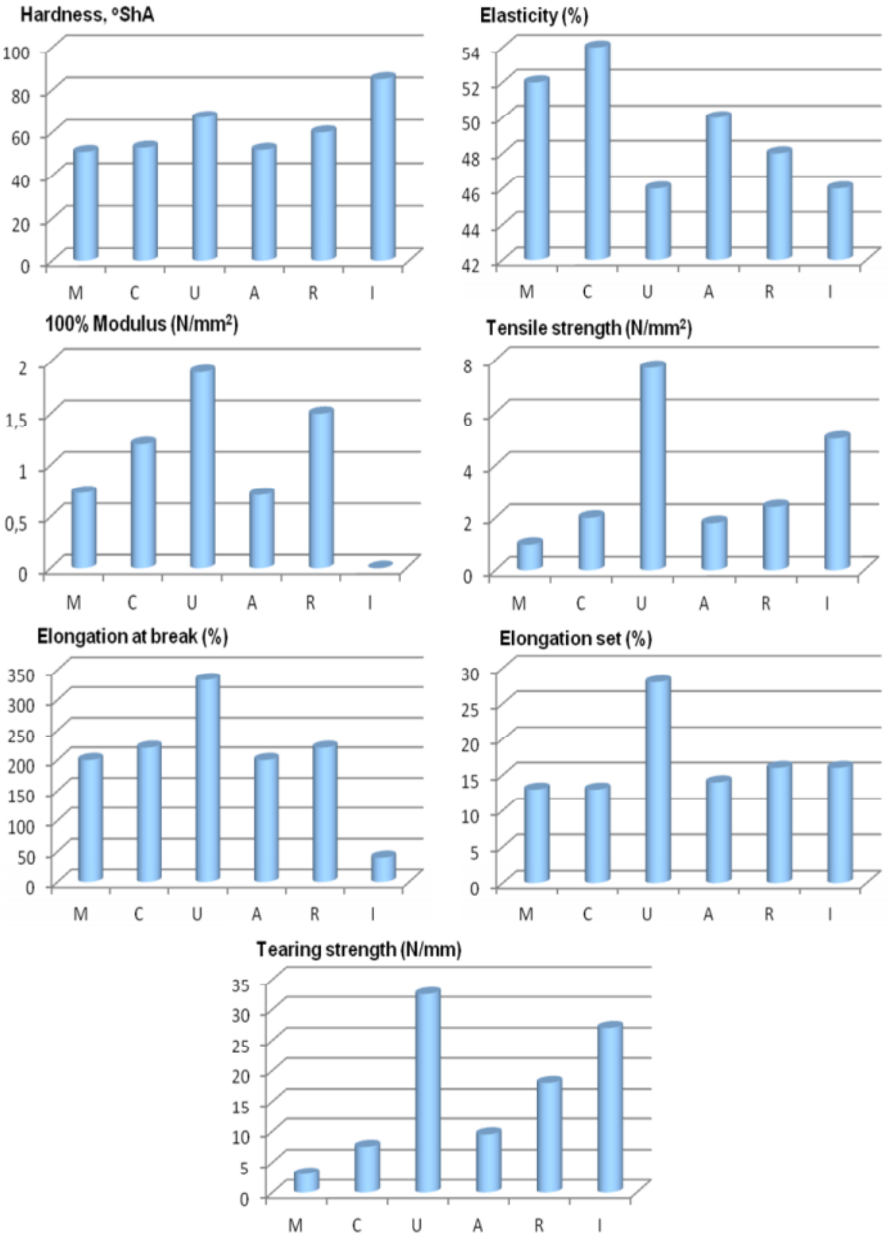

Figure 1. Physical-mechanical characterisation

CONCLUSIONS

The objective of this research is to compare the effect of different types of fillers, mineral or organic, on the characteristics of natural rubber mixtures. From the data it was observed that the mixture filled with starch had a different behavior from the other mixtures, that may be determined from the existence of the plasticizer - glycerine, which led to a plasticized mixture. For other mixtures, it was noticed that introducing the filler leads to an increase in CRI and a decrease in optimum vulcanization time (highest/lowest values were seen when using precipitated silica, followed by sawdust and hemp). The values of hardness, tensile strength and tearing strength have increased by introducing fillers in rubber mixture because the latter have a reinforcing effect on natural rubber. It is noticed that the mixtures filler with sawdust and hemp had better characteristics than those achieved for the mixture with chalk filler. The mixture filled with plasticized starch shows similar physical and mechanical characteristics to those obtained for mixtures filled with chalk and are also superior to the control mixture.

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REFERENCES

Arroyo, M., Lopez-Manchado, M.A. and Herrero, B. (2003), “Organo-montmorillonite as substitute of carbon black in natural rubber compounds”, *Polymer*, 44(8), 2447–2453.

Evans, L.R. (2001), “Introduction to mineral fillers for rubber”, *Rubber World*, USA.

Franta, I. (1989), “Elastomers and Rubber Compounding Materials”, *Studies in Polymer Science*, Elsevier, SNIL, Publishers of Technical Literature and Elsevier, Czechoslovakia.

Lopez-Manchado, M.A., Herrero, B. and Arroyo, A. (2003), “Preparation and characterization of organoclay nanocomposites based on natural rubber”, *Polymer International*, 52(7), 1070–1077.

Mănăilă, E., Crăciun, G., Stelescu, M.D., Ighigeanu, D. and Ficai, M. (2014), “Radiation vulcanization of natural rubber with polyfunctional monomers”, *Polymer Bulletin*, 71, 57–8.

Stelescu, M.D., Mănăilă, E., Crăciun, G. and Dumitrascu, M. (2014), “New green polymeric composites based on hemp and natural rubber processed by electron beam irradiation”, *Scientific World Journal*, Article ID 684047, http://dx.doi.org/10.1155/2014/684047.

INFLUENCE OF CROSSLINKING METHOD ON THE PROPERTIES OF NATURAL RUBBER MIXTURES

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The most important stage in the rubber processing technology is vulcanization / crosslinking, which confers mechanical and thermal stability to rubbers. During crosslinking, rubber molecules with chain configuration are linked by chemical bridges / bonds, and the rubber mass turns from its plastic mass into an elastic one. This is normally done by sulphur and accelerator for general purpose rubbers. The chemistry of vulcanization is complex and the resulting crosslinks may be mono-, di-, tri- or higher poly-sulphides, with a proportion which is among others largely determined by the vulcanization system, the cure time and the temperature. These compounds and their reaction products could be responsible for cytotoxicity and allergy-causing compounds, such as nitrosamines and nitrosatable materials. For these reasons, the aim was to eliminate or reduce toxic ingredients in elaborating and developing crosslinking technologies for natural rubber mixtures, so that the resulting materials may be used in the pharmaceutical or food industry, as well as in manufacturing other rubber goods (toys, various components for footwear, rubber plates, seals and hoses for various purposes, etc.). This paper presents a study on the influence of the vulcanization method on the characteristics of a natural rubber blend. The tested methods have been: (1) with sulphur and vulcanization accelerators, (2) with benzoyl peroxide in the presence of a polyfunctional monomer. Modification of characteristics was analysed depending on the crosslinking method.

Keywords: natural rubber, vulcanization, characteristics

INTRODUCTION

Natural rubber is the most frequently used elastomer worldwide due to its low cost, renewability, non-toxicicty and excellent physical properties. This elastomer is obtained from a series of laticifers, such as *Hevea brasiliensi, Sf Ficus elastica*, etc. Natural rubber is a diene polymer with the structure of cis-poly(isoprene). Natural rubber is used in the carcass of passenger car cross-ply tires for its building tack, ply adhesion, and good tear resistance. It is also used in the sidewalls of radial ply tires for its fatigue resistance and low heat buildup. In tires for commercial and industrial vehicles, natural rubber content increases with tire size. Almost 100% natural rubber is used in the large truck and earthmover tires which require low heat buildup and maximum cut resistance. Natural rubber is also used in industrial goods, such as hoses, conveyor belts, and rubberized fabrics; engineering products, for resilient load bearing and shock or vibration absorption components; and latex products such as gloves, and adhesives. The macromolecular chains that make up the rubber structure harden at temperatures below 10°C, when a "frozen" structure with low elasticity is obtained. To prevent the slip of macromolecular chains and to improve elastic properties, natural rubber is vulcanized. Physical properties of vulcanized rubber systems are influenced by the curing process and chemical nature of the formed crosslinks. The bond strength of linkages can help predict the performance of a given vulcanizate in specific applications. Peroxide cured systems are often employed in static or compressive conditions where low set and heat resistance are required. A network derived from polysulfidic linkages is preferred for components subject to dynamic strain where improved flexural fatigue and tear properties are desired. Bond dissociation energies are often used to quantify the strength of the crosslinks and differentiate the systems (Henning and Shapot, 2005; Palys and Callais, 2003; Dluzneski, 2001). Carbon-

carbon linkages possess high dissociation energies and resist failure to a limiting point, then fail catastrophically. Having lower dissociation energies, polysulfidic bonds break more readily under strain, but due to their chemical nature, also possess the ability to reform and alleviate stresses (Cooper, 1958; Bateman, 1963).

This paper presents a study on the influence of the vulcanization method on the characteristics of a natural rubber blend.

EXPERIMENTAL

Materials

To obtain rubber mixtures the following materials were used:

- Natural rubber (NR) for pharmaceutical use, Crep from Sangtvon Rubber Ltd, in the form of white rubber sheets, Mooney viscosity 67.64 ML (1'+4') 100°C, volatile matter content of 0.5%, nitrogen content 0.45%, ash content of 0.25%, impurity content of 0.026%;
- Starch - produced by Lach-Ner - soluble potato starch (water insoluble substances 0.28%; loss on drying 16.9%, easily biodegradable: BOD_5 – 0.6 g/g – and COD – 1.2 mg/g) plasticized with glycerin: produced by SC CHIMREACTIV SRL (free acidity 0.02%, density 1.26 g/cm^3, purity 99.5%); the starch was dried at 80°C for 24 h, then mixed with glycerine for 7 min at 2000 rpm and 70°C until obtaining a homogeneous mixture.
- Richon IPPD antioxidant (4010 NA) N-isopropyl - N-phenyl - phenylene diamine, 98% purity, molecular mass 493.6374;
- Curing activator: zinc oxide quality I in the presence of stearin;
- Polyethylene glycol coupling agent PEG 4000;
- For crosslinking blends, the following were used: (a) Perkadox 40 benzoyl peroxide (density 160 g/cm^3, 3.8% active oxygen content, 40% peroxide content, pH 7) and two types of polyfunctional monomers with the characteristics presented in Table 1, (b) sulphur and 4 types of curing accelerators presented in Table 2.
- Maleated natural rubber (NR-g-AM) was obtained by roll mixing NR with 5 phr (parts per 100 parts of rubber) of maleic anhydride for synthesis, S6855208, produced by Merck KGaA, Germany (melting point 52°C) and 0.75 phr of Perkadox 40; the resulting mixture was kept at a temperature of 160°C for 30 minutes and then used as such.

Table 1. Chemical structure, molecular weight and boiling points of the polyfunctional monomers used in the experiments

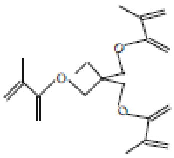
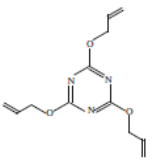
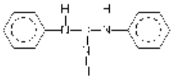
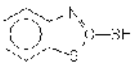
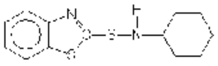
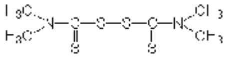
Polyfunctional monomer	Chemical structure	Chemical characteristics
Trimethylolpropane trimethacrylate Luvomaxx TMPT DL 75 (TMPT)		Molecular weight: 338.4 Boiling point: 200°C 75 ± 3 % active ingredient 22 % of ash pH 9.2 Density: 1.36 g/cm^3 Volatility: moderate
Triallylcyanurate Luvomaxx TAC DL 70 (TAC)		Molecular weight: 249.27 Boiling point: 119-120°C Density: 1.34 g/cm^3 26 % of ash 30 % active synthetic silica Volatility: moderate

Table 2. Accelerators used in sulfur curing

Accelerator	Chemical Structure	Property
a) Diphenyl guanidine (DPG) Group: Guanidines. Speed: Scorchy & Slow cure rate.		Molecular weight: 211.27 Melting point: 148°C Density: 1.15 g/cm^3
b) 2- Mercaptobenzothiazole (MBT) Group: Thiazoles. Speed: Scorchy Ultra fast		Molecular weight: 167.2 Melting point: 177-182°C Boiling point: 302°C Density: 1.42 g/cm^3
c) N-Cyclohexyl-2-benzothiazole sulfenamide (CBS) Group: Sulfenamides Speed: Delayed Action. Ultra fast.		Molecular weight: 264.4 Melting point: 97-105°C Density: 1.31 g/cm^3
d) Tetramethylthiuram disulfide (TMTD) Group: Thiurams Speed: Ultra fast		Molecular weight: 240.4 Melting point: 155-158°C Boiling point: 129°C Density: 1.43 g/cm^3

Obtaining Mixtures

Mixtures have been made by means of blending technique, on a laboratory roll. Table 3 presents mixture formulations. The operating mode was: (1) natural rubber (NR) and maleated natural rubber (NR-g-AM) are bound on the roll and homogenized for 3', (2) zinc oxide, stearin, PEG 4000 and antioxidant are added and embedded in the blend, the time needed being 2', (3) glycerine-plasticized starch is added and embedded for 10' (4) curing agents are added for 2', (5) homogenization of the blend and taking it off the roll in the form of a sheet, time needed about 3'. Work parameters on the roll were: friction of 1:1.1 and roller temperature of 30-50°C.

Table 3. Formulations

Mixture symbol	P (phr)	PT1 (phr)	PT2 (phr)	S1 (phr)	S2 (phr)	S3 (phr)	S4 (phr)	S5 (phr)
NR	95	95	95	95	95	95	95	95
NR-g-AM	5	5	5	5	5	5	5	5
Glycerine-plasticized starch	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8
Perkadox 40	8	8	8	-	-	-	-	-
TMPT	-	3	-	-	-	-	-	-
TAC	-	-	3	-	-	-	-	-
ZnO	1	1	1	5	5	5	5	5
Stearic acid	0.1	0.1	0.1	0.5	0.5	0.5	0.5	0.5
PEG 4000	3	3	3	3	3	3	3	3
Sulphur	-	-	-	2.5	2.5	2.5	2.5	2.5
DPG	-	-	-	-	0.5	0.5	-	-
MBT	-	-	-	0.5	0.5	0.5	0.5	0.5
TMTD	-	-	-	0.5	-	0.5	-	0.5
CBS	-	-	-	-	-	-	0.5	0.5
Antioxidant 4010 (g)	1	1	1	1	1	1	1	1

Preparation of Tensile Test Sample

For quality control of elastomer blends, plates have been made by compression, using a hydraulic press; work parameters were: work temperature 165°C, pressure of 150 MPa and vulcanization time was determined with Monsanto rheometer.

Testing

Tensile measuring and tearing strength test was carried out with a Schoppler strength tester with testing speed 460 mm/min, using dumb-bell shaped specimens according to ISO 37/2012, respectively angular test pieces (type II) in according to ISO 12771/2003. The hardness was determined using a hardness tester according to ISO 7619-1/2011 on samples with thickness of 6 mm. The unit of hardness was expressed in A Shore. Elasticity was estimated with a Schob test instrument on 6 mm thick samples, according to ISO 4662/2009. Curing characteristics were determined by an oscillating disk rheometer (Monsanto), according to ISO 3417/1997. Delta torque or extent of crosslinking is the maximum torque (MH) minus the minimum torque (ML). Optimum cure time (t_{90}) is the time to reach 90% of the delta torque above minimum.

RESULTS AND DISCUSSIONS

Rheological Characteristics

Rheological characteristics of blends crosslinked with peroxide and with sulphur respectively, obtained by means of Monsanto rheometer are presented in Table 4 and Figures 1 and 2. In the case of mixtures crosslinked with peroxide, when introducing polyfunctional monomers / curing co-agents, TAC and TMPT, respectively, there is an increase in the maximum torque, and in the variation of torque, and a decrease of the optimal curing time. The increase in torque level during vulcanization is proportional to the number of cross links formed per unit volume of rubber.

Table 4. Rheological characteristics of blends

Characteristic/Mixture symbol	P	PT1	PT2	S1	S2	S3	S4	S5
M min (dNm)	10.4	10.2	7.5	32.9	11.4	39.3	14.1	32.6
M max (dNm)	21.1	25.6	36.8	34.3	32.5	42.4	28.6	37.1
ΔM (dNm)	10.7	15.4	29.3	1.4	21.1	3.1	14.5	4.5
T_{90} (minutes)	18.29	17.43	16.87	3.71	5.29	1.68	2.8	1.95

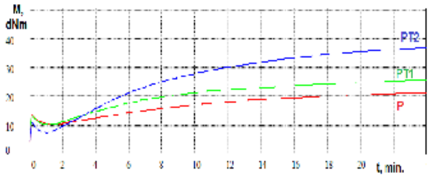


Figure 1. Rheograms of blends crosslinked with peroxide

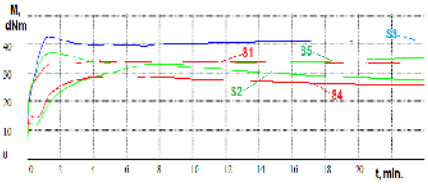


Figure 2. Rheograms of blends crosslinked with sulphur

NR compounds crosslinked with sulfur and curing accelerators show different cure behaviors, as Figure 2 shows. In these types of mixtures, the reversion phenomenon was noticed. Reversion is defined by a loss in physical properties associated with degradation of network integrity. The reversion process is thermally initiated and primarily associated with overcure or high temperature service conditions. Reversion involves reactions that lead to the desulfuration of polysulfidic linkages and main-chain modification (cis-to-trans isomerization) which results in weaker network structures (Mori, 2003; Chen *et al*., 1981). As desulfuration progresses, the distribution shifts from polysulfidic to mono- and disulfidic crosslinks and eventually crosslink density is also lost. The result is a degradation of physical properties and a decrease in the performance of the rubber article (Shankar, 1952). No such standard procedure exists for the quantification of reversion. To evaluate the value of reversion, the following equation was used in this paper:

$$\textit{Degree of reversion (\%)} = (M_H - M_{R'})*100/M_H \qquad (1)$$

where M_H is the rheometer maximum torque and M_R is rheometer cure torque at final (measurement) time.

It was noticed that the reversion of natural rubber blends cured with sulphur and curing accelerators varies as follows: S1 (0.01%) < S3 (3.3%) < S5 (5.66%) < S4 (9.09%) < S2 (16.92%). Therefore, there was a negligible reversion in mixtures with MBT and TMTD accelerators, and low reversion values for systems of three types of accelerators that also contain TMTD. The optimal curing time was lower than in the case of blends crosslinked with peroxide (Bristow, 1991; Sloan, 1992).

Physical-Mechanical Characteristics

Physical-mechanical characteristics of samples are presented in Table 5.

Table 5. Physical-mechanical characteristics

Characteristic/Mixture symbol	P	PT1	PT2	S1	S2	S3	S4	S5
Hardness, °Sh A	38	49	49	42	43	47	41	47
Elasticity, %	32	30	30	42	32	40	38	46
Tensile strength, N /mm^2	6.3	1.4	2.6	3.3	10.5	8.5	4.3	11.2
Elongation at break, %	620	200	440	660	780	660	780	740
Residual elongation, %	16	16	19	15	26	20	18	60
Tear strength, N /mm	16	9	10	20	20	22.5	21.5	2.3

The data shows that the crosslink type of a rubber vulcanizate determines the physical properties such as hardness, resilience, elongation at break etc.

The carbon-carbon bond is created by a free-radical mechanism, which can be initiated by a peroxide vulcanizing agent. The sulphur crosslinks are developed as previously outlined. The monosulfidic crosslinks are created by using sulphur donors (thiuram disulfide or morpholine disulfide). The di- and polysulfidic crosslinks are created by varying sulphur-accelerator. The results on crosslink type vs. crosslink mobility can be summarized as follows: (1) C-C crosslink limited rotation, (2) C-S-C bond increased rotation, (3) C-S-S-C bond increased rotation, limited linear motion. (4) C-Sx-C bond substantial rotation and linear motion. Crosslink mobility (ability to realign under strain) should further enhance tensile strength (Hertz, 1984; Choi and Choi, 2006; Stelescu *et al*., 2010; Hofmann, 1967; Van Duin, 2002; Volintiru and Ivan, 1974). Thus, mixtures crosslinked with peroxide had lower values for elasticity, tensile and tear strength. Blends containing polyfunctional monomers have led to higher values of hardness. In the case of mixtures crosslinked with sulphur and curing accelerators, the best values of physical-mechanical characteristics were those for S5 and S3 blends that contain three types of curing accelerators. The best tear strength and hardness were obtained in the case of these mixtures, as well as very good elasticity, tensile strength and elongation at break. Residual elongation has good values for all types of blends, indicating a good return to the initial shape after applying a force, and therefore, good sample vulcanization.

CONCLUSIONS

The study leads to the following conclusions: the advantages of peroxide cure over sulphur cure include simple formulation, long term compound storage stability and possibility of using higher processing temperatures and yet no reversion. Another advantage of peroxide vs. sulphur cures is that the peroxide cures can more easily be made to impart better heat aging resistance. The reason is that the carbon–carbon bond strength is about 350 kJ/mol vs. sulphur–sulphur bond strength of only 115–270 kJ/mol and carbon–sulphur bond strength of only 285 kJ/mol (Palys, 2009). In contrast, in the case of curing with sulphur and curing accelerators, better physical-mechanical properties were obtained due to crosslink mobility, and the optimal curing time had lower values compared to peroxide crosslinking.

Acknowledgements

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REFERENCES

Bateman, L. (1963), *The Chemistry and Physics of Rubber-Like Substances*, MacLaren, London.

Bristow, G.M. (1991), “Influence of Grade of Natural Rubber on Reversion Behavior”, *Journal of Natural Rubber Research*, 6(3), 137-151.

Chen, C.H., Koenig, J.L., Shelton, J.R. and Collins E.A. (1981), “Characterization of the Reversion Process in Accelerated Sulfur Curing of Natural Rubber”, *Rubber Chemistry and Technology*, 54(4), 734-750.

Choi, S.S., Choi, S.J. (2006), “Influence of Silane Coupling Agent Content on Crosslink Type and Density of Silica-Filled Natural Rubber Vulcanizates”, *Bulletin of the Korean Chemical Society*, 27(9), 1473-1476.

Cooper, W. (1958), “Copolymers of butadiene and unsaturated acids: Crosslinking by metal oxides”, *Journal of Polymer Science Part A: Polymer Chemistry*, 28(116), 195-206.

Dluzneski, P.R. (2001), “Peroxide Vulcanization of Elastomers”, *Rubber Chemistry and Technology*, 74(3), 451-492.

Henning, S.K. and Shapot, S.A. (2005), *Multifunctional Acrylates as Anti-Reversion Agents in Sulfur Cured Systems*, The Fall 168th Technical Meeting of the Rubber Division, American Chemical Society, Pittsburgh.

Hertz, D. (1984), “Vulcanization - In Theory and Practice”, *Elastomerics*, 116(11), 17-21.

Hofmann, H. (1967), *Vulcanization and vulcanizing agents*, MacLaren and Sons Ltd, London, Palmerton Publishing Co. Inc., New York.

Mori, M. (2003), “Study of Vulcanization and Degradation Chemistry in Natural Rubber by Solid-State C NMR and Physical Property Measurements”, *Rubber Chemistry and Technology*, 76(5), 1259-1275.

Palys, L.H. (2009), “Peroxide Cure Systems”, in: J.S. Dick (ed.), *Rubber Technology, Compounding and Testing for Performance*, Hanser Publications, Munich, 434.

Palys, L.H. and Callais, P.A. (2003), “Understanding organic peroxides to obtain optimal crosslinking performance”, *Rubber World*, 229(3), 35-41.

Shankar, U. (1952), “Investigations of the Reversion of Vulcanized Rubber under Heat”, *Rubber Chemistry and Technology*, 25(2), 241-250.

Sloan, J.M. (1992), “Reversion Studies of Natural and Guayule Rubbers”, Accesion number ADA257501, U.S. Army Materials Technology Laboratory: Polymer Research Branch.

Stelescu, M.D., Georgescu, M. and Mănăilă, E. (2010), “Aspects regarding crosslinking of a natural rubber blend”, *Proceedings of the 3rd International Conference on Advanced Materials and Systems*, Bucharest, 313-318.

Van Duin, M. (2002), “Chemistry of EPDM Cross-linking”, *Kautschuk Gummi Kunststoffe*, 55(4), 150-156.

Volintiru, T. and Ivan, Gh. (1974), *Technological Bases for Processing Elastomers*, Technical Press, Bucharest.

HIGH-DENSITY POLYETHYLENE/RED MUD POLYMER COMPOSITES: EFFECT OF UV ANNEALING

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An investigation was carried out on the effects of red mud ratio and UV annealing treatment on the mechanical, thermal and morphological properties of high-density polyethylene (HDPE)/red mud (RM) polymer composites. Red mud, in six different concentrations (0, 10, 20, 30, 40, and 50 wt %), was added to HDPE to produce composites. The annealing UV treatment was carried out at 100°C for three different holding times: 100, 200 and 300 h. The mechanical properties, including the elastic modulus, tensile strengths, strain at tensile strength, Izod impact resistance, hardness, and melt flow index with and without an annealing UV treatment, and the thermal properties, such as the Vicat softening point, heat deflection temperature and melt-flow index, of the composites were investigated.

Keywords: high-density polyethylene, red mud, UV annealing

INTRODUCTION

Nowadays there is a significant interest for the development of environmentally friendly polymers and polymer composites based on renewable resources due to the increasing environmental concerns and the decreasing fossil resources (Kalia *et al.*, 2011). Utilization of waste materials has become more pressing than ever. Red mud is accumulating at a rate of 30 million ton annually throughout the world. Under normal conditions when 1 ton of alumina is produced from bauxite, an equal amount of red mud is generated as a waste. Disposal of red mud is a severe problem as it is highly alkaline and produced in huge quantities (Bhat *et al.*, 2011; Milacic *et al.*, 2012).

The higher cost of composites is the only factor hampering its use in majority of industrial application in spite of possessing customized properties specific to given application. Some researchers have found that adding low cost and readily available filler is the easiest way to bring down the cost of composites. However, mechanical properties of the composites should not be affected adversely in the attempt of reducing the cost. Therefore, fillers are added firstly to improve the mechanical and tribological properties and secondly to reduce the cost of the components (Rothon, 1997). Red mud has been suggested as filler for polymer reinforcement or as a cheap adsorbent for removal of toxic metals or an acid by several researchers. Chand and Hashmi (1999) tried to improve the mechanical properties and abrasive wear properties of polymer blend filled with red mud. Pradhan *et al.* (1999) had reported that activated red mud as a good adsorbent was used for adsorption of phosphate or chromium. In addition, the mechanical and thermal properties of polymers are generally improved by the addition of inorganic fillers. The challenges in this area of high-performance organic–inorganic hybrid materials are to obtain significant improvements in the interfacial adhesion between the polymer matrix and the reinforcing material since the organic matrix is relatively incompatible with the inorganic phase.

Thermoplastics have a big potential for applications in the industry as well as in construction, electrical applications and food packaging. One of the few disadvantages associated with the use of nano fillers, is their high cost. The present research work has been undertaken with an objective to explore the use of red mud as a reinforcing material as a low cost option. This is due to the fact that red mud alone contains all these reinforcement elements and is plentifully available (Bhat *et al.*, 2011).

EXPERIMENTAL PROCEDURES

Six different polymer composites were prepared. Compositions of HDPE/RM polymer composites that were formed are given in Table 1.

Table 1. Composition of the HDPE/RM polymer composites formulations

Groups	HDPE Content (wt %)	RM Content (wt %)
1	100	-
2	90	10
3	80	20
4	70	30
5	60	40
6	50	50

High-density polyethylene (HDPE) (I 668 UV) was supplied by Petkim (Izmir-Turkey). Specific gravity is 0,970 g/cm^3. Melt flow rate is 5.2 g/10 min (190°C–2.16 kg). Yield strength is 28,0 MPa and notched Izod impact (23°C) is 12 kJ/m^2. Red Mud was supplied Güray Seramik Company (Avanos-Turkey).

Sample Preparation

Red mud was first heat treated at 150°C for decomposition of hydrates combined with red mud and then dry grinded with Siemens simatic C7-621 control system device to obtain unsegregated powders. The size of red mud particles varied between 10–80 µm and the mean particle size was 35 µm. HDPE and Red mud were dried overnight at 105°C in a vacuum oven prior to melt blending. Mechanical premixing of solid compositions was done using a LB-5601 liquid-solids blender (The Patterson-Kelley Co., Inc. east Stroudsburg, PA - USA) brand batch blender for 15 min. Samples with various proportions of HDPE/RM polymer composites were produced between 190-220°C at 12 bar pressure, and a rotation rate of 20-25 rpm, with a Microsan extruder (Microsan Instrument Inc. Kocaeli - Turkey). Polymer composites were also dried in vacuum oven at 105°C for 24 hours after extrusion. Subsequently, test samples were manufactured by injection molding. Injection temperature was 190-220°C, pressure was 100-120 bar and screw speed was 23-25 rpm.

Mechanical Characterization

The tensile modulus, tensile strength and strain at tensile strength of the compressed plates were measured by using a tensile testing machine (Zwick Z010, Germany) according to ASTM D638 at room temperature and crosshead speed of 50 mm/min. For every composition, six samples were tested, and the averages of the six measurements were reported. The hardness test was done according to the ASTM D2240 method with Zwick hardness tester. To investigate fracture behavior, Izod impact test (notched) was done at room temperature according to the ASTM D256 method with Zwick B5113 impact tester (Zwick, Germany). Flow behavior testing of all the mixtures was done according to ISO 1133 standard with Zwick 4100 MFI equipment. Heat deflection temperature (HDT) and Vicat softening point tests were done according to ISO 75 and ISO 307 standard with determined by CEAST 6521 (Ceast SpA, Italy) HDT-Vicat test equipment.

UV Annealing

The UV annealing test was done according to the ASTM D 5208 method with Devotrans pre-heated UV oven treatment device. UV holding time is 100, 200 and 300 hours and UV holding temperature is 100°C. The distance of the light is 50 mm and Osram 300 W ultra-vıtalux type lamp was used.

Microscopy

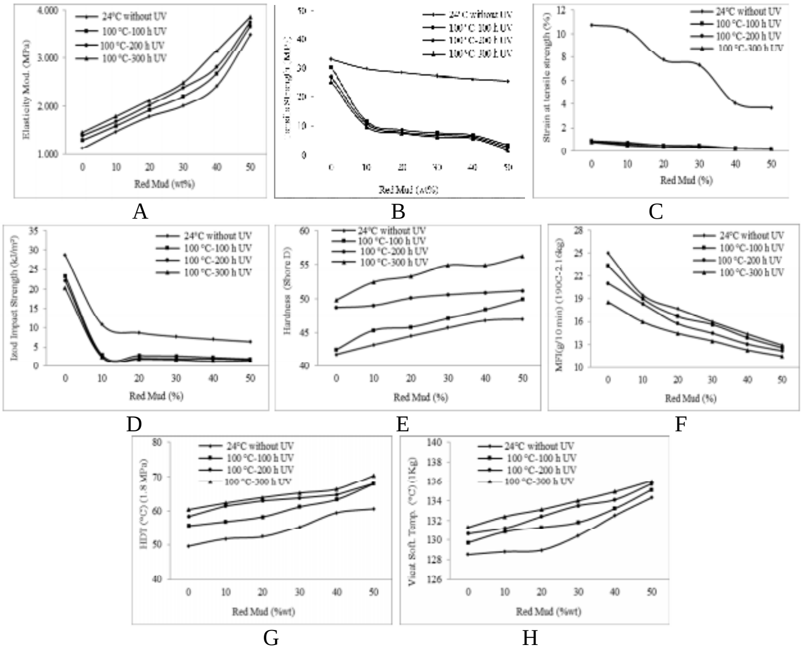
The fractured surfaces of the HDPE/RM polymer composites were coated to thickness of 20 Å of a gold (Au) to prevent electrical charging by Polaron SC7640 (high resolution sputter coater) (United Kingdom). The surfaces of the prepared samples were observed by the FEI Sirion XL30 FEG (Nederland) scanning electron microscopy (SEM) at an acceleration voltage of 15 kV.

RESULTS AND DISCUSSION

Elasticity modulus of HDPE/Red Mud specimens measured in tensile test. Figure 1/A shows the variation of the elasticity modulus as a function of the red mud content of the composites. Since red mud has much higher stiffness values than the HDPE matrix, the elasticity modulus of the HDPE/RM polymer composites increased as the red mud concentration increases from 0 to 50 wt %. On the other hand, the elasticity modulus of the HDPE/RM polymer composites increased as the UV holding time increases from 100 hours to 300 hours. The maximum elasticity modulus is observed at Group 6 (100°C-UV 300h). In comparison with the elasticity modulus of virgin HDPE, the elasticity modulus increased by 241% for the composites with a 50 wt % red mud concentration. This was probably due to the improved dispersion quality of red mud particles in the HDPE matrix. The results showed that the elasticity modulus of composites improved with increasing red mud concentration. The tensile strength of the HDPE/RM polymer composites is shown in the Figure 1/B. With the addition of red mud in HDPE has been a change in tensile strength values. A systematic decrease in tensile strength was observed as red mud contents increase. At high weight fractions of red mud, tensile strength decreases due to the filler high volume incorporated into the HDPE matrix For example, the tensile strength of virgin HDPE and HDPE/RM (50/50) polymer composites (without UV) were 33.2 MPa and 25,6 MPa respectively. In comparison with the tensile strength of virgin HDPE, the tensile strength decreased by 23% for the composites with a 50 wt % red mud concentration.

Similar situation was seen in tensile strength values (with UV). For example, the tensile strength of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV, holding time: 100 hours) were 30,3 MPa and 3,1 MPa respectively. On the other hand, the tensile strength of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV, holding time: 300 hours) were 25,3 MPa and 1,5 MPa respectively. The agglomeration and the poor dispersion of the red mud into the HDPE matrix had a significant impact on the mechanical properties of the composite in comparison to the neat matrix strength. This behavior might be attributed to the grinding method applied which affects the red mud granule size as well as their physical and morphological characteristics.

The elongation at break of red mud filled composites was measured, as shown in Figure 1/C. The minimum elongation at break is observed at the 50 wt % red mud powder concentration for HDPE. In comparison with the elongation at break of virgin HDPE, the elongation at break decreased by approximately 98 % for the composites with a 50 wt % red mud concentration. A decrease in elongation at break as the red mud content increases is observed indicating the presence of a poor interfacial adhesion between the red mud and the HDPE which does not allow efficient stress transfer between the two phases of the composite. For red mud, agglomerates induce a decrease in strength. On the other hand, the elongation at break of the HDPE/RM polymer composites did not significant change as the UV holding time increases from 100 hours to 300 hours.



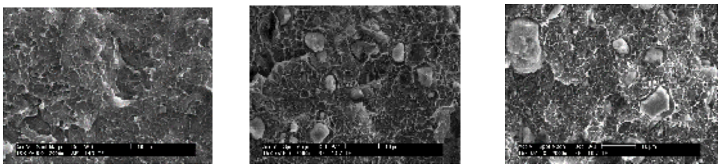
A B C

D E F

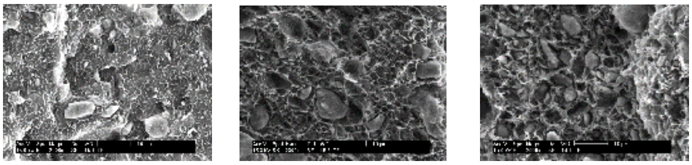
G H

Figure 1. Mechanical properties of HDPE/RM polymer composites

The measured values of the Izod impact strengths are presented in Figure 1/D. The impact strength decreased as the red mud concentration increased from 0 to 50 wt %. This means that the energy not absorbed by the specimen decreases and with it its toughness also decreases. For example, the Izod impact strength of virgin HDPE and HDPE/RM (50/50) polymer composites (without UV) were 28,7 kJ/m^2 and 6,4 kJ/m^2 respectively. Similar situation was seen in Izod impact strength values with UV. For example, the Izod impact strength of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV aging 100 hours) were 23,4 kJ/m^2 and 1,9 kJ/m^2 respectively. On the other hand, the Izod impact strength of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV, holding time: 300 hours) were 20,2 kJ/m^2 and 1,5kJ/m^2 respectively. The minimum Izod impact strength is observed at the 50 wt % red mud (UV 300 h) concentration. In comparison with the Izod impact strength of virgin HDPE, the Izod impact strength decreased by approximately 94% for the composites with a 50 wt % red mud (UV 300 h) concentration. The impact strength is the ability of a material to withstand fracture or the amount of energy required to propagate a crack. It depends on certain factors such as fiber and matrix strength, load transfer efficiency, resistance to crack propagation, bonding strength, volume fraction, fiber distribution, and geometry (Bax and Mussig, 2008). The relationship between the red mud content and the hardness of the polymer composites is shown in Figure 1/E. The hardness of the composites increased (from 0 to 50wt %) linearly with an increase weight percentage of filler. The Hardness of the HDPE/RM polymer composites increased as the UV aging time increases from 100 hours to 300 hours as well. The maximum and minimum Hardness are observed at Group 6 (100°C- UV 300h) and Group 1 (24°C- UV 0h) respectively. The relationship between the red mud content and the MFI of the polymer composites is shown in Figure 1/F. The MFI of the composites decreased (from 0 to 50wt %) linearly with an increase weight percentage of red mud. For example, the MFI of virgin HDPE and HDPE/RM (50/50) polymer composites (without UV) were 24,9 g/10 min and 12,8 g/10 min respectively. Similar situation was seen in MFI values (with UV). For example, the MFI of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV aging 100 hours) were 23,3 g/10 and 12,5 g/10 respectively. On the other hand, the MFI of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV holding time: 300 hours) were 18,5g/10 and 11,4 g/10 respectively. The relationship between the red mud content and the HDT of the polymer composites is shown in Figure 1/G. The HDT experiment was started at room temperature with a heating rate of l20°C/h and under a load of 1.8 MPa. The HDT of the composites increased (from 0 to 50wt %) linearly with an increase weight percentage of red mud. For example, the HDT of virgin HDPE and HDPE/RM (50/50) polymer composites (without UV) were 49,6°C and 60,5°C respectively. Similar situation was seen in HDT values (with UV aging). For example, the HDT of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV, holding time: 100 hours) were 55,6°C and 67,7°C respectively. On the other hand, the HDT of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV aging 300 hours) were 60,3°C and 70,3°C respectively. The maximum HDT is observed at the 50 wt % red mud concentration (UV holding time: 300 h) for HDPE. In comparison with the HDT of virgin HDPE, the HDT increased by 41% for the composites at a 50 wt % red mud (UV holding time: 300 h) concentration. The relationship between the red mud content and the Vicat softening temperature of the polymer composites is shown in Figure 1/H. The Vicat softening temperature experiment was started at room temperature with a heating rate of 120°C/h and under a load of 1 Kg. The Vicat softening temperature of the composites increased (from 0 to 50wt %) linearly with an increase weight percentage of red mud. For example, the Vicat softening temperature of virgin HDPE and HDPE/RM (50/50) polymer composites (without UV) were 128,5°C and 134,3°C respectively. Similar situation was seen in Vicat softening temperature values (with UV aging). For example, the Vicat softening temperature of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV, holding time: 100 hours) were 129,7°C and 135,1°C respectively. On the other hand, the Vicat softening temperature of virgin HDPE and HDPE/RM (50/50) polymer composites (with UV aging 300 hours) were 131,3°C and 136°C respectively. The maximum Vicat softening temperature is observed at the 50 wt % red mud concentration (UV holding time: 300 h) for HDPE.



100% HDPE 90%HDPE-%10 RM 80%HDPE-%20 RM



70%HDPE-%30 RM 60%HDPE-%40 RM 50%HDPE-%50 RM

Figure 2. Scanning electron micrographs of HDPE/RM polymer composites

The fractured surfaces of polymer composites were examined with SEM are presented in Figure 2. Interfacial tension between polymer and filler is very important for phase morphology. It is clear that the filler at low loading level (10%) morphology slightly differs from that of pure HDPE polymer. Low level pullout was observed which reflects a relative good tensile stress performance of the polymer composite compared to that of pure HDPE. At high red mud loading, (%50) as shown in Figure 2, more filler pullout and deponding were observed. This was probably due to poor adhesion between red mud and HDPE polymer matrix.

CONCLUSIONS

In the present work a novel composite material containing red mud dispersed in HDPE matrix was manufactured and studied. The aim of this study was to investigate the effect of red mud at different weight fractions without coupling agent on mechanical, morphological, and thermal properties of HDPE matrix composite. The results indicate the variation of the composite properties with varying the filler content. Substantial improvements in the mechanical properties were obtained by the addition of red mud in the HDPE polymer matrix. The results showed that the elasticity modulus, hardness, Vicat softening temperature, and HDT of composites improved with increasing powder content. The annealing UV treatment increased the elastic modulus, hardness, Vicat softening temperature and HDT values, and different holding times showed similar effects on the increased elastic modulus values as well. In contrast, the tensile stress reduction at high red mud loading may be due to the poor interfacial bonding between red mud powder and HDPE. The annealing UV treatment decreased the tensile strength and strain at tensile, different holding times showed similar effects on the decreased these values. MFI decreased as the red mud content and UV holding times increased. At high red mud loading (%50), more filler pullout and deponding were observed. This was probably due to poor adhesion between red mud and HDPE polymer matrix.

Acknowledgement

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REFERENCES

Bax, B. and Mussig, J. (2008), “Impact and tensile properties of PLA/cordenka and PLA/flax Composites”, *Composites Science and Technology*, 68, 1601–1607.

Bhat, A.H., Abdul Khalil, H.P.S. and Banthia, A.K. (2011), “Thermoplastic polymer based modified red mud composites materials”, in Attaf, B. (ed.), *Advances in composites materials - Ecodesign and analysis*, 3, 55-86.

Chand, N. and Hashmi, S.A.R. (1999), “Effect of red mud addition on abrasive wear rate of isotactic polypropylene/low density polyethylene blend under low stress conditions”, *Journal of Scientific and Industrial Research*, 58, 795.

Kalia, S., Kaith, B.S. and Kaur, I. (eds.) (2011), *Cellulose Fibers: Bio- and Nano-Polymer Composites*, Green Chemistry and Technology, Springer.

Milacic, R., Zuliani, T. and Scancar, J. (2012), “Environmental impact of toxic elements in red mud studied by fractionation and speciation procedures”, *Science of the Total Environment*, 426, 359–365.

Pradhan, J., Das, S.N. and Thakur, R.S. (1999), “Adsorption of hexavalent chromium from aqueous solution by usin activated red mud”, *Journal of Colloid and Interface Science*, 217, 137.

Rothon, R.N. (1997), “Mineral fillers in thermoplastics”, *The Journal of Adhesion*, 64, 87–109.

FATIGUE BEHAVIOR OF HONEYCOMB SANDWICH COMPOSITES UNDER FLEXURAL AND BUCKLING LOADING

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This study experimentally investigated the fatigue behaviors of honeycomb-sandwich composites under buckling and three-point bending loads. The ASTM C 365 and ASTM C 393 standards were used as references to prepare test specimens and conduct experiments. The investigation looked at how the cell diameter, core thickness and thickness of the skin material affected the fatigue behavior. It was observed that the most significant parameter affecting fatigue under buckling loads was the cell diameter, and the least significant parameter affecting fatigue under three-point loads was the thickness of the skin material.

Keywords: honeycomb, sandwich composites, fatigue

INTRODUCTION

Honeycomb-sandwich structures are formed by putting thick cell structures between thin, tough plates. Bonding between the plates and the honeycomb cells is provided by an adhesive, as shown in Figure 1. Honeycomb structures are known to have low weight and high flexural rigidity, and they are widely used under tensile and bending loads. The use of honeycomb structures in place of conventional materials under high loading has increased (Allen, 1969).

Aluminum alloys 3003, 2024 and 5052 are commonly used. The 5052 aluminum alloy is commonly used in aerospace applications, while the 2024 alloy is preferred for high-temperature uses. Although different methods are used to form honeycomb cells, the most common one is to use adhesive bonding (http://www.flightglobal.com).

Honeycomb composites are used for airfoils, spoilers, passenger floors and shells. Critical loads in airplanes can be created by bird impact, hail damage and other foreign objects. It is important to determine the damage caused by impacts to the airfoils and to the airframe. The resistance of sandwich composites to high-velocity impacts is low due to their thin layers and easily deformable core materials. The damage types most often observed – matrix cracking, separation and fiber damage – can be seen occurring individually or occurring in combination.

If the velocity of the impact is low, sandwich cells can absorb the load by being bent. If the kinetic energy of the impcat is lower than the elastic strain energy of the plate, the impact results in light damage (Bitzer, 1997). In high-velocity impacts, when the local contact stress exceeds the local strength, honeycomb cells may suffer from skin bending, debonding between skin and core and crush damage to the honeycomb cells (Aktay *et al*., 2008). Thus, energy absorption abilities of honeycomb composites depend on core damage and deformation (Abrate, 1998).

Because honeycomb composites have been developed and extensively used, many researchers have studied them to determine and enhance their mechanical properties. Yamashita and Gotoh (2005) investigated quasi-static behaviors of aluminum honeycomb composites under flatwise impact load. Miller *et al*. (2011) experimentally investigated and calculated the effects of eight different cell structures on buckling behaviors in composites. They determined that the conventional model had a buckling strength 13% less than the recessed model. Giglio *et al*. (2012) experimentally investigated and numerically determined the strength of honeycomb sandwich composites that consisted of aluminum surface layers and nomex cells by using three-point bending tests. Numerical and experimental results were compared using curves of load-shape changes and detailed photographic images of cell crushing under loads. They determined differences of 1.8% and 0.29% between numerical and experimental results for the energy absorption and maximum load respectively, and reported that the numerical and experimental curves of the load-shape change showed a similar tendency. Belouettar *et al*. (2009) experimentally investigated mechanical and fatigue behaviors of honeycomb composite structures consisting of aramid fibers and aluminum cells under static loads by using four-point bending tests. They determined that the damage formation in the cell structure was due to the decrease in rigidity and stated that an aluminum L model was more suitable in terms of structural lifetime. Solmaz and Topkaya (2012) experimentally and numerically investigated critical buckling loads of sandwich composites having ellipsoid cells. Copper and aluminum were used as the core materials and it was found that aluminum cells had greater buckling strength.

The fatigue behavior of sandwich composites with aluminum honeycomb cells were experimentally investigated in this study by investigating the effects of different cell diameters (D), cell heights (T) and thicknesses of skin material (h) under different loads.

MATERIALS AND METHODS

In the study, skin plates were produced using the 5754 aluminum alloy. The 3003 aluminum alloy was used as the honeycomb cell material. The 3M 2216 gray branded two-component, epoxy-based adhesive was used to bond honeycomb cells to skin materials. To enhance the quality of the bonding, skin material surfaces were ground using 180 grid sand paper (P180) and materials were cleaned by acetone to remove grease, dirt and impurities. The 3M 2216 gray can be cured at room temperature. However, to cure the adhesive faster, models were kept at 93°C for 30 minutes. The sandwich composite model is shown in Figure 1.

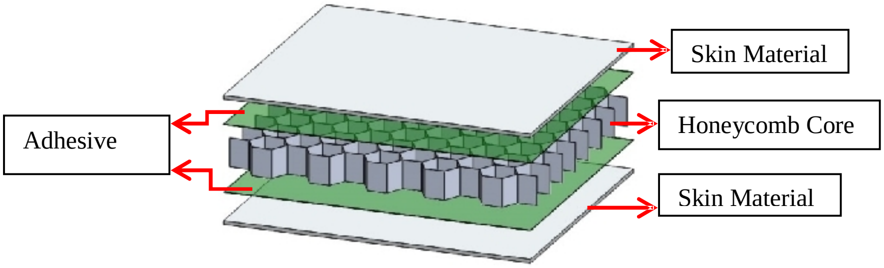

Figure 1. Sandwich Composite Model

The mechanical properties of materials used in the study are shown in Table 1. The basic reason for using honeycomb sandwich composites is that they have high strength values despite their low weights. Low weight values can be achieved by using large cell diameters and small cell heights; however, this results in strength decreases. The parameters used to determine strength decreases with respect to weight decreases are shown in Figure 2. Dimensions of the specimen – W and L values – were set to be 80 and 135 mm respectively. The cell diameter of the honeycomb was 6.35 and 9.525 mm respectively; cell height (T) was 10, 15, and 20 mm respectively; the thickness of skin material was 0.5, 1 and 1.5 mm respectively.

Table 1. Material Properties

Material	Density [kg/m^3]	Young's Modulus [MPa]	Poissons Ratio
AL 5754 Alloy	2670	70300	0.33
Adhesive 3M 2216	1296	565.6	0.47
AL 3003 Alloy	2730	68900	0.33

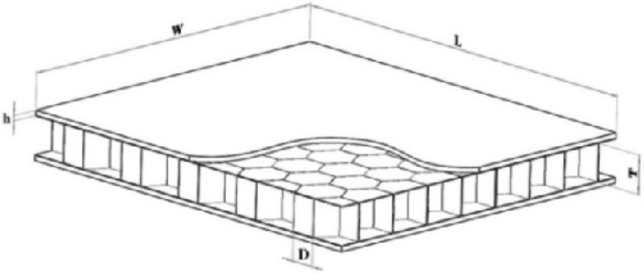

Figure 2. Dimensions of Model

Before conducting the fatigue experiments, static tests were performed to determine the specimen strengths. Three-point bending and buckling loads were used in the tests. Experiments were conducted using Shimadzu Universal test equipment with a load cell capacity of 250 kN.

After determining the static strength values of specimens, they were subject to fatigue experiments. During fatigue experiments, R=Fmin/Fmax was set to 0.1, and the test frequency was 5 Hz. Maximum load values were 90, 85, 80, 75, 70, 60, and 50% of the static damage loads (ultimate strengths) of specimens. Loads were applied to specimens under three-point bending and buckling. The ASTM C 365 and ASTM C 393 standards were used to determine specimen and boundary conditions. The specimens placed in the test equipment to undergo buckling and three-point bending loads are shown in Figure 3.

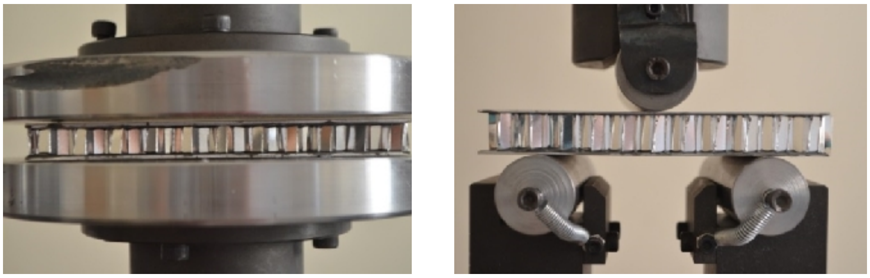

Figure 3. Application of Load: a. Buckling b. Three Point Bending

RESULTS AND DISCUSSION

Static strengths were found before the fatigue experiments. For buckling, maximum fatigue loads were applied as 90, 85, 80, 75, and 70% of the ultimate strength; for three-point bending, maximum fatigue loads were applied as 90, 80, 70 and 50% of the ultimate strength. Fatigue cycle numbers, with respect to buckling loads, are shown in Figure 4.

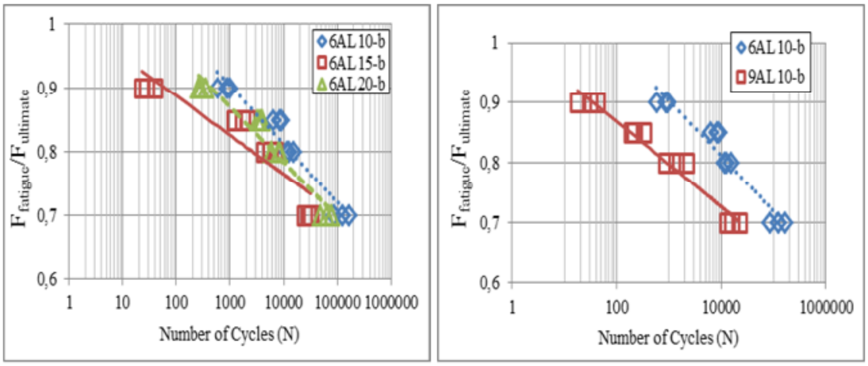

Figure 4. Applied Force-Number of Cycles Graphs for Compression Loading

It was observed that the lowest cell height (10 mm) had the highest fatigue cycle number, while the lowest fatigue cycle number was seen in the specimens with a cell height of 15 mm. As the ratio of applied load to static load decreases, fatigue cycle numbers were closer to each other. Increasing the cell diameter decreased the fatigue strengths.

Fatigue curves for three-point bending loads are shown in Figure 5. Increasing the thickness of the honeycomb core material increased the bending fatigue strengths of the specimens. Decreasing the $F_{fatigue}/F_{ultimate}$ value caused cycle numbers to be closer to each other. Increasing the thickness of the skin material increased the fatigue strengths, as in the case of static loading. When the thickness of the skin material was increased from 0.5 mm to 1 mm, the increase in the cycle number was more pronounced than increase from 1 mm to 1.5 mm. When the effects of cell diameter on fatigue strengths were examined, it was observed that higher cell diameters had lower fatigue cycle numbers for all loads.

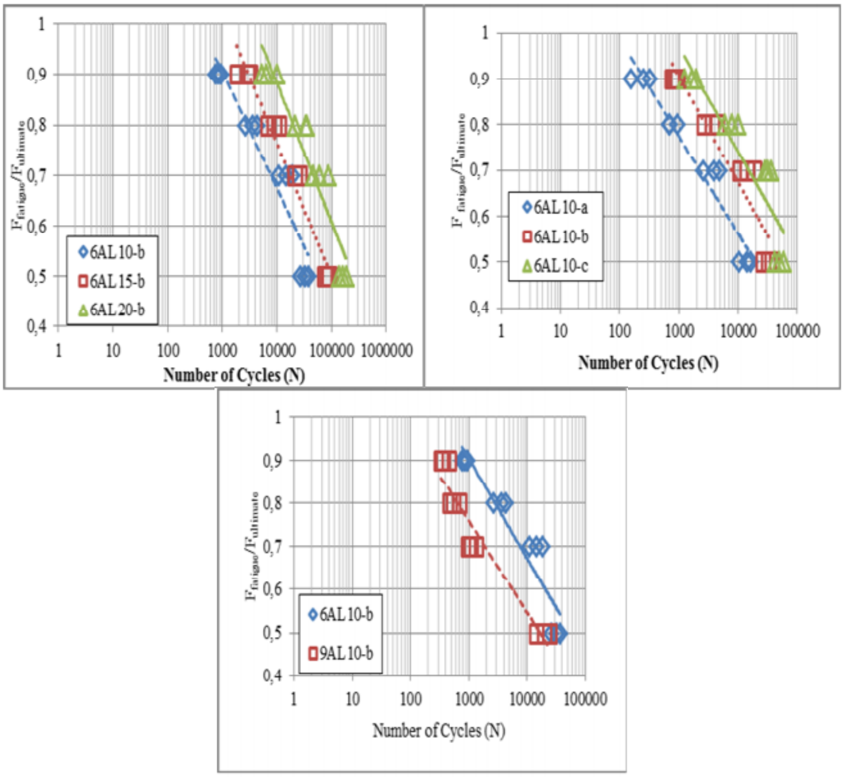

Figure 5. Applied Force-Number of Cycles Graphs for 3 Point Bending Loading

Damaged specimens having different cell thicknesses under three-point bending loads are shown in Figure 6. It was seen that the damage to the specimen having 10 mm cell thickness was a complete bending of the composite structure, as shown at the top of the figure. Partial local buckling damage was observed in the model with 15 mm thickness, while the damage in the model with 20 mm thickness was a complete local buckling (wrinkling).

Figure 6. Damaged samples after three point bending test

CONCLUSIONS

In this study, the fatigue behaviors of honeycomb sandwich composites were experimentally investigated under buckling and three-point bending loads and the results are shown below:

- Increasing cell diameter decreased the strength of specimens under both buckling and three-point bending loads. However, it was observed that it was more pronounced under buckling loadings.
- Increasing the thickness of the skin material increased the fatigue strength of specimens under three-point bending loads. The effect of the thickness of the surface material was not seen under buckling loads, where the load was carried only by the honeycomb core.
- Increasing the thickness of honeycomb cells decreased the fatigue strength of specimens under buckling loads, but increased it under three-point bending loads. The inertia increase occurring in specimens was considered the reason for this phenomenon.
- The greatest difference observed because of increasing cell thickness was in the damage-type and the damage in the specimen with 10 mm cell thickness resulting in a complete bending of the structure. This was partially local in the specimen with 15 mm thickness but only local in the specimen with 20 mm thickness.

Acknowledgement

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REFERENCES

Abrate, S. (1998), *Impact on composite structures*, Cambridge: Cambridge University Press.

Aktay, L., Johnson, A.F., Kröplin, B.H. (2008), “Numerical modelling of honeycomb core crush behaviour”, *Engineering Fracture Mechanics*, 75, 2616-2630.

Allen, G. (1969), *Analysis and Design of Structural Sandwich Panel*, Pergamon Press Oxford, England.

Belouettar, S., Abbadi, A., Azari, Z., Belouettar, R. and Freres, P. (2009), “Experimental investigation of static and fatigue behaviour of composites honeycomb materials using four point bending tests”, *Composite Structures*, 87, 265-237.

Bitzer, T. (1997), *Honeycomb Technology*. Springer-Science+Business Media, B. V. USA.

Giglio, M., Gilioli, A. and Manes, A. (2012), “Numerical investigation of a three point bending test on sandwich panels with aluminum skins and Nomex™ honeycomb core”, *Computational Materials Science*, 56, 69–78.

Miller, W., Smith, C.W. and Evans, K.E. (2011), “Honeycomb cores with enhanced buckling strength”, *Composite Structures*, 93, 1072–1077.

Solmaz, M.Y. and Topkaya, T. (2012), “Elipsoit petek yapılı sandviç yapıların burkulma dirençlerinin sayısal ve deneysel olarak araştırılması”, *3. Ulusal Tasarım İmalat ve Analiz Kongresi Proceedings*, 53-61.

Yamashita, M. and Gotoh, M. (2005), “Impact behaviour of honeycomb structures with various cell specifications numerical simulation and experiment”, International Journal of Impact Engineering, 32, 618–630.

*** Creating a Titan, http://www.flightglobal.com/news/articles/creating-a-titan-199071, Date of Access: 23.08.2015.

THE INFLUENCE OF MICROWAVE IRRADIATION ON THE COLLOIDAL PROPERTIES OF VEGETABLE TANNIN EXTRACT SOLUTIONS

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Vegetable tannin extract has an important use as a tanning agent in leather industry. The colloidal properties of vegetable tannin extract solutions, as a critical factor, greatly affect vegetable tanning process. In this work, we have investigated the particle size and Zeta potential of Commercial Acacia Mangium extract solutions (CAME) and Commercial valonia extract solutions (CVE) under water bath heating and microwave irradiation. The selected conditions were as follows: time, 15 min, 30 min, 60 min and 120 min; temperature, 30°C, 40°C and 50°C. It was found that the particle size of both CAME and CVE under microwave irradiation decreased while Zeta potential increased compared with water bath heating. Particularly, the extent of the decreased particle size and increased Zeta potential of CVE is bigger than CAME. Furthermore, the feature becomes more and more significant with the rise of temperature. In the vegetable tanning process, tannin extracts with small particle size can easily penetrate into the skin and then crosslink well with the collagen of skin. Therefore, as a novel, environmentally friendly and efficient thermal method, microwave irradiation may be used for the well-performance leather, less pollution and cleaner tanning process.

Keywords: dynamic light scattering, microwave irradiation, vegetable tannin extract

INTRODUCTION

Microwave is a kind of electromagnetic wave in the frequency from 300 MHz - 300 GHz. It can inspire molecular rotational level transition, thus directly act on the condensed matter molecules in the reaction system, and then cause dipole steering polarization and interfacial polarization to heat materials (Jin *et al.*, 1999). As a novel, mild, environmentally friendly and efficient thermal technology, microwave is widely used in pharmaceutical chemicals (Meng *et al.*, 2010), food chemicals (Kim *et al.*, 2011), life sciences field (Panagopoulos, 2012) and so on. Furthermore, these applications have already achieved fruitful research results.

Vegetable tannin extracts occupy a very important position in the leather industry, and their solutions and actions are extremely complex whether in physics or chemistry. From the composition point of view, vegetable tannin extracts are a complex mixture of polydisperse colloid contains tannins, non tannins and insolubles. In chemistry, vegetable tannins are classified into two categories: hydrolysable tannin and condensed tannin. Hydrolysable tannin is a compound formed by polyphenol acid and its derivatives, glucose or polyol through the ester bonds, which is easily hydrolyzed to the polyols and phenolic acids. In general, condensed tannin is a kind of oligomers or polymers formed by the condensation of flavanol-3-alcohol which is the structural unit through 4→8 (or 4→6) C—C bond, and it shows a different feature from hydrolysable tannin (Zhang *et al.*, 2012). The structures of hydrolysable tannin and condensed tannin are exhibited in Figure 1. There is a reversible equilibrium between the molecular dispersions and colloidal dispersions, thus this system is often referred to as semicolloid (Chen and Li, 2011). The micelle of vegetable tannin extracts is a structure with double electrical layer, namely, adsorption layer directly connected with colloidal nucleus and diffusion layer outside the adsorption layer where about colloidal nucleus. In the electric field, when tannin molecules move towards the anode, positively charged ions in the adsorption layer move together with colloidal particles, while the positively charged ions in the diffusion layer move out of the colloidal particles. Meanwhile, the formed potential difference between colloidal particles and the dispersion medium is called Zeta potential (Chen *et al.*, 2007). The smaller the colloidal particle size is, the greater the diffusion coefficient is. Then tannin particles diffuse easily with great diffusion coefficient and this is calculated by Brownian motion diffusion coefficient calculation formula as follows by (1) (Zhou, 2002):

$$D = \frac{RT}{6\pi\eta\gamma L} \tag{1}$$

Therefore, in the tanning process, the speed of tannin particles penetration is closely related to the size of tannin particles. Thus, with small tannin particles, tannins more easily penetrate into skin in actual tanning process. The colloid chemical properties of vegetable tannin extracts will undergo some changes under microwave irradiation, hence it will lead to transformation in the tanning process, technology and even tanning mechanism. According to Brownian motion, the particles in the form of colloid can be investigated by using dynamic light scattering and the electrophoresis properties of colloid which dynamically measure Zeta potential and particle size distribution of colloidal particles. These properties for the evaluation of the significance of the penetration ability, filling performance and the degree of combination between vegetable tannin extract solutions and hide fibers are more than the chemical characters of the extract solutions themselves (Thorstensen, 1985). In this work, we have investigated the colloidal properties of vegetable tannin extract solutions by using Zetasizer Nano-ZS series equipment and evaluated the stability of the solutions under microwave irradiation and water bath heating in different time and temperature. Finally, the penetration, filling and bonding performance of the tannin extract solutions were explained by using the variation tendency of particles size distribution and Zeta potential measured in this experiment. Therefore, it may provide the experimental basis for revealing electrochemical change in the vegetable tanning process.

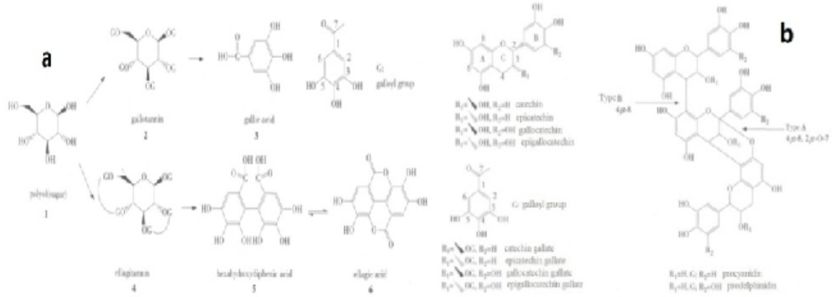

Figure 1. The structure of hydrolysable tannin (a) and condensed tannin (b)

EXPERIMENTAL

Material

Commercial Acacia Mangium extract and Commercial valonia extract were industrial products, commercially purchased from Wu Ming tannin extract factory in Guangxi, China. Microwave irradiation was produced by a Xian Yuhui MCR-3 microwave chemistry reactor. Water bath heating was achieved by a Shanghai

Jihengshiye magnetic stirring water bath pot. Zeta potential and particle size were measured on a Zetasizer Nano-ZS series equipment (Malvern Instruments, UK).

Procedure

Preparation of the original vegetable tannin extract solutions (mass concentration, 4g/L) was achieved according to the literature (Nanjing technological college of forest products, 1980). The gained CAME and CVE solutions were centrifuge at 3000 r/min for 30 minutes. Then, the supernate was collected with clean beaker as a stand-by.

60 mL of CAME and CVE solutions were heated by water bath and microwave irradiation, respectively. The selected conditions were as follows: time, 15 min, 30 min, 60 min and 120 min; temperature, 30°C, 40°C and 50°C. After that, Zeta potential and particle size of the treated tannin extract solutions were measured by Zetasizer Nano-ZS series equipment at the same temperature as mentioned.

RESULTS AND DISCUSSIONS

In leather-making, consideration of oxidation of tannins and denaturation of the collagen caused by a long rotation time under the high temperature, the temperature of vegetable tanning is generally controlled at below 38°C, and 35°C to 38°C is appropriate (Shi and Di, 1998). In order to explore the change law of particle size and Zeta potential of tannin extract solutions in the process of rising temperature, the temperature range for 30-50°C was set up. By the Stokes-Einstein equation, it is known that hydrodynamic diameter of colloidal particles is proportional to the temperature, namely, rise in temperature will increase colloidal particle size. Simultaneously, high temperature can accelerate the Brownian motion of colloidal particles, and more frequent collisions appear among the particles. Thus, there is a substantial reduction in the stability of the solutions (Fu *et al.*, 1990).

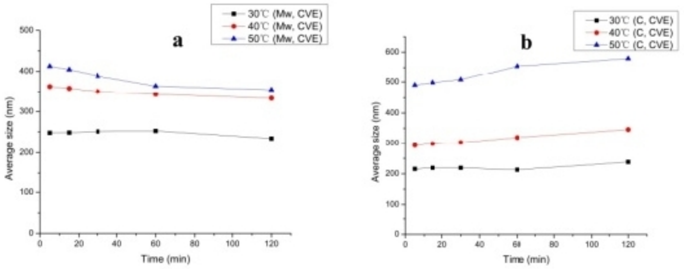

Figure 2. Particle size of CVE under microwave irradiation and water bath heating

The particle size of CVE under microwave irradiation and water bath heating is shown in Figure 2. Under microwave irradiation, the particle size of CVE decreased gradually with irradiation time, moreover, it reduced more significantly when the temperature rose up. As seen in Figure 2b, under water bath heating, the particle size of CVE is smaller within 60 minutes than microwave irradiation. This is because, in the water bath heating process, thermal energy transfer to the external surface of material by convection, conduction and radiation in the existence of the thermal gradient so that the material is heated slowly and unevenly. Nevertheless, in the microwave field, electromagnetic energy directly turns into thermal energy that can generate heat at different depths inside the materials, therefore, the materials are heated more quickly and evenly (Barba *et al.*, 2008). In contrast to Figure 2a and 2b, the particle size of CVE increased with the rising of temperature. But when the treatment is performed for a long time (such as 120 min), compared with water bath heating, the particle size reduced about 70 nm under microwave irradiation. What is more, the reduction was more obvious with the rise of temperature. In a word, the results showed that microwave irradiation may induce the reduction of CVE particle size.

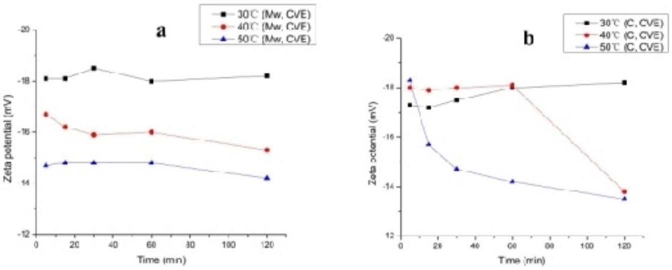

Figure 3. Zeta potential of CVE under microwave irradiation and water bath heating

Zeta potential is an important index value to determine whether the colloidal solution is stable. In general, solution which the absolute value of Zeta potential is more than 30 mV is a stable system, on the contrary, the solution which the absolute value of Zeta potential is within 30 mV is an unstable system (Chen *et al.*, 2007). When Zeta potential value is 0, the solution arrives at the isoelectric point that the solution is most likely to occur micelle aggregation and precipitation phenomenon. So the greater absolute value of Zeta potential indicates more stable vegetable tannin extract solution. Zeta potential of CVE under microwave irradiation and water bath heating is shown in Figure 3. Under microwave irradiation, there was no change in Zeta potential with irradiation time (seen in Figure 3a). However, the absolute value of Zeta potential declined as irradiation temperature increased, and it declined about 3.5 mV from 30°C to 50°C. Nevertheless, under water bath heating, the Zeta potential of CVE showed a falling trend when the heating time increased, and it decreased approximately 4.5 mV while the heating temperature increased from 30°C to 50°C as seen in Figure 3b. Owing to the slowness and unevenness of water bath heating, within 30 minutes, Zeta potential of CVE did not substantially change with the increase of heating temperature, but it showed a regular change under microwave irradiation from the beginning of irradiation. At the same temperature, the absolute value of Zeta potential of CVE irradiated by microwave increased about 1.5 mV compared with water bath heating. From the variation trend point of view, microwave irradiation is better for CVE to maintain a high degree of stability.

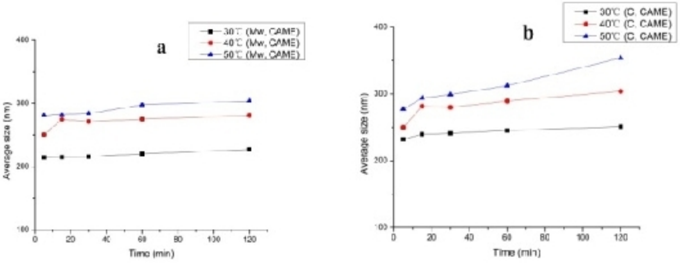

Figure 4. Particle size of CAME under microwave irradiation and water bath heating

The particle size of CAME under microwave irradiation and water bath heating is shown in Figure 4. Under microwave irradiation, the particle size kept essentially unchanged with the irradiation time. However, it increased gradually under water bath heating, and increased more remarkablely when the temperature rose up as shown in Figure 4a. In contrast to Figure 4a and 4b, the particle size increased with the rising temperature, and this is because the hydrodynamic diameter of the colloidal particles is proportional to temperature. Moreover, under microwave irradiation, it increased about 75nm from 30°C to 50°C while increased approximately 95 nm under water bath heating. At the same temperature, the particle size of CAME irradiated by microwave reduced about 30 nm compared with water bath heating. Besides, the higher the temperature was, the particle size reduced more. Therefore, the results showed that microwave irradiation may induce the reduction of CAME particle size.

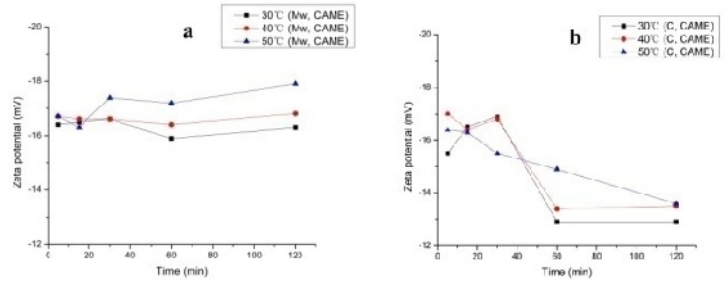

Figure 5. Zeta potential of CAME under microwave irradiation and water bath heating

Zeta potential of CAME under microwave irradiation and water bath heating is shown in Figure 5. Under microwave irradiation, the Zeta potential of CAME remained essentially unchanged with the irradiation time. With the rise of irradiation temperature, the absolute value of Zeta potential increased slightly (about 1.5 mV) from 30°C to 50°C as shown in Figure 5a. The absolute value of Zeta potential of CAME declined 3 mV under water bath heating after 120 minutes treatment (seen in Figure 5b). However, it was obvious that the Zeta potential of CAME remained about the same whatever the different heating temperature was. Under the same temperature, the absolute value of Zeta potential of CAME irradiated by microwave increased about 3.5 mV compared with water bath heating, namely, microwave irradiation might make the properties of CAME more stable.

As seen in Figures 2-5, the particle size of CVE and CAME decreased gradually with the microwave irradiation, and furthermore, it decreased sharply as the temperature rose up. However, the particle size of CVE reduced more under microwave irradiation compared with CAME. As to the Zeta potential, both CVE and CAME kept stable under microwave irradiation. Compared to water bath heating, the absolute value of Zeta potential of both CVE and CAME increased under microwave irradiation, nevertheless, the absolute value of Zeta potential of CVE increased more in comparison to CAME. Since there is some differences in the structures of CVE and CAME (seen in Figure 1), the influence of microwave irradiation on the colloidal properties of CVE is more significant. In microwave electromagnetic field, the polar molecules will produce induced dipole. From the structure point of view, both CVE and CAME are polar molecules with different polarity. Therefore, the effect of microwave irradiation on hydrolysable tannin and condensed tannin is quite different owing to the disparate polarity.

CONCLUSIONS

In this work, we have investigated the influence of microwave irradiation on the colloidal properties of vegetable tannin extract solutions with water bath and microwave heating. And then the colloidal properties were characterized by the particle size and Zeta potential. The results showed as follows: (1) compared with water bath heating, the particle size of CVE (hydrolysable tannin) and CAME (condensed tannin) will decrease as well as the Zeta potential will increase under microwave irradiation. Moreover, the effect is more significant with high irradiation temperature and long irradiation time. (2) Under microwave irradiation, the extent of the decreased particle size and increased Zeta potential of CVE is bigger than CAME. Hence it may be proved that the influence of microwave irradiation on the colloidal properties of hydrolysable tannin is greater than condensed tannin. Notably, as a novel, environmentally friendly and efficient thermal method, microwave irradiation is likely to bring about reduction in the particle size and increase on the absolute value of Zeta potential of tannin extract solutions. Therefore, microwave irradiation may be used for the well-performance leather, less pollution and cleaner tanning process.

Acknowledgements

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REFERENCES

Barba, A.A., Calabretti, A., d’Amore, M., Piccinelli, A.L. and Rastrelli, L. (2008), “Phenolic constituents levels in cv. Agria potato under microwave processing”, LWT-Food Science and Technology, 41(10), 1919-1926.

Chen, J.P., Gong, Y. and Chen, W.Y. (2007), “Dynamic light scattering and electrophoresis studies of stability of tannin extract solutions”, Chemistry and Industry of Forest Products, 27(1), 19-24.

Chen, W.Y. and Li, G.Y. (2011), Tanning Chemistry, China Light Industry Press(the third edition), Beijing.

Fu, X.C., Shen, W.X. and Yao, T.Y. (1990), Physical Chemistry, Higher Education Press, Beijing.

Jin, Q.H., Dai, S.S. and Huang, K.M. (1999), Microwave Chemistry, Science press, Beijing.

Kim, T.H., Choi, J.H., Choi, Y.S., Kim, H.Y., Kim, S.Y., Kim, H.W. and Kim, C.J. (2011), “Physicochemical properties of thawed chicken breast as affected by microwave power levels”, Food Science and Biotechnology, 20(4), 54-64.

Meng, T., Wang, J., Peng, H.L., Fang, G.H., Li, M., Xiong, B., Xie, X., Zhang, Y.L., Wang, X. and Shen, J.K. (2010), “Discovery of benzhydrylpiperazine derivatives as CB1 receptor inverse agonists via privileged structure-based approach”, European Journal of Medicinal Chemistry, 45(3), 1133-1139.

Nanjing technological college of forest products (1980), The Forest Chemical Industry Manual (I), China Forestry Publishing House, Beijing.

Panagopoulos, D.J. (2012), “Effect of microwave exposure on the ovarian development of drosophila melanogaster”, Cell Biochemistry and Biophysics, 63(2), 121-132.

Shi, B. and Di, Y. (1998), “The application of plant polyphenol in leather industry”, Leather Chemistry and Engineering, 8(3), 5-25.

Thorstensen, T.C. (1985), Practical Leather Technology, Krieger Publishing Co, Malabar, Florida.

Zhang, L.L., Wang, Y.M., Xu, M., Wu, D.M. and Chen, J.H. (2012), “Research process in analytical method of vegetable tannins”, Chemistry and Industry of Forest Products, 32(3), 107-116.

Zhou, L. (2002), Physical Chemistry Course, Science press, Beijing.

THE INFLUENCE OF MICROWAVE DRYING ON LEATHER PROPERTIES

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The microwave irradiation and oven were used to dry goat garment leather crust, then the influence of different drying method on leather was compared by testing the mechanical properties, shrinkage temperature, softness, microstructure, dielectric constant and the uniformity of chrome tanning agent and fatliquoring agent. The results indicated that microwave drying would not damage the collagen structure, however the shrinkage temperature, softness and mechanical properties of microwave dried leather were improved, and the uniformity of chrome tanning agent and fatliquoring agent were also promoted. Moreover, the higher dielectric constant of microwave dried leather was observed compared with traditional dried leather together with much more order and dispersive arrangement of collagen weaving. The difference between microwave dried leather and oven dried leather proved not only thermal effect but also non-thermal effect existed during microwave drying process which could promote the interaction and combination between collagen and polar chemical materials. These effects both had positive contribution to leather comprehensive properties.

Keywords: microwave drying, leather comprehensive property, non-thermal effect

INTRODUCTION

Microwave is a fast, time and energy saving heat resources with the remarkable advantage of selective heating ability, so it had been used to dry leather and leather coat (Gilet, 1987; Komanowsky, 1990; Zhang *et al*., 2013). Several models were established to explain the kinetics of leather microwave drying to understand and utilize microwave in the process efficiently and systematically (Monzó-Cabrera *et al*., 2000, Monzó-Cabrera *et al*., 2001; Monzó-Cabrera *et al*., 2004). The difference between chrome tanned leather and vegetable tanned leather with various fat contents under microwave was found to be the primary evidence that microwave had extra effect more than thermal (Bajza, 1997).

Microwave could improve the leather color rub fastness (Gong *et al*., 2011) and fat distribution uniformity without damaging the leather (Gong *et al*., 2012). The chrome tanning process assisted by microwave irradiation could increase the shrinkage temperature of wet blue and improve the tear strength (Wang *et al*., 2011). These researches had proven the microwave had effect besides thermal also existed in leather making process with microwave, it was non-thermal effect.

Microwave was used in leather drying, but the detail influence of microwave on leather properties had not been studied yet except the microwave would not damage the leather. In this paper, the comparison on chemical and mechanical properties between microwave dried leather (MDL) and oven dried leather (ODL) were studied to clarify the thermal and non-thermal effect in microwave drying and the influence on leather properties, and provide reference for using microwave in leather industry further.

EXPERIMENTAL

Materials

The goat wet blue with the thickness of 1.0±0.1mm was prepared in the lab and finished retanning, neutralizing, dyeing and fatliquoring according to conventional garment leather process to get wet crust. The chemicals used for leather manufacturing were commercial grade and used for analyzing were research grade.

Leather Sampling and Drying Method

The wet crust was divided into two pieces along the spine after 12h standing, one was used for microwave drying which was cut as 30×30cm samples from belly and back respectively, the other one was used for oven drying with the same sampling method at the symmetric position.

The weight of each sample before drying was recorded and the water content was estimated about 80% (based on the total weight, and all water content mentioned in the paper was illustrated as the same). A MCR-3S microwave reactor (Xi'an Yuhui instrument Co. Ltd. China) was used for MDL drying with the 100W heating power, the leather sample was heated 2min every 2min to prevent high temperature at the beginning to damage the leather. The ODL was dried in a DHG-9070A drying oven (Shanghai Feiyue instrument Co. Ltd. China) at 45°C. The drying was stopped when the water content reduced to 20%. Then the MDL and ODL samples were placed into a temperature humidity chamber with 25°C and 65% relative humidity for 24h.

Test Methods

Physical Properties and Shrinkage Temperature (Ts) Measurement

Both MDL and ODL samples were conditioned as the standard method before mechanical properties testing. The mechanical properties like tensile and tear strength were tested by tensile machine (AI-7000S), and the softness was tested by measuring apparatus for leather softness (GJ9E1) according to standard. The shrinkage temperature was tested by Shrinkage Temperature Tester (MSW-YD4, China) according to standard.

Chrome Content and Chrome Uniformity Measurement

The samples were split into 3 uniform layers (about 0.3mm) and cut into about 1×1mm fragments, then dried in 102±2°C for 6h to constant weight. Each sample was digested with HNO_3 and H_2O_2 and the total chromium content in digestion solution was determined by Optima 2100DV Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) following the manufacturer's direction and the content of Cr_2O_3 in leather was calculated. Each value was an average of two tests. The uniformity of chrome distribution was calculated as following formula:

$$\text{chrome uniformity}(\%) = \frac{2 \times \text{Cr2O3 in middle layer}}{\text{Cr2O3 in grain layer} + \text{Cr2O3 in fresh layer}} \times 100\% \tag{1}$$

Fat Content and Fat Uniformity Measurement

The constant weighted leather fat content was determined by extraction with dichloromethane. The uniformity of fat distribution was calculated as follows:

$$\text{fat uniformity}(\%) = \frac{2 \times \text{fat in middle layer}}{\text{fat in grain layer} + \text{fat in fresh layer}} \times 100\% \qquad (2)$$

Scanning Electron Microscope (SEM) Measurement

A JSM-5900LV scanning electron microscope (Japan) was used for the analysis. The micrographs for the leather cross sections were obtained by operating the SEM at low vacuum (10^{-4}Pa) with an accelerating voltage of 20kV at 1000 magnification levels.

Dielectric Constant Measurement

The 35mm×35mm samples were cut from MDL and ODL and a DZ5001 dielectric constant meter (China) was used for the test at 1MHz frequency level according to manufacturer's advice.

RESULTS AND DISCUSSION

Influence of Microwave Drying on Leather Mechanical Properties and Softness

Table 1. The mechanical properties and softness of ODL and MDL

Sample	Tensile strength: MPa		Tear strength: N/mm		Softness: mm	
	Back	Belly	Back	Belly	Back	Belly
MDL	25.65	21.50	56.52	38.64	6.69	8.91
ODL	23.10	20.10	55.13	36.51	6.23	8.30

As shown in Table 1, the tensile strength, tear strength and soft of MDL were slightly better than ODL, indicating the microwave had positive effect on leather mechanical properties and softness. Because water has much better absorptive capacity of microwave than leather itself, the drying rate of microwave slows down at the end of drying process with the leather moisture reducing obviously. In addition, there is no temperature gradient during microwave heating, leading the uniform water evaporation at the inner and surface of leather while the surface would be over-dried due to oven drying process is outside-in. The characteristics of microwave drying would not make the collagen fiber adhesion to prevent the stress concentration. These effects improved leather mechanical properties and softness.

Influence of Microwave Drying on Leather Shrinkage Temperature

Table 2. The shrinkage temperature of ODL and MDL

Sample	Back Ts: °C	Belly Ts: °C
MDL	116.0	114.4
ODL	114.1	113.5

Table 2 demonstrated the Ts of MDL back and belly were 1.9°C and 0.9°C higher than ODL respectively. The higher shrinkage temperature means better hydrothermal stability and better crosslink between collagen and tanning agents. It could infer that microwave improved the crosslink degree of leather as a result of the polar chromium complex molecules and active amino acid residues in collagen were affected by the high frequency conversion microwave electromagnetic field, then the extra collision, turn and oscillate between molecules except for temperature happened to accelerate the reaction rate and degree.

Influence of Microwave Drying on Chrome and Fat Migration

Table 3. Chrome uniformity and fat uniformity of ODL and MDL

Sample	Chrome uniformity: %		Fat uniformity: %	
	Back	Belly	Back	Belly
MDL	80.60	85.18	63.04	82.32
ODL	56.32	82.31	39.75	64.61

The chrome uniformity of MDL samples were much more even than ODLs, especially the back was 24.28% higher than the control (showed in Table 3). High chrome uniformity represents low difference of chrome content between inner and surface of leather. The better chrome uniformity of ODL was caused by the no temperature gradient and fast heating of microwave which could mostly avoid the migration of free and weak combined chromium with water evaporation to the surface occurred in the oven drying. Moreover, the crosslink between collagen and chrome promoted by microwave would reduce the content of free and weak combined chromium and decrease the potential of its migration.

Table 3 also indicated the microwave drying could get better fat uniformity leather. Prior study had verified that microwave could decrease the viscosity and particle size of fatliquoring agent to increase the permeability, leading fat was well dispersed during microwave drying. As fatliquoring agent uniform existed in leather, it could lubricate fibers sufficiently to get a better softness and improve the mechanical properties of leather.

Influence of Microwave Drying on Leather Microstructure

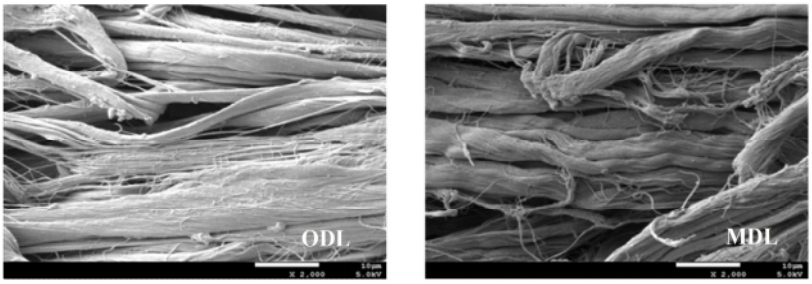

Figure 1. The SEM images of MDL and ODL back (A: MDL, B: ODL)

According to Figure 1, there was no significant difference between ODL and MDL in collagen weaving condition, but collagen fiber of MDL with a slight improvement in dispersity and orderliness. Collagen has diamagnetic anisotropy which trends to form a parallel arrangement which is perpendicular to magnetic field. The polar groups in collagen were affected by microwave electromagnetic field during drying process, then the groups with same charge had tendency to form parallel arrangement, leading repulsion force generation. So the tight section of leather such like butt and back had better dispersion and orderliness after microwave drying than traditional.

Influence of Microwave Drying on Leather Dielectric Constant

Table 4. The dielectric constant of MDL and ODL

Sample	Back	Belly
ODL	1.82	1.55
MDL	2.06	1.69

Table 4 presented the dielectric constant of leather at different sections. The MDL dielectric constant was higher than DOL, which was a direct proof of the non-thermal effect existing in microwave drying process because of the leather polarity increasing during the process. The induced dipole moment of polar molecule like collagen, tanning agent and fatliquoring agent produced with microwave electromagnetic field, making leather polarity increase to enlarge the dielectric constant.

CONCLUSIONS

The microwave drying would not damage the collagen structure; however, the leathers with higher shrinkage temperature together with better mechanical properties and softness were obtained comparing with oven drying. Moreover, the higher dielectric constant of microwave dried leather was observed compared with traditional dried leather together with much more order and dispersive arrangement of collagen weaving, The difference between microwave dried leather and oven dried leather proved both uniform thermal effect and non-thermal effect existing during microwave drying process; they have both positive contributions to leather comprehensive properties.

Acknowledgements

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REFERENCES

Bajza, Z. (1997), “The influence of fatliquor concentration on microwave drying kinetics”, *Journal of the Society of Leather Technologists and Chemists*, 81, 227-230.

Gilet, J. (1987), “Drying without heat”, *Journal of the American Leather Chemists Association*, 82, 61-64.

Gong, Y., Cheng, K., Zhang, T. and Chen W.Y. (2011), “Automated Clean Leather Dyeing Assisted by Wringing, Ultrasound and Microwave”, *Journal of the American Leather Chemists Association*, 106, 127-132.

Gong, Y., Zhang, T. and Chen W.Y. (2012), “Behaviour of fatliquored leather in a microwave field”. *Journal of the American Leather Chemists Association* , 107, 60-67.

Komanowsky, M. (1990), “Drying of leather with microwaves”, *Journal of the American Leather Chemists*

Association, 85, 131-141.

Monzó-Cabrera, J., Catalá-Civera, J. M., Plaza-González, P. and Sánchez-Hernández, D. (2004), “A model for microwave-assisted drying of leather: Development and validation”, *Journal of Microwave Power and Electromagnetic Energy*, 39, 53-64.

Monzó-Cabrera, J., Díaz-Morcillo, A., Catalá-Civera, J.M. and Delos-Reyes, E. (2000), “Heat flux and heat generation characterization in a wet-laminar body in microwave-assisted drying: An application to microwave drying of leather”, *International Communications in Heat and Mass Transfer*, 27, 1101-1110.

Monzó-Cabrera, J., Díaz-Morcillo, A., Catalá-Civera, J.M. and Delos-Reyes, E. (2001), “Effect of dielectric properties on moisture leveling in microwave-assisted drying of laminar materials”, *Microwave and Optical Technology Letters*, 30, 165-168.

Wang, H., Chen, W.Y., Gong, Y. and Sun, H.B. (2011), “Based on the studies of chrome tanning chemistry with microwave”, *Leather Science and Engineering*, 21, 10-14.

Zhang, C.L., Teng, B. and Chen, W.Y. (2013), “Effect of microwave drying on properties of leather coating”, *Leather Science and Engineering*, 23, 12-14.

II.
BIOMATERIALS

ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OILS ON COTTON FABRICS

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Pathogenic microorganisms, bacteria and fungi such as *Trichophyton interdigitale* may easily develop inside footwear (cotton linings), causing “foot fungus” or “athlete's foot”, a fungal infection that affects the skin, especially in the interdigital space and nails. Previous studies have shown that some essential oils have antibacterial and antifungal effect. The aim of the present study was to test antifungal activity of five essential oils (origanum, clove, orange, lavender and cinnamon) applied onto the cotton fabric used for shoe lining, with the purpose of preventing mycoses caused by *Trichophyton interdigitale*. The antifungal activities of the tested essential oils against *Trichophyton interdigitale* revealed that origanum and cinnamon oils, as well as the combination of origanum-clove-orange and clove-lavender-cinnamon completely inhibited the growth of *Trichophyton interdigitale* on cotton fabrics, with potential application in footwear production and/or exploitation.

Keywords: essential oil, cotton fabrics, *Trichphyton interdigitale*

INTRODUCTION

The warm and humid environment inside the footwear facilitates the development of various harmful microorganisms that may degrade the material; therefore it is necessary to develop new products with low toxicity and environmentally friendly treatment of cotton used as shoe linings.

Pathogenic fungi that grow on human skin are *Trichophyton interdigitale, Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum gypseum.* These fungi cause *tinea pedis*, condition also known as “foot fungus” or “athlete's foot”. These fungi are located between the toes, on the soles or nails, causing onychomycosis. Foot fungus is highly contagious and can be spread easily by previously worn shoes of a person affected because the spores remain on the material it is lined with. Through the same mechanism “foot fungus” can relapse after being removed by treatment. A shoe lined with a material with both antifungal properties could prevent the spread of fungal infections, and reinfection of the wearer.

To date, treatments for disinfection of footwear based on ultraviolet (Ghannoum *et al*., 2012) and ozone (Gupta *et al.,* 2013) have been successfully tested, but to prevent colonization bacteria and fungi lining, the application of substances is required which at the same time have a low toxicity for both the wearer and the environment. Other studies developed for incorporating antimicrobial technologies into leather compounds matrix to control and eradicate microorganisms associated with the surface of leather (Deselnicu *et al*., 2005). Cotton fabrics exposed to oxidizing plasma and subsequently treated with silver nanoparticles and titanium dioxide showed antifungal effect (Surdu *et al*., 2014; Chirila *et al*., 2014).

Synthetic fungicides presently used are potentially harmful to both human health and for the environment and therefore, production and marketing is regulated and monitored continuously by European directives and regulations of the European Parliament and Council (Deselnicu, D.C. *et al*., 2014; Deselnicu, V. *et al.,* 2014). There is no EU legislation in regulating specifically the content of chemicals in footwear and other leather products, but the use of chemicals is restricted by REACH (EC1907/2006).

Essential oils extracted from plants may be an alternative to conventional fungicides. Antimicrobial activities of essential oils extracted from plants have been reported by: Nzeako *et al*., 2006, showing antimicrobial activities of clove and thyme extracts; Chee and Lee (2007) showed antifungal activity of clove essential oil and its volatile vapour against Dermatophytic Fungi; Radwan *et al*. (2014) showed in a study regarding antifungal activity of thyme, clove and cinnamon essential oils on *Candida albicans* and moulds isolated from different sources that thyme oil completely inhibited the growth of different fungal isolates at concentrations of 0.25, 0.5 and 1%; Surdu *et al*. (2014) studied improvement of the resistance to *Candida albicans* and *Trichophyton interdigitale* of some woven fabrics based on cotton.

The aim of this work is to test antifungal activity against *Trichophyton interdigitale* of five essential oils - cinnamon, lavender, origanum, orange and clove - on cotton fabrics used as lining in footwear production.

MATERIALS AND METHODS

Materials

Cotton fabrics used as footwear lining; samples of 1.5/1.5 cm were used.

Essential oil isolated from cinnamon *(Cinnamomum verum)*.

Essential oil isolated from lavender *(Lavandula angustifolia)*.

Essential oil isolated from origanum *(Origanum vulgare)*.

Essential oil isolated from orange *(Citrus sinensis)*.

Essential oil isolated from clove *(Eugenia caryophyllata)*.

The essential oils were obtained by hydro distillation in a continuous extractor type Clevenger (Berechet, 2015).

Biologic Material: *Trichophyton interdigitale*

Methods

Chemical composition of essential oils was determined by gas chromatography-mass spectrometry (GC-MS) with Agilent 6890 N.

Antifungal activity against *Trichophyton interdigitale* was performed following Standard SR EN ISO 20645:2004 - Textile fabrics – Determination of antibacterial activity - Agar diffusion plate test.

Two series of experiments were made: a) with each essential oil, and b) with a combination of 3 essential oils (with a rate of 1:1:1).

Applying essential oils on cotton fabric samples was made by dropping 0.2 ml oil on the surface of 1.5 x 1.5 cm^2.

In each Petri dish Dextrose Agar Sabouraud culture medium was poured. Both cotton samples treated with essential oils alone, with a combination of essential oils and untreated were placed in each Petri dish in the center of the surface of the culture medium, then the culture medium was seeded in 3 points around the sample, as an equilateral triangle. There have been two parallel samples. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analized after 3, 7, 14, 21 and 28 days.

Optical microscopy images were captured using a Leica stereomicroscope S8AP0 model with optic fiber cold light source, L2, with three levels of intensity, and magnification 40X.

RESULTS AND DISCUSSION

Composition of Essential Oils

Essential oil of clove (Eugenia caryophyllata). The essential oil has 13 components and the major one is eugenol (96.999%).

Essential oil of cinnamon (Cinnamomum verum). The essential oil has 30 components and the major one is cinnamaldehyde (84.125%).

Essential oil of lavander (Lavandula angustifolia). Analysis allowed the identification of 32 components and the majors ones are geraniol (26.349%), camphor (23.193%) and eucalyptol (20.821%).

Essential oil of origanum (Origanum vulgare). Analysis allowed the identification of 30 components and the majors ones are thymol (64.413%) and carvacrol (27.624%).

Essential oil of orange (Citrus sinensis). The essential oil has 13 components and the major one is limonene (97.825%).

Biological Activity

Tests of Each Essential Oil

Table 1 presents the images of antifungal activity of essential oils against *Trichophyton interdigitale* after 3, 7, 14, 21 and 28 days.

After 3 days a zone of inhibition of 2.5 cm appeared around samples treated with essential oil of clove. After 7 days colonies of *Trichophyton interdigitale* have appeared, keeping the inhibition zone of 2.5 cm. No inhibition zone diameter has changed by the end of the experiment.

The essential oil of lavender has not inhibited the growth, while orange essential oil has favored the growth of *Trichophyton* colonies.

Essential oils of origanum and cinnamon completely inhibited the growth of *Trichophyton* by the end of the experiment.

On the control sample, *Trichophyton interdigital* grew all over the culture medium after 3 days, after which no changes have occurred until the end of the experiment.

Tests of Combinations of Three Essential Oils

Four combinations of tree essential oils in the rate of 1:1:1 were tested, as follows:

- b1) origanum-clove-cinnamon;
- b2) origanum-clove-orange;
- b3) clove-lavender-cinnamon;
- b4) clove-lavender-orange.

Table 2 presents the images of antifungal activity of the fourth combination (b1-b4) of the essential oils against *Trichophyton interdigitale* after 3, 7, 14, 21 and 28 days.

The combination of esential oils b1) origanum-clove-cinnamon partially inhibited the growth of dermatophytes, with an inhibition zone of 1.5 cm.

The combination of esential oils b2) origanum-clove-orange and b3) clove-lavender-cinnamon completely inhibited the growth of dermatophytes

The combination of esential oils b4) clove-lavender-orange has a strong inhibiting effect on the growth of *Trichophyton* with an inhibition zone of 1 cm.

Combinations of essential oils b2 and b3 were the most effective in controlling *Trichophyton interdigitale,* and can be used to treat cotton lining or other footwear components to prevent transmission of mycoses.

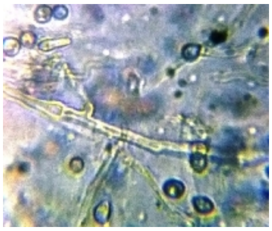


Figure 1. *Trichophyton interdigitale* (image 40x with Leica stereomicroscope S8AP0)

Table 1. Antifungal activity of essential oils against *Trichophyton interdigitale* on cotton fabrics

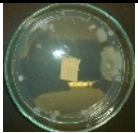
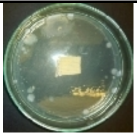
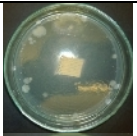
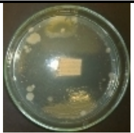
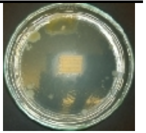
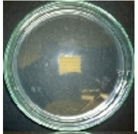
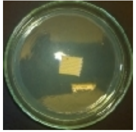
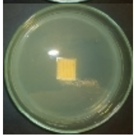
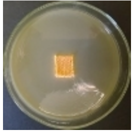
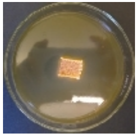
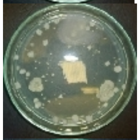
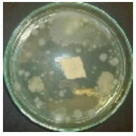
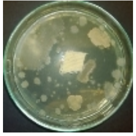
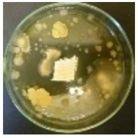
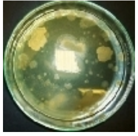
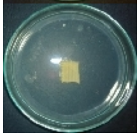
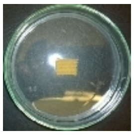
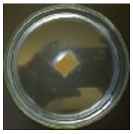
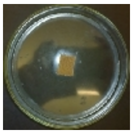
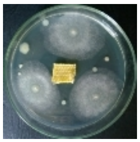
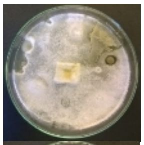
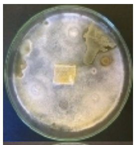
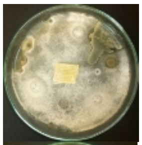
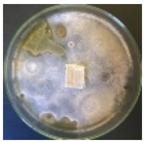
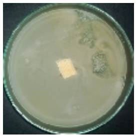
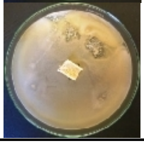
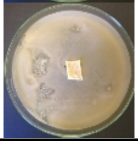
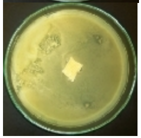
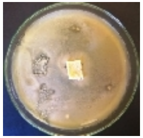
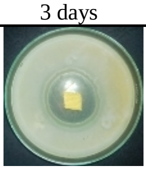
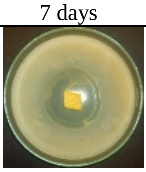
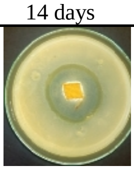
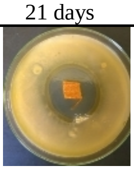
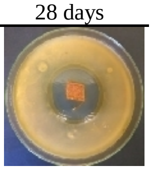
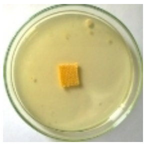
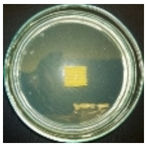
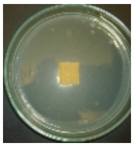
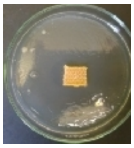
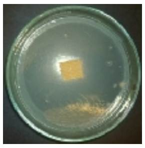
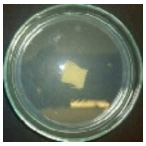
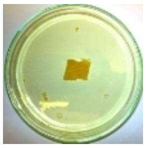
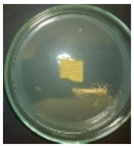
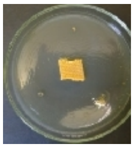
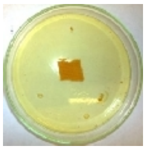
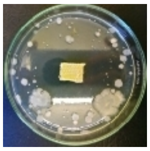
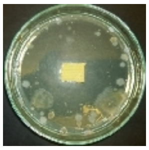
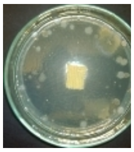
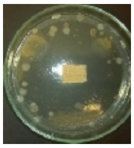
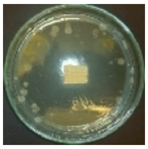
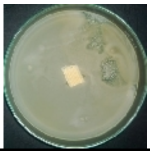
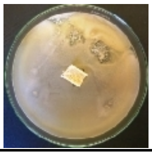
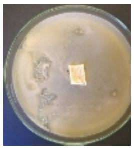
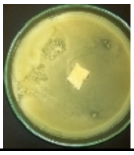
	3 days	7 days	14 days	21 days	28 days
Essential oil of clove					
Essential oil of cinnamon					
Essential oil of lavender					
Essential oil of origanum					
Essential oil of orange					
Control					

Table 2. Antifungal activity of some combination of essential oils against *Trichophyton interdigitale* on cotton fabrics

	3 days	7 days	14 days	21 days	28 days
b1) combination Essential oil of origanum, clove and cinnamon					
b2) combination Essential oil of origanum, clove and orange					
b3) combination Essential oil of clove, lavender and cinnamon					
b4) combination Essential oil of clove, lavender and orange					
Control					

CONCLUSIONS

According to this study, among the tested essential oils, origanum and cinnamon have the highest long-term antifungal activity as they inhibited the growth of *Trichophyton interdigitale* completely. Antifungal activity of essential oils is due to active components: thymol and carvacrol and cinnamaldehyde.

Certain combinations of the oils led to a synergistic effect, which is interesting in view of potentiating their inhibition of *Trichophyton interdigitale* colonization and infectiousness.

The results of this study may have potential for use in the development of applications in footwear component sanitation.

Acknowledgements

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REFERENCES

Berechet, M.D. (2015), “Essential oils – Their Use in Practice and Research”, Doctoral Thesis.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), “Comparative study regarding resistence of wet-white and wet-blue leather to the growth of fungi”, *Leather and Footwear Journal*, 14(2), 107-120.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), “Study regarding the resistance to the growth of fungi of wet-white leather tanned with Titanium – Aluminum”, *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, RO, 31-36.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), “Study regarding the resistance of wet-white leather organic tanned to the growth of fungi”, *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, 37-42.

Deselnicu, D.C. (2014), *European Policies for Products and their Relevance for the Footwear Sector* (RO), ISBN: 978-973-720-555-1 (245pg), Agir Press.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), “Sustainable consumption and production in the footwear sector”, *Leather and Footwear Journal*, 14(3), 159-180.

Deselnicu, V., Deselnicu, D.C., Vasilescu, A.M. and Militaru, G. (2014), “EU Policy for Sustainable Consumption and Production – EU Ecolebal for footwear”, *Proceedings of the 5th International Conference on Advanced Materials and Systems ICAMS 2014*, 23-25 October 2014, Bucharest, 641-646.

Deselnicu, V., Maier, S.S., Deselnicu, O.C. and Florescu, M. (2005), “Impact of technological changes on increased health and comfort efficiency”, *Proceedings of The 4th International Conference in Management of Technological Change*, Book 1, Chania, Greece, 87-92.

Ghannoum, M.A., Isham, N. and Long, L. (2012), “Optimization of an infected shoe model for the evaluation of an ultraviolet shoe sanitizer device”, *Journal of the American Podiatric Medical Association*, 102(4), 309-13.

Gupta, A.K. and Brintnell, W.C. (2013), “Sanitization of contaminated footwear from onychomycosis patients using ozone gas: a novel adjunct therapy for treating onychomycosis and tinea pedis?”, *Journal of Cutaneous Medicine and Surgery*, 17(4), 243-9.

Chee, H.Y. and Lee, M.H. (2007), “Antifungal Activity of Clove Essential Oil and its Volatile Vapour Against Dermatophytic Fungi”, *Mycobiology*, 35(4), 241–243.

Inouye, S., Uchida, K. and Yamaguchi, H. (2001), “In-vitro and in-vivo anti-Trichophyton activity of essential oils by vapour contact”, *Mycoses*, 44(3-4), 99-107.

Nzeako, B.C., Al-Kharousi, Z.S.N. and Al-Mahrooqui, Z. (2006), “Antimicrobial Activities of Clove and Thyme Extracts”, *Sultan Qaboos University Medical Journal*, 6(1), 33–39.

Radwan, I.A., Abed, A.H., Abeer, M.R., Ibrahim, R.A. and Abdallah, A.S. (2014), “Effect of thyme, clove and cinnamon essential oils on Candida albicans and moulds isolated from different sources”, *American Journal of Animal and Veterinary Sciences*, 9(4), 303-314.

Surdu, L., Stelescu, M.D., Manaila, E., Nicula, G., Iordache, O., Dinca, L.C., Berechet, M.D., Vamesu, M., Gurau, D. (2014), “The improvement of the resistance to Candida albicans and Trichophyton interdigitale of some woven fabrics based on cotton”, *Bioinorganic Chemistry and Applications*, Article ID 763269, http://dx.doi.org/10.1155/2014/763269.

ANTIFUNGAL ACTIVITY OF THYME ESSENTIAL OIL ON WOOLEN SHEEPSKINS

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This work covers the chemical composition of thyme essential oil analyzed using GC-MS and its antifungal activity on woolen sheepskins. The main components of thyme oil were determined: carvacrol (57.355%), Tymol (32.430%) and o-Cymol (3.993%). The woolen sheepskins treated with thyme essential oil in a rate of 30g/1000g tanned furs were assayed for antifungal activity against *Candida albicans* and *Aspergillus niger.* These pathogens are very contagious and results in severe damage of leather/skins/furs. The woolen sheepskins treated with thyme essential oil were investigated by ASTM D4576–86 (1996) method against *Aspergillus niger* and by Standard SR EN ISO 20645:2004 against *Candida albicans*. The essential oil of *Thymus vulgaris* displayed a moderate antifungal activity against *Candida albicans* and *Aspergillus niger* on woolen sheepskins.

Keywords: woolen sheepskin, thyme essential oils, antifungal activity, *Candida albicans, Aspergillus niger*

INTRODUCTION

Both in woolen sheepskins production and during use and storage, finished fur products may be damaged due to external factors and insects. These effects can be controlled or corrected by finishing and maintenance treatment of fur and fur products.

For protection against insects and fungi, insecticide and fungicide have to be applied very early. Several chemicals, so called fungicides and insecticides, have been used to prevent and kill fungi and insects in various environments. Although several synthetic preservatives have been used with good results, the disadvantages of using them are their potential negative effects on the environment, for instance toxicity, accumulation in soil and water and build-up of resistance in fungal and insect populations, limited their applications. In relation with that, the availability and legal acceptance of suitable preservatives differ from country to country (Deselnicu, D.C., 2014; Deselnicu, V. *et al*., 2014).

On the other hand, various different natural substances from plant extracts have been investigated in terms of fungal activities. The plants can act as the potent sources of biologically active substances which can be applied as preservative to protect woolen sheepskins and its products from biological deterioration (Niculescu *et al*., 2015).

Essential oils are liquid substances, with oily appearance, insoluble in water, soluble in alcohol and organic solvents, with the characteristic smell of volatile substances they contain. In terms of chemistry, volatile oils are complex mixtures of aliphatic and aromatic hydrocarbons, aldehydes, alcohols, esters and other constituents. Aromatic essences are extracted using three procedures: cold pressing, solvent extraction and water vapour distillation.

Volatile oils can be extracted from various parts of the plant, from flowers, seeds, leaves, stems, peels, roots, rhizomes, tubers, flower buds etc. Essential oils are very concentrated in active chemical elements and have various properties: they are antiseptic, antibacterial, immuno-stimulant, etc. (Constantinescu *et al.*, 2004; Stanescu *et al.*, 2004).

Due to the main disadvantages of the current preservative agents, the public demand has grown for more environmental friendly methods (Deselnicu *et al*., 2005; Deselnicu, V., *et al*., 2014). Therefore, it is important that more effective and less toxic naturally-occurring preservative agents with novel mechanisms of action be discovered and developed.

Many papers reported antifungal activity of Thyme, e.g.: effect of thyme on *Candida albicans* and moulds isolated from different sources (Radwan *et al*., 2014; Nzeako *et al*., 2006; Janssen *et al*., 1987); the use of essential oils for inhibition of fungi growth (Stević *et al*., 2014).

The aim of this work is to test antifungal activity of essential oils extracted from thyme (*Thymus vulgaris*) against *Candida albicans* and *Aspergillus niger* on woolen sheepskins and possibility for their application in the woolen sheep skins preservation. The research activity will be directed to discover a new, effective and environmentally-acceptable plant-derived preservative and the suitable preservative treatment method.

MATERIALS AND METHODS

Materials

Essential oil isolated from thyme *(Thymus vulgaris)* was obtained by hydro distillation in a continuous extractor type Clevenger (Berechet, 2015).

Woolen Sheep skins treated during fatliquoring operation with 30g thyme oil /1000g fur tanned weight;

Biologic material: *Candida albicans 10231* and *Aspergillus niger ATCC 6275.*

Methods

Chemical composition of essential oils was determined by Gas Chromatography-Mass Spectrometry (GC-MS) with AGILENT 6890 N.

Antifungal activity against *Candida albicans:* The tests were performed in accord with Standard SR EN ISO 20645:2004 - Textile fabrics – Determination of antibacterial activity - Agar diffusion plate test. In each Petri dish was poured Dextrose Agar Sabouraud culture medium. Both samples treated with thyme essential oil and control samples were placed in each Petri dish in the center of the surface of the culture medium, and then the culture medium was seeded in 3 points around the sample, as a equilateral triangle. There have been two parallel samples. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14 and 21 days.

Antifungal activity against *Aspergillus niger:* The tests were made following standard ASTM D4576–86 (1996) - Standard Test Method for Mold Growth Resistance of Wet Blue. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14, 21 and 28 days.

Optical microscopy images were captured using a Leica stereomicroscope S8AP0 model with optic fiber cold light source, L2, with three levels of intensity, and magnification 40X.

RESULTS AND DISCUSSION

Chemical Composition of Essential Oil

Thyme essential oil was analysed by GC and GC/MS (Figure 1). Analysis allowed the identification of 16 components presented in Table 1 of which, carvacrol (57.355%), Tymol (32.430%) and o-Cymol (3.993%) were detected as major constituents (see Figure 2).

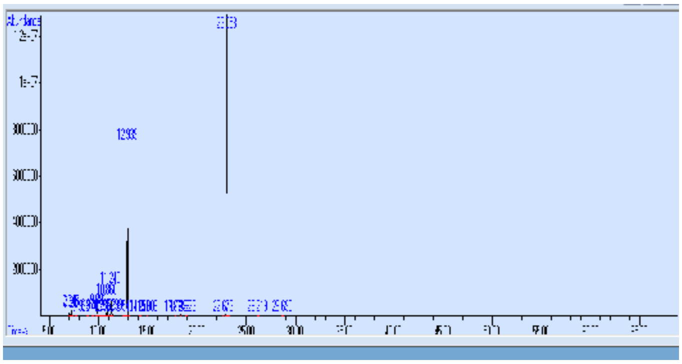
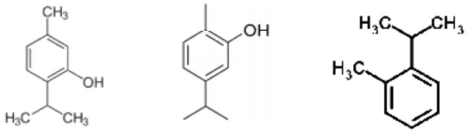


Figure 1. Thyme essential oil chromatogram

Table 1. Chemical compounds identified in *Thymus vulgaris* essential oil by GC-MS

No	Retention time, min	Compound	Formula	Area, %	Kovats Indices, KI
1	7,045	α-Phellandrene	$C_{10}H_{16}$	0,660	650,501
2	7,241	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	$C_{10}H_{16}$	0,596	655,987
3	9,449	cis-2-Ethyl-2-hexen-1-ol	$C_8H_{16}O$	0,058	709,19
4	9,882	β-Pinene	$C_{10}H_{16}$	1,008	718,147
5	10,533	3-Carene	$C_{10}H_{16}$	0,044	$1{,}039\text{x}10^3$
6	10,860	Terpinolen	$C_{10}H_{16}$	2,319	$1{,}048\text{x}10^3$
7	11,240	o-Cymol	$C_{10}H_{14}$	3,993	$1{,}057\text{x}10^3$
8	11,400	Limonene	$C_{10}H_{16}$	0,318	$1{,}061\text{x}10^3$
9	12,542	2,7-Bis(spirocyclopropane) bicyclo[2.2.1]heptan-5-one	$C_{11}H_{14}O$	0,060	$1{,}086\text{x}10^3$
10	12,940	γ-Terpinen-	$C_{10}H_{16}$	32,430	$1{,}095\text{x}10^3$
11	17,774	3,4-Dimethyl-1H-pyrrole-2-carboxylic acid	$C_7H_9NO_2$	0,042	$1{,}18\text{x}10^3$
12	18,292	4-Terpineol	$C_{10}H_{18}O$	0,213	$1{,}578\text{x}10^3$
13	18,925	1,2,3,5,6,7-Hexahydro-inden-4-one	$C_9H_{12}O$	0,050	$1{,}591\text{x}10^3$
14	23,060	Carvacrol (Antioxine)	$C_{10}H_{14}O$	57,355	$2{,}291\text{x}10^3$
15	26,220	Neoclovene	$C_{15}H_{24}$	0,127	$2{,}595\text{x}10^3$
16	28,690	Seychellene	$C_{15}H_{24}$	0,156	$2{,}855\text{x}10^3$



Tymol Carvacrol o-Cymol

Figure 2. Main components of thyme essential oil

Thymol is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol; Carvacrol, or cymophenol, $C_6H_3CH_3(OH)(C_3H_7)$, is a monoterpenoid phenol; o-cymol is *o*-Isopropyltoluol.

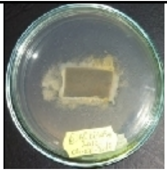
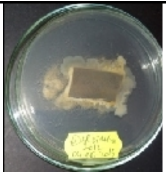
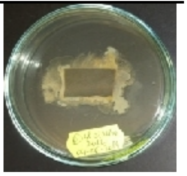
Antifungal Activity

The thyme essential oil was screened for its *in vitro* antifungal activity against *Candida albicans* 10231 and *Aspergillus niger* ATCC 6275.

Antifungal Activity against Candida albicans

During microbiological tests in the presence of *Candida albicans* it was observed that the first growth occurred only after 14 days in the control samples. *Candida albicans* formed pseudo-micelles (Table 2 and Figure 3). Samples treated with thyme essential oil completely inhibited the growth of *Candida albicans.*

Table 2. Antifungal activity of thyme essential oil against *Candida albicans*

	3 days	7 days	14 days	21 days
Thyme essential oil				
Control				

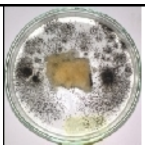
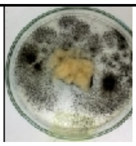
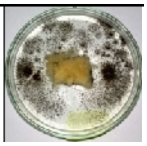
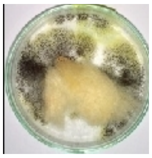
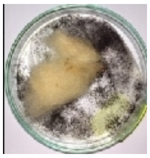
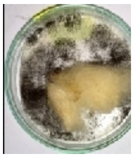
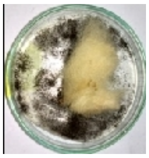
In conclusion, thyme essential oil may inhibit the growth of *Candida albicans* colonies for 14 days.

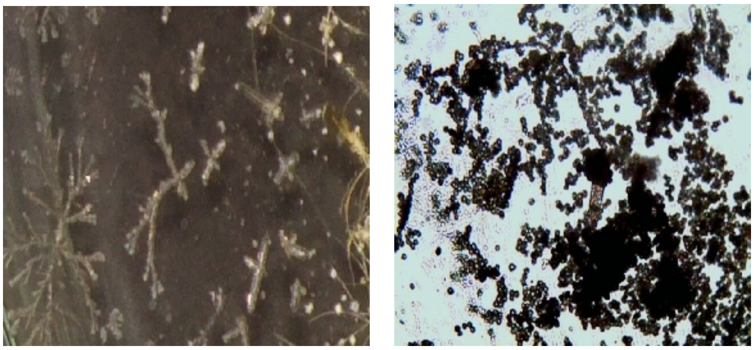
Antifungal Activity against Aspergillus niger

After 3 days, growth of *Aspergillus niger* occurred in Petri plates, but not on fur samples treated with thyme oil; there is an area of 5 mm inhibition around, which was kept till the end of the experiment (Table 3 and Figure 3b).

In conclusion, thyme oil has a mild inhibitory effect against *Aspergillus niger* on fur skins treated.

Table 3. Antifungal activity of thyme essential oil against *Aspergillus niger*

	3 days	7 days	14 days	21 days	28 days
Thyme essential oil					
Grade	0	0	0	0	0
Control					
Grade	2	3	4	5	5



(a) (b)

Figure 3. *Candida albicans* filaments after 21 days (a), and *Aspergillus niger* colonies (b)

CONCLUSIONS

Thyme essential oil has high contents of cavacrol, thymol which displayed antifungal activity against yeasts and molds. The antifungal activity of thyme may be interpreted by reduction of ergo-sterol content: the major sterol content in fungal cell membrane (Pinto *et al.*, 2006).

In the field experiment, the active compound from thyme essential oil displayed potent activity against woolen sheepskins degrading fungi. The research results open the possibility to apply extract of *Thymus vulgaris* and their active compounds as natural preservatives. The extract and active compounds offer a safer application for human and animals and are more environmentally friendly than chemical synthetic preservatives because they are easily degradable in nature during disposal.

Acknowledgements

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REFERENCES

Bayramoğlu, E.E., Gülümser, G. and Karaboz, I. (2008), "The Investigation of Antibacterial Activities of Some Essential Oils in Wet Blue Leather", *International Journal of Natural and Engineering Sciences*, 2 (1), 33-36.

Berechet, M.D. (2015), "Essential oils – Their Use in Practice and Research", Doctoral Thesis.

Constantinescu, D.G., Hatieganu, E. and Busuricu, F. (2004), *Medicinal Herbs Used in Therapeutics*, Medical Press, Bucharest.

Deselnicu, D.C. (2014), *European Policies for Products and their Relevance for the Footwear Sector* (RO), ISBN: 978-973-720-555-1 (245pg), Agir Press.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), "Sustainable consumption and production in the footwear sector", *Leather and Footwear Journal*, 14(3), 159-180.

Deselnicu, V., Deselnicu, D.C., Vasilescu, A.M. and Militaru, G. (2014), "EU Policy for Sustainable Consumption and Production – EU Ecolebal for footwear", *Proceedings of the 5th International Conference on Advanced Materials and Systems* ICAMS 2014, 23-25 October 2014, Bucharest, 641-646.

Deselnicu, V., Maier, S.S., Deselnicu, O.C. and Florescu, M. (2005), "Impact of technological changes on increased health and comfort efficiency", *Proceedings of The 4th International Conference in Management of Technological Change*, Book 1, Chania, Greece, 87-92.

Janssen, A.M., Scheffer, J.C. and Baerheim Svendsen, A. (1987), "Antimicrobial Activity of Essential Oils: A 1976—1986 Literature Review. Aspects of the Test Methods", *Planta medica*, 395-398.

Nzeako, B.C., Al-Kharousi, Z.S.N. and Al-Mahrooqui, Z. (2006), "Antimicrobial Activities of Clove and Thyme Extracts", *Sultan Qaboos University Medical Journal*, 6(1), 33–39.

Pinto, E., Pina-Vaz, C., Salgueiro, L., Concalves, M.J., Costa-De-Oliveira, S. *et al*. (2006), "Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species", *Journal of Medical Microbiology*, 55, 1367-1373, DOI: 10.1099/jmm.0.46443-0.

Radwan, I.A., Abed, A.H., Abeer, M.R., Ibrahim, R.A. and Abdallah, A.S. (2014), "Effect of thyme, clove and cinnamon essential oils on Candida albicans and moulds isolated from different sources", *American Journal of Animal and Veterinary Sciences*, 9(4), 303-314.

Stanescu, U., Miron, A., Hancianu, M. and Aprotosoaie, C. (2004), *Medicinal Herbs from A to Z*, Gr. T. Popa Press, Iasi.

Stević, T., Berić, T., Šavikin, K., Soković, M., Gođevac, D., Dimkić, I. and Stanković, S. (2014), "Antifungal activity of selected essential oils against fungi isolated from medicinal plant", *Industrial Crops and Products*, 55, 116–122.

COLLAGEN-FIBROIN-HYDROXYAPATITE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Bone regeneration is a serious problem nowadays because of the increased number of people suffering from infections, arthritis and bond loss. The aim of the present work was to develop and characterize collagen – fibroin - hydroxyapatite matrices for hard tissue regeneration. In our study we wish to develop biomaterials which mimic bone composition and prevent allergic or toxic effects. The composites matrices obtained by freeze drying were characterized by FT-IR analysis, water uptake capacity and optic microscopy. The results obtained from analyses confirmed that collagen – fibroin - hydroxyapatite matrices exhibit proper characteristics for bone mineralization.

Keywords: collagen hydroxyapatite, fibroin, bone regeneration

INTRODUCTION

Bone problems can appear from different reasons such as medications, bacteria or unstable lifestyle. Bone regeneration approaches require a biocompatible material such as a scaffold to support cell proliferations as well as to deliver drugs needed for a proper recuperation.

Among natural polymers, collagen is one of the most common proteins in mammals so it can be successfully used as a biomaterial for medical application, because it has excellent biocompatibility, permeability, hydrophilicity and it is stable *in vivo* (Mederle *et al.*, 2016). Collagen scaffolds could also be used for tissue regeneration being proper for cell deposition and proliferation (Marin *et al.*, 2014). In order to obtain mechanical strength and elasticity comparable to those of extracellular matrix collagen gels are crosslinked (Aziz *et al*., 2005).

Hyroxyapatite (HAP) is a natural mineral found in bones that is responsible for mechanical properties of hard tissue (Kobayashi *et al*., 2001). Hyroxyapatite, as a scaffold, can improve, enhance sinterability and densification which may improve fracture toughness. Hydroxyapatite can be obtained by different methods such as hot pressing, isostatic hot pressing and slip casting, tape casting or injection molding (Rodriguez *et al.*, 2001). Among all methods the most advantages is gel casting.

Silk fibroin (SF) has outstanding properties including biocompatibility, water vapor permeability, biodegradability and little inflammatory reaction. Generally, fibroin is isolated from silkworm cocoons and purified from the sericin content. The studies showed that fibroin maintain the adhesion and proliferation of the fibroblasts and it helps the regeneration of the bone. The regenerative proprieties of fibroin were improved by using hydroxyapatite. Moisenovich *et al.* introduced nano-hydroxiapatyte and collagen to enhance their compatibility, maintain the adhesion and proliferation of fibroblasts (Moisenovich *et al*., 2014).

Collagen and HAP are the most important composites studied for bone tissue. Azami *et al.* prepared the nanocomposite trough a freeze-drying technique. The scaffolds are well defined with interconnected pours. Cells exhibited good proliferation which indicates a high level of biocompatibility (Azami *et al.*, 2006).

Fibroin caught the attention in tissue engineering field because of its outstanding biocompatibility, biodegradability and minimal inflammatory reaction. (Pascu *et al.*, 2014).

The aim of this paper was to develop and characterize collagen – fibroin - hydroxyapatite scaffolds in order to be used in medical applications such as bone mineralization and regeneration.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.85% (w/w) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). The hydroxyapatite nano-powder was purchased from Sigma Aldrich, the fibroin was obtained in the Science and Engineering of Polymers Department laboratory, from University Politehnica of Bucharest (Zaharia, 2016).

Preparation of Collagen Scaffolds

The concentration of each collagen gel was adjusted at 1% and 7.2-7.4 pH using 1M sodium hydroxide (the pH of the physiological medium). 2% fibroin was added to collagen gel and 70% HAP (w/w), then the collagen gels were cross-linked with 0.05% glutaraldehyde (GA)(reported to collagen dry substance) as Table 1 presents

Table 1. Composition and name of collagen gels

Code of gels	Col, %	SF, %	HAP, %	GA, %
Coll	1	0	0	0.05
Coll-SF	1	2	0	0.05
Coll-HAP	1	0	70	0.05
Coll-SF-HAP	1	2	70	0.05

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer and spongious forms was obtained. All the samples were characterized by FT-IR analysis, water absorption and optic microscopy.

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Absorption

In order to determine the water absorption, the scaffolds were first immersed in water and at well defined time intervals; the samples were withdrawn and weighted. The water absorption was calculated using the following equation:

$$\%\ Water\ up\text{-}take = (Wt - Wd)/Wd\ g/g \qquad (1),$$

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

All images were captured with a Leica Stereomicroscope S8AP0 model and 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used.

RESULTS AND DISCUSSION

After lyophilization the 3D porous collagen sponges based on collagen, fibroin and hydroxyapatite, were obtained, with the appearance presented in Figure 1.

Figure 1. A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

The results of FT-IR spectra are presented in Figure 2.

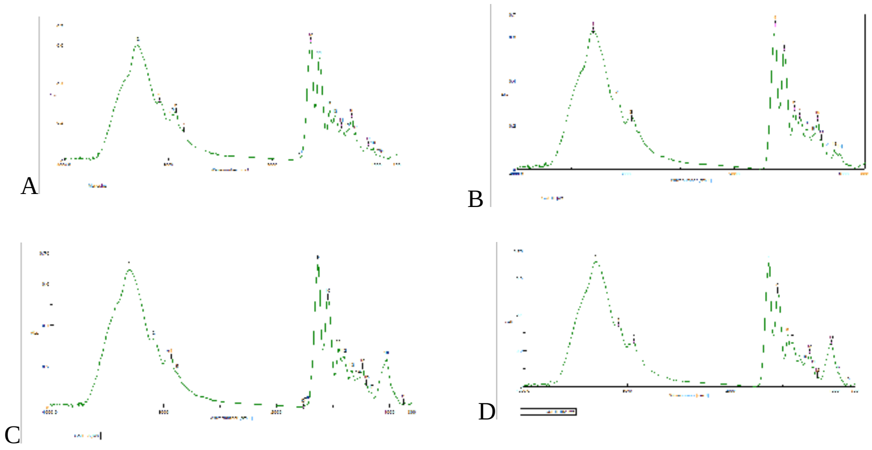

Figure 2. FT-IR spectra of spongious matrices:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

The FTIR spectra of collagen with and without silk fibroin and hydroxyapatite matrices are represented in Figure 2. The spectrum of collagen matrix (Fig. 2A) exhibited typical amide bands of proteins i.e. 3303 cm^{-1} and 2924 cm^{-1} for amide A and B respectively, 1630 cm^{-1} was ascribed to amide I (C=O stretching), 1544 cm^{-1} to amide II (N-H deformation) and 1238 cm^{-1} to amide III (N-H deformation) (Albu, 2011). When silk fibroin was added only amide B shifted to 2950 cm^{-1}, maybe because its small amount. More changes were visible in matrix Collagen-Fibroin-hydroxypatite, such as the 1030 and 961 cm^{-1} bands corresponded to v3 and v1 mode vibration of PO4^{3}.

Figure 3 presents the water up-take during 72 hours for the studied samples.

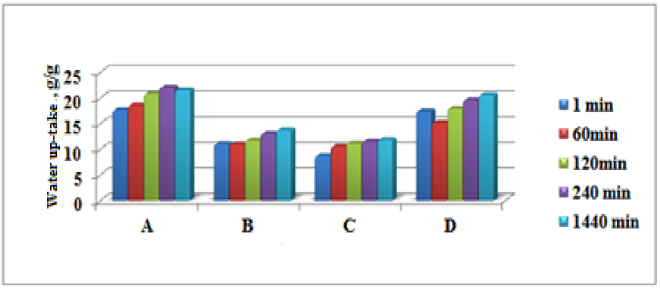

Figure 3. Water up-take for collagen scaffolds:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

From Figure 3 can be observed that the reference collagen sample (Coll) absorbs the heights amount of water, namely up to 20g/g in 72 hours. Fibroin addition leads to a lower absorption capacity, Coll-SF sample absorbing 15 g/g, less than the blank sample, which indicates density increasing. Hydroxyapatite addition contribute to density increasing, sample Coll-HAP having a water absorption capacity of 10 g/g. The last sample, containing collagen, fibroin and hydroxyapatite presented a high water absorption capacity due to the structure formed, porous structure favoring the uptake of water.

The optical microscopy results are presented in the Figure 4.

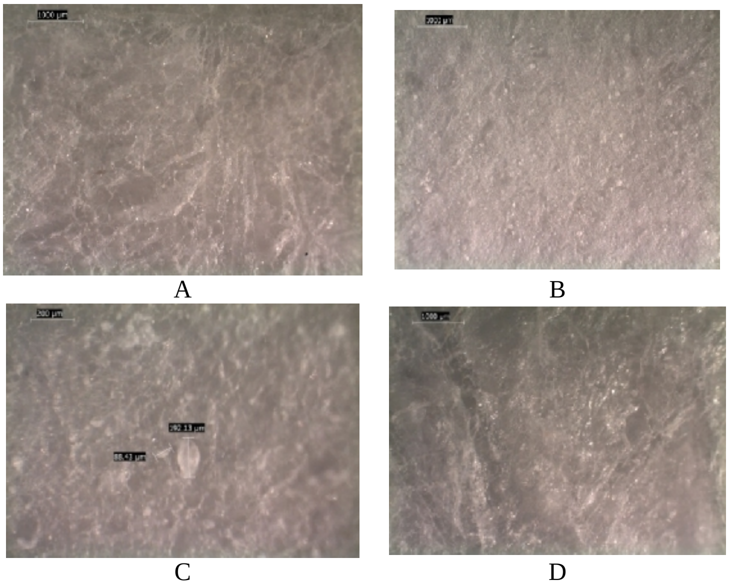
A B
C D

Figure 4. Optical microscopy for obtained matrices:
A-Coll, B-Coll-SF, C-Coll-HAP, D-Coll-SF-HAP

From optical microscopy images it can be notice the porous structure of obtained samples. Sample Coll and Coll-SF-HAP present a porous structure with interconnected pores, associated with collagen structure, while sample Coll-SF and Coll-HAP show a denser structure, with lower pore size that result from addition of fibroin and hydroxyapatite. Obtained results after optical microscopy analysis are in agreement with result obtained following water up-take analysis. Water absorption capacity is correlated with samples structure and pores morphology.

CONCLUSIONS

Type I collagen with silk fibroin and hydroxyapatite and their combinations were used in order to obtain matrices for bone tissue regeneration. The combination between components were highlighted by FT-IR spectra changes when fibroin and hydroxyapatyte was added. The matrices absorbed between 20 and 10 g/g water, being less absorbable the one with fibroin. The most compact samples were the one with hydroxyapatite. The results were proved by optical microscopy images and showed that collagen-silk fibroin-hydroxyapatite are potentially novel candidates as scaffolds for bone tissue engineering applications.

Acknowledgements

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REFERENCES

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Azami, M., Orang, F. and Moztarzadeh, F. (2006), “Nanocomposite bone tissue-engineering scaffolds prepared from gelatin and hydroxyapatite using layer solvent casting and freeze-drying technique”, *Conference on Biomedical and Pharmaceutical Engineering*, Singapore, 259–264.

Aziz, O., Atanasiou, T. and Darzi, A. (2005), *Collagen Interactions: Drug Design and Delivery*, Tufts University, Medford, 35-40.

Kobayashi, T., Nakamura, S. and Yamashita, K. (2001), “Enhanced osteobonding by negative surface charges of electrically polarized hydroxyapatite”, *Journal of Biomedical Materials Research*, 57(4), 477–484.

Lin, W., Li, Q. and Zhu, T. (2011), “Study of solvent casting/particulate leaching technique membranes in pervaporation for dehydration”, *Journal of Industrial and Engineering Chemistry*, 941-947.

Marin, S., Marin, M., Ene, A-M., Kilic, T.I., Chelaru, C., Albu, M. and Ghica, M.V. (2014), “Collagen-Doxycycline Spongious Forms For Infected Tissue Treatment”, *Proceedings of the 4th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 249-254.

Merdele, N., Marin, S., Marin, M.M., Danila, E., Merdele, O., Albu Kaya, M.G. and Ghica, M.V. (2016), “Innovative Biomaterials Based on Collagen-Hydroxyapatite and Doxycycline for Bone Regeneration”, *Advances in Materials Science and Engineering*, Article ID 3452171, 5 pages.

Moisenovich, M.M., Arkhipova, A.Y., Orlova, A.A. and Agapov, I.I. (2014), “Composite Scaffolds Containing Silk Fibroin, Gelatin, and Hydroxyapatite for Bone Tissue Regeneration and 3D Cell Culturing”, *Acta Nature*, 96-101.

Pascu, E.I., Stokes, J. and McGuinness, G.B. (2013), “Electrospun composites of PHBV, silk fibroin and nano-hydroxyapatite for bone tissue engineering”, *Materials Science and Engineering. C, Materials for Biological Applications*, 33(8), 4905-4916.

Rodriquez-Lorenzo, R.M., Vallet-Regi, M. and Ferreira, J.M.F. (2005), “Fabrication of hydroxyapatite bodies by uniaxial pressing from a precipitated powder”, *Biomaterials*, 583-588.

DEVELOPMENT AND CHARACTERIZATION OF COLLAGEN – CARBOXYMETHYLCELLULOSE MATERIALS FOR LENSES

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Cataract is one of the most common causes of blindness for people over 40 years old. Hydrogels are three – dimensional structures with increased properties which can be successful used in medical application such prosthesis or intraocular soft contact lenses. Sodium carboxymethylcellulose (CMC - Na) is the cheaper option currently available on the market with properties like biocompatibility similar with pHEMA. The aim of this study was to develop polymeric crystalline materials based on collagen (COL) and carboxymethylcellulose cross-linked with EDC/NHS for cataracts or other eye-disease. Type I fibrillar collagen gel with various ratios of CMC-Na hydrogels were lyophilized and tests tested by optic and scanning electronic microscopy, FT-IR spectroscopy and water absorption. The obtained gels were crosslinked with EDC/NHS and lyophilized in order to obtain spongious forms. They are porous structures with pore sizes between 25-81 µm. The samples with CMCNa presented more uniform and dense matrices and the crosslinked ones are more resistant, being more proper as material for lenses for be used in cataract.

Keywords: collagen, carboxymethylcellulose, EDC/NHS.

INTRODUCTION

Cataract is the most common cause of vision loss in people over age 40 and is the principal cause of blindness in the world. In fact, there are more cases of cataracts worldwide than there are of glaucoma or other eyes diseases combined, according to Prevent Blindness America (PBA) (Gretchyn *et al*., 2016).

Poly(hydroxyethyl methacrylate), pHEMA, hydrogels are highly biocompatible and transparent materials, with a high thermal stability, resistance to acid and alkaline hydrolysis and tuneable mechanical properties. These properties make them particularly useful as a basis of biomedical devices, such as catheters, intrauterine inserts, prosthesis or intraocular and soft contact lenses (pHEMA being the main material for lenses).

Collagen is the most widely used tissue-derived natural macromolecule, and it exhibits attractive properties including good biodegradability, weak antigenicity, excellent biocompatibility and unique fibril-forming properties (Engel *et al*., 2005; Lee *et al*., 2001; Madhan *et al*., 2002).

Carboxymethylcellulose (CMC) is one of the major low-cost, commercially available derivatives of cellulose used in industrial applications (Heinze and Koschella, 2005). An interesting potential application is the preparation of hydrogels with superabsorbent properties (Chang and Zhang, 2011). CMC has several advantageous properties for gel synthesis, such as good water solubility and the presence of reactive hydroxyl and carboxymethyl groups. The non-toxic nature and biocompatibility of such gels is advantageous for biomedical applications (Caló and Khutoryanskiy, 2015).

1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride / N-Hydroxysuccinimide (EDC/NHS) is a crosslinking agent complex for collagen and it offers transparent lens and is non-toxic, while glutaraldehyde is yellowish and toxic in a certain amount.

The aim of this study was to develop polymeric crystalline lens made of collagen and carboxymethylcellulose cross-linked with EDC/NHS for people who suffer from cataracts or other eye-disease.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel (Col) having a concentration of 2,85% (w/v) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu *et al*., 2011). EDC/NHS was purchased from Sigma-Aldrich (China), Sodium Carboxymethylcellulose (CMC-Na) from Fluka and collagenase from Sigma-Aldrich, China. The other reagents as HCl or NaOH were of analytical grade.

Preparation of Collagen Sponges

The concentration of each collagen gel was adjusted at 1% and 5.5 pH using 1M sodium hydroxide. 2% carboxymethylcellulose was added to half of collagen gel (w/v), then the collagen gels were cross-linked with 2:1 and 1:1 ratios of EDC/NHS as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Col, %	CMC-Na, g	EDC/NHS
AX 3.1	1	-	1:1
AX 3.2	1	-	2:1
AX 3.3	1	8	1:1
AX 3.4	1	8	2:1

The collagen-CMC-Na gels were cast in polystyrene Petri dishes of 3 cm diameter at 20°C. The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) and spongious forms were obtained.

Methods

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Absorption

In order to determine the water absorption, the collagen gels were first immersed in water. At scheduled time intervals, the samples were withdrawn and weighed. The water absorption was calculated using the following equation:

% Water up-take = (Wt –Wd)/Wd (g/g) (1),

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity.

Scanning Electron Microscopy

The scanning electron microscopic (SEM) images of sponge samples were registered using Quanta 200 FEI.

RESULTS AND DISCUSSION

The collagen gels with compositions according to Table 1, based on collagen and CMC-Na, cross-linked with EDC/NHS were freeze-dried and spongious matrices with the appearance and codification presented in Figure 1(a-d) were obtained.

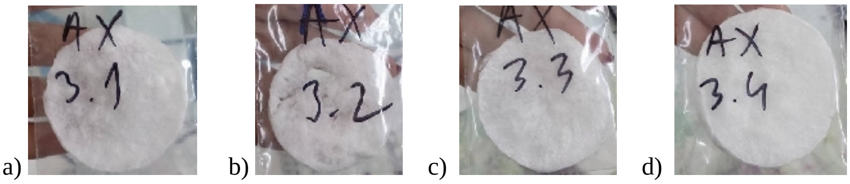


Figure 1. Collagen spongious forms:
a) Coll-R(1:1) b) Coll-R(2:1) c) Coll-R(1:1)-CMC-Na d) Coll-R(2:1)-CMC-Na

The samples from Table 1 were analyzed by FT-IR spectroscopy, water absorption, optical and scanning electron microscopy.

From the FT-IR spectra (Figure 2a, b) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011) at 3291 cm^{-1}, 3070 cm^{-1}, 1631 cm^{-1}, 1549 cm^{-1} and 1237 cm^{-1}, respectively.

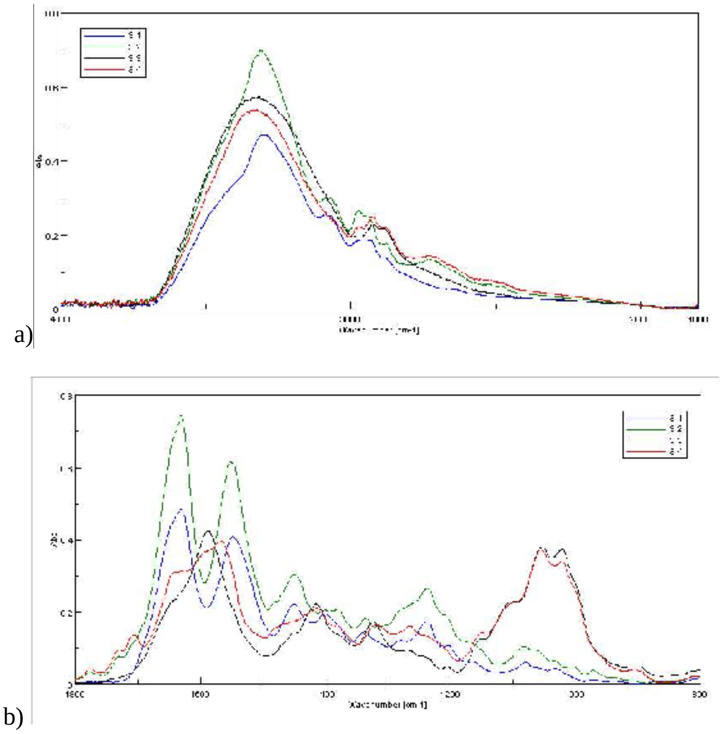


Figure 2. FT-IR spectra of matrices: a) 4000-1800 cm^{-1} and b) 1800 – 800 cm^{-1}

The cross-linking agent, EDC/NHS modify the collagen structure as it can be observed from amide A which shifted from 3292 cm^{-1} to 3306 cm^{-1}, amide II from 1549 cm^{-1} to 1553 cm^{-1} and also the appearance of new peak at 1730 cm^{-1}. The CMCNa presents characteristic peaks at 1595 cm^{-1} (stretching vibration of carboxylate group), 3402 cm^{-1} (O–H stretching vibration), 2912 cm^{-1} (C–H stretching vibration), 1421 cm^{-1} (–CH2 scissoring vibration), 1322 cm^{-1} (–OH bending vibration), 1060 cm^{-1}, (>CH–O–CH2 stretching vibrations), respectively (Liu *et al*., 2016). The structure of Coll-CMCNa presented many changes compared with control one (Coll – AX 3.1.) such as: there is no amide I and amide A shift from 3291 cm^{-1} to 3316 cm^{-1}, amide II from 1549 cm^{-1} shifted to 1588 cm^{-1} because of carboxylate group from CMCNa and specific peaks of CMCNa appeared at 1322 cm^{-1} and 1052 cm^{-1}. The crosslinking from sample A.X. 3.4 are highlighted by shifted of amide A (from 3292 to 3323 cm^{-1}) and the peak from 1705 cm^{-1}.

The water up-take for all the studied samples is presented during 1, 2, 4 and 24 hours in Figure 3.

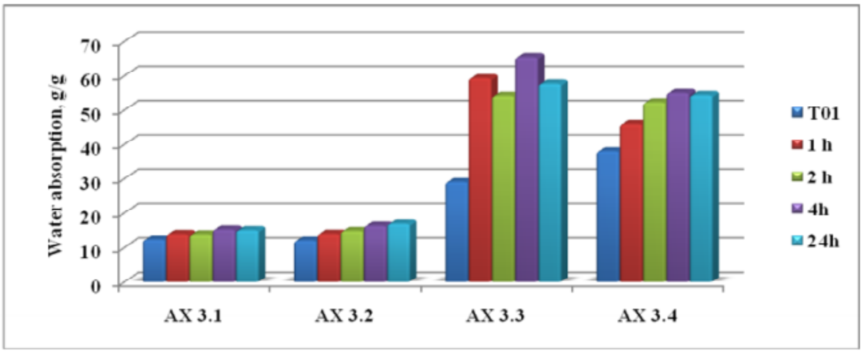
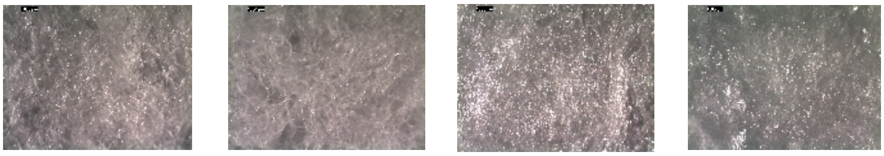


Figure 3. Water up-take during 24 hours for spongious forms

It can be observed that samples AX 3.1 and AX 3.2 have the capacity of water absorption under 20 g/g, compared to AX 3.3 and AX 3.4 that have a water absorption capacity over 50 g/g. Therefore samples with CMC-NA are about 2.5 times more hydrophilic then the others.

Figure 4 presents optical microscopy images for obtained matrices.



AX 3.1 AX 3.2 AX 3.3 AX 3.4

Figure 4. Optical microscopy (20x)

It can be observed a denser structure for samples AX 3.1 and AX 3.2 which explain why the lower amount of water was absorbed. The samples with CMC – Na (AX 3.3 and AX 3.4) present interconnected pores and a higher homogeneity.

Figure 5 presents the scanning electron microscopy for AX 3.2 sample.

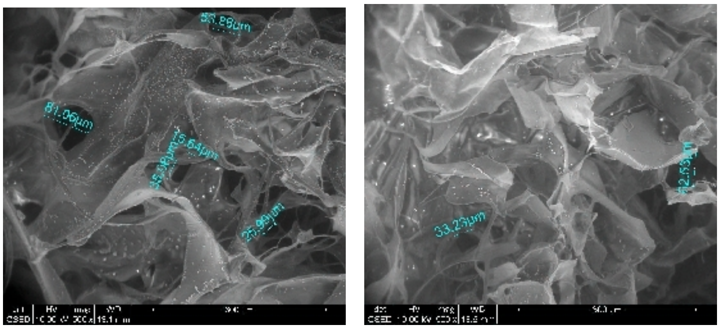


Figure 5. SEM images for AX 3.2

It presents a denser structure with interconnected pores of about 25 – 81 µm.

CONCLUSIONS

Type I fibrillar collagen and sodium carboxymethylcellulose were chosen as polymer for lens materials because of their transparency and biocompatibility. The obtained gels were crosslinked with EDC/NHS and lyophilized in order to obtain spongious forms. They are porous structures with pore sizes between 25-81 µm. The samples with CMCNa presented more uniform and dense matrices and the crosslinked ones are more resistant, being more proper as material for lenses for be used in cataract.

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REFERENCES

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Caló, E. and Khutoryanskiy, V. (2015), “Biomedical applications of hydrogels: A review of patents and commercial products”, *European Polymer Journal*, 65, 252-267.

Chang, C. and Zhang, L. (2011), “Cellulose-based hydrogels: Present status and application prospects”, *Carbohydrate Polymers*, 84, 40-53.

Engel, J. and Bachinger, H.P. (2005), “Structure, stability and folding of the collagen triple helix”, In J. Brinckmann, H. Notbohm, & P.K. Muller (Eds.), *Collagen* (pp. 7–33). Netherlands: Springer.

Gretchyn, B., Heiting, G. and Lee, J. (2016), “Cataracts”, available at http://www.allaboutvision.com/conditions/cataracts.htm, accessed in July 2016.

Horak, D., Jayakrishnan, A. and Arshady, R. (2003), “Poly(2-hydroxyethyl methacrylate) hydrogels: Preparation and properties”, In: Arshady R, editor, *Introduction to polymeric biomaterials*, London: Citus Books, pp 65–107.

Lee, C.H., Singla, A. and Lee, Y. (2001), “Biomedical applications of collagen”, *International Journal of Pharmaceutics*, 221, 1–22.

Liu, J., Chu, H., Wei, H., Zhu, H., Wang, G., Zhu, J. and He, J. (2016), “Facile fabrication of carboxymethyl cellulose sodium/graphene oxide hydrogel microparticles for water purification”, *RSC Advances*, DOI: 10.1039/C6RA06438H.

Madhan, B., Muralidharan, C. and Jayakumar, R. (2002), “Study on the stabilisation of collagen with vegetable tannins in the presence of acrylic polymer”, *Biomaterials*, 23, 2841–2847.

Ratner, B.D. and Hoffman, A.S. (1976), “Synthetic hydrogels for biomedical applications”, *ACS Symposium Series*, 31, Washington: American Chemical Society, 1–36.

MICROORGANISMS FOUND IN THE TANNERY AIR

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Past research has shown that there may be different types of microorganisms in the tannery air. These microorganisms can be harmful for the health of the employees and for the leather. Pathogenic bacteria in genus *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Nocardiopsis* and other putrefaction microorganisms and fungi that can damage the leather enter the tannery with the raw hides. During the experiment, a Petri plate with Sabouraud culture medium was exposed to the tannery air, being kept open for 13 minutes in the wet operations area of a tannery. After incubation at 30°C, the plates were analyzed after 3, 4, 5, 6 and 7 days for the identification of the microorganisms in the air. At the end of the experiment the mould colonies that have grown were analysed using the optical microscope. Multiple colonies of *Penicillium* and a colony of *Aspergillus flavus* have grown on the nutrient medium. Our experiment proved that there are spores of *Penicillium* and *Aspergillus flavus* in the tannery air. These moulds can damage the leather and *Aspergillus flavus* produces afflation, being harmful for the health.

Keywords: tannery, aerosols, fungal spores

INTRODUCTION

Bio aerosols are living particles in the air, such as viruses, bacteria, fungi and even mites and pollen grains (Deselnicu *et al.,* 2005). Regarding the size of the particles, these are very small and vary from less than one micrometer to 100 micrometers. Bio aerosols react to the air flow, moving fast or slow depending on environmental factors such as air density or gravity. Bio aerosols can lead to the spreading of atypical and very severe diseases.

The tannery air contains microorganisms from the hides (Deselnicu *et al.,* 2005; Deselnicu *et al.,* 2007; Chirila *et al.,* 2014), from the workers or from the external environment. With the raw hide, bacteria in genus *Staphylococcus, Bacillus, Pseudomonas, Enterobacter, Nocardiopsis* and other putrefaction microorganisms enter the tannery air (Daliborca, 2009).

From the past research is known that the tannery workers can inhale diverse types of biological agents, most of them bacteria (50-92%) (Skóra *et al.*, 2014).

The species *Acinetobacter calcoaceticus*, *Acinetobacter johnsonii*, *Nocardiopsis dassonvillei*, *Pantoea agglomerans*, *Pseudomonas putida*, *Staphylococcus gallinarum*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus cereus*, *Corynebacterium lubricantis*, *Cladosporium cladosporioides*, *Penicillium commune*, *Penicillium echinulatum*, *Penicillium chrysogenum*, *Penicillium crustosum*, *Candida parapsilosis* and *Cryptococcus albidus* have been identified in the tannery air (Skóra *et al.,* 2014; Castellanos-Arévalo *et. al.,* 2016).

Acinetobacter calcoaceticus is a gram-positive aerobic coccobacillus which can be found in the human intestinal flora and can cause opportunistic infections in immune-compromised patients.

Nocardiopsis dassonvillei is an aerobic bacterium in the *Actinomicetae* group which lives in the soil where animals are grown, in the marine sediments and other natural mediums, but it can be pathogenic causing shin and lung infections. The bacterium produces resistance spores and a branched mycelium. *Nocardiopsis dassonvillei* can decompose adenine, casein, tyrosine, xanthan, hypoxanthine, gelatin and urea. The bacteria can hardly be identified, being necessary to analyze the fat acids in the cell wall and ARNr 16S s sequencing (Beau *et al.*,1999; Sun *et al.*, 2010).

Pantoea agglomerans or *Enterobacter agglomerans* is a gram-negative bacteria in the *Enterobacteria* family and it can cause opportunistic infections in immune-compromised patients. The bacteria are found commonly on the substrate plant (leaves, seeds or fruits) and faces. The bacteria is hard to be differentiated from the genus *Enterobacter*, *Klebsiella* and *Serratia*, the only difference being the fact it does not use the amino acids lysine, arginine and ornithine. This bacterium is part of the intestinal flora grasshoppers and mosquitoes, the latter being protected from malaria by this bacterium.

Pseudomonas putida is a gram-negative rod shaped bacteria that can be found in the soil. This bacteria can degrade toluene and other organic solvents, which makes it useful for the soil bioremediation. It can rarely cause infections, but a lethal case has been reported in a malnourished paralyzed patient with peripheral circulatory problems (Thomas *et al.*, 2013; https://microbewiki.kenyon.edu/).

Staphylococcus gallinarum is a gram-positive bacteria which, like *Staphylococcus aureus*, forms grape like structures, and that is why it is called staphylococcus (Gr. Staphylo = grape). The bacterium was first identified in chickens and pheasants but is present in human saliva. Although usually not pathogenic, bacteria were found in infected wounds of patients in hospitals and in a patient with hepatitis B (Castellanos-Arévalo *et. al.*, 2016).

Bacillus pumillus is a gram-positive aerobic bacterium which lives in the soil, near the roots of plants, forming spores. The bacteria can stop the growth of other bacteria or fungi due to some plasmid genes (Castellanos-Arévalo *et. al.*, 2016).

Bacillus subtilis called the grass bacillus is a gram-positive bacteria that lives in the soil and in the animal gut. The bacteria measures 4-10 µm length and 0.25–1.0 µm diameter and shows flagella that can move. The optimum temperature for bacterial growth is between 25 and 35°C, but it can resist in extreme conditions due to its ability to form endospores. The genome of this species consists of approximately 4,100 genes (Castellanos-Arévalo *et. al.*, 2016).

Bacillus cereus is a gram-positive β-hemolytic bacteria which lives in the soil. Some strains are pathogenic while others are benefic for the animals. At 30°C, a *Bacillus cereus* population can double in 20 minutes to 3 hours, depending on the substrate. Like other species of the *Bacillus* genus, *Bacillus cereus* can cause endospores resistance. In 2006 in this species were discovered enzymes involved in AlkD AlkC and DNA repair. In the intestine, *Bacillus cereus* is in competitive relationship with *Salmonella* and *Campylobacter*, and its presence reduces the number of these bacteria. *Bacillus cereus* is used to inhibit the replication of *Salmonella* bacteria in the intestine and cecum in birds, rabbits and pigs (Skóra *et al.*, 2014).

Cladosporium cladosporioides is a dark colored mold which can grow on many types of materials and it can be often found in the air, its spores can cause allergies. The fungus is not pathogenic for humans and animals, but it is harmful for plants, attacking the leaves and the fruits. Species asexually reproduce by spores form branched chain and can grow in conditions of low humidity and very low temperatures (Lewis *et al.*, 2005).

Penicillium commune is a fungus that grows on dry meat and some cheeses and it produces toxins such as cyclopiazonic acid, rugulovasin and viridicatin.

Penicillium echinulatum is a fungus in the genus *Penicillium* which produces celulases, 5-Hydroxymaltol and mycophenolic acid.

Penicillium chrysogenum is a mold in the genus *Penicillium* living in temperate areas and subtropical areas, resistant in high salinity conditions, which can develop on salted food. This mold can develop inside buildings where there is high humidity. There have been isolated cases where this species was pathogenic to humans. *Penicillium chrysogenum* β-lactam antibiotics produce penicillin is the best known of these antibiotics. *Penicillium chrysogenum* was used in the pharmaceutical industry to produce penicillin. *Penicillium glaucum* and *Penicllium Rubens* and they produce penicillin and were used in early research for getting antibiotics. In 1928 Alexander Fleming discovered *Penicillium Rubens* that can inhibit the growth of *Staphylococcus aureus* colonies with bactericidal effect. Like other species of *Penicillium, Penicillium chrysogenum* reproduces by spores (conidia) that form long chains on the conidiophores form of brush. The color is blue-green colonies with areas where there is a yellow pigment. This yellow pigment gives the name of the species. In ancient Greek *chrysos* means gold (Lewis *et al.,* 2005).

Penicillium crustosum is a mold in the genus *Penicillium* found frequently in the places populated by humans, in soil and on some food products. Some strains produce neurotoxins while others produce penicillin. The colonies have a dark bluish green color, they grow fast and have a crust made of conidia (Moldes-Anaya *et al.,* 2012).

Candida parapsilosis is a yeast similar to *Candida albicans* that can cause infections in immune-compromised patients with open wounds. In contrast, *Candida parapsilosis* is not pathogenic for immune-compromised patients than being a commensal to the human species, seen most often in the skin on the hands. In patients with low immunity *Candida parapsilosis* can cause onychomycosis. The species mentioned above can cause nosocomial infections, being present in hospitals. This species grows naturally in soil or parasitic insects and various animals (Castellanos-Arévalo *et. al.,* 2016).

Cryptococcus albidus is a fungus that can grow on human skin, the vagina or in the lungs, which can cause ringworm. On Sabouraud culture medium colonies have a smooth, glossy, creamy-white specific yeast colonies. Under the microscope, appearance of the yeast as globular or ovoid blast conidia can be observed (http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Yeasts/Cryptococcus/C_albidus.html).

In this study we aimed to highlight the pathogenic microorganisms in the air of a tannery that processes cattle leather in wet operations - soaking, liming, deliming to tanning.

MATERIALS AND METHODS

Data Regarding the Wet Operations Workplace

Built by the year: 1951; Surface: 100 m^2; Volume: 500 m^3; No. of workers: 5; Ventilation: natural; Temperature: 24°C; Humidity of the air: 60%.

Method

A Petri plate with Sabouraud culture medium has been left open for 13 minutes in a tannery in the wet operations area, and then it has been put into the incubator at 30°C and analyzed after 3, 4, 5, 6 and 7 days to identify the microorganisms grown on the plate. The samples have been analyzed using an optic microscope Biolux NV - BRESSER la Magnitude 100.

RESULTS

Identification of the Microorganisms

The samples were tracked daily on colony growth to 7 days, after which they were analyzed with light microscopy to identify them. After 3 days colonies of micro-organisms first appeared. Two colonies of fungi spore began, and after four days, most colonies began to spore.

Figure 1. Colonies appeared on the Sabouraud culture medium after 7 days

Penicillium colonies predominated, but an *Aspergillus flavus* colony appeared isolated, the first colony that sporulated. *Aspergillus flavus* colony presented a large number of bodies of fructification, even after 3 days from the beginning of the experiment when other colonies did not start sporulation at all.

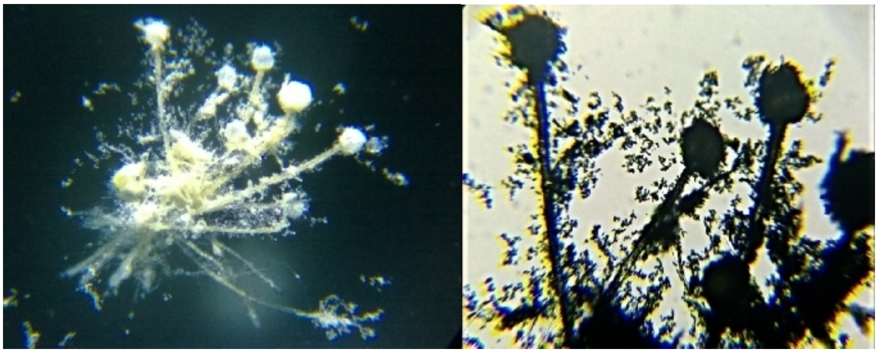

Figure 2. *Aspergillus flavus* (magnitude 100)

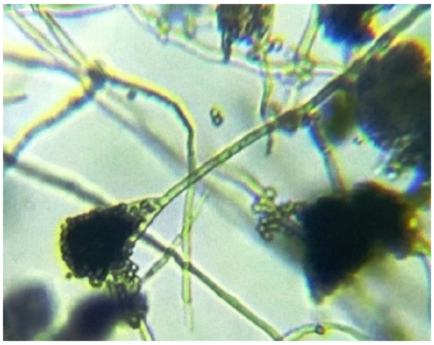

Figure 3. *Penicillium* (magnitude 100)

Aspergillus flavus is one of the 180 species of the genus Aspergillus, is akin to *Aspergillus niger* and *Aspergillus fumigates*. *Aspergillus flavus* mold saprophyte is widespread in the environment that can grow in soil and on certain plants or animals even if they are in a state of immune-suppression. *Aspergillus flavus* develops very quickly on cereal, vegetables and on various food products stored in improper conditions. Colonies are yellow or yellow-green, depending on the strain. The spores develop on conidiophores form globular features like *Aspergillus*.

Besides the fact that it can be allergen and pathogen for immune-compromised patients, *Aspergillus flavus* produces a toxin and carcinogen afflation.

Aspergillus flavus colonies sporulated faster than those of the species of the genus *Penicillium*. The species of the genus *Aspergillum* have an invasive and aggressive nature certainly developing rapidly at the expense of other species. This phenomenon was observed in colony-forming *Aspergillus niger* growing even faster than the *Aspergillus flavus* and in less than seven days covering the entire surface of the culture medium, leaving no space for other species.

CONCLUSIONS

In the tannery air *Penicillium* spores are predominant, harmless for the human health, but harmful for the leather which can be deteriorated by this mold; there is also *Aspergillus flavus*, which is harmful for the objects that may be degraded by this mold and also for the human health because it produces afflation.

Results indicate that potential health risks arise from the fungal small bio aerosol particles presence in relation to the outdoor air.

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REFERENCES

Beau, F., Bollet, C., Coton, T., Garnotel, E. and Drancourt, M. (1999), “Molecular Identification of a *Nocardiopsis dassonvillei,* Blood Isolate”, *Journal of Clinical Microbiology*, 37(10), 3366-3368.

Castellanos-Arévalo, A.P., Camarena-Pozos, D.A., Castellanos-Arévalo, D.C., Rangel-Córdova, A.A., Peña-Cabriales, J.J., Arévalo-Rivas, B., Guzmán de Peña, D. and Maldonado-Vega, M. (2016), “Microbial contamination in the indoor environment of tanneries in Leon, Mexico", *Indoor and Built Environment*, 25(3), 524-540.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), "Comparative study regarding resistance of wet-white and wet-blue leather to the growth of fungi", *Leather and Footwear Journal*, 14(2), 107-120.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), "Study regarding the resistance to the growth of fungi of wet-white leather tanned with Titanium – Aluminum", *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, 31-36.

Chirila, C., Deselnicu, V. and Crudu, M. (2014), "Study regarding the resistance of wet-white leather organic tanned to the growth of fungi", *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, 37-42.

Daliborca, C.V. (2009), "Determination of species and number of pathogens in the air inside public buildings and education institutions in Timisoara", available at: http://www.dmmt.ro/uploads/files/proiecte%20si%20studii/Aeroflora_2.pdf

Deselnicu, V., Maier, S.S., Deselnicu, O.C. and Florescu, M. (2005), "Impact of technological changes on increased health and comfort efficiency", *Proceedings of The 4th International Conference in Management of Technological Change*, Book 1, Chania, Greece, 19-20 August, 87-92.

Deselnicu, V., Maier, S.S., Albu, L. and Buruntea, N. (2007), "Antimicrobial and antifungal leathers for increasing the health and the comfort of the individuals", *CORTEP*, 18-21 Oct., Iasi, RO.

Lewis, P.R., Donoghue, M.B., Cook, L., Granger, L.V. and Hocking, A.D. (2005), "Tremor syndrome associated with a fungal toxin: sequelae of food contamination", *Medical Journal of Australia*, 182(11), 582-584.

Moldes-Anaya, M., Rundbergetc, T., Fæstec, C.K., Eriksenc, G.S. and Bernhoft, A. (2012), "Neurotoxicity of *Penicillium crustosum* secondary metabolites: Tremorgenic activity of orally administered penitrem A and thomitrem A and E in mice", *Toxicon*, 60(8), 1428–1435.

Skóra, J., Gutarowska, B., Stepień, Ł., Otlewska, A. and Pielech-Przybylska, K. (2014), "The evaluation of microbial contamination in the working environment of tanneries", *Medycyna pracy*, available at http://www.ncbi.nlm.nih.gov/pubmed/24834690.

Sun, H., Lapidus, A., Nolan, M., Lucas, S., Del Rio, T.G., Tice, H., Cheng, J.F., Tapia, R., Han, C., Goodwin, L., Pitluck, S., Pagani, I., Ivanova, N., Mavromatis, K., Mikhailova, N., Pati, A., Chen, A., Palaniappan, K., Land, M., Hauser, L., Chang, Y.J., Jeffries, C.D., Djao, O.D., Rohde, M., Sikorski, J., Göker, M., Woyke, T., Bristow, J., Eisen, J.A., Markowitz, V., Hugenholtz, P., Kyrpides, N.C. and Klenk, H.P. (2010), "Complete genome sequence of *Nocardiopsis dassonvillei* type strain (IMRU 509)", *Standards in Genomic Sciences*, 3(3), 325-36, doi: 10.4056/sigs.1363462.

Thomas, B., Okamoto, K., Bankowski, M.J. and Seto, T.B. (2013), "A Lethal Case of *Pseudomonas putida* Bacteremia Due to Soft Tissue Infection", *Infectious Diseases in Clinical Practice* (Baltimore, MD), 21(3), 147–213, doi: 10.1097/IPC.0b013e318276956b.

*** http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Yeasts/Cryptococcus/C_albidus.html

THYME ESSENTIAL OIL AS NATURAL LEATHER PRESERVATIVE AGAINST FUNGI

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This work covers the chemical composition of essential oil isolated from thyme (*Thymus vulgaris)* analyzed using GC-MS and its antifungal activity. The main components of *Thymus vulgaris* oil were tymol (32.43%) and carvacrol (57.355%). The thyme oil was assayed for antifungal activity against *Candida albicans* and *Aspergillus niger* on sheepskins for footwear lining treated with thyme oil at a rate of 30g/1000g wet blue leather, during fatliquoring process. These pathogens are very contagious and result in severe damage of leather. Compounds were investigated by ASTM D4576–86 (1996) method against *Aspergillus niger* and by Standard SR EN ISO 20645:2004 against *Candida albicans*. The essential oil of thyme showed the best antifungal activity on sheepskins for footwear lining.

Keywords: lining leather, thyme essential oil, antifungal activity, fungi

INTRODUCTION

The hides, skins and leather can be easily contaminated with different species of bacteria and fungi. These microorganisms can damage the material or can be pathogenic for the user. Natural skins are very sensitive to the destructive action of fungi. Thus, once with raw skin can enter in tannery the diverse bacteria that may be pathogenic as *Staphylococcus, Bacillus, Pseudomonas, Enterobacter, Nocardiopsis* and other microorganisms decay. Also during processing and commercialization, particularly in transport over seas the wet blue or finished leather must ensure protection against the main species of fungi that can grow on the skin (Ţârlea *et al*., 2009; Chirilă *et al*., 2014). These microorganisms include the following species: *Aspergillus niger, Aspergillus flavus, Trichoderma viride, Penicillium glaucum, Penicillium cyclopium, Paecilomyces variotii, Candida albicans, Scopulariopsis brevicaulis* which develop on various leather items, degrading the material (Figure 1).

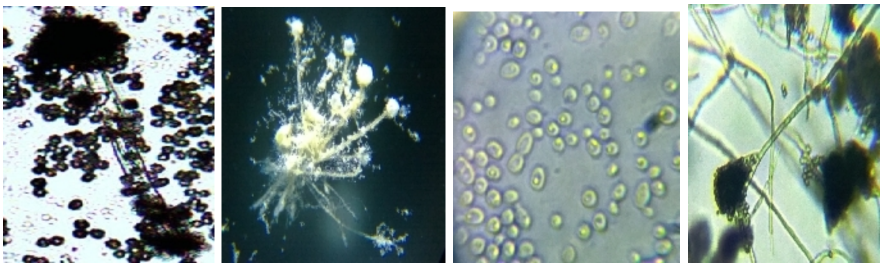

(a) (b) (c) (d)

Figure 1. *Aspergillus niger* (a), *Aspergillus flavus* (b), *Candida albicans* (c), *Penicillium* (d)

Biocides are used for the protection of leather, fur, footwear and leather objects against microorganisms (Deselnicu *et al*., 2005; Deselnicu *et al*., 2007). “Biocide” is a generic term that refers to both bactericidal (which are effective against bacteria) and fungicides (which are effective against fungi). They act as oxidizing agents or by distorting or crosslinking proteins, through a number of mechanisms including inhibition of bacterial enzyme systems (Radwan *et al*., 2014).

Bactericides are used mainly at the beginning of leather processing during conservation and soaking phases.

Fungicides are commonly used from the pickling to the drying steps, because the pH conditions of these processes are ideal for mold growth.

Biocides are among chemicals potentially harmful to humans and the environment, therefore their production and marketing is regulated and monitored continuously by European directives and regulations of European Parliament and Council (Deselnicu, 2014; Deselnicu *et al*., 2014). There is no EU legislation in regulating specifically the content of chemicals in footwear and other leather products, but the use of chemicals is restricted by REACH (EC 1907/2006).

Directive 94/11/EC, also called the EU Directive on Labeling of Footwear is specifically linked to the European market harmonization of laws and administrative provisions of the Member States relating to labeling of the materials used in the main components of footwear for sale by consumers. In Romania, Directive 98/8/EC was adopted by HG 956/2005, complete with HG 545/2008 on the placing on the market of biocidal products.

The biocides used in the leather industry are classified as quaternary ammonium compounds, isothiazole, thiocarbamates and the like, such as sulfur-containing heterocycles, for example benzothiazole derivatives of 2-(tiocianatmetiltio)-1,3) benzothiazole and glutaraldehyde. Fungicides include phenol derivatives (ortho-phenylphenol), TCMTB, carbamates, etc. Halogenated organic compounds are also used, for example bronopol [2-bromo-2-nitropropane-1,3-diol]. Due to their toxicity and negative effects they have on the environment and human health it is necessary to replace these chemicals with natural, environmentally friendly and low toxicity compounds.

Many studies have been made that aimed at replacing potentially hazardous substances used in the processing of hides, skins and furs:

- Synthesis of new classes of biocides based on derivatives of 2-amino-benzothiazole-6-substituted with methyl, methoxy, chloro, nitro, modified chemically by sulphonation (Ţârlea *et al*., 2009a) and use in leather processing cattle (Ţârlea *et al*., 2009b), which have been shown to be effective against *Aspergillus niger* species, but not against the *Trichoderma viride* species;
- Synthesis of new classes of tanning agents to replace chromium in tanning hides and furs (Crudu *et al*., 2008a,b; Crudu *et al*., 2010; Crudu *et al*., 2012; Deselnicu *et al*., 2008; Pruneanu *et al*., 2010; Pruneanu *et al*., 2011; Albu *et al*., 2011).

Several studies have been conducted on the use of natural products derived from plants treating leather and fur:

- Bayramoglu *et al*. (2010) from Ege University in Turkey used *Origanum* species to extract essential oils from plants through steam distillation process. It has been found that utilization of the essential oil of *Origanum minutiflorum* during pickling has antifungal activity and that its effect is improved with increasing concentration. As a result of this study, it was found that the wet blue leather specimens that were treated with 1% *Origanum sp.* essential oil showed antibacterial effect to Gram-positive bacteria. *Origanum minutiflorum* essential oil had an antifungal effect on wet blue. The bacteria are more resistant to the essential oils than yeasts and moulds. A concentration of 21% relative to the fleet of the essential oils of oregano from three different species and fennel oil was tested for their antimicrobial activity along with 7-25% of phenol and 4-chloro-3-methyl-phenol as a commercial bactericides commonly used in the leather industry. The results showed that the three essential oils of oregano had a much stronger bactericidal activity than commercial and may find use as antibacterial agents in the leather industry.

- Use of essential oils in finishing leather and fur (Niculescu *et al*., 2015a; 2015b);
- Use of essential oils to stop the growth of fungi (Stević *et al*., 2014).

The aim of this work is to test antifungal activity of essential oils extracted from thyme (*Thymus vulgaris*) against *Candida albicans* and *Aspergillus niger* on sheepskin leather for footwear lining.

MATERIALS AND METHODS

Materials

Essential oil isolated from thyme *(Thymus vulgaris)* was obtained by hydro distillation in a continuous extractor type Clevenger (Berechet, 2015).

Sheep skins for footwear linings treated during fatliquoring operation with thyme oil 30g/1000g wet blue.

Biologic material: *Candida albicans 10231* and *Aspergillus niger ATCC 6275*.

Methods

Chemical composition of essential oils was determined by Gas Chromatography-Mass Spectrometry (GC-MS) with AGILENT 6890 N.

Antifungal activity against *Candida albicans:* The tests were performed in accord with Standard SR EN ISO 20645:2004 - Textile fabrics – Determination of antibacterial activity - Agar diffusion plate test. In each Petri dish Dextrose Agar Sabouraud culture medium was poured. Both samples treated with thyme essential oil and control samples were placed in each Petri dish in the center of the surface of the culture medium, and then the culture medium was seeded in 3 points around the sample, as an equilateral triangle. There have been two parallel samples. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14 and 21 days.

Antifungal activity against *Aspergillus niger:* The tests were made following standard ASTM D4576–86 (1996) - Standard Test Method for Mold Growth Resistance of Wet Blue. Petri dishes were placed in thermo-hygrostat at 30°C temperature and were analyzed after 3, 7, 14, 21 and 28 days.

Optical microscopy images were captured using a Leica stereomicroscope S8AP0 model with optic fiber cold light source, L2, with three levels of intensity, and magnification 40X.

RESULTS AND DISCUSSION

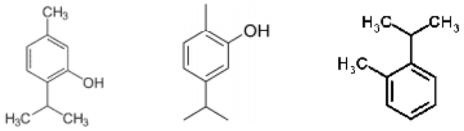
Chemical Composition of Essential Oils

Table 1 presents the components of thyme essential oil determined by GC/GC-MS chromatography.

Table 1. Main chemical compounds identified in *Thymus vulgaris* essential oil

No	Retention time, min	Compound	Formula	%	Kovats Indices, KI
1	23,060	Carvacrol (Antioxine)	$C_{10}H_{14}O$	57.355	2,291x10^3
2	12,940	Tymol	$C_{10}H_{16}$	32.430	1,095x10^3
3	11,240	o-Cymol	$C_{10}H_{14}$	3.993	1,057x10^3

Analysis allowed the identification of 16 components of which, carvacrol (57,355%) and Tymol (32,430%) and o-Cymol (3.993%) were detected as major constituents.



Tymol Carvacrol o-Cymol

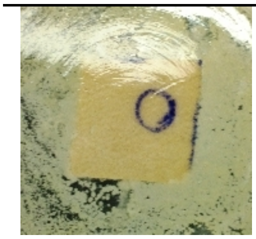
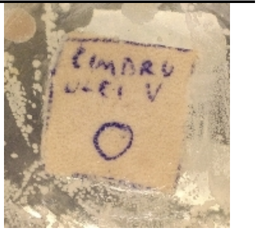
Figure 2. Main components of thyme essential oil

Thymol is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol; Carvacrol, or cymophenol, $C_6H_3CH_3(OH)(C_3H_7)$, is a monoterpenoid phenol; o-cymol is *o*-Isopropyltoluol.

Antifungal Activity

Table 2 presents antifungal activity against *Candida albicans* after 3 days of incubation at 37°C of sheepskin leather treated with essential oil of thyme.

Table 2. Antifungal activity after 3 days of incubation at 37°C on sheepskin leather for lining treated with essential oil of thyme against *Candida albicans*

Control sample	Sheepskin leather for lining treated with essential oil of thyme
	
+++	++

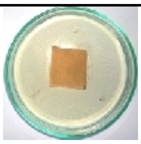
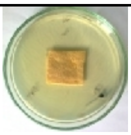
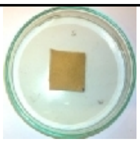
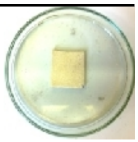
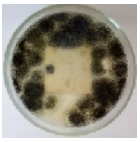
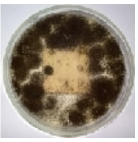
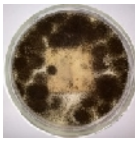
- : No growth, +: Mild growth, ++: Moderate growth, +++: Heavy growth

The results of tests made on sheepskins for footwear linings treated during fatliquoring operation with thyme oil 30g/1000g wet blue revealed that the number of *Candida albicans* colonies appeared on the culture medium is lower in treated leather with essential oil of thyme compared to the untreated control leather.

Table 3 presents antifungal activity of thyme essential oil against *Aspergillus niger* on sheepskin leather for footwear lining.

On the treated specimens with thyme essential oil no growth of *Aspergillus niger* can be observed anymore; thyme essential oil totally inhibited the grows of fungi.

Table 3. Antifungal activity of thyme essential oil against *Aspergillus niger* on sheepskin leather for lining

	3 days	7 days	14 days	21 days
Thyme essential oil				
Grade	0 + inhibition zone	0 + inhibition zone	0 + inhibition zone	0 + inhibition zone
Control				
Grade	2	3	3	4

CONCLUSION

The results obtained in this study show that *Thymus vulgaris* oil exhibited high antifungal activity against *Candida albicans* and *Aspergillus niger*. Thyme oil proved to be the best inhibitor of fungi conferred by high thymol and/or carvacrol content. This study suggests the possibility of using the thyme oil as natural leather preservative.

Acknowledgements

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REFERENCES

Albu, L., Popescu, M., Deselnicu, V., Albu, E. and Zainescu, G. (2011), "Study and practice on alternative eco-friendly processes for leather manufacture", *Leather and Footwear Journal*, 11(3), 211-220.

Bayramoğlu, E.E., Gülümser, G. and Karaboz, I. (2008), "The Investigation of Antibacterial Activities of Some Essential Oils in Wet Blue Leather", *International Journal of Natural and Engineering Sciences*, 2 (1), 33-36.

Berechet, M.D. (2015), "Essential Oils – Their Use in Practice and Research", Doctoral Thesis.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), "Comparative study regarding resistance of wet-white and wet-blue leather to the growth of fungi", *Leather and Footwear Journal*, 14(2), 107-120.

Chirila, C., Crudu, M. and Deselnicu, V. (2014), "Study regarding the resistance to the growth of fungi of wet-white leather tanned with Titanium – Aluminum", *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, 31-36.

Chirila, C., Deselnicu, V. and Crudu, M. (2014), "Study regarding the resistance of wet-white leather organic tanned to the growth of fungi", *Proceedings of The 5th ICAMS 2014*, 23-25 October 2014, Bucharest, 37.

Crudu, M., Deselnicu, V., Ioannidis, I. and Crudu, A. (2012), "New Wet White Tanning Agents and Technology", *Proceedings of the 4th International Conference on Advanced Materials and Systems, ICAMS 2012*, 27-34.

Crudu, M., Deselnicu, V., Mutlu, M.M., Gulumser, G., Bitlisli, B.O., Basaran, O. and Adiguzel Zengin, A.C., (2010), "New tanning agents based on titanium and zirconium", *Proceedings of the 3rd International Conference on Advanced Materials and Systems ICAMS 2010*, Bucharest, 27-32.

Crudu, M., Deselnicu, V., Mutlu, M.M., Gulumser, G., Bitlisli, B.O., Basaran, B., Adiguzel Zengin, A.C. (2010), "New tanning agents based on titanium and zirconium", *Proceedings of The 3rd ICAMS 2010*, 27-32, 16-18 September 2010, Bucharest.

Crudu, M., Deselnicu, V., Rosca, I., Sutiman, D., Cailean, A., Ghiga, M., Bocu, V., Boca, N. and Capac, D., (2008), "Obtaining new eco-friendly tanning agents by valorization of wastes", *Ecology & Safety Symposium*, June 2008, Sunny Beach, Bulgaria, vol.2, 171-176.

Crudu, M., Deselnicu, V., Rosca, I., Sutiman, D., Cailean, A., Ghiga, M., Bocu, V., Boca N. and Capac, D. (2008), "Eco-friendly tanning agents to be used in leather manufacture", *CHEMPOR – 10th International Chemical and Biological Engineering Conference, Proceedings*, 498-499, September 2008, Braga, Portugal.

Deselnicu, D.C. (2014), *European Policies for Products and their Relevance for the Footwear Sector* (RO), ISBN: 978-973-720-555-1 (245pg), Agir Press.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), "Sustainable consumption and production in the footwear sector", *Leather and Footwear Journal*, 14(3), 159-180.

Deselnicu, V., Arca, E., Badea, N., Maier, S.S. and Deselnicu, D.C. (2008), "Alternative process for tanning leather", *Leather and Footwear Journal*, 8(4), 25-34.

Deselnicu, V., Deselnicu, D.C., Vasilescu, A.M. and Militaru, G. (2014), "EU Policy for Sustainable Consumption and Production – EU Ecolebal for footwear", *Proceedings of the 5th International Conference on Advanced Materials and Systems ICAMS 2014*, 23-25 October 2014, Bucharest, 641-646.

Deselnicu, V., Maier, S.S., Albu, L. and Buruntea, N. (2007), "Antimicrobial and antifungal leathers for increasing the health and the comfort of the individuals", *CORTEP*, 18-21 Oct., Iasi, RO.

Deselnicu, V., Maier, S.S., Deselnicu, O.C. and Florescu, M. (2005), "Impact of technological changes on increased health and comfort efficiency", *Proceedings of The 4th International Conference in Management of Technological Change*, Book 1, Chania, Greece, 19-20 August, 87-92.

Niculescu, O., Leca, M., Moldovan, Z. and Deselnicu, D.C. (2015), "Obtaining and characterizing of a product with antifungal properties based on essential oils and natural waxes for finishing natural leathers", *Revista de chimie* (Bucharest), 66(11), 1733-1736.

Niculescu, O., Leca, M., Moldovan, Z. and Deselnicu, D.C. (2015), "Research on Obtaining Products for Fragrance and Biological Protection on Natural Leathers and Furs", *Revista de Chimie* (Bucharest), 66(12), 1956-1959.

Pruneanu, M., Maier, S.S., Maier, V., Deselnicu, V., Mutlu, M.M., Gulumser, G., Bitlisli, B.O., Basaran, B. and Adiguzel Zengin, A.C. (2010), "Oligomeric melamine-formaldehyde resin as pre-tanning agent", *Proceedings of The 3rd ICAMS 2010*, 16-18 September 2010, Bucharest, 113-118.

Pruneanu, M., Maier, S.S., Niculescu, O., Vitan, F. and Deselnicu, V. (2011), "Obtaining and characterization of a benzensulfonate melamine-formaldehyde resin, with applications in leather and fur industry", *Revista de chimie*, 62(1), 60-63.

Radwan, I.A., Abed, A.H., Abeer, M.R., Ibrahim, R.A. and Abdallah, A.S. (2014), "Effect of thyme, clove and cinnamon essential oils on Candida albicans and moulds isolated from different sources," *American Journal of Animal and Veterinary Sciences*, 9(4), 303-314.

Stević, T., Berić, T., Šavikin, K., Soković, M., Gođevac, D., Dimkić, I. and Stanković, S. (2014), "Antifungal activity of selected essential oils against fungi isolated from medicinal plant", *Industrial Crops and Products*, 55, 116–122.

Ţârlea, M.M., Mutlu, M.M., Bitlisli, B.O., Basaran, B. and Adiguzel Zengin, A.C. (2009a), "Antifungal compounds for Leather. Part I - Synthesis of Benzothiazoles Biocides for Bovine Leathers", *Leather and Footwear Journal*, 9(2), 104-117.

Ţârlea, M.M., Mutlu, M.M., Macovescu, G., Radulescu, C.H., Adiguzel Zengin, A.C., Bitlisli, B.O. and Basaran, B. (2009b), "Antifungal compounds for Leather. Part II - Testing of antifungal activity on bovine leather", *Leather and Footwear Journal*, 9(4), 284-295.

COLLAGEN NETWORKS OBTAINED BY LEATHER INDUSTRY BY-PRODUCTS

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Chemically cross-linked collagen polymeric networks with different structures and properties were obtained from collagen hydrolysates and glutaraldehyde and were designed for potential applications in bioeconomy. The swelling behavior of these networks was tested in distilled water at constant temperature and the equilibrium swelling degree was calculated. The influence of different cross-linking parameters used to tailor the structure and properties of these networks (collagen-glutaraldehyde ratio and the aminic nitrogen content of the collagen hydrolysate) upon the collagen networks was investigated by FTIR spectroscopy, thermal analysis, swelling tests and contact angle measurements. Different amounts of glycerol were added in order to further improve the general properties of the collagen networks. The general properties of these materials leaded to the conclusion that they could be used for potential applications in bioeconomy.

Keywords: Collagen hydrolysate, cross-linking, bioeconomy

INTRODUCTION

Hydrogels are three-dimensional cross-linked polymeric networks which exhibit the ability to swell and retain large volumes of water in their structure, without dissolution. Their resistance to dissolution is given by the cross-links between network chains. Based on the type of these cross-links, hydrogels can be classified in two categories: chemical or permanent hydrogels, which exhibit chemical junctions between polymeric chains, and physical hydrogels which presents temporary junctions that arise from weak physical interactions, such as hydrogen bonds, chain entanglements, and ionic or hydrophobic interactions. Hydrogels can be also divided in two main categories, based on the nature of the polymeric chains: natural, synthetic polymers, or combination of these two categories. Natural hydrogels are made up from polymers like collagen, gelatin, starch, agarose, and alginate. Synthetic hydrogels are obtained from chemically synthesized polymers (Ahmed, 2015).

Collagen is one of the most important and abundant proteins present in animals and humans bodies and plays a key role in the extracellular matrix and in many tissues like skin, bone, cartilage, tendons (Niculescu and Gaidau, 2014; Zhang *et al.*, 2014; Fiorani *et al.*, 2014). Collagen is currently extracted from this tissues using chemical-enzymatic or chemical methods (Niculescu and Gaidau, 2014; Fiorani *et al.* 2014). Collagen hydrolysate is a polypeptide compound made by further hydrolysis of collagen, which displays the ability to form thin films on different surfaces, has a hydrophilic character and is not dangerous for human health. Due to these remarcable properties, it has been used in various applications, including as a natural fertilizer in agriculture due to the release of free amino acids with a considerable nitrogen content (Niculescu and Gaidau, 2014; Pei *et al.*, 2013). Other applications of collagen hydrolyates are in regenerative medicine, in pharmaceutics, in cosmetics, as a food additive and as a biodegradable packing material (Pei *et al.*, 2013). Unlike gelatin, collagen hydrolyates displays a lower molecular weight and better biodegradability and absorbency (Pei *et al.*, 2013). The mechanical and thermal stability of the collagen physical hydrogels can be further improved by employing various cross-linking agents, such as: formaldehyde (Zhang *et al.*, 2014; Tan *et al.*, 2015), glutaraldehyde (Zhang *et al.*, 2014; Osborne *et al.*, 1997; Tan *et al.*, 2015; Rault *et al.*, 1996), glycosaminoglycan (Osborne *et al.*, 1997), chondroitin-6-sulphate (Osborne *et al.*, 1997), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Osborne *et al.*, 1997; Sionkowska *et al.*, 2010, Rault *et al.*, 1996), 1,1-carbonyldiimidazole (Osborne *et al.*, 1997), hexamethylene diisocyanate (Zhang *et al.*, 2014; Rault *et al.*, 1996), cyanamide (Rault *et al.*, 1996), N-hydroxysuccinimide (Sionkowska *et al.*, 2010; Tan *et al.*, 2015), diepoxypropylether (Sionkowska *et al.*, 2010), hydrazine (Rault *et al.*, 1996), diphenylphosphorylazide (Rault *et al.*, 1996), dialdehyde carboxymethyl cellulose (Tan *et al.*, 2015) and genipin (Zhang *et al.*, 2014; Biswal *et al.*, 2016). Among these cross-linkers, glutaraldehyde was widely used for the collagen/gelatine hydrogels preparation due to its high reactivity, high water solubility and low cost. The reaction mechanism of collagen cross-linking by glutaraldehyde consists of the chemical reaction between the free amino functional groups of different amino acids from collagen and the carbonyl functional group from glutaraldehyde, with the formation of a Schiff base type structure (Tian *et al.*, 2016).

The collagen hydrogels display high biocompatibility and biodegradability, whereas their mechanical and thermal properties were relative low (Nistor *et al.*, 2015). In order to overcome this disadvantages, the collagen hydrogels were additivated with various compounds, such as cellulose (Pei *et al.*, 2013), glycerol or poly(ethylene glycols) of different molecular weights (Langmaier *et al.*, 2008).

The aim of this research is to obtain new 3-D structures by reusing collagen by-products from the leather industry. Moreover, the target of this research is to transform low-economical value by-products from leather industry into value-added products for bioeconomy, following the concept of economical and environmental sustainability.

EXPERIMENTAL

The bovine collagen hydrolysates were obtained according the procedure described (Niculescu and Gaidau, 2014), and their characteristics are presented in Table 1.

Table 1. Physico-chemical characteristics for the bovine collagen hydrolysates employed for the 3-D collagen structures synthesis

No.	Characteristics, MU	HCN2	HCN3	Standard
1	Dry substance, %	9.80	9.59	SR EN ISO 4684:2006
2	Total ash, %	5.92	7.09	SR EN ISO 4047:2002
3	Total nitrogen, %	16.52	16.79	SR EN ISO 5397:1996
4	Free amino nitrogen, %	1.84	1.55	ICPI internal standard
5	Organic substance, %	92.90	94.36	SR EN ISO 5397:1996
6	Calcium oxide, %	4.90	3.43	ICPI internal standard
7	Chromium oxide, %	Values below the detection limit (0.3%)		SR EN ISO 5398-1:2008
8	pH	8.50	8.77	STAS 8619/3:1990

The bovine collagen hydrolysate HCN3 was employed for the formation of HCG-5 and HCG-9 hydrogels, whereas HCG-11 was obtained from the HCN2 collagen hydrolysate (which displays a higher free amino N_2 content). In all cases the crosslinking agent was a 25% aqueous solution of glutaraldehyde. The HCG-9 and HCG-11 hydrogels were additivated with 25% and 2.5% glycerol, respectively, whereas HCG-5 was not additivated.

The FTIR spectra of the collagen xerogels were recorded in the transmission mode (FTIR-ATR) on a ABB MB3000 spectrometer. The IR spectra was recorded in the wavenumbers range of 4000-550 cm^{-1} and consists of 64 scans at 4 cm^{-1} spectral resolution. The DSC measurements of the collagen xerogels were performed on a Netzsch DSC200 F3 Maia thermal analysis system. The measuring conditions were the following: temperature range: -20 - 400°C, the heating rate was 10°C/min, and the Argon flow rate was $1Lh^{-1}$. The contact angle of a water droplet on the xerogel surface was measured with the VCA Optima XE device. The surface energy was determined by the means of the VCA Optima XE device software. For the swelling tests, the xerogel thickness was measured using a micrometer.

RESULTS AND DISCUSSION

The free amino nitrogen content provides valuable information for the free amino groups available for the cross-linking reaction with glutaraldehyde. The HCN2 hydrolysate had a free amino nitrogen content of 1.84%, whereas HCN3 had 1.55%. After pH adjustment at the value of 7.4, the reaction mixture (collagen hydrolysate, glutaraldehyde and glycerol) was very well stirred and injected through a 2.5 mm thick circular rubber gasket between two parallel glass square plates (as described in Cursaru *et al.*, 2008), as one can see in Figure 1A. This device was kept in an oven at 40°C for 1 hour. At the end of the time, the device was opened and the hydrogel circular plate was cut into 10 mm diameter disks. These disks were after immersed into a determined volume of distilled water (considered in excess), and kept 6 days at room temperature with water changing every day, in order to remove the unreacted species. Figure 1B presents the increased volume of the collagen hydrogel at equilibrium swelling, as compare with the initial dry disk (xerogel).

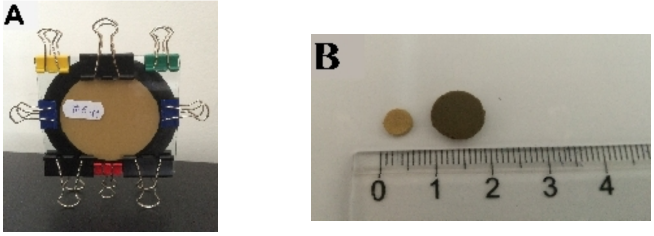

Figure 1. The device employed in the synthesis of the collagen networks (A); Hydrogel disks at the equilibrium swelling at 28°C (B)

Figure 2 comparatively presents FT-IR spectra of collagen hydrolysate (HCN3) and of the corresponding hydrogel (HCG-5). In these spectra, only HCG-5 displayed the two characteristic peaks of a Schiff base: at 3325 cm^{-1} (assigned to stretching vibration of N-H group) and at 1670 cm^{-1} (assigned to carbonyl groups). The presence of these peaks demonstrates the occurrence of the cross-linking reaction between collagen hydrolysate and glutaraldehyde. Both the collagen hydrolysate (HCN3) and its corresponding collagen network (HCG-5) displayed the following peaks, respectively:

ν_{O-H} stretching vibration at 3069 and 3204 cm^{-1}; asymmetric stretching vibrations of -CH$_2$ group at 2947-2874 cm^{-1} and 2945-2872 cm^{-1}; characteristic bands of amide I (1637-1657 cm^{-1} in the case of HCN3 and 1655 cm^{-1} for HCG-5) due to $\nu_{C=O}$; amide II (at 1540 and 1560 cm^{-1}) due to ν_{C-N} and δ_{N-H}; amide III at 1248 cm^{-1} for HCN3 and 1240 cm^{-1} in the case of HCG5 (due to ν_{C-N} and δ_{N-H}).

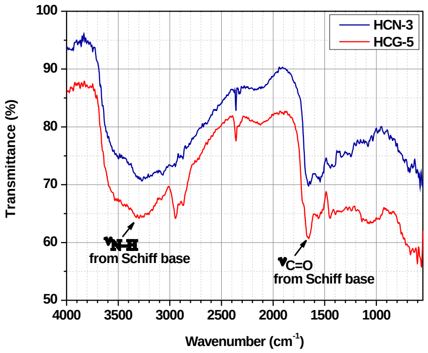

Figure 2. FT-IR spectra of collagen hydrolysate (HCN3) and of the corresponding hydrogel (HCG-5)

Swelling studies were done, in order to comparatively analyse the swelling behavior of the 3-D collagen networks. The swelling degree was determined by immersing a weighted xerogel disk (W_0) (with a diameter around 6 mm and a thickness in the range of 1.25-1.5 mm) into a determined volume of distilled water at a constant temperature (28ºC). The disks were extracted from the water at certain time intervals, superficially wiped with filter paper, weighted (W_t) and sank again. The swelling experiments were carried out until the swelling degree of the hydrogel reached a constant value, corresponding to the equilibrium swelling degree. The swelling degree was determined according to equation (1) (Cursaru *et al.*, 2008):

$$SD = (W_t - W_0)/W_0 \qquad (1)$$

Evolution of the swelling degree in time for the HCG-5, HCG-9 and HCG-11 hydrogels is presented in Figure 3.

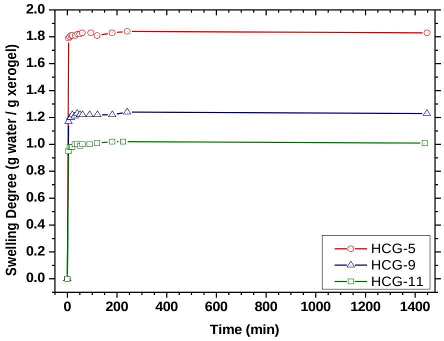

Figure 3. Evolution of the swelling degree in time for the HCG-5, HCG-9 and HCG-11 hydrogels

The equilibrium swelling degree (ESD) values were 1.83 for HCG-5, 1.23 in the case of HCG-9 and 1.02 corresponding to HCG-11.

Both the contact angle of a water droplet on a xerogel surface and the calculated surface energy are depicted in Figure 4, and together reveals the hydrophilic character of the hydrogels analysed.

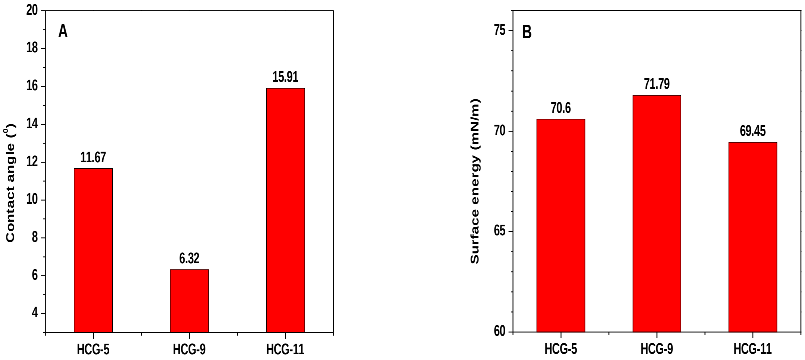

Figure 4. Contact angle of collagen networks (A) and the calculated surface energy (B) on HCG-5, HCG-9 and HCG-11 disks surface

From DSC curves depicted in Figure 5, one can observe a superior thermal stability of the xerogel (HCG-11) compared with the precursor hydrolysate (HCN2). This could be due to the cross-links present in the hydrogel network. Moreover, thermal degradation occurs in only two steps (at 180° and 333.8°C), comparing to collagen hydrolysate precursor, where more endothermic peaks can be observed.

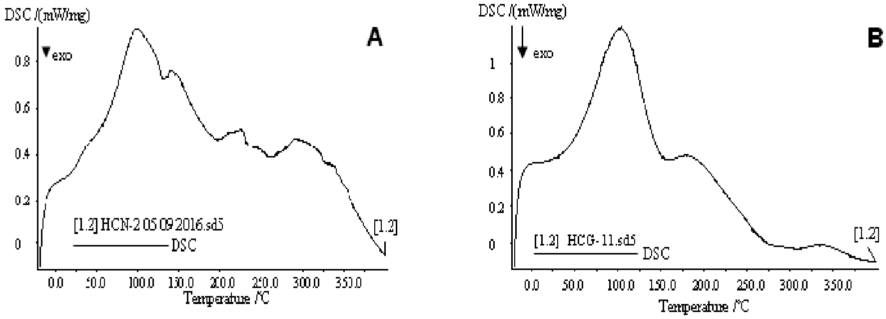

Figure 5. DSC curve for HCN2 hydrolysate and for HCG-11 xerogel (plasticized with 2.5% glycerol)

CONCLUSIONS

Some new collagen 3-D networks with potential applications in bioeconomy were obtained from two different bovine collagen hydrolysates and glutaraldehyde.

The FT-IR spectra revealed the characteristic peak of a Schiff-base compound only on collagen hydrogels spectra, which demonstrated the occurrence of cross-linking reaction between free amino groups present in collagen hydrolysates and the carbonyl function of glutaraldehyde.

The DSC thermograms revealed higher thermal stability in the case of the collagen hydrogels additivated with glycerol in comparison with the precursor collagen hydrolysate.

The contact angle determination demonstrated the hydrophilic character of the collagen hydrogels.

The HCG-5 hydrogel exhibited a higher swelling degree in comparison with the two others hydrogels, which contains glycerol.

Acknowledgement

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REFERENCES

Ahmed, E.M. (2015), “Hydrogel: Preparation, characterization, and applications: A review”, *Journal of Advanced Research*, 6, 105-121.

Biswal, D., Anupriya, B., Uvanesh, K., Anis, A., Banerjee, I. and Pal, K. (2016), “Effect of mechanical and electrical behavior of gelatin hydrogels on drug release and cell proliferation”, *Journal of the Mechanical Behavior of the Biomedical Materials*, 53, 174-186.

Cursaru, B., Stănescu, P. and Teodorescu, M. (2008), “Compression properties of hydrogels synthesized from diepoxy-terminated poly(ethylene glycol)s and aliphatic polyamines”, *Materiale Plastice*, 45(4), 314-319.

Fiorani, A., Gualandi, C., Panseri, S., Montesi, M., Marcacci, M., Focarete, M.L. and Bigi, A. (2014), “Comparative performance of collagen nanofibers electrospun from different solvents and stabilized by different crosslinkers”, *Journal of Materials Science: Materials in Medicine*, 25, 2313-2321.

Langmaier, F., Mokrejs, P., Kolomaznik, K., and Mladek, M. (2008) “Plasticizing collagen hydrolysate with glycerol and low-molecular weight poly(ethylene glycols)”, *Thermochimica Acta*, 469, 52-58.

Niculescu, M.-D. and Gaidau, C. (2014), “New collagen extracts conditioned for applications in crop protection against pests”, *Revista de Chimie (Bucharest)*, 1457-1461.

Nistor, M.T., Vasile, C. and Chiriac, A.P. (2015), “Hybrid collagen-based hydrogels with embedded montmorillonite nanoparticles”, *Materials Science and Engineering*, C53, 212-221.

Osborne, C.S., Reid, W.H. and Grant, M. H. (1997), “Investigation into cell growth on collagen/chondroitin-6-sulphate gels: the effect of crosslinking agents and diamines”, *Journal of Materials Science: Materials in Medicine*, 8, 179-184.

Pei, Y., Yang, J., Liu, P., Xu, M., Zhang, X. and Zhang, L. (2013), “Fabrication, properties and bioapplications of cellulose/collagen hydrolysate composite films”, *Carbohydrate Polymers*, 92, 1752-1760.

Rault, I., Frei, V. and Herbage, D. (1996), “Evaluation of different chemical methods for cross-linking collagen gel, films and sponges”, *Journal of Materials Science: Materials in Medicine*, 7, 215-221.

Sionkowska, A., Skopinska-Wisniewska, J., Gawron, M., Kozlowska, J. and Planecka, A. (2010), “Chemical and thermal cross-linking of collagen and elastin hydrolysates”, *International Journal of Biological Macromolecules*, 47, 570-577.

Tan, H., Wu, B., Li, C., Mu, C., Li, H. and Lin, W. (2015), “Collagen cryogel cross-linked by naturally derivated dialdehyde carboxymethyl cellulose”, *Carbohydrate Polymers*, 129, 17-24.

Tian, Z., Liu, W. and Li, G. (2016), “The microstructure and stability of collagen hydrogel cross-linked by glutaraldehyde”, *Polymer Degradation and Stability*, 130, 264-270

Zhang, X., Chen, X., Yang, T., Zhang, N., Dong, L., Ma, S., Liu, X., Zhou, M. and Li, B. (2014), “The effects of different crossing - linking conditions of genipin on type I collagen scaffolds: an in vitro evaluation”, *Cell Tissue Bank*, 15, 531-541.

DERMATOCOSMETICS FACIAL MASKS FOR TOPICAL TREATMENT OF ACNE

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Acne is one of the most common skin diseases affecting mostly adolescents, but can occur also into adulthood. Acne can have profound psychological and social effects, not only for high severity acne, but even in less severe cases. *Staphylococcus epidermidis* (*S. epidermidis*) bacteria and *Propionibacterium acnes* (*P. acnes*) bacteria are considered to cause this disease. Over time they have used many treatments for acne especially antibiotics, metronidazole showing positive effects and long-lasting. Thus, the purpose of this study was to design and investigate some facial masks in form of membranes with collagen and metronidazole to reduce and prevent adverse effects of conventional treatments using for acne. Type I fibrillar collagen gel was the main component of all masks. Hydrogels based on collagen, metronidazole, starch and polyvinilpyrolidone showed a pseudoplastic behavior with yield stress facilitating their flow and allowing their good manipulation. The membranes were obtained by drying the hydrogels in controlled environment and characterized by water absorbtion and enzymatic degradation. The results relieves that the presence of polymers (starch and polyvinylpirolidone) influence the stability and integrity of the membranes obtain. Based on these results, we could conclude that the obtained masks are potentially usable as a favorable solution in acne disease.

Keywords: acne disease, collagen, masks.

INTRODUCTION

Acne is one of the most common skin diseases affecting mostly adolescents, but can occur and adulthood. Worldwide, about 85% of young people aged between 12 and 25 years are affected by acne (Kim and Michaels, 2012). In Romania, over 90% of adolescents have acne, 50% of adult women, 25% of all adults, regardless of gender, suffer from acne, according to studies by Romanian Society of Dermatology. This disease affects the skin of the face, the neck and the upper torso, forming non-inflammatory lesions (comedones) or inflammatory lesions (papules, pustules and nodules). *Staphylococcus epidermidis* (*S. epidermidis*) and *Propionibacterium acnes* (*P. acnes*) bacteria that are considered main cause of this disease (Sawarkar *et al.*, 2010). Genetic and environmental factors contribute to the pathogenesis of acne (Ballanger *et al.*, 2006).

Nowadays different topical therapies are available for patients with acne, including comedolytic agents, anti-inflammatory medications, antibiotics, and even herbal preparations. Metronidazole, an imidazole, is an antibacterial agent that has been shown to be effective in reducing acne inflammation when is administered orally or in form of an aqueous gel for topical application. Its mechanism of action in acne is thought to be associated with its anti-inflammatory, immunosuppressive, and antimicrobial properties (Khodaeiani *et al.*, 2012). Studies have shown that metronidazole can be released in a sustained manner for a period of one week. This study aimed to evaluate the efficacy of 0.95% metronidazole in combination with 1% collagen in a facial mask for the treatment of acne. Collagen has become an important component in cosmetics formulations providing numerous benefits. In particular, collagen is a natural humectant efficient because of the hydration orderly sphere that surrounds the molecule (Peng *et al.*, 2004). Regarding acne, there are studies that claim that collagen degradation is responsible for acne scars, healing them being related to the rearrangement of collagen fibers (Fabbrocini *et al.*, 2012).

The aim of this study is to obtain and characterize collagen-metronidazole masks for treatment of acne.

MATERIALS AND METHODS

Materials

Type I fibrillar collagen gel (Coll) was extracted from calf hide with initial concentration of 2.84 % (w/w), using technology previously described (Albu, 2011). Glycerine (GL) was purchased from Romaqua Holdings, Romania, Ethanol (ET) from Chemreactiv S.R.L., Romania, Metronidazole (MN) from Hubei Hongyuan Pharmaceutical technology Co.,Ltd., China, Starch (ST), Polyvinylpirolidone (PVP) and Glutaraldehyde (GA) from Merck, Germany, *Collagenase* from Sigma Aldrich, Germany and Sodium hydroxide from Lach-Ner, Czech Republic.

Collagen Hydrogels and Masks Preparation

Collagen hydrogels were obtained by continuous stirring of gels adjusted at 1% and 7.2 pH, using 1M sodium hydroxide, with glycerine, ethanol, and added different concentrations of metronidazole, starch and polyvinylpirolidone, reported to collagen dry substance, as it is shown in Table 1. Then, all the gels were crosslinked with a specific amount of glutaraldehyde (0.5% reported to collagen dry substance), cast in Petri dishes resulting hydrogels which were dry in oven at 37°C, obtaining collagen membranes - masks.

Table 1. Collagen hydrogel compositions

Code of gels	Coll, %	GL, %	ETH, %	MN, %	ST, %	PVP, %	GA, %
G1	1	2.53	7.2	-	-	-	0.5
G2	1	2.53	7.2	1.26	-	-	0.5
G3	1	2.53	7.2	1.26	0.26	-	0.5
G4	1	2.53	7.2	1.26	-	0.26	0.5

Methods of Analyses

Rheological Analysis

The rheological measurements were conducted at 37^{o}C with a rotational viscometer MultiVisc-Rheometer, Fungilab equipped with a standard spindle TR 9 and an

Ultrathermostat ThermoHaake P5 to keep constant the temperature during the experiments. The operational conditions for rheological determinations were detailed in our previous studies (Ghica *et al*., 2012).

The rheograms shear stress versus shear rate was built. For the quantification of the flow behaviour, different rheological models were used: *Casson* (eq. 1), *Bingham* (eq. 2), *Ostwald-de Waelle* (eq. 3) and *Herschel-Bulkley* (eq. 4) (Albu, 2009) and the determination coefficients (R^2) values were used as an indicator to chose the one that best fitted the flow profiles (Paunica-Panea *et al*., 2016).

$$\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5} \cdot \dot{\gamma}^{0.5} \qquad (1)$$

$$\tau = \tau_0 + \eta \cdot \dot{\gamma} \qquad (2)$$

$$\tau = K \cdot \dot{\gamma}^n \qquad (3)$$

$$\tau = \tau_0 + K \cdot \dot{\gamma}^n \qquad (4)$$

where, τ is the shear stress (Pa), $\dot{\gamma}$ – shear rate (s^{-1}), η– plastic viscosity (Pa·s), τ_0– yield stress (Pa) related to the minimum stress to be applied for determining the start of hydrogel flow, K – consistency index ($Pa\cdot s^n$) associated with the hydrogel viscosity, n – flow behavior index indicating the flow profiles ((Paunica-Panea *et al*., 2016; Ghica *et al*., 2016).

Water Up-take

Collagen membranes obtained by drying in oven were tested by water up-take. They were firstly immersed in water at 36°C then withdrawn and weighed at fixed time intervals. The equation used (eq. 1) for water absorption determination was:

%Water up-take = (Wt – Wd)/ Wd (g/g) (5)

where Wt is the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Enzymatic Degradation

Enzymatic degradation of collagen membranes was investigated by monitoring the weight loss depending on exposure time to collagenase solution. Small pieces of collagen membranes were accurately weighed, placed in collagenase (1 μg/mL) and incubated at 36°C. At regular time intervals, the swollen membranes were removed from degradation solution and weighted. The percentage of membrane degradation was determined by the following relation:

*% weight loss = (Wi- Wt)/Wi*100* (6)

where *Wi* is the initial weight and *Wt* is the weight after time t.

RESULTS AND DISCUSSION

The influence of the hydrogels composition on the rheological profiles plotted as shear stress as a function of shear rate is presented in Figure 1a and 1b.

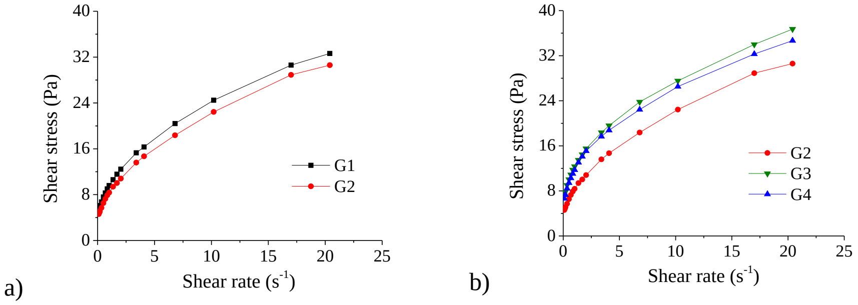

Figure 1. The flow profiles recorded at 37°C for hydrogels

a) G1 and G2 b) G2, G3 and G4

The rheograms recorded in Figures 1a and b shown a non-Newtonian behaviour, the shear stress increasing with shear rate increase. The determination coefficients R2 obtained for the rheological models (eqs. 1-4) mentioned at Materials and Methods section are given in Table 1.

Table 1. The determination coefficients values of different rheological models for hydrogels G1-G4 analyzed at 37°C

Rheological models	R2 values			
	G1	G2	G3	G4
Casson	0.9878	0.9901	0.9856	0.9881
Bingham	0.9421	0.9504	0.9317	0.9227
Ostwald-deWaele	0.9937	0.9939	0.9937	0.9959
Herschel-Bulkley	0.9994	0.9994	0.9998	0.9996

As can be seen in Table 1, the highest values for R2 were obtained for Herschel-Bulkley, indicating that this model best fitted the rheological experimental data. The parameters characteristic for this model are presented in Table 2.

Table 2. Rheological parameters specific to Herschel-Bulkley model for hydrogels G1-G4 tested at 37°C

Rheological parameters	Hydrogels			
	G1	G2	G3	G4
Yield stress (Pa)	3.207	2.472	4.277	3.358
Consistency index (Pa·sn)	6.421	5.588	7.962	8.358
Flow index	0.509	0.538	0.465	0.438

The values recorded for flow index are smaller than 1, indicating a pseudoplastic behavior. The adding of metronidazole in formulation G2 determined a decrease of yield stress about 23% and of consistency index about 13% respectively, in comparison with hydrogel G1. The presence of starch and PVP in hydrogels formulation G3 and G4 led to an increase of yield stress about 1.73 and 1.36 times respectively comparing to hydrogel G2, while for the consistency index the increase was aproximately 1.42 and 1.50 times respectively.

The results of water up-take studies are presented in Figure 2.

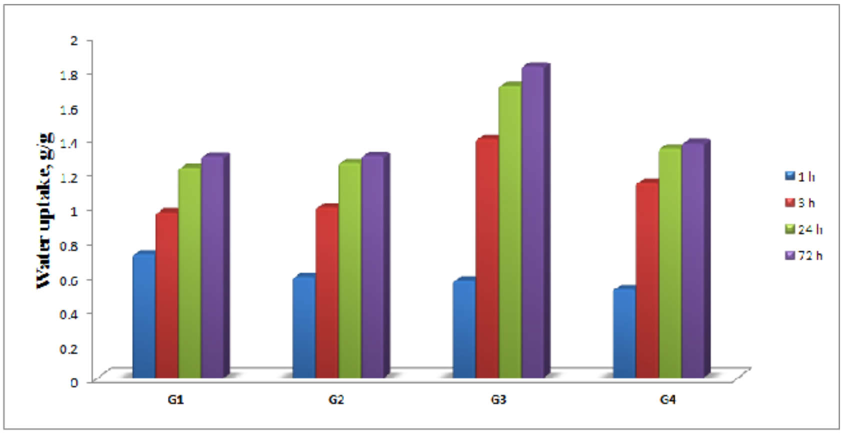

Figure 2. Water up-take for collagen membranes

Figure 2 showed that swelling was influenced by the presence of polymers added in each membrane. The highest water up-take was recorded for membranes which have starch in composition, this being the most hydrophilic one. The metronidazole and PVP did not influence too much the water up-take, having similar values. Also the difference of water up-take after one and three days is not too high, showing the stability of the membranes.

The results of enzymatic degradation presented in Figure 3 showed that de membranes degraded up to 15.23% (G3) in first hour.

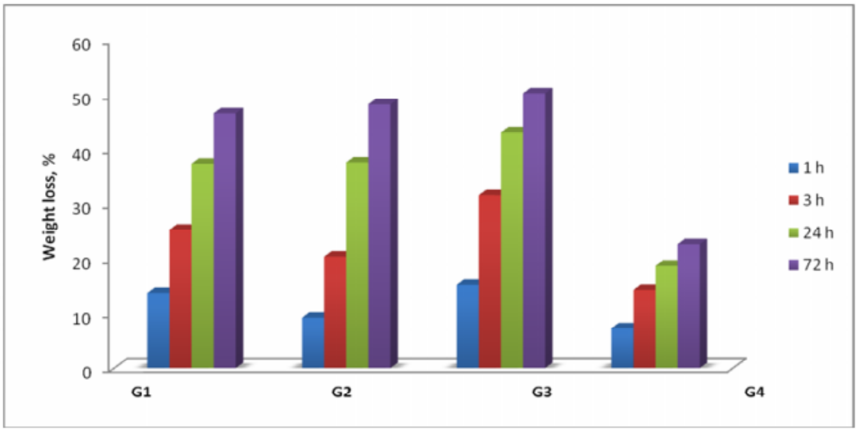

Figure 3. Enzymatic degradation for collagen membranes

After one day, G1 and G2 degraded about 37% and G3 about 43%. These results are in accordance with water up-take and it was expected for sample G3, which was the most hydrophilic to be fast degradable. The samples containing PVP showed the best resistance to collagenase solution, PVP being a good stabilizer in cosmetics. The results of degradation are in correlation with rheological and water up-take results.

CONCLUSIONS

Collagen hydrogels with metronidazole and polymers (starch and polyvinil-pyrolidone) were prepared and rheologically analysed in ordor to be used for masks preparation. Rheological studies show that all the designed hydrogels presented a pseudoplastic character with yield stress facilitating their flow and allowing their good manipulation. Absorbtion and enzymatic degradation studies relieves that the presence of polymers (starch and polyvinylpirolidone) influence the stability and integrity of the membranes obtain. Based on these results, we could conclude that the membranes obtained are potentially usable as a dermatological masks for treating acne disease.

Acknowledgements

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REFERENCES

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Albu, M.G., Ghica, M.V., Popa, L., Leca, M. and Trandafir, V. (2009), “Kinetics of *in vitro* release of doxycycline hyclate from collagen hydrogels”, *Revue Roumaine de Chimie*, 54(5), 373-379.

Ballanger, F., Baudry, P., N’Guyen, J.M., Khammari, A. and Dreno B. (2006), “Heredity: a prognostic factor for acne”, *Dermatology*, 212(2), 145-149.

Fabbrocini, G., De Vita, V., Cozzolino, A., Marasca, C., Mazzella, C., and Monfrecola, A. (2012) “The management of atrophic acne scars: overview and new tools”, *Journal of Clinical and Experimental Dermatology Research*, 34, 874-879.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C., Moisescu, S. (2012), “*In vitro* kinetic release and flow behaviour of some collagen-minocycline topical hydrogels”, *Revista de Chimie*, 63(9), 929-935.

Ghica, M.V., Hîrjău, M., Lupuleasa, D., Dinu-Pîrvu, C.E. (2016), “Flow and thixotropic parameters for rheological characterization of hydrogels”, *Molecules*, 21(6), 17 pages.

Khodaeiani, E., Rohollah, F.F., Neda, Y., Mehdi, A., Shahla B. and Javad, S. (2012), “Efficacy of 2% metronidazole gel in moderate acne vulgaris”, *Indian Journal of Dermatology*, 54(4), 279-281.

Kim, G.K. and Michaels B.B. (2012), “Post-adolescent acne in women: more common and more clinical considerations “, *Journal of Drugs in Dermatology*, 11(6), 708-713.

Paunica-Panea, G., Ficai, A., Marin, M.M., Marin, S., Albu, M.G., Constantin, V.D., Dinu-Pîrvu, C., Vuluga, Z., Corobea, M.C., Ghica, M.V. (2016), “New collagen-dextran-zinc oxide composites for wound dressing”, *Journal of Nanomaterials*, 2016, 7 pages.

Peng, Y., Glattauer, V., Werkmeister, J.A. and Ramshaw, J.A.M. (2004), „Evaluation for collagen products for cosmetic application”, *Journal of Cosmetic Science*, 55, 327-341.

Sawarkar, H.A., Khadabadi, S.S., Mankar, D.M., Farooqui I.A. and Jagtap N.S. (2010), “Development and biological evaluation of herbal anti-acne gel“, *International Journal of Pharmacy and Technology Research*, 2(3), 2028-2031.

SYNERGISTIC ACTIVITIES OF THE ESSENTIAL OILS HYPERICUM PERFORATUM WITH METHOTREXATE ON HUMAN BREAST CANCER CELL LINE MCF-7

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Hypericum perforatum (Hypericaceae) is a perennial plant usually known as “Sarikantaron” that has been reported to have various important biological activities.In this present study, it was aimed to (I) identify the components (ii) show the synergistic activities of the essential oils *Hypericum perforatum* with methotrexate on human breast cancer cell line MCF-7. A normal [Madin-Darby Bovine Kidney (MDBK) cell line] and a cancer cell line [Human breast adenocarcinoma cell line (MCF-7)] were used in this study. The cell culture were treated with various concentrations of *Hypericum perforatum*’s oils. The cytotoxic activity of the essential oils of *Hypericum perforatum* on the cell lines was measured using the MTT method and the results were evaluated as IC_{50}. In this study, the presence of trans-caryophyllene, germacrene-D, α-pinene, trans-cadina-1,4-diene, β-Selinene, caryophyllene oxide and α-Selinene were identified as major constituents of *Hypericum perforatum*’s oils. The essential oils of *Hypericum perforatum* also exhibited anticancer activities against MCF-7 cells. The IC_{50} values of the essential oils, MTX and the essentialoilsplus MTX weredetermined as 0.78, 6.25 and 0.195 µg/ml, respectively, But, the essential oils of *Hypericum perforatum* was found to be non-cytotoxic for MDBK cells. In conclusion, the essential oils of *Hypericum perforatum* was chemically characterized and γ-muurolene, δ-cadinene, germacrene B, α-copaene, germacrene D, bicyclogermacrene, and (E)-caryophyllene were found to be major constituents. The essential oils of *Hypericum perforatum* possess significant *in vitro* anticancer potential. The essential oils of *Hypericum perforatum* with MTX found to be significant effective against breast cancer cells. Further studies especially *in vivo* anticancer properties of *Hypericum perforatum* should be searched. These compounds found to be very promising compounds in the treatment of cancer therapy.

Keywords: MCF-7 cell line, anticancer, *Hypericum perforatum*, essential oil, synergistic activity.

INTRODUCTION

Breast cancer is one of the most common types of cancer in women. Also, it can be seen in both women and men. Breast cancer is the major cause of death among women (Siegel *et al*., 2014).

Cancer is one of the most important diseases of this of this century.Chemotherapy for the treatment of breast cancer is one of the most effective treatment options. Unfortunately, there is no effective drug for the treatment of certain cancers. There are common side effects of current drugs used in cancer treatment, as well as low efficiency in treatment. furthermore, drug resistance in cancer treatment is another serious problem (Raguz, 2008).

Therefore, the new drug researches for cancer chemotherapy continue. An important part of the studies on this topic has focused on natural products (Burmaoglu *et al*., 2016). Because its many pharmacological features, *Hypericum perforatum* L. is interesting plant species for cancer research. *Hypericum perforatum* L. is a member of the *Hypericaceae* is reported to identify more than 400 species in the world (Mabberley, 1987). It can grow in many countries the world as well as in our country. *H. perforatum* is reported to have very important pharmacological properties among medicinal plants (Wills and Bone, 2000). Hypericum species reported to use in the folk medicine for the treatment of many diseases such as skin wounds, burns, eczema, gastrointestinal disorders and psychological disorders (Butterweck, 2003).

In this study, we aimed to investigate the anticancerogen activity of *Hypericum perforatum* on human breast adenocarcinoma (MCF-7) cell line. Also, it was aimed to search the synergistic activities of the essential oils *Hypericum perforatum* with MTX (Methotrexate) on MCF-7cells.

MATERIALS AND METHODS

Isolation Of The Essential Oils

Herba of *Hypericum perforatum* were collected from Amanos Mountain (Anatolian region) in blooming period and dried at room temperature. Essential oils were obtained from dried leaves and flowers. The aerial parts of *Hypericum perforatum* (100 g) were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and then stored in dark color glass bottles, at -4 ^{o}C ready for GC-MS analysis.

Analysis of GC/MS

Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, autosampler and TR-5MS (5% Phenyl Polysilphenylenesiloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 ^{o}C, MS Ionization source temperature was 220 ^{o}C, the injection port temperature was 220 ^{o}C. The samples were injected with 250 split ratio. The injection volume was 1 µL. Oven temperature was programmed in the range of 50 to 220 ^{o}C at 3 ^{o}C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley 9 library). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

Table 1. Essential oil components of *Hypericum perforatum*

RT	RI	Compound Name	Cas #	Area %
2,36	902	Nonane	111-84-2	1,28
2,93	970	Nonane, 3-methyl	5911-04-6	0,54
3,68	1031	α-Pinene	80-56-8	10,59
4,14	1060	Decane, 2-methyl	6975-98-0	0,81
4,94	1101	Undecane	1120-21-4	0,52
5,19	1114	β-Pinene	127-91-3	0,64
6,56	1172	β-Myrcene	123-35-3	0,66
9,15	1260	cis-Ocimene	6874-10-8	1,16
16,58	1465	α-Longipinene	5989-08-2	0,31
17,20	1480	α-Ylangene	14912-44-8	0,3
18,53	1514	β-Bourbonene	5208-59-3	0,26
20,41	1564	β-Sesquiphellandrene	20307-83-9	1,36
21,45	1590	trans-Caryophyllene	87-44-5	17,13
23,05	1634	β-Chamigrene	18431-82-8	0,46
23,19	1637	Valencene	4630-07-3	0,25
24,12	1662	α-Humulene	6753-98-6	1,05
24,38	1669	β-Farnesene	18794-84-8	2,69
25,00	1685	Junipene	475-20-7	0,21
25,59	1700	Germacrene-D	23986-74-5	11,22
25,91	1709	β-Selinene	17066-67-0	6,99
26,05	1714	trans-Cadina-1,4-diene	87-44-5	8,84
26,12	1716	α-Selinene	473-13-2	4,63
26,49	1726	Bicyclogermacrene	100762-46-7	1,36
26,59	1729	α-Bergamotene	17699-05-7	0,98
27,47	1754	β-Elemene	515-13-9	2,53
34,89	1969	Caryophyllene oxide	1139-30-6	5,26
37,26	2043	Nerolidol	7212-44-4	1,06
39,58	2126	Spathulenol	77171-55-2	0,77
39,84	2139	1-Dodecanol	112-53-8	3,93
39,99	2146	junipercamphor	NA	0,31
41,33	2206	1-Hexadecanol	36653-82-4	3,55
41,91	2221	Cubenol	21284-22-0	0,23
42,10	2226	α-Bisabolol	72691-24-8	0,39
42,42	2234	Longipinane	NA	0,56
42,84	2245	Veridiflorol	552-02-3	0,71
43,39	2259	Globulol	51371-47-2	1,6
47,01	2370	Junipene	475-20-7	0,38

Determination of Non-Toxic DMSO Concentration

To solve the plant essential oils, DMSO (dimethylsulfoxide) was used as a potent solvent. To determine the non-toxic concentration of DMSO on MDBK, 1×10^6 cells were inoculated into each well of flat bottomed plates. RPMI-1640 was selected as a growth medium. Plates were incubated 96 hours in the presence of decreasing amounts of DMSO (8%, 4%, 2%, 1%, 0.5%).

To determine the non-cytotoxic concentration of *Hypericum perforatum* Madin-Darby Bovine Kidney (MDBK) cell line was selected. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v). Cells were incubated in a humidified atmosphere at 37 ºC in 5% CO_2.

Cytotoxic Tests

For this purpose, the MTT assay was selected. MTT assay was performed as described previously (Mosmann, 1983). The normal and cancer cells were cultured in RPMI-1640 medium with 10% (w/v) fetal bovine serum. Incubation of the cells was performed at 37°C with 95% air and 5% carbon dioxide. The essential oils of the plants were dissolved in DMSO at the concentration of lower than 1%. The culture cell is inoculated in 96-well plates overnight. After 96 hours of incubation with plants esssential oils, the cells were washed with PBS. And then, 100 µL of 0.5 mg/mL MTT were added to all wells and incubated at 37 °C. The plate was incubated for 30 minutes at the same conditions. MTT reduction ratio was determined by measuring the difference in absorbance at 570 and 650 nm using a microplate reader. All tests were performed in triplicate.

Statistical Analysis

All data were obtained in the experiments repeated three times. Statistical analyses were performed using Student t-test. The p value<0.05 was considered significant. All statistics in the present study were done using SPSS program.

RESULTS AND DISCUSSION

The chemical composition of the essential oils investigated in this study is shown in Table 1. *Hypericum perforatum* has been a rich constituents such as trans-Caryophyllene (17.13%), Germacrene-D (%11.22), α-Pinene (10.59%), trans-Cadina-1,4-diene (8.84%), β-Selinene (6.94%), Caryophylleneoxide (5.26%) and α-Selinene (4.63%), 1-Hexadecanol (3.93%), β-Farnesene (2.69%), β-Elemene (2.53%), Nonane (1.28%) and Nerolidol (1.06%).

Trans-Caryophyllene (17.13%), Germacrene-D (%11.22), α-Pinene (10.59%), trans-Cadina-1,4-diene (8.84%), β-Selinene (6.94%), Caryophylleneoxide (5.26%) and α-Selinene (4.63%) were identified as the major constituents in the essential oil of *Hypericum perforatum*. In studies conducted in previous years in the literature, caryophyllene, germacrene-D and α-pinene have been reported that place among the important components of various medical plants. The activities of these components were investigated on some cancer cell lines (Amiel *et al*., 2007; Ofir *et al*., 2012; Quintans *et al*., 2013).

Today, there are a limited numbers and drug activity in cancer chemotherapy. Methotrexate is a commonly used drug for the treatment of many cancers types such as breast cancer (http://www.breastcancer.org). Due to the many side effects of MTX and the low treatment success, new active molecules investigation continues intensively. We have investigated the anticancer activity and the existence of a synergistic effect of *Hypericum perforatum*'s oils with MTX in this study.

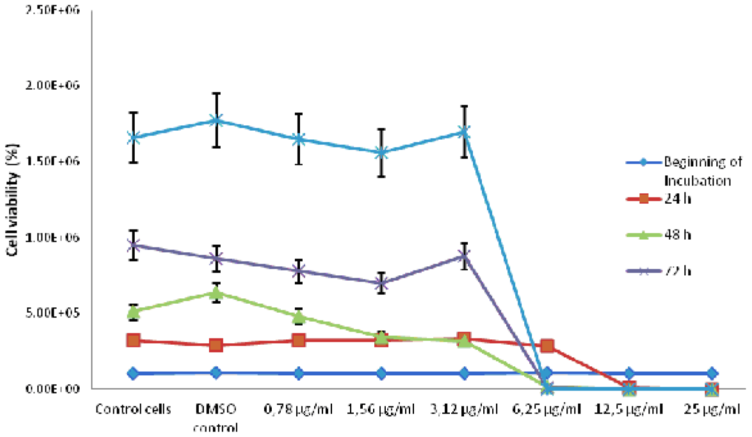

Figure 1. Effects of the essential oils of *Hypericum perforatum* on the proliferation of MCF-7 cells

The Inhibition concentrations of the essential oils of *Hypericum perforatum* against cancer (MCF-7) and normal cells (MDBK) cells were evaluated. The IC_{50} values were determined as 6.25 µg/ml and 25 µg/ml, respectively. The IC_{50} value for MTX against MCF-7 cells was determined to be 0.78 µg/ml. This value was calculated for the essential oils of *Hypericum perforatum* (EO of HP) plus MTX as 0.195 µg/ml. It had been found that the essential oils of *Hypericum perforatum* enhanced synergistically the effect of MTX against MCF-7 cells (Figure 2).

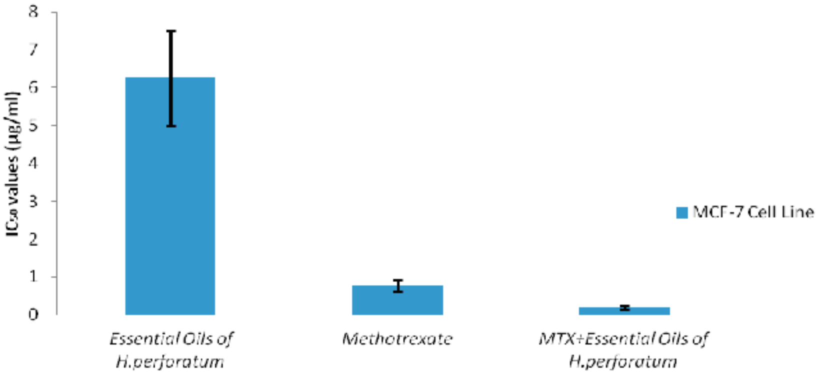

Figure 2. Effects of the essential oils of *Hypericum perforatum* on the proliferation of MCF-7 cells

In Figure 2, the synergistic effects of the methotrexate with the essential oils of *Hypericum perforatum* were investigated. As is clear in the figure 2, the essential oils of *Hypericum perforatum* significantly raised the effectiveness of the standard anticancerogen drug. A synergistic activity was determined between *the essential oils of Hypericum perforatum* and methotrexate.

CONCLUSIONS

In conclusion, *Hypericum perforatum* collected from the South of Turkey (Hatay region) quite inhibited human lung adenocarcinoma cells, and was not toxic to normal cells. Besides this, the strong synergistic activity between methotraxate and *Hypericum perforatum* was determined. We think that *Hypericum perforatum* L. may be a promising natural product for the breast cancer chemotherapy. However, further studies are needed on this issue.

REFERENCES

Amiel, E., Legault, J. and Pichette, A. (2007), "Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel", *Journal of Pharmacy and Pharmacology*, 59, 1643-7.

Burmaoglu, S., Algul, O., Anıl, D.A. *et al*. (2016), "Synthesis and anti-proliferative activity of fluoro-substituted chalcones", *Bioorganic & Medicinal Chemistry Letters*, 26, 3172-6.

Butterweck, V. (2003), "Mechanism of action of St. John'sWort in depression. What is known?", *CNS Drugs*, 17, 539-62.

Mabberley, D.J. (1987), *The Plant Book*, Cambridge University Press, Cambridge.

Mosmann, T. (1983), "Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays", *Journal of Immunological Methods*, 65, 55-63.

Ofir, R., Dudai, N. and Soloway, E. (2012), "β-Caryophyllene, a Compound Isolated from the Biblical Balm of Gilead (*Commiphora gileadensis*), Is a Selective Apoptosis Inducer for Tumor Cell Lines", *Evidence-Based Complementary and Alternative Medicine*, Article ID 872394-12.

Quintans, J.S., Soares, B.M., Ferraz, R.P. *et al*. (2013), "Chemical constituents and anticancer effects of the essential oil from leaves of Xylopialaevigata", *Planta Medica*, 79, 123-30.

Raguz, S. and Yague, E. (2008), "Resistance to chemotherapy: New treatments and novel insights into an old problem", *British Journal of Cancer*, 99, 387-91.

Siegel, R., Ma, J., Zou, Z., *et al*. (2014), "Cancer Statistics, 2014", *CA: A Cancer Journal for Clinicians*, 64, 9-29.

Wills, R.B.H., Bone, K. and Morgan, M. (2000), "Herbal products: active constituents, models of action and quality control", *Nutritional Research Reviews*, 13, 47-77.

*** http://www.breastcancer.org/treatment/druglist/methotrexate.

IN VITRO CYTOTOXIC ACTIVITY OF *NIGELLA SATIVA* L. ON HUMAN MALIGNANT MELANOMA CELL LINES

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Nigella is a medicinal plant that has various pharmacological properties. It is widely used in folk medicine to treat many diseases in the world. It was aimed to investigate the essential oils *Nigella sativa* L. on two human melanoma cells (A-375 and A-2058 cell lines). In this study, two different cancer cell lines (A-375and A-2058 cell lines) and one normal cell culture (vero cells) were used. Firstly, the non-toxic concentrations the essential oils of *Nigella sativa* L. were determined on normal cells (Vero cell line). And then, the cytotoxicity test were applied at these non-toxic concentrations. The cytotoxic activity of the essential oils of *Nigella sativa* L. on the cell lines was measured by MTT and Trypan blue assays. The chemical components analyses of the *Nigella sativa* L. oils were carried out by GC-MS. Medically important various components of *Nigella sativa* L. were determined in the GC-MS analysis. Some of these components are as follows; anethole (22.97%), thymoquinone (21.36%), α-thujene (6.22%), longifolene (5.76%), trans-isoeugenol (3.55%), carvacrol (2.23%). A total of 24 compounds were identified. The essential oils of *Nigella sativa* L. were found to inhibit the cell proliferation of human malignant melanoma cells. The IC50 values of the essential oils of *Nigella sativa* L. It were investigated as compared with a standard drug (methotrexate). Statistically significant decrease on the cell proliferations was found in the cells treated with the essential oils of *Nigella sativa* L. It was found to be non-toxic on normal cells (on Vero cell line) at the effective concentrations of the essential oils. While the non-toxic concentrations of essential oils on Vero cell line were found to be 12.5 µg/ml, the effective concentrations for A-375 and A-2058 cell lines were as 1.56 µg/ml and 3.12 µg/ml, respectively. In conclusion, the essential oils of *Nigella sativa* L. were found to be significantly inhibited the cell proliferation on the human melanoma cells. The effect may arise from the components situated in the structure of *Nigella sativa* L. such asanethole, thymoquinone, α-thujene, longifolene, trans-isoeugenol and carvacrol. These components of this plant have been found promising in the treatment for human melanomas. But further studies, especially further animal studies should be needed to explain the action mechanisms of these components.

Keywords: A-375 cell line, A-2058 cell line, melanoma, cytotoxicity, proliferation, MTT.

INTRODUCTION

Turkey has a rich flora in terms of medicinal plants. There is plenty of endemic plant species especially in the Hatay region located in the south of Turkey. Nigella is a useful plant that naturally grown or cultured in Hatay commonly used in the treatment of various diseases. The seeds of *Nigella sativa L.* have been reported to use in the treatment of many different diseases and disorders. In Islamic literature, it is considered as one of the major herbal medicine (Ali and Blunden, 2003).

In folk medicine, Nigella oil has been widely used to treat many diseases such as peritonitis, cough, bronchitis, oedama, headache, rheumatism, fever, influenza and eczema for centuries in many countries (Burits and Bucar, 2003).

Some studies were carried out about the anti-carcinogenic effects of *Nigella spp.*, although the number of studies on human malignant melanoma is quite limited (Alenzi *et al*., 2010; Alhebshi *et al*., 2013; El-Mahdy *et al*., 2005; Ulasli *et al*., 2013; Woo *et al*., 2011).

Even, we did not find any studies that investigate the effectiveness of *Nigella sativa* L. against human melonoma cells. Melanoma is a skin malignant tumor that occurred as a result of the melanocyte transformation and one of the important cancer types for human health. Studies indicated that melanoma begin to appear more often than the other cancer types all over the world (Sun and Schuchter, 2001).

The incidence of melanoma has been reported to increase day by day. As in other cancers, there is no powerful drug choice for pharmacological treatment in melanoma chemotherapy. In addition to this, the drugs used in the treatment of human malignant melanoma have been reported to show high toxicity and low effectiveness. Hence, new drug research for the treatment of melanoma continues rapidly (Atallah and Flaherty, 2005; Goldberg *et al*., 2007).

In this study, we aimed to investigate *in-vitro* cytotoxic and antiproliferative activities of *Nigella sativa* L. on two human malignant melanoma (A-375 and A-2058 cell lines) cells compared to normal cells (Vero cell line).

MATERIALS AND METHODS

Isolation of the Essential Oil

Black Cumin (*Nigella sativa* L.) seeds were obtained from the spice bazaar of Antakya. Essential oils were obtained from seeds after powdered. The seeds of black cumin (100 g) were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. The oils were dried over anhydrous sodium sulfate and then stored in dark color glass bottles, at -4 °C ready for GC-MS analysis.

Analysis of GC/MS

Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylenesiloxane, 0.25 mm x 30 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250 °C, MS Ionization source temperature was 220 °C, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1 µL. Oven temperature was programmed in the range of 50 to 220 °C at 3 °C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley9 library). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

Table 1. Essential oil components *Nigella sativa* L.

RT	RI	Compound Name	Cas #	Area %
3,48	1016	α-Thujene	2867-05-2	6,22
3,65	1029	α-Pinene	80-56-8	3,71
4,06	1056	Camphene	79-92-5	0,93
5,06	1107	Sabinene	3387-41-5	1,83
5,19	1114	β-Pinene	127-91-3	1,46
6,56	1172	β-Myrcene	123-35-3	0,43
6,88	1183	Limonene	138-86-3	4,55
7,06	1189	Eucalyptol	470-82-6	0,74

RT	RI	Compound Name	Cas #	Area %
7,12	1191	β-Phellandrene	555-10-2	0,13
8,21	1230	γ-Terpinene	99-85-4	0,44
9,15	1260	p-Cymene	99-87-6	17,16
18,89	1524	trans-Sabinene hydrate	17699-16-0	0,31
19,36	1537	Linalyl acetate	115-95-7	0,22
19,94	1552	Endobornyl acetate	76-49-3	0,96
20,38	1563	Caryophyllene	87-44-5	0,67
20,89	1576	Terpinen-4-ol	562-74-3	0,3
25,38	1695	β-Bisabolene	495-61-4	0,21
29,03	1796	Anethole	4180-23-8	22,97
29,90	1822	p-Cymenol	1197-01-9	0,86
36,65	2023	Thymoquinone	490-91-5	21,36
40,09	2151	trans-Isoeugenol	5932-68-3	3,55
41,68	2215	Carvacrol	499-75-2	2,23
43,22	2255	Longifolene	475-20-7	5,76
44,65	2290	Longifolenaldehyde	19890-84-7	1,39

Cell Culture Studies

In the present study, as cancer cell cultures two different human malignant melanoma cell lines (A375 and A-2058 cell lines) and as a normal cell culture Vero cell line were selected. Human melanomas and Vero cell cultures were obtained Mustafa Kemal University, Virology laboratory's culture collections, Hatay, Turkey. The cells were cultured in RPMI-1640 medium, supplemented with 10% (v/v) fetal bovine serum, L-glutamine and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). The incubation of the cell cultures was performed at 37°C with 5% CO_2 in a humidified incubators.

Effect of DMSO

Dimethyl sulfoxide (DMSO) was selected as a solvent to solve the essential oils of *Nigella sativa* L. In order to test the effects of DMSO (Sigma, USA), $1x10^6$ cells were inoculated into each well of micro plates containing RPMI-1640 medium (Gibco-BRL). And then, the cultures were incubated for 72 hours in the presence of decreasing amounts of DMSO (4, 2, 1.5, 1.0, 0.8, 0.4, 0.2, 0.1%). The non-toxic concentration was determined equals and lower than 2%. In the experiments, the essential oils of *Nigella sativa* L. were dissolved in 1% DMSO.

Trypan Blue Exclusion

The cell viabilities in the cultures, trypan blue dye assay method was used. For this dying, 100 µl of *Nigella sativa* L.'s oils was mixed with 800 µl of PBS (phosphate buffer saline) and 100 µl cell suspension was added. Each concentration of the essential oils was performed in triplicate. Then, the prepared mixtures were incubated at 37°C in an incubator for 30 min. Finally, 100 µl of trypan blue dye was added to this mixture. The viability of the cells was determined in a haemocytometer by a light microscope (Gupta, 2002).

MTT Test

MTT assay was performed as previously described by Mossman (Mosmann, 1983). The Vero (normal cells), A375 (human melanoma) and A-2058 (human melanoma) cell lines were cultured in RPMI-1640 medium with 10% (w/v) fetal bovine serum. Incubation of the cells was performed above mentioned conditions. The culture cells inoculated in 96-well plates overnight. The plaques were assessed at the incubation of

96 hours. The cells in the plaques were washed at least three times with PBS after incubation, and then, 100 µL of 0.5 mg/mL MTT were added to all wells and incubated at 37 °C. The plaques were incubated for 30 minutes at the same conditions. MTT reduction ratio was determined by measuring the difference in absorbance at 570 nm using a microplate reader. All tests were performed in triplicate.

Statistical Analysis

All measurements were expressed as mean ± standard deviation. Statistical analyses of data were performed using Student t-test. The p value<0.05 was considered significant. All data were obtained in the experiments repeated three times. For statistical evaluation, SPSS, a Windows based program was used to assess data.

RESULTS AND DISCUSSION

The chemical composition of the essential oils of *Nigella sativa* L. is given in Table 1. The chemical components analyses of the *Nigella sativa* L. oils were carried out by GC-MS. A total of 24 compounds of *Nigella sativa* L.'s oils were identified. In the GC-MS analysis, *Nigella sativa* L. was found to have many medically important components. Some of these these valuable components and their percentages as follows; anethole (22.97%), thymoquinone (21.36%), α-thujene (6.22%), longifolene (5.76%), trans-isoeugenol (3.55%), carvacrol (2.23%). The essential oils of *Nigella sativa* L. were found to inhibit the cell proliferation of human malignant melanoma cells. The IC_{50} values of the essential oils of *Nigella sativa* L. were compared with a standard drug (Methotrexate).Statistically significant decrease on the cell proliferations was found in the cells treated with the essential oils of *Nigella sativa* L. It was found to be non-toxic on Vero cell line at these effective concentrations of the essential oils.

In the present study, while the non-toxic concentrations of essential oils on Vero cell line were found to be 12.5 µg/ml, the effective concentrations for A-375 and A-2058 cell lines were as 1.56µg/ml and 3.12 µg/ml, respectively (Figure 1).

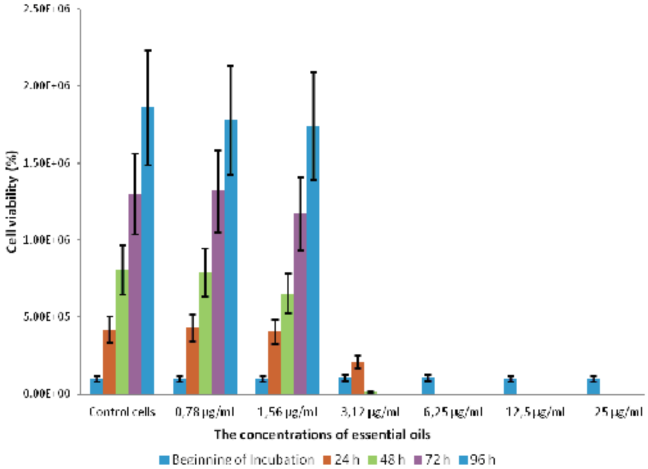

Figure 1. Cytotoxicity of the essential oils of *Nigella sativa* L. on A-375 cell line

When the essential oils of *Nigella sativa* L. compared to MTX, the efficacy of *Nigella sativa* L. found to be lower than MTX. But, cancer cell proliferation significantly inhibited by the essential oils of *Nigella sativa* L. (Figure 2).

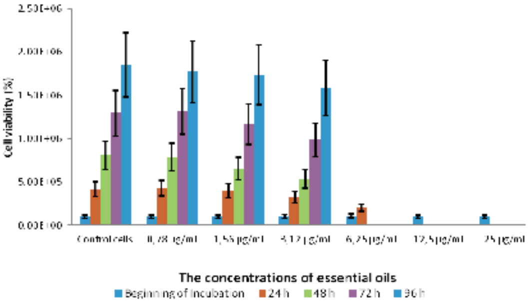

Figure 2. Cytotoxicity of the essential oils of Nigella sativa L. on A-2058 cell line

In the GC-MS analyses, anethole (22.97%), thymoquinone (21.36%), α-thujene (6.22%), longifolene (5.76%), trans-isoeugenol (3.55%), carvacrol (2.23%) were identified as the major constituents of *Nigella sativa* L. The studies conducted previously in the literature reported that the phytochemical anethole possesses the anticancer activity against breast cancer cells (Muthukumari *et al*., 2013). Thymoquinone is one of the most common components of the *Nigella sativa* L. Similarly, thymoquinone has reported to have a significant anticancerogen agent for mouse colon carcinoma (MC38) cells (Agbaria *et al*., 2015). Before the studies, it's reported that carvacrol has an anti-proliferative and pro-apoptotic effects on human hepatocellular carcinoma cell line HepG-2 (Yin *et al*., 2012).

The components of *Nigella sativa* L. on various cancer cell lines were studied, though, we have not found any study that performed on human melanoma cells. As we know, this study is one of the first studies carried out on human melanoma cells in the south of Turkey.

Besides, in the experiment, the inhibition concentrations of the essential oils of *Nigella sativa* L. against as cancer cell cultures in two different cell lines (A-375 and A-2058 cell lines) and a healthy cell line (Vero cell line) were evaluated. While the IC_{50} values of the essential oils of *Nigella sativa* L. against A-375 and A-2058 cell lines were determined as 1.56 µg/ml and 3.12 µg/ml, respectively, this value for Vero cells was determined as 12.5µg/ml. Although, the IC_{50} values for MTX against A-375 and A-2058 cell lines were determined to be lower than *Nigella sativa* L., the essential oils of *Nigella sativa* L. were found to be highly active to inhibit the proliferation of melanoma cells (Figure 3).

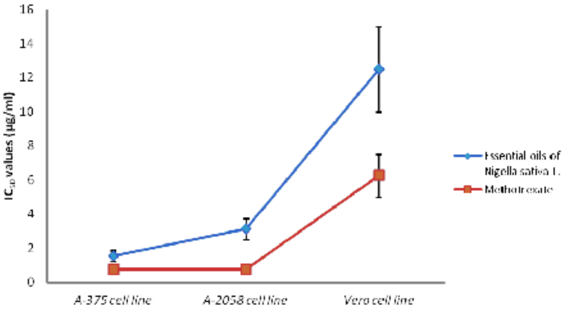

Figure 3. IC_{50} values of the essential oils of *Nigella sativa* L. on three different cell lines

CONCLUSIONS

In conclusion, the essential oils of *Nigella sativa* L. were found to be significantly inhibited the cell proliferation on the human melanoma cell lines. The effect may arise from the components situated in the structure of *Nigella sativa* L. such as anethole, thymoquinone, α-thujene, longifolene, trans-isoeugenol, carvacrol. The components of this plant have been found promising in the treatment of human melanoma. But further studies, especially further animal studies should be needed to explain the action mechanism of action. To explain the mechanism of the effect, additional studies are required.

REFERENCES

Agbaria, R., Gabarin, A., Dahan, A. and Ben-Shabat, S. (2015), "Anticancer activity of *Nigella sativa* (black seed) and its relationship with the thermal processing and quinone composition of the seed", *Journal of Drug Design, Development and Therapy*, 9, 3119-24.

Alenzi, F.Q., El-Bolkiny, Y. and Salem, M.L. (2010), "Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anti -cancer drug cyclophosphamide", *British Journal of Biomedical Science*, 67, 20-28.

Alhebshi, A.H., Gotoh, M. and Suzuki, I. (2013), "Thymoquinone protects cultured rat primary neurons against amyloid beta-induced neurotoxicity", *Biochemical and Biophysical Research Communications*, 433, 362-7.

Ali, B.H. and Blunden, G. (2003), "Pharmacological and toxicological properties of *Nigella sativa*", *Phytotherapy Research*, 17, 299-305.

Atallah, E. and Flaherty, L. (2005), "Treatment of metastatic malignant melanoma", *Current Treatment Options in Oncology*, 6, 185-93.

Burits, M. and Bucar, F. (2000), "Antioxidant activity of *Nigella sativa* essential oil", *Phytotherapy Research*, 14, 3238.

El-Mahdy, M.A., Zhu, Q., Wang, Q.E. *et al*. (2005), "Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells", *International Journal of Cancer*, 117, 409-17.

Goldberg, M.S., Doucette, J.T., Lim, H.W. *et al*. (2007), "Risk factors for presumptive melanoma in skin cancer screening: American Academy of Dermatology National Melanoma/Skin Cancer Screening Program experience 2001–2005", *Journal of the American Academy of Dermatology*, 57, 60-6.

Mosmann, T. (1983), "Rapid colorimetric assay for cellular growth and survivalapplication to proliferation and cytotoxicity assays", *Journal of Immunological Methods*, 65, 55-63.

Muthukumari, D., Padma, P.R. and Sumathi, S. (2013), "In Vitro Analysis of Anethole as an Anticancerous Agent for Triple Negative Breast Cancer", *International Journal of Pharmaceutical Sciences Review and Research*, 50, 314-8.

Sun, W. and Schuchter, L.M. (2001), "Metastatic melanoma", *Current Treatment Options in Oncology*, 2, 193-202.

Ulasli, S.S., Celik, S., Gunay, E. *et al*. (2013), "Anti - cancer effects of thymoquinone, caffeic acid phenethyl ester and resveratrol on A549 non-small cell lung cancer cells exposed to benzo(a)pyrene", *Asian Pacific Journal of Cancer Prevention*, 14, 6159-64.

Woo, C.C., Loo, S.Y., Gee, V. *et al*. (2011), "Anti-cancer activity of thymoquinone in breast cancer cells: possible involvement of PPAR-gamma pathway", *Biochemical Pharmacology*, 82, 464-75.

Yin, Q.H., Yan, F.X., Zu, X.Y. *et al*. (2012), "Anti-proliferative and pro-apoptotic effect of carvacrol on human hepatocellular carcinoma cell line HepG-2", *Cytotechnology*, 64, 43-51.

ENCAPSULATED CHLORHEXIDINE DIGLUCONATE USAGE ON THE DIABETIC FOOTWEAR LINING LEATHERS

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It is important for a therapeutic shoe to have a wearable quality and its comfortable use is also prominent in terms of providing an increase in user's quality of life. In long-term treatment of diseases, medical products which are to be manufactured with medical leather materials might be a prominent alternative via local effect application as an adjuvant to the treatment. It is aimed in this study to produce microparticles (MPs) which contain the active agent chlorhexidine digluconate and application of these MPs on lining leather for manufacturing diabetic shoes while providing them a functional quality. Within the scope of the project, MPs loaded with drugs were obtained via spraying chlorhexidine digluconate (CHD) active agent and through spraying with ethyl cellulose polymer and ustulation. *In vitro* characterisation studies were performed on the acquired MPs. Additionally, active agent quantitation and *in vitro* drug delivery studies were also performed. Following the studies, the determined optimum MP formulations were applied on the leather, then existence and efficiency of MPs within the leather was shown in the subsequent studies.

Keywords: Chlorhexidine digluconate, encapsulation, diabetic shoes, leather

INTRODUCTION

Diabetic foot disease is a serious problem, with a life-time prevalence of 15–25% in the diabetic population (Boulton, 2000). While in the UK up to 100 people/week have a limb amputated as a result of diabetes, it is indicated that up to 80% of these amputations could have been prevented with correct management (Anonymous, 2011). Foot ulcers in people with diabetes are multi-factorial and linked to a variety of clinical risk factors, like peripheral neuropathy and vascular insufficiency, as well as biomechanical risk factors, such as increased plantar pressure (Ledoux, 2013; Crawford, 2007). In shoe plantar pressure assessment is becoming increasingly popular in both research and clinical practice to evaluate the effects of prescribed footwear in diabetic patients who have a foot ulcer or who are at risk for ulceration (Singh, 2005). Diabetic footwear plays an important role for the reduction of plantar pressure in people with diabetes (Panagiotis, 2015).

Microencapsulation is one of the most important forms of controlled release of active ingredient. This technology allows heat, temperature or pH sensitive components to be physically enveloped in a protective matrix or wall material in order to protect these ingredients or core materials from adverse reactions, loss or against light, heat and prolonged contact with air. It is also one of the most important forms of controlled release of substances and allows the utilization of some that otherwise would be unfeasible (Nirmala, 2013; Sanchez-Navarro, 2015). These systems offer some advantages over conventional dosage forms, including improved efficiency, reduced toxicity and improved patient compliance (Grattard, 2002).

In the footwear industry, the incorporation of microencapsulated substances into materials or components allows the concept of active shoes to be realized, which contributes to improve the welfare of users, satisfying their needs and expectations (Morace, 2012). Fragrances applied to footwear, both directly and through packaging, cover one of the main consumer demands regarding the solution of bad odours generated during footwear use (Misher, 2007). Along the same lines, in shoe packaging, microencapsulation allows the development of active issues with different purposes: trapping undesirable odours or incorporation of antimicrobial agents, to be released over time in order to improve the useful life of the packed shoe or the incorporation of controlled released scents to avoid their degradation and to improve the durability of the aroma (Sanchez-Navarro, 2012; Sanchez-Navarro, 2011).

It is aimed in this study to produce microparticles (MPs) which contain the active agent Chlorhexidine digluconate (CHD) and application of these MPs on lining leather for manufacturing diabetic shoes while providing them a functional quality.

MATERIAL AND METHOD

Material

CHD 20% solution was purchased from Sigma-Aldrich. Aquacoat ethyl cellulose dispersion (Aquacoat® ECD) was gift from FMC BioPolymer (Philadelphia, PA). All other materials were of analytical grade.

Method

Preparation of MPs

MPs were carried out in a spray dryer model SD-Basic (Lab-Plant, Huddersfield, U.K). Aquacoat® ECD was used as a polymeric system. CHD and Aquacoat® ECD were mixed in distilled water. The drug to polymer ratios in the microencapsulating compositions were maintained in 1:1, and 1:2, respectively (Table 1).

Table 1. The composition of the formulations

Formulation Code	CD: EC*	Pump Speed (mL/dk)	Inlet Temperature (˚C)	Outlet Temperature (˚C)
F1	1:1	10	120	80
F2	1:2	10	120	80

(*): Aquacoat® ethyl cellulose

Particle Morphology

The morphology of the MPs was examined by a scanning electron microscope (SEM, FEI Quanta 250 FEG). The sample was mounted onto an aluminum stub and sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator.

In Vitro Drug Release of the MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37ºC. MPs were suspended in tubes containing 10 ml of PBS. At the appropriate time intervals, the medium in the corresponding tube was filtered through 0.22 µm filter and released CHD amount determined by validated UPLC method. Sink conditions were maintained in the receptor compartment during *in vitro* release studies (n=5).

Application of the MPs on Lining Leathers

MPs were applied on the lining leathers for diabetic footwear in the finishing process by using spraying pistol. MPs that are containing antimicrobial material were added into finishing recipe (Table 2) as 20 g per m^2 (Kleban, 2002).

Table 2. Basic finishing recipe with MPs

Material	Quantity	Application
Water	100 part	3x Spray
Anionic wax	50 part	
Nonionic aliphatic polyurethane binder	25 part	
MPs	12 part	

SEM of the Lining Leathers with the MPs

The morphology of the samples was examined by a SEM (HITACHI TM 1000).

In Vitro Drug Release of the Lining Leathers with MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37ºC. Lining leathers with 10 cm^2 area were placed in beaker containing 125 ml of PBS. The samples withdrawn directly at appropriate time intervals were analyzed by a validated UPLC method as previously described (n=5).

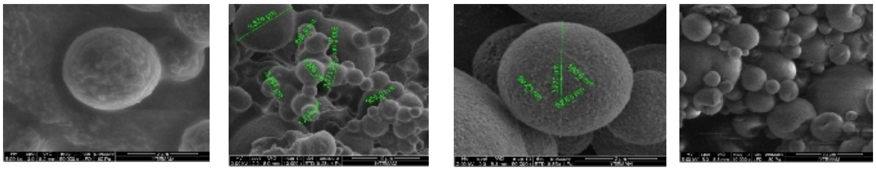
Microbiologic Studies on Lining Leathers with MPs

Agar disc diffusion method was used to examine the effect of drug, MPs and lining leather with MPs against to the test microorganisms. Test microorganisms were incubated at 37^oC for 18 hours in the Muller Hinton Broth (MHB) medium. After incubation, microorganisms were inoculated into petri dishes containing Muller Hinton Agar (MHA) medium as 10^5 CFU/mL. Lining leather samples with 12.7 mm diameter were placed into the petri dishes. All petri dishes were incubated at 37^oC for 24 hours. Finally, inhibition zones were measured for determining the antibacterial activity.

RESULTS AND DISCUSSION

Particle Morphology

According to the SEM images, MPs had a spherical shape with a rough surface morphology. The MPs exhibited irregular shape also. They do not show the presence of free drug on their surfaces. These morphological characteristics point out that the CHD is dispersed all over the MPs (Figure 1). In other spray-drying studies performed by using aqueous polymeric dispersions, MPs with similar morphological characteristics were also obtained (Rattes, 2007; Arici, 2014).



F1 formulation (a) F1 formulation (b) F2 formulation (a) F2 formulation (b)

Figure 1. SEM images of MPs at 50000x magnification (a) and at 10000x magnification (b)

In Vitro Drug Release of the MPs

In vitro drug release studies showed that in CHD release from MPs was very fast which is probably the consequence of very good swelling properties or it could be related with burst effect (Figure 2). In general, the initial rates of release for many drug delivery systems are high during the first period, most likely due to the release of drug enriched on the sample surfaces (Kenawy, 2002). The same behaviour, defined as the initial burst release, is presented in the MPs. MPs prepared by using spray drying method generally have a matrix structure. For this reason, besides drug substance being in the particles, depending on the loading concentration, it can also be on the outer surface of the particles. When the MPs are exposed to the dissolution media, the drug on the outer surface (non-encapsulated drug) causes a sudden drug release (Ghorab, 1990; Saravanan, 2003).

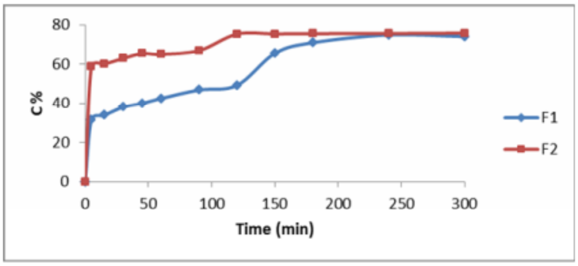
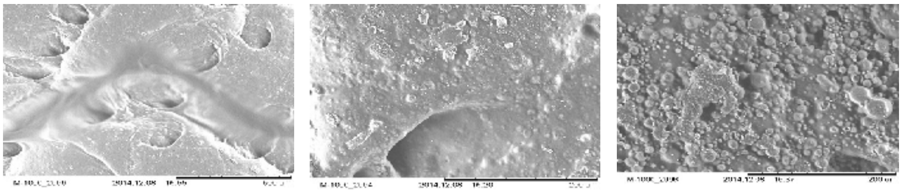


Figure 2. *In vitro* release of MPs

SEM of the Lining Leathers with the MPs

Lining leather samples that were applied finishing recipes with MPs containing antibacterial drug and without MPs was examined by SEM. As seen in Figure 3, there were fewer MPs on the lining leather surface because of the F1 formulation's polymer quantity is half of the F2 formulation.



Without MPs F1 formulation F2 formulation

Figure 3. SEM images of the lining leather after finishing process

In Vitro Drug Release of the Lining Leathers with MPs

The *in vitro* release results of the leathers in pH 7.4 PBS are presented in Figure 4. As seen in Figure 4, there was a steady release of the CHD into the PBS for all formulations. CHD entrapped deep within the MPs sustained the release to more than 24h. Comparing the formulations among each other, the drug release ratio of lining leather with F1 was higher than the release of lining leather with F2.

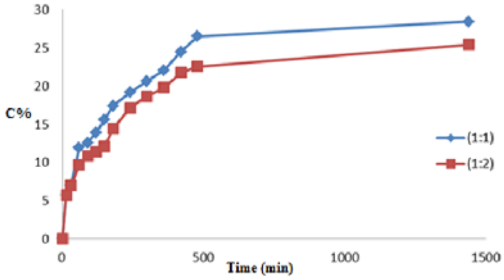


Figure 4. *In vitro* release of lining leathers with MPs

Microbiologic Stuies on Lining Leathers with MPs

It was not seen any clear inhibition zone around the lining leather samples with MPs in Table 3. On the other hand, slightly inhibition zone was seen on some leather samples examining by microscope. This situation could be interpreted that MPs don’t show antimicrobial property comparing the non-capsulated drug. Effect of applying the MPs on the lining leather of the diabetic shoes was positive considering that encapsulation is used the permanence of the drug on the material and controlled release.

Table 3. Microbiologic test results of lining leathers with MPs

Formulation Code	***Staphlylococcus aureus*** **ATCC 6538-P**	***Escherichia coli*** **ATCC 12228**	***Pseudomonas aeruginosa*** **ATCC 27853**	***Candida albicans*** **ATCC 10239**	***Klebsiella pneumoniae*** **CCM 2318**	***Enterococcus faecalis*** **ATCC 29212**	***Staphlylococcus epidermidis*** **ATCC 12228**
F1							
F2							

CHD diffusion was not occurred on the lining leather surface, because of this inhibition zone was not seen. However, antimicrobial effect can be evaluated with proliferation or without proliferation in the area under the leather samples. This effect is expressed as contact inhibition. It was not seen any proliferation on the contact surface of the lining leathers in Table 3. Also, there was not seen any proliferation surface or edge of the lining leathers.

CONCLUSION

In this study, CHD was microencapsulated by the spray drying method using an Aquacoat® ECD, followed by its application on lining leather for manufacturing diabetic shoes. Aquacoat® ECD has proved to be a useful polymer for formulating CHD MPs using spray drying technology in an aqueous system. Using MPs produced with an

Aquacoat® ECD have the main advantage of being ecofriendly, due to the fact that organic solvents can be avoided. Water was the only solvent used. SEM photographs showed smooth shaped MPs and good adhesion between the leathers and the MPs. In a conclusion, diabetic shoes with MPs will be an adjuvant therapy to the oral therapy by releasing drug on the applying area for a long time.

Acknowledgments

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REFERENCES

Arici, M., Topbas, O., Karavana, S.Y., Ertan, G., Sariisik, M. and Ozturk, C. (2014), "Preparation of naproxen–ethyl cellulose microparticles by spray-drying technique and their application to textile materials", *Journal of Microencapsulation*, 31(7), 654–666.

Boulton, A.J. (2000), "The diabetic foot: A global view", *Diabetes/Metabolism Research and Reviews*, 16(Suppl 1), 2-5.

Crawford, F., Inkster, M., Kleijnen, J. and Fahey, T. (2007), "Predicting foot ulcers in patients with diabetes: A systematic review and meta-analysis", *QIM*, 100, 65-86.

Ghorab, M.M., Zia, H. and Luzzi, L.A. (1990), "Preparation of controlled release anticancer agents. I: 5-Fluorouracil-ethyl cellulose microspheres", *Journal of Microencapsulation*, 7, 447–54.

Grattard, N., Pernin, M., Marty, B., Roudaut, G., Champion, D. and Le Meste, M. (2002), "Study of release kinetics of small and high molecular weight substances dispersed into spray-dried ethylcellulose microspheres", *Journal of Controlled Release*, 84, 125–135.

Kenawy, el-R., Bowlin, G.L., Mansfield, K., Layman, J., Simpson, D.G., Sanders, E.H. and Wnek, G.E. (2002), "Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend", *Journal of Controlled Release*, 81, 57–64.

Kleban, M., Weisser, J., Koch, F. and Schwaiger, W. (2002), "Leather Finished with Scent-Containing Microcapsules", *United States Patent Application Publication*, US 2002/0198392 A1, USA.

Ledoux, W.R., Shofer, J.B., Cowley, M.S., Ahroni, J.H., Cohen, V. and Boyko, E.J. (2013), "Diabetic foot ulcer incidence in relation to plantar pressure magnitude and measurement location", *Journal of Diabetes and its Complications*, 27, 621-626.

Misher, B.D., (2007), *J Int Soc Sports Nutr*, 4, 3.

Morace, F. and Ferrarini, P. (2012), "Real Footwear Trends", *Futura Concept Lab*, Milan.

Nirmala, D. and Dilip, K.K. (2013), "Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex", *Journal of Food Engineering*, 117, 193–204.

Panagiotis, E., Chatzistergos, R.N. and Nachiappan, C. (2015), "A method for subject-specific modelling and optimisation of the cushioning properties of insole materials used in diabetic footwear", *Medical Engineering and Physics*, 37, 531-538.

Rattes, A.L.R. and Oliveria, W.P. (2007), "Spray drying conditions and encapsulating composition effects on formation and properties of sodium diclofenac microparticles", *Powder Technology*, 171, 7–14.

Sanchez-Navarro, M.M., Cuesta-Garrote, N., Aran-Ais, F. and Orgiles-Barcelo, C. (2012), *Progress in Colloid and Polymer Science*, 139, 73-77.

Sanchez-Navarro, M.M., Cuesta-Garrote, N., Aran-Ais, F. and Orgiles-Barcelo, C. (2011), *Journal of Dispersion Science and Technology*, 32, 1722-1727.

Sanchez-Navarro, M.M., Perez-Liminana, M.A., Aran-Ais, F. and Orgiles-Barcelo, C. (2015), "Scent properties by natural fragrance microencapsulation for footwear applications", *Polymer International*, 64, 1458–1464.

Saravanan, M., Bhaskar, K., Srinivasa Rao, G. and Dhanaraju, M.D. (2003), "Ibuprofenloaded ethylcellulose/ polystyrene microspheres: An approach to get prolonged drug release with reduced burst effect and low ethylcellulose content", *Journal of Microencapsulation*, 20, 289–302.

Singh, N., Armstrong, D.G. and Lipsky, B.A. (2005), "Preventing foot ulcers in patients with diabetes", *Journal of the American Medical Association*, 293, 217–228.

*** (2011), *Diabetes in the UK 2011-12 Key Statistics on Diabetes*, UK.

PREPARATION AND CHARACTERIZATION OF CHITOSAN/HYDROXYAPATITE SOL-GEL COATING ON Ti-6Al-4V

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A biocomposite coating containing chitosan and hydroxyapatite was developed on Ti-6Al-4V substrate by a sol-gel method in order to obtain a biocompatible and antimicrobial implant surface which can be used to create a smooth transmucosal region for a faster and better wound healing and an increased bioactivity. The coatings characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis and differential thermal analysis (DTG). Scratch test was carried out to evaluate the adhesion between the coated film and the substrates. The critical load at which the coating film was peeled off from the substrate is determined by using the spherical diamond stylus. The results revealed that the coating roughness is approximately 0.23. The critical load for debonding the coating film from the substrate was determined between 6 to 11N. Only a little wear debris was come out from the coating film in the initial stage of a small-applied load. With increasing the load, the coating film became crushed and finally worn away.

Keywords: Chitosan, hydroxyapatite, sol-gel.

INTRODUCTION

Biomaterials can be classified as synthetic and natural biomaterials. Synthetic biomaterials have some disadvantages because of their structure, composition that is not similar to original tissues/organs and due to their biocompatibility. Naturally obtained biomaterials like chitosan are generally applied to replace or restore structure and function of damaged tissues/organs. They have properties in order to improve cell adhesion, migration, proliferation, and differentiation. Especially, when natural biomaterials implanted into a defective area, they can increase the attachment and migration of cells from the surrounding environment (Bao *et al*., 2013).

Chitosan property of osteoconductive is suitable for engineering applications in hard tissues, but its mechanical properties and biological activities need to be enhanced (Yun *et al*., 2013). In order to increase the mechanical properties, chitosan can be modified by blending with other natural polymers like silk, alginate, gelatin or ceramics, such as, tricalcium phosphate and hydroxyapatite (Thein-Han and Misra, 2009; Silva *et al*., 2007). The development on the production of hybrid HA/Chitosan coatings is an area of increasing interest. Hybrid HA coatings with chitosan can enhance the adhesive strength of the HA coatings. Chitosan was selected in the fabrication of hybrid HA coatings is due to its magnificent film forming properties and the cationic nature in aqueous solutions. It has an antimicrobial activity, good chemical stability, good biocompatibility, good advanced mechanical and other properties that has been utilized in biomedical implants (Isa *et al*., 2012).

Titanium and its alloys are the most common metallic biomaterials that used for medical implants due to their low elastic modulus, high strength, excellent biocompatibility and excellent resistance against corrosion (Adell *et al*., 1981). However, because of the differences in chemical composition and structure, there is lots of inflammatory effect around metallic implants observed (Lee *et al*., 2009). One of the solutions is coating the titanium substrates with Hydroxyapatite (HA). Hydroxyapatite is commonly used in bioceramic due to similarities with human skeletal system, which increase the osteoconductive bonding of implants with surrounding tissues (Milella *et al*., 2001).

HA coatings, produced by plasma spraying (Zhang *et al*., 2006), have been shown clinical problems such as delamination, which leads to the premature loosing of implants (Bloebaum *et al*., 1994). The sol-gel process ensures that an attractive method to others due to the easy occurring of crystalline films at relatively lower processing temperatures, higher purity and homogeneity, thin films, simple and easy method of preparation (Im *et al*., 2007). It is convenient for complex shapes like coatings and the main advantage of this process is that the properties of the surface not change at all (Brinker and Scherer, 1990).

The purpose of this research was to reveal the effect of HA/Chitosan on the properties of the deposited coating on Ti-6Al-4V alloys. The composition, phase structure, microstructure and morphology as well as the mechanical features of the HA/Chitosan sol-gel coating are presented and discussed.

EXPERIMENTAL PROCEDURE

Materials and Sample Pre-treatment

The Ti-6Al-4V alloys in this study was cut into 10 mm x 10 mm x 2 mm discs. These discs were then chemically stigmatized with sand blasting with Al_2O_3 (250 µm) particles (MKK-975 sand blaster) and ultra-sonically cleaned, for 15 min, in distilled water, absolute ethanol and acetone, respectively, in order to remove the macro-level surface defects and contaminants. Finally, all samples were dried at 60°C in an incubator for 1h.

Preparation of the HA/Chitosan Sol and Coating

HA powders were synthesized by a sol-gel method. Precursors of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and phosphoric pentoxide (P_2O_5) were added into absolute ethyl alcohol in order to prepare the solutions with a certain molar ratio. These two powders were dissolved separately in absolute ethyl alcohol with different amounts to obtain a theoretical Ca/P ratio of 1.67. Phosphoric pentoxide solution was added at the calcium nitrate tetra hydrate mixture in a drop wise manner after stirring for 6 hours. The final mixture was aged until it turned to opaque. After aging, the mixture was left in water bath for 1h at 60°C. In addition to precursors of $Ca(NO_3)_2 \cdot 4H_2O$ and P_2O_5 samples, Chitosan added to the final mixture with a certain molar ratio. The viscosity of the solution was adjusted by evaporating ethanol solvent at 80°C before dipping. Titanium substrates were immersed in the solution for 1 minute, dried at 80°C in air for 12h. This step repeated several times to meet the requirement of the thickness.

Analysis Techniques

Ti alloys, HA/Chitosan composite coating were examined by means of both SEM and XRD measurements. Scanning electron microscopy (SEM) was performed with a JEOL JSM–6335F, JSM 6600 microscopes. The procedure employed with an electron probe which has a voltage of 20 kV. Before the SEM observations, the samples were coated with Au-Pd. The phase transformation and compound formation were investigated by X-ray diffraction (XRD) measurements were performed by means of a Bruker D8. The XRD measurements were performed on a stage using a radiation of wavelength λ=1.5406 Å X-ray source with a step rate of 0.02° per s. HA powder, which used in coating procedures observed with SEM for determine elements ratio. The EDS analysis confirmed that calcium and phosphorous are present in the coating in a ratio of 1.67.

RESULT AND DISCUSSION

Microstructure and Composition Characterization

The XRD patterns of the synthesized HA/Chitosan powder are represented by the Figure 1 compared with standard HA (ICDD 01-074-9780). XRD analysis of the obtained coating was made on titanium has been proven (ICDD 00-044-1294). The results revealed the presence of HA characteristic peak at 25.99°, 31.75°, 33.65° and 40.68°.

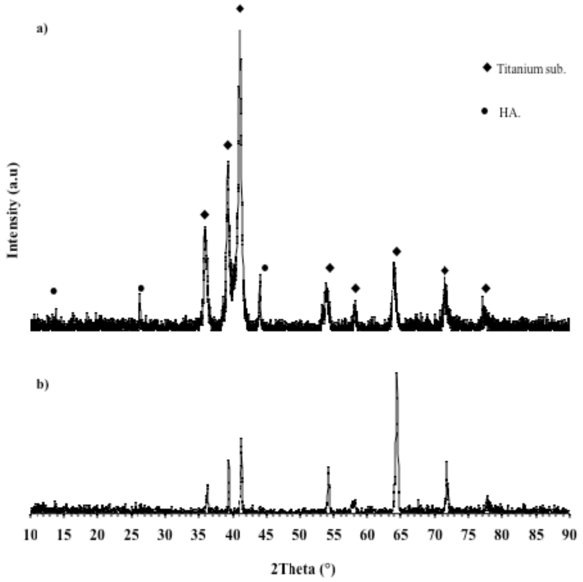

Figure 1. XRD of HAp-chitosan coating a) HA/Chitosan b) Ti Alloy

In Figure 2 the scanning electron micrograph of HA/Chitosan coating at different magnifications is given. When chitosan was added into the HA solution, the morphological structure of the coating was significantly changed from flower-like structure to flake-like structure interconnected with net-like structure of chitosan.

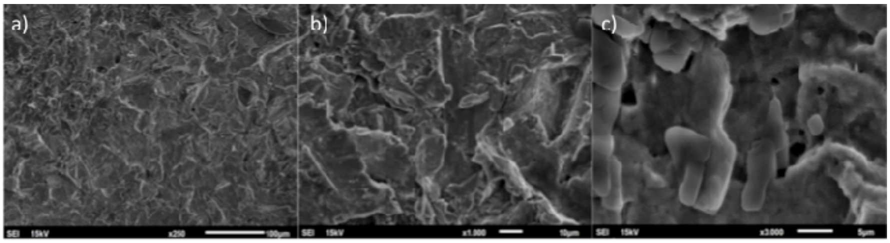

Figure 2. SEM of HA/Chitosan coatings

In Figure 3 as a result of scratch test coating roughness is approximately 0.23. The critical load for debonding the coating film from the substrate was determined between 6 to 11N. Only a little wear debris was come out from the coating film in the initial stage of a small-applied load. With increasing the load, the coating film became crushed and finally worn away.

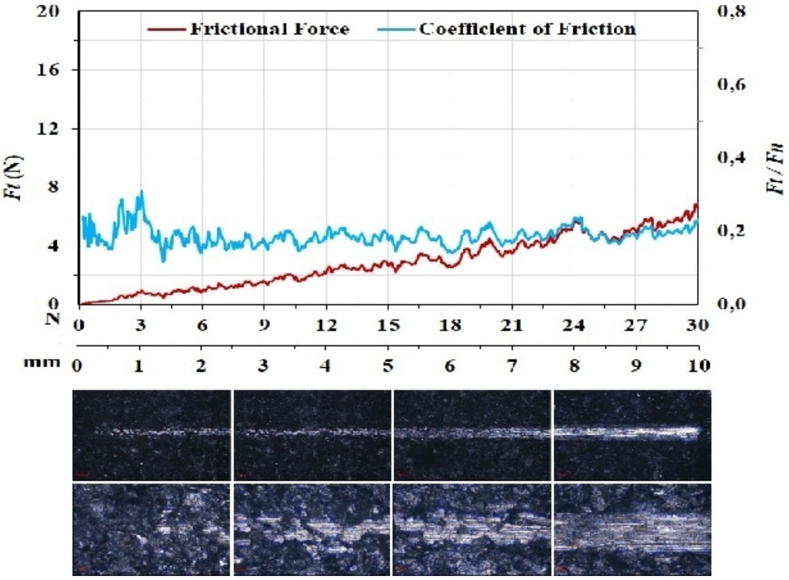

Figure 3. Scratch Test results of the HA/Chitosan coated Ti substrates

CONCLUSION

The preparation of HA/Chitosan coatings on the Ti substrates was achieved by sol-gel method with Ca/P ratio being 1.67. Surface observations, XRD pattern, were investigated, respectively. XRD pattern of HA/Chitosan coating proved that thin film obtained successfully on substrate. The insertion of chitosan has extremely affected the surface morphology and structural characteristics of HA coatings deposited on Ti substrate. The chitosan content in the formed hybrid HA/Chitosan coatings will be increased with increasing chitosan concentration in the deposition solution. In this research, it is obvious that the presence of high amount of chitosan in the deposition solution has prevented the HA formation and makes the chitosan determinant in the coatings formation. In addition to this, doping of hybrid HA coatings with chitosan improved the adhesive strength of the HA coatings.

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REFERENCES

Adell, R., Lekholm, U., Rockler, B. and Branemark, P. (1981), “A 15-year study of osseointegrated implants in the treatment of the edentulous jaw”, *International Journal of Oral Surgery,* vol. 10, 387-461.

Bao, T.L., Quan, T.M., Vu, D.N. and Si, D.M. (2013), “Naturally Derived Biomaterials: Preparation and Application”, *Intech*, Open Access Publisher.

Bloebaum, R., Beeks, D., Dorr, L., Savory, C., DuPont, J. and Hofmann, A. (1994), “Complications with hydroxyapatite particulate separation in total hip arthroplasty”, *Clinical Orthopaedics and Related Research*, 298.

Brinker, C.R. and Scherer, G.W. (1990), *Sol-Gel Science - The Physics and Chemistry of Sol-Gel*, Academic Press, London.

Im, K., Lee, S., Kim, K. and Lee, Y. (2007), “Improvement of bonding strength to titanium surface by sol-gel derived hybrid coating of hydroxyapatite and titania by sol-gel process”, *Surface & Coatings Technology*, 202, 1135-1138.

Isa, N.N.C.I., Mohd, Y. and Yury N. (2012), “Electrochemical Deposition and Characterization of Hydroxyapatite (HAp) on Titanium Substrate”, *APCBEE Procedia*, 3, 46–52.

Lee, H., Jeong, Y., Park, S., Jeong, S., Kim, H. and Cho, C. (2009), “Surface properties and cell response of fluoridated hydroxyapatite/TiO_2 coated on Ti substrate”, *Current Applied Physics*, 9, 528-533.

Milella, E., Cosentino, F., Licciulli, A. and Massaro, C. (2001), “Preparation and characterization of titania/hydroxyapatite composite coatings obtained by sol-gel process”, *Biomaterials*, 22, 1425-1431.

Silva, G.A., Ducheyne, P. and Reis, R.L. (2007), “Materials in particulate form for tissue engineering. Basic concepts”, *Journal of Tissue Engineering and Regenerative Medicine*, 1, 4–24.

Thein-Han, W.W. and Misra, R.D.K. (2009), “Biomimetic chitosan–nanohydroxyapatite composite scaffolds for bone tissue engineering”, *Acta Biomaterialia*, 5, 1182–1197.

Yun, Y.P., Lee, S.Y., Kim, H.J., Song, J.J. and Kim, S.E. (2013), “Improvement of osteoblast functions by sustained release of bone morphogenetic protein-2 (BMP-2) from heparin-coated chitosan scaffold”, *Journal of Tissue Engineering and Regenerative Medicine*, 10, 183–191.

Zhang, S., Xianting, Z., Yongsheng, W., Kui, C. and Wenjian, W. (2006), “Adhesion strength of sol-gel derived fluoridated hydroxyapatite coatings”, 200, 6350-6354.

ADVANCED COLLAGEN-INSULIN SYSTEMS FOR DIABETICS

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Diabetes is the most common disease in our world. In our country over 50.000 of people are diagnosed with diabetes every year. Diabetes is a metabolic disorder generated by the pancreas inability to produce insulin in order to complete glucose metabolic process. Therefore, the life of the patients is sustained by the insulin administration. Existing methods on the market for insulin administration are not too various and there are a lots of disadvantages. Insulin glargine is a long-action, man-made version of human insulin. It replaces the insulin that is not produced by the body and helps the metabolic process of glucose. To increase insulin stability and releasing time, zinc oxide (ZnO) can be used for insulin encapsulation. Collagen is a natural protein that can be used as a support for controlled releases system because of its high biocompatibility. The aim of this study was to develop and characterize a controlled released subdermal support, for diabetics patients, made of a composite material based on collagen, zinc oxide and insulin. Obtained matrices were characterized by FT-IR spectroscopy, optical and scanning electronic microscopy, water up-take, degradation in collagenase solution. The results showed that the combination between collagen, ZnO and insulin could be suitable active supports for subdermal support with controlled delivery of insulin for diabetics.

Keywords: diabetes, collagen, insulin, zinc oxide.

INTRODUCTION

Nowadays diabetes represents one of the most common metabolic diseases for our modern world. It is estimated that diabetes affects around 5% of the grown-up population around the world and annually provokes the death of 3.2 million people. At present about 246 million and by 2025, 380 million people will need medical support that means a percentage of 7.1% of the grown-up population. In Romania 50.000 people are diagnosed with diabetes and 5% of them have the disease confirmed (helpnet.ro).

Diabetes mellitus is a chronic metabolic disorder caused by the incapacity of the pancreas to produce enough insulin (a hormone who controls blood sugar) to complete the metabolic process of the glucose. This disease left untreated will have bad consequences causing kidney failure, retinopathy with potential blindness, cardio-vascular and cerebrovascular problems.

The absolute lack of insulin, also known as Type I Diabetes refers to an auto-immune action of the body that destroys his own β-cells, the producing cells of insulin. In this case, it is necessary to sustain patients' life by administrating a dietary intake of insulin (Dansinger, 2015).

For patients with Type I Diabetes, the main methods available on market to administrate insulin are: insulin injections (pre-filled pen systems); insulin pumps and inhaled insulin. The main disadvantages of those methods are: lumps or scars where patients had to many injections, problems with batteries and catheters for insulin pumps and contraindications regarding inhaled insulin for smokers and people who have asthma or COPD (Alberti *et al.*, 1998). Insulin glargine is a long-acting, man-made version of human insulin. Insulin glargine works by replacing the insulin that is normally produced by the body and by helping move sugar from the blood into other body tissues where it is used for energy. It also stops the liver from producing more sugar (medlineplus.gov). Zinc oxide is used to enhance insulin stability during release because studies showed that when insulin was encapsulated with a zinc salt, EE encapsulation efficiency increased significantly, secondary structure was unaltered, and no degradation or aggregation products were found. Initial burst release and release kinetics were markedly changed with the addition of zinc salts. More than 87% of the encapsulated insulin was released over a 2-week period with the addition of a zinc salt (Manoharan *et al.*, 2009). Collagen is a natural protein found in skin, bones and tendons. Because of its high biocompatibility is widely use in tissue engineering, pharmaceutical and cosmetic industries which makes it a good candidate for controlled releases system.

The main purpose of this article is to find an alternative of the already existing products on the market and to find a solution to overcome daily treatment by creating a controlled released subdermal support for diabetic patients. The drug release system is made of a composite material based on collagen, zinc oxide and insulin.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.84% (w/v) was extracted from calf hide as we previously described (Albu, 2011). Zinc oxide (ZnO) was purchased from Merck, Germany; the insulin glargine from LANTUS SoloStar (Germany). Sodium hydroxide and hydrochloric acid were of analytical grade. Type I collagenase obtained from *Clostridium histolyticum* was purchased from Sigma-Aldrich, Germany and glutaraldehyde (GA) from Sigma-Aldrich (Germany).

Preparation of Gel Composites and Their Corresponding Matrices

The concentration of each collagen gel was adjusted at 1% and 7.4 pH using 1M sodium hydroxide (the pH of the physiological medium in human body). ZnO and insulin glargine were added to collagen gel (w/v) and then the composite gels were cross-linked with 0.025% glutaraldehyde (reported to collagen dry substance) as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Code of matrices, %	Coll, %	Insuline, %	ZnO, %
Coll	N1	1	0	0
Coll-Insuline	N2	1	0.25	0
Coll-ZnO	N3	1	0	0.25
Coll-insuline-ZnO	N4	1	0.25	0.25

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer using the freeze-drying program presented in Figure 1.

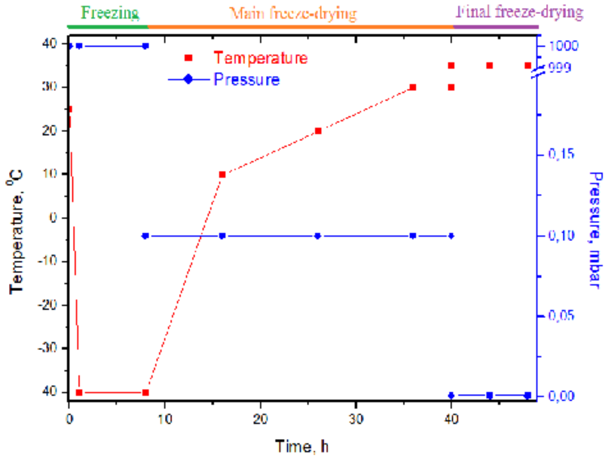


Figure 1.Graph chart of freeze-drying process (Marin *et al*., 2014)

The resulted matrices were named as shown in Table 1 and were characterized using the following methods.

Methods

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Up-take Capacity and Enzymatic Degradation

The water uptake capacity and enzymatic degradation were performed using the protocol as we previously described (Albu, 2012) on the obtained matrices with compositions according with Table 1.

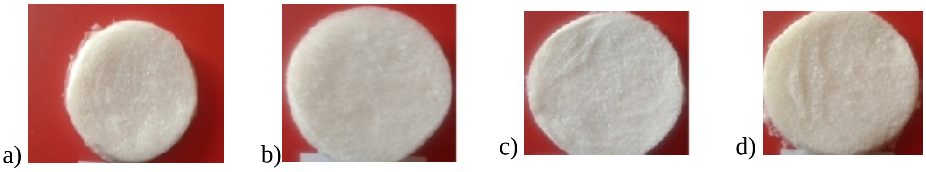
Optical and Scanning Electron Microscopy (SEM)

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used and optical images were obtained.

SEM analysis was performed using a Quanta 200 FEI microscope in order to obtain high-resolution images of collagen discs surfaces to study the structure of collagen fibers and the absorption of ZnO.

RESULTS AND DISCUSSION

After composite gels lyophilization, the 3D porous collagen sponges based on collagen, insulin and ZnO, cross-linked, were obtained, with the aspect presented in Figure 2.



a) b) c) d)

Figure 2. Collagen spongious forms: a) N1; b) N2; c) N3; d) N4

The samples from Table 1 were analysed by FT-IR spectroscopy, water up-take, optical and SEM microscopy, enzymatic degradation.

From the FT-IR spectra (Figure 3) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011) at 3300 cm^{-1}, 2936 cm^{-1},1630 cm^{-1},1544 cm^{-1}, and 1238 cm^{-1}.

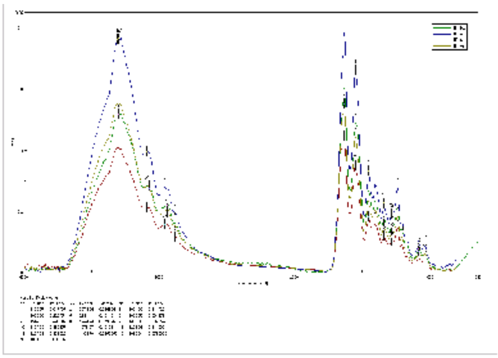


Figure 3. ATR-FTIR spectra of overlayed spectrum of collagen samples from Table 1

There are not significant changes when insulin and ZnO was added, possible because too less amount of them.

The water up-take for all the studied samples is presented as kinetics during 24 hours in Figure 4.

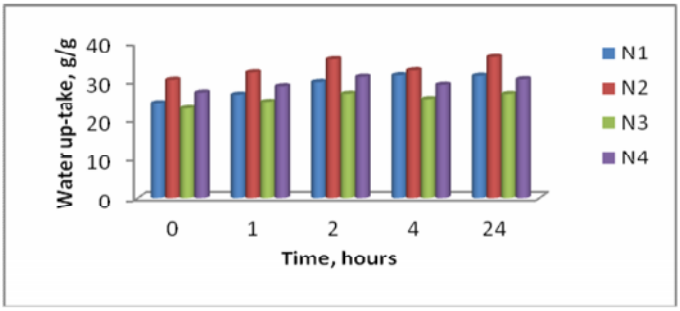


Figure 4. Water up-take during 24 hours for spongious forms

Figure 4 presents the water up-take during 24 hours for the studied samples. The samples with insulin absorbed the higher amount of water than the others, due to higher content of protein. The samples with ZnO absorbed lower amount of water due to their more compact structure.

Optical images showed porous structures with interconnected pores. In Figure 5 is presented as example the Coll-Insulin-Zn.

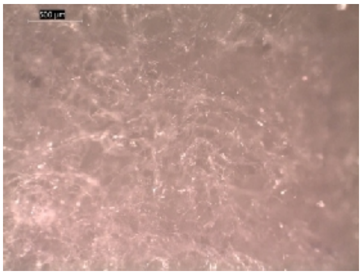


Figure 5. The optical microscopy image for Coll-Insulin-Zn

Figure 6 (a and b) presents SEM images of Coll and Coll-Insulin-ZnO samples and can be observed the fibrilar structure of collagen, the porosity of the samples and the adsorption of zinc salts on fibrilar structure.

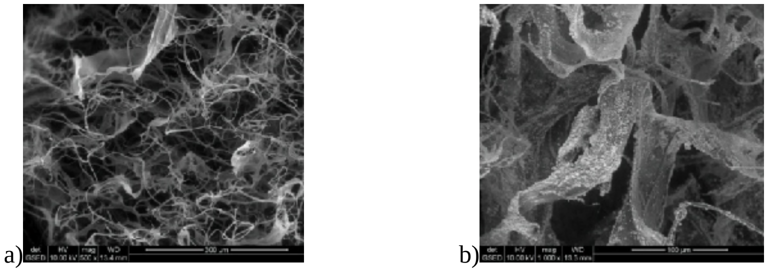


Figure 6. SEM image for: a) Coll (x500) and b) Coll-Insulin-ZnO (x1000)

It is clearly evidenced that ZnO deposited on collagen fibers crosslinked them in a more compact structure. The pore sizes are between 50 and 250 µm.

The *in vitro* behaviour of samples in collagenase solution during 24 hours for all samples and during 2 weeks for samples with ZnO is presented in Figure 7 a and b.

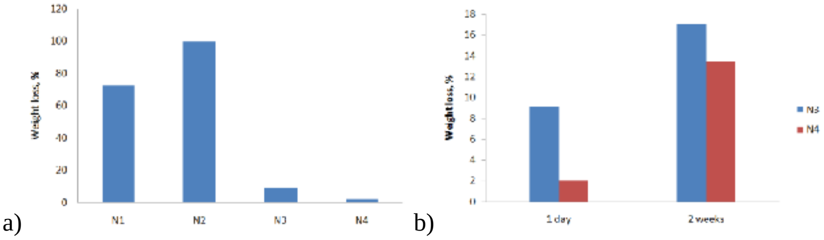


Figure 7. Weight loss of samples in collagenase solution: a) during 24 h and b) comparative study for 1 day and 2 weeks for samples N3 and N4

The degradation results are in correlation with water up-take ones. The sample N2, which contain collagen and insulin and which had the higher absorbance capacity is totally degraded during 24 hours, meanwhile the collagen as alone is degraded in 80%. The samples with ZnO are very slowly degraded, 9.12% N3 and 2.05% N4 during 24 hours. They kept stability also after 2 weeks, the weight loss being of 17.01% for N3 and 13.43% for N4.

CONCLUSIONS

Insulin was incorporated in collagen and collagen with ZnO in order to obtain advanced delivery systems for diabetics. Spongious lyophilized forms based on collagen, ZnO and insulin and their combination (together and two by two) were obtained and physical-chemical characterized. The results showed that the combination between collagen, ZnO and insulin could be suitable active supports for subdermal support with controlled delivery of insulin for diabetics.

Acknowledgements

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REFERENCES

Alberti, K.G. and Zimmet, P.Z. (1998), “Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation”, *Diabetic Medicine*, 15, 539-553.

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Dansinger, M. (2015), “The Facts About Insulin for Diabetes”, WebMD, available at http://www.webmd.com/diabetes/guide/overview.

Manoharan, C. and Singh, J. (2009), “Insulin Loaded PLGA Microspheres: Effect of Zinc Salts on Encapsulation, Release, and Stability”, *Journal of Pharmaceutical Science*, 98, 529-542.

Marin, S., Marin, M., Ene, A-M., Kilic, T.I., Chelaru, C., Albu, M. and Ghica, M.V. (2014), “Collagen-Doxycycline Spongious Forms For Infected Tissue Treatment”, *Proceedings of the 4th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 249-254.

*** http://www.helpnet.ro/metabolice-nutritie.

*** https://medlineplus.gov/druginfo/meds/a600027.html.

DEVELOPMENT AND EVALUATION OF SOME METRONIDAZOLE-LOADED COLLAGEN SUPPORTS DESIGNED FOR PERIODONTITIS

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Periodontitis is one of the most common chronic disease in the world affecting both the adults and young people, having severe consequences at cardiovascular level, and causing the premature birth. Moreover, the untreated periodontal disease is the major cause for tooth loos. Thus, the purpose of this study was to design and investigate some collagen supports with metronidazole and strontium ranelate as a treatment option for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease. Type I fibrillar collagen gel was extracted from calf hide. Collagen hydrogels with various ratios of metronidazole and strontium ranelate were rheologically tested at two temperatures: 23°C and 37°C. The collagenic supports obtained by hydrogels lyophilization were investigated by goniometric analysis. The in vitro metronidazole release from spongious matrices was conducted with a sandwich device adapted to a dissolution equipment. The hydrogels presented a pseudoplastic behaviour facilitating the formulation flow and their good manipulation. The Power law model fitted well the kinetic data indicating a non-Fickian drug transport mechanism. The physico-chemical properties were in relation with the drug release patterns from spongious supports. Based on the results obtained, we could conclude that the designed formulations are potentially usable as a favorable solution in periodontal disease.

Keywords: collagen supports, metronidazole release, strontium ranelate.

INTRODUCTION

Periodontal disease is a general term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingival), supporting bone (alveolar bone), periodontal ligament and cementum (Vyas *et al*., 2000). One of the most important clinical features of periodontitis is periodontal pocket (Divya and Nandakumar, 2006). The epithelium of the gingiva migrates along the tooth surface forming "periodontal pocket" that provides an ideal environment for the growth and proliferation of microorganisms (Vyas *et al*., 2000).

Antimicrobial agents have been suggested for use as an adjunctive therapy to eliminate pathogenic bacteria and improve the clinical outcome. The oral administration of an antimicrobial agent does not guarantee that an adequate concentration of drug is delivered to the periodontal pocket (Addy and Martin, 2003). Topical antimicrobial agents are more suitable because the drug can be delivered specifically to the action site at a high concentration, and patients have no systemic side effects (Southard and

Godowski, 1998). Metronidazole is a nitroimidazole compound, frequently used in the treatment of periodontal diseases since it can be effective against several Gram-negative anaerobic rods, the pathogenesis of periodontitis, by inhibiting bacterial nucleic acid synthesis (Pejcic *et al.*, 2010). Many reports suggested that local delivery of metronidazole has been suitable for periodontitis treatment (Pichayakorn and Boonme, 2012). Collagen is one of the main components of the bone and teeth being one of the most used material for bone grafting scaffolds (Ficai *et al.*, 2010). Strontium is an important trace element in human bone, stimulating bone formation and reduce bone resorption by increasing osteoblast activity (Wu *et al.*, 2012; Er *et al.*, 2008).

Thus, the purpose of this study was to design and investigate some collagen supports with metronidazole and strontium ranelate as a treatment option for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease.

MATERIALS AND METHODS

Materials

Type I fibrillar collagen (C) gel having a concentration of 2.85% (w/w) and pH 2.5 was extracted from calf hide by the currently used technology as previously described (Albu, 2011). Metronidazole (MTZ) was supplied from Hubei Hongyuan Pharmaceutical technology Co., Ltd., China. Strontium ranelate was obtained from OSSEOR. Glutaraldehyde (GA) was purchased from Sigma-Aldrich (Germany). Sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were obtained from Merck (Germany). All the chemicals were of analytical grade and the water was distilled.

Collagen Hydrogels Preparation

Reference hydrogel (Coll) having the concentration 1.1% and pH 7.4 was prepared from the initial collagen gel under stirring with distilled water and NaOH 1M solution. 2% metronidazol and 2% strontium ranelate reported to the collagen hydrogel was added and collagen according with composition from Table 1. The obtained hydrogels were then cross-linked with glutaraldehyde and stored for 24h at 4°C for cross-linking. The composition of the collagen hydrogels is given in Table 1.

Table 1. Composition of collagen hydrogels

Hydrogel	Collagen Coll (%)	Metronidazole MTZ (%)	Strontium ranelate Sr (%)	Glutaraldehyde GA (%)
Coll	1.1	0	0	0.0020
Coll-MTZ	1.1	2	0	0.0020
Coll-Sr	1.1	0	2	0.0020
Coll-Sr-MTZ	1.1	2	2	0.0020

*the amounts of Coll, MTZ, Sr and GA are reported to 100g hydrogel

The obtained hydrogels were then lyophilized using the Delta LSC 2-24 Martin Christ lyophilizer (Germany) and the method previously detailed (Albu, 2011) and the corresponding collagen spongious matrices were obtained.

Stationary Shear-Rheometry

The flow behaviour of the designed hydrogels was performed with a rotational viscometer MultiVisc-Rheometer (Fungilab) equipped with standard spindle TR 9 and an ultrathermostat ThermoHaake P5 to maintain constant the sample temperature during the measurements. All the experiments were carried out in triplicate at 23°C±0.5°C and 37°C±0.5°C.

Evaluation of Sponges Surface Wettability

The wetting behaviour of spongious matrices was determined with a KSV Scientific Instrument equipped with a video camera for images capturing and a CAM-101 software for data acquisition, using the pendant drop dynamic method. All the goniometric experiments were conducted in triplicate, on both sides of the porous supports.

In vitro Metronidazole Kinetics Release

The kinetic studies were carried out using a "sandwich" device adapted to a dissolution apparatus (EssaDissolver) as previously described in our studies (Ghica et al 2014). The receiving medium was the phosphate buffer pH 7.4, maintained at 37°C. The amount of metronidazole released at different period of time was spectrophotometrically assessed at 319 nm (Perkin-Elmer UV-Vis spectrophotometer).

RESULTS AND DISCUSSION

The flow patterns for the designed hydrogels tested at 23°C and 37°C and recorded as viscosity versus shear rate are presented for exemplification in Figure 1 a-b and Figure 2 a-b.

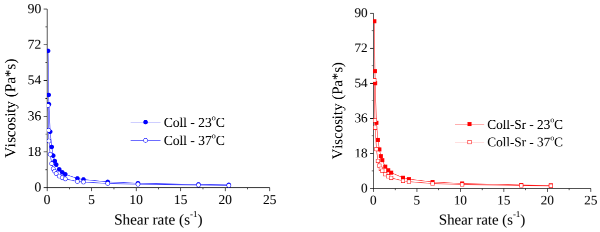

Figure 1. Rheological profiles viscosity versus shear rate for the collagen hydrogels: a) Coll; b) Coll-Sr, evaluated at 23°C and 37°C

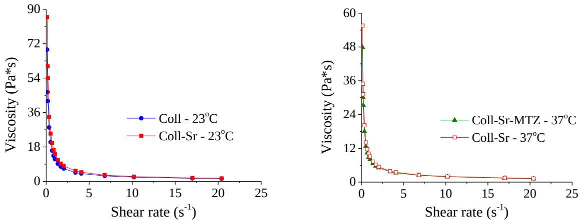

Figure 2. Rheological profiles viscosity versus shear rate for the collagen hydrogels: a) Coll and Coll-Sr analyzed at 23°C; b) Coll-Sr and Coll-Sr-MTZ analyzed at 37°C

As it can be remarked from the flow patterns given in Figures 1-2, all the tested samples presented a decrease of viscosity with the shear rate increase at both temperatures, indicating a shear thinning character which facilitates the formulations flow and their good manipulation.The above rheograms show both the influence of composition and work temperature on the hydrogels rheological behaviour.

The quantification of the stationary shear-rheometry data was realized through Power law rheological model (eq. 1):

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1),$$

where, m and n are parameters correlated with the hydrogels composition (Ghica *et al.*, 2015) and computed through the linearization of eq. (1) by double logarithmic method. m" parameter is associated with the viscosity obtained for the shear rate of $1\cdot s^{-1}$. The values of the determination coefficients (R^2) were higher than 0.99 indicating that this model fitted well the experimental data. The values of the aforesaid parameters and of R^2 specific to Power law model are listed in Table 2.

Table 2. The m and n parameters values and the determination coefficients specific to the Power law rheological model applied to hydrogels tested at 23°C and 37°C

Hydrogel	Temperature, 23°C			Temperature, 37°C		
	m	n	R^2	m	n	R^2
Coll	12.171	0.756	0.9995	7.381	0.748	0.9983
Coll-MTZ	10.626	0.759	0.9994	6.845	0.687	0.9974
Coll-Sr	15.043	0.765	0.9984	8.752	0.795	0.9983
Coll-Sr-MTZ	12.007	0.778	0.9997	7.912	0.774	0.9980

As expected, the temperature increase conducted to a viscosity decrease for all hydrogels about 1.52-1.72 times. The presence of strontium ranelate in formulation determined an increase of m parameter about 1.13-1.24 times, while the addition of metronidazole led to a decrease about 8-10%.

The wettability characteristics of the spongious matrices surface was further determined. The surface properties allow a better understanding of the drug delivery patterns from collagen sponges (Ghica *et al.*, 2013) and also represent an indicator of the interactions established between drug release support and gingival crevicular fluid.

The surface characteristics of the collagenic spongious matrices were quantified through contact angle (CA) and evaluated from Young-Laplace equation (eq. 2) (Popa et al. 2013):

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cdot \cos\theta \quad (2)$$

where, γ_{SG} is the solid-vapour interfacial tension, γ_{SL} – the solid-liquid interfacial tension, γ_{LG} – the liquid-vapor superficial tension, and θ – the contact angle.

The influence of the spongious matrices composition on surface wettability, as well the values recorded for the contact angle (expressed in degrees) are illustrated in Figure 3.

As it can be seen from Figure 3 a-d, for all the tested samples the contact angles are smaller than 90° indicating a good hydrophilicity of the spongious surfaces and consequently a proper wetting by the biological enviroment, favoring its diffusion in the porous structure.

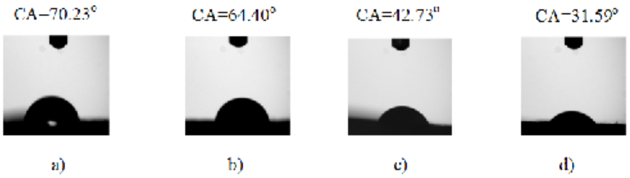

Figure 3. Images of the drop shape recorded at 0.064s for the collagen spongious matrices: a) Coll; b) Coll-MTZ; c) Coll-Sr; d) Coll-Sr-MTZ

The addition of metronidazole in both samples without (Coll-MTZ) and with strontium ranelate (Coll-Sr-MTZ) induced a decrease of the contact angle and consequently an increase of hydrophilicity in comparison with the samples without drug. Also, the presence of strontium ranelate in formulation determined an obvious increase of surface wettability comparing with the samples without mineral.

The influence of the formulation factors on the MTZ release from the spongious matrices was than evaluated and the kinetic patterns are presented in Figure 4.

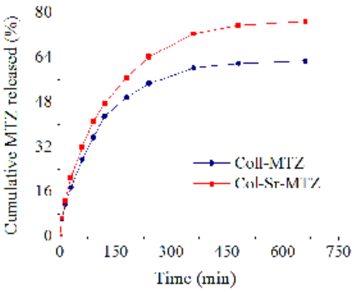

Figure 4. Cumulative release profiles of metronidazole from collagen spongious matrices as a function of time

The MTZ released percentage after 11 hours of experiments was higher for the sample with Sr, the increase being about 1.22 times (Table 3).

The Power law kinetic model (eq. 3) was applied to the experimental data and the fitting parameters are given in Table 3.

$$\frac{m_t}{m_\infty} = k \cdot t^n \tag{3}$$

where, m_t/m_∞ represents the fraction of drug released at time t, k – the kinetic constant, n – the release exponent indicatind the drug kinetic release mechanism.

Table 3. Fitting parameters for the Power law kinetic model; MTZ released percentage

Collagen sponges	Correlation coefficient	Kinetic constant ($1/\text{min}^n$)	Release exponent	MTZ Released percent (%)
Coll-MTZ	0.9762	0.063	0.371	62.76
Coll-Sr-MTZ	0.9822	0.066	0.394	76.72

The values obtained for the release exponent indicated for both spongious supports a non-Fickian drug release mechanism.

The kinetic results are in line with the ones obtained for the goniometric studies, the samples proving a good wettability and facilitating the permeation of the receiving medium into the porous structures, and consequently the drug diffusion through the gel network.

CONCLUSIONS

The collagen hydrogels designed in this paper presented a non-newtonian pseudoplastic behaviour facilitating the formulation flow and their good manipulation. The spongious matrices composition influenced their surface wettability properties which were corelated with the drug release profiles. Based on the results obtained, we conclude that the designed formulations could represent a favorable starting solution for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease.

Acknowledgements

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REFERENCES

Addy, M., Martin, M.V. (2003), “Systemic Antimicrobials in the Treatment of Chronic Periodontal Diseases: A Dilemma”, *Oral Diseases*, 9, 38-44.

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Divya., P.V. and Nandakumar, K. (2006), “Local Drug Delivery - Periocol In Periodontics”, *Trends in Biomaterials and Artificial Organs*, 19, 74-80.

Er, K., Polat, Z.A., Ozan, F., Tasdemir, T., Sezer, U., Siso, S.H. (2008), “Cytotoxicity Analysis of Strontium Ranelate on Cultured Human Periodontal Ligament Fibroblasts: A Preliminary Report”, *Jorunal of the Formosan Medical Association*, 107, 609-615.

Ghica, M.V., Kaya, D.A., Albu, M.G., Popa, L., Dinu-Pirvu, C.E., Cristescu, I. and Udeanu, D.I. (2011), “Ibuprofen-Collagen Sponges for Wound Healing, *Proceedings of the 5th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 213-218.

Ghica, M.V., Ficai, A., Marin, S., Marin, M.M., Ene, A.M. and Pătraşcu, J.M. (2015), “Collagen / Bioactive Glass Ceramic / Doxycycline Composites for Bone Defects”, *Revista Română de Materiale*, 45(4), 307-314.

Ghica, M.V., Albu, M.G., Popa, L. and Moisescu, S. (2013), “Response Surface Methodology and Taguchi Approach to Assess the Combined Effect of Formulation Factors on Minocycline Delivery from Collagen Sponges”, *Pharmazi*e, 68(5), 340-348.

Ficai, A., Andronescu, E., Voicu, G., Albu, M.G. and Ilie, A. (2010), “Biomimetically Synthesis of Collagen / Hydroxyapatite Composite Materials”, *Materiale Plastice*, 47, 205-208.

Pejnic, A., Kesic, L., Obradovic, R. and Mirkovic, D. (2010), “Antibiotics in the Management of Periodontal Disease”, *Scientific Journal of the Faculty of Medicine in Nis*, 27, 85-92.

Pichayakorn, W. and Boonme, P. (2012), “Evaluation of Cross-Linked Chitosan Microparticles Containing Metronidazole for Periodontitis Treatment”, *Materials Science and Engineering*, 33, 1197-1202.

Popa, L., Ghica, M.V., Albu, M.G., Ortan, A. and Dinu-Pirvu, C.E. (2013), “Hysteresis of Contact Angle. Dynamic Wettability Studies of Collagen and Doxycycline Porous Matrices Crosslinked with Tannic Acid, *Digest Journal of Nanomaterials and Biostructures*, 8(3), 937-943.

Southard, G.L. and Godowski, K.C. (1998), “Subgingival Controlled Release of Antimicrobial Agents in the Treatment of Periodontal Disease”, *International Journal of Antimicrobial Agents*, 9, 239-253.

Vyas, S.P., Sihorkar, V. and Mishra, V. (2000), “Controlled and Targeted Drug Delivery Strategies Towards Intraperiodontal Pocket Diseases”, *Journal of Clinical Pharmacy and. Therapeutics*, 25, 1-42.

Wu, C., Zhou, Y., Lin, C., Chang, J. and Xiao, Y. (2012), “Strontium-Containing Mesoporous Bioactive Glass Scaffolds with Improved Osteogenic/Cementogenic Differentiation of Periodontal Ligament Cells for Periodontal Tissue Engineering”, *Acta Biomaterialia*, 8, 3805-3815.

DEVELOPMENT AND CHARACTERIZATION OF INDOMETHACIN LOADED POLYVINIL ALCOHOL-COLLAGEN SMART HYDROGELS FOR BURNS INJURIES

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Hydrogels play a very important role in burns treatments, alone or in combination with other products, having the capacity to calm and moisturize the wound. Hydrogels are three – dimensional structures with interesting properties, having the ability to induce autolysis debridation, to absorb the exudates and to keep the moist of wound healing. The aim of this study was to develop and characterize some new composite materials based on collagen (COLL), polyvinyl alcohol (PVA) and indomethacin (IND), designed to be used for burns injuries and wound healing. Type I fibrillar collagen gel was extracted from calf hide. Indomethacin was added because of anti-inflammatory effect. Hydrogels with various ratios of collagen, PVA and indomethacin were rheologically tested. The collagenic supports obtained by hydrogels lyophilization were investigated by antimicrobial and cytotoxicity tests. The hydrogels presented a pseudoplastic behavior, and the Herschel-Bulkley model best fitted the rheological data. The biological properties, cytotoxicity and antimicrobial tests revealed the possibility of using them in medical application. Based on the hydrogels performance, we could conclude that the anti-inflammatory spongious matrices based on collagen and PVA are potentially usable for burn injuries and wound healing.

Keywords: collagen hydrogels, PVA, indomethacin, burns

INTRODUCTION

Hydrogels are three-dimensional networks derived from natural or synthetic polymers and are characterized by the ability to retaining large amounts of water or fluids. They have a soft consistency similar to that of human tissue (Ullah *et al*., 2015).

Collagen, the most common protein in the human body, has the ability to take over biological tissues functions. It is widespread in many applications such as vascular grafts or matrices for tissue regeneration (Davidenko *et al*., 2012).

The polyvinyl alcohol is a synthetic polymer that may be used in combination with collagen - a natural polymer in order to avoid glutaraldehyde cross-linking, which at certain concentrations is toxic for the body.

Indomethacin is a non-steroidal and anti-inflammatory drug. It is used to relieve pain and inflammation. It works by blocking cyclooxygenase, a substance present in the human body which is involved in producing chemical irritants as a response to surgery or illness. By blocking the action of this, indomethacin is able to reduce pain and inflammation (Fitzpatrick, 2004; Rasekh *et al*., 2014).

The aim of this study was to prepare and characterize some collagen - indomethacin – PVA hydrogels. In order to obtain a dressing with anti-inflammatory properties, hydrogels of collagen, polyvinyl alcohol and indomethacin were performed with the purpose of treating and healing burns.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel (COLL) having a concentration of 2.85% (w/w) was extracted from calf hide using technology developed at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). Polyvinyl alcohol (PVA), with molecular weight of 60 000 Da was purchased from Sigma-Aldrich and glutaraldehyde (GA) from Merck (Germany). The indomethacin (IND) was obtained from Fluka.

Preparation of Collagen Hydrogels

The collagen gel with the initial concentration of 2.85% and acid pH was adjusted using 1M sodium hydroxide at pH 7.3 for a better biocompatibility. The final concentration of used collagen gel was 1% (w/v). Thereafter PVA with a concentration of 0.5% and 0.2% indomethacin were dispersed in the collagen gels in different proportions, according to the compositions shown in Table 1. For cross-linking 0.025% glutaraldehyde solution was used.

Table 1.Composition of collagen hydrogels

	COLL, %	PVA, %	IND, %	GA, %
S1	0	100	0.2	0.025
S2	100	0		
S3	50	50		
S4	75	25		
S5	25	75		

The collagen gels, in order to be analyzed, were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer, using a 48 hours lyophilization programme.

Rheological Analysis

The flow properties of the designed hydrogels were determined with a rotational viscometer MultiVisc-Rheometer (Fungi lab) equipped with standard spindle TR 9 and an ultrathermostat ThermoHaake P5. The rheological measurements were carried out at 37°C±0.5°C. The operational conditions were detailed in our previous studies (Paunica-Panea *et al*., 2016; Ghica *et al*., 2012a). The viscosity of the hydrogels was plotted as a function of shear rate and the corresponding curves were obtained. To quantify the hydrogels rheological behavior, the Power law model was applied (eq. 1):

$$\eta = m \cdot \dot{\gamma}^{-n} \qquad (1)$$

where, m and n are flow parameters correlated with the designed hydrogels composition and determined through the linearization of eq. (1) by double logarithmic method (Ghica *et al*., 2012b).

Hydrogels Antimicrobial Activity

For testing the antimicrobial activity, Nutrient Agar culture medium was used sterilized at 121°C for 15 minutes and poured into Petri dishes. After solidification, the Petri dishes were inoculated with 100 mL of cell suspension of *Staphylococcus aureus* ATCC 25923, all over nutrient agar. In each case were carried out 2 holes (with a diameter of about 6 mm) with the help of sterile glass tubes and through these holes were inserted 40 µL of each sample. The test plates were thermostatted for 18 hours at 37°C, after which they were analyzed. For a preliminary assessment of the possible cytotoxic effects, human osteosarcoma MG 63 cells were cultured in the same well with tested samples. First, the samples were cut to obtain samples with the same size and then were sterilized by maintaining for 24 hours in 70% (v/v) ethanol. Subsequent, the samples were washed with sterile distilled water and further maintained for 24 hours in specific cell culture conditions: 37°C, 5% CO_2, relative humidity < 95%, pH 7.2-7.4, in DMEM medium with 1‰ glucose, supplemented with 10 % heat-inactivated fetal bovine serum and antibiotics: penicillin 300 UI/mL, streptomycin 300 µg/mL and neomycin 150 µg/mL. After these steps the samples were seeded with a 25.000 cells/mL cell suspension and placed in culture condition. Phase contrast microscopy images were taken at day 2 and day 4 of the culture, in order to evaluate the culture state surrounding tested samples. For comparison it was used MG 63 cells cultured only in culture medium.

RESULTS AND DISCUSSION

After lyophilization the 3D porous matrices based on collagen-polyvinyl alcohol - indomethacin were obtained, with the appearance presented in Figure 1.

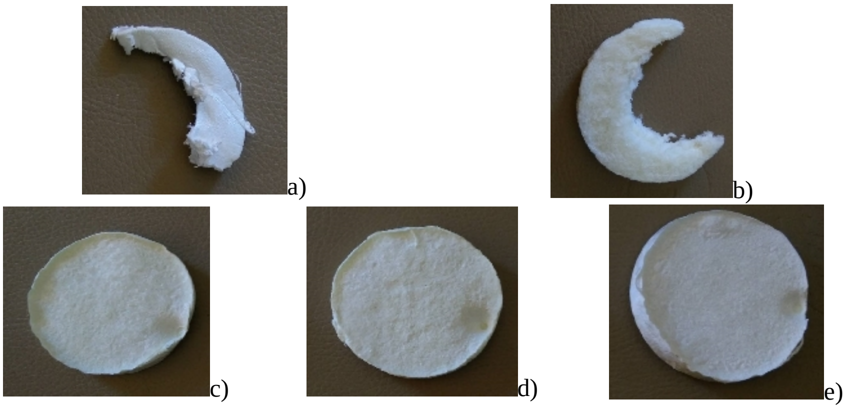

Figure 1. Collagen spongious forms: a) S1; b) S2; c) S3; d) S4; e) S5.

The influence of formulation factors, namely the ratio PVA: COLL on the rheological behavior of the tested hydrogels, plotted as viscosity versus shear rate, is shown in Figure 2.

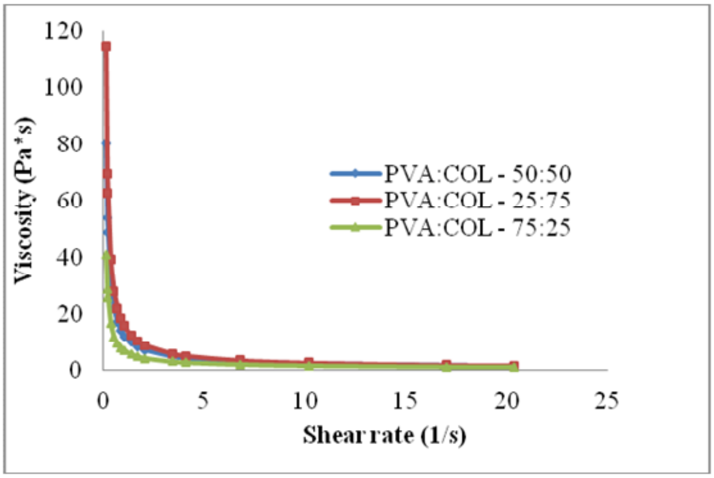

Figure 2. Plots of viscosity as a function of shear rate for the collagen hydrogels evaluated at 37°C

As can been seen in Figure 2 similar flow profiles were recorded for the designed hydrogels, the viscosity decreasing with shear stress increase. This behavior conducts to pseudoplastic properties of the samples which facilitate the formulations flow and consequently their suitable manipulation (Ghica *et al.*, 2015).

The Power law equation applied to rheological data conducted to flow parameters, m and n, mentioned to Materials and Methods section, and listed in Table 2. The determination coefficients values, ranging between 0.9988 and 0.9993 indicate that this rheological model fitted very well the experimental data.

Table 2. Fitting parameters of the Power law rheological model for designed collagen hydrogels

Hydrogel	m	n	R^2
Gel PVA:COLL - 50:50 (S3)	13.037	0.794	0.9989
Gel PVA:COLL - 25:75 (S4)	15.718	0.857	0.9993
Gel PVA:COLL - 75:25 (S5)	7.432	0.748	0.9988

From Table 2 it is observed that the ratio PVA: COLL markedly influence the parameter m, (associated with the viscosity recorded for the shear rate of $1 \cdot s^{-1}$). Thus, the highest value of this parameter was recorded for a ratio PVA: COLL of 25:75 (S4), while a ratio PVA: COLL 50:50 (S3) causes a decrease in the value of m to about 1.20 times. The increase of PVA concentration in the formulation at a ratio PVA: COLL 75:25 (S5) leads to the lowest values of the parameter m, recordings decrease of approximately 43% compared it with the gel S3, and respectively 53% compared to the gel S4.

The results of antimicrobial activity of tested hydrogels are presented in Figure 3.

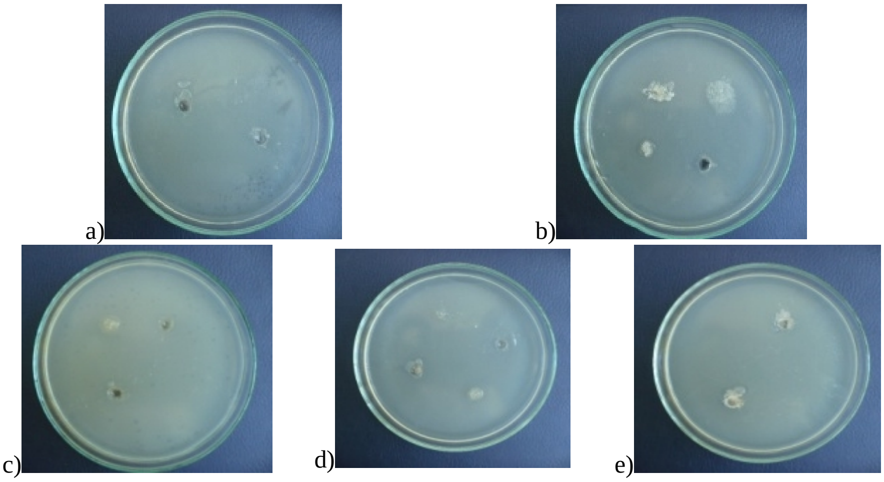

Figure 3. Results for antimicrobial activity: a) S1; b) S2; c) S3; d) S4; e) S5

Following the analysis of antimicrobial activity, no zones of inhibition were observed around any samples that increased bacterial strain evenly across the entire culture medium. Therefore, no evidence of antibacterial activity against strain used as indicator was shown. This was expected because there was used not any antimicrobial agent. Is recommended that lyophilized samples to be sterilized by γ radiation or to obtain hydrogels under sterile conditions.

In the Figure 4 is presented the phase contrast images of MG 63 osteosarcoma cells after 2 and 4 days of culture of the matrices S1 – S5.

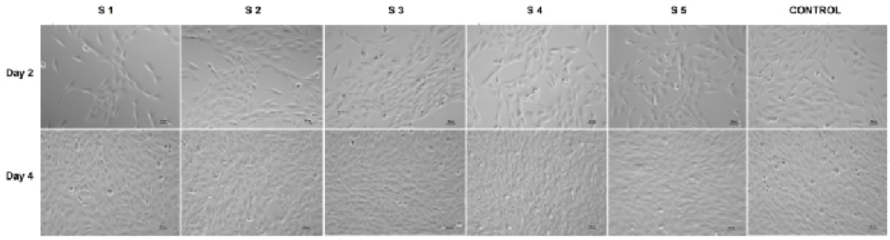

Figure 4. Phase contrast images of MG 63 osteosarcoma cells after 2 and 4 days of culture in presence of samples S1-S5. As control MG 63 cells cultured only in culture medium were used. Scale bar 20μm.

Both on the second and fourth day after initiating the cell culture no cytotoxic effects were observed by phase contrast microscopy. Cell morphology seems to be normal indicating that no cytotoxic compound was released from tested samples into the medium to change the cell morphology. The number of rounded cells was relatively equal for all samples. For a more precisely characterization of samples effects on osteosarcoma cells other assay are needed for evaluating proliferation level, oxido-reductase enzymes activity, alkaline phosphatase activity, gene transcription level etc.

CONCLUSIONS

Collagen - polyvinyl alcohol - indomethacin (COLL-PVA-IND) hydrogels with different ratios of COLL: PVA were obtained and characterized in order to be used as burn wound dressing. The increase of PVA concentration in the formulation at a ratio PVA: COLL 75:25 (S5) leads to the lowest values of the parameter m, recording a decrease of approximately 43% compared it with the gel S3, and respectively 53% compared to the gel S4.The hydrogels were rheologically tested, the ratio PVA: COLL strongly influencing their flow properties. The collagenic supports obtained by hydrogels lyophilization were investigated by antimicrobial and cytotoxicity tests. The biological properties, cytotoxicity and antimicrobial tests revealed the possibility of using them in medical application. Based on the hydrogels performance, we could conclude that the anti-inflammatory spongious matrices based on collagen and PVA are potentially usable for burn injuries and wound healing.

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REFERENCES

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Davidenko, N., Gibb, T., Schuster, C., Best, S.M., Campbell, J.J., Watson, C.J. and Cameron, R.E. (2012), “Biomimetic Collagen Scaffolds with Anisotropic Pore Architecture”, *Acta Biomaterialia*, 8, 667-676.

Fitzpatrick, F.A. (2014), “Cyclooxygenase Enzymes: Regulation and Function”, *Current Pharmaceutical Design*, 10, 577-588.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C. and Moisescu, Şt. (2012a), “*In vitro* Kinetic Release and Flow Behavior of some Collagen-Minocycline Topical Hydrogels”, *Revista de Chimie*, 63(9), 929-935.

Ghica, M.V., Albu, M.G., Coară, Gh. and Dinu-Pîrvu, C. (2012b), “The Influence of Crosslinking Agent on Kinetic Release and Rheological Behaviour of some Collagen-Niflumic acid Hydrogels”, *Proceedings of the 4th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 267-272.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C. and Moisescu, Şt. (2012), “*In vitro* kinetic release and flow behavior of some collagen-minocycline topical hydrogels”, *Revista de Chimie*, 63(9), 929-935.

Ghica, M.V., Ficai, A., Marin, Ș., Marin, M., Ene, A.M. and Pătraşcu, J.M. (2015), “Collagen / Bioactive Glass Ceramic / Doxycycline Composites for Bone Defects”, *Romanian Journal of Materials*, 45, 307-314.

Lu, J., Lin, X., Jiang, B., Li, X., Chen, J. and Zhang, X. (2005), “Preparation and Characterization of Collagen by Hydrogel Formation Method”, *Key Engineering Materials*, 288-289, 377-380.

Paunica-Panea, G., Ficai, A., Marin, M.M., Marin, S., Albu, M.G., Constantin, V.D., Dinu-Pîrvu, C., Vuluga, Z., Corobea, M.C. and Ghica, M.V. (2016), “New Collagen-Dextran-Zinc Oxide Composites for Wound Dressing”, *Journal of Nanomaterials*, 2016, 7 pages.

Rasekh, M., Karavasili, C., Soong, Y.L., Bouropoulos, N., Morris, M., Armitage, D., Li, X., Fatoutous, D.G. and Ahmad, Z. (2014), “Electrospun PVP-Indomethacin Constituents for Transdermal Dressings and Drug Delivery Devices”, *International Journal of Pharmaceutics*, 473, 95-104.

Ullah, F., Othman, M.B.H., Javed F., Ahmada, Z., Akil, H.Md. (2015), “Classification, Processing and Application of Hydrogels: A Review”, *Materials Science and Engineering C*, 57, 414-433.

EFFECTS OF DIFFERENT DRYING METHODS ON COMPONENTS OF THYMBRA SPICATA L. ESSENTIAL OIL FROM FLORA OF HATAY (TURKEY)

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Zahter (*Thymbra spicata*), a perennial member of the *Labiatae* family is one of the most important spices in Hatay's cuisine. Its dry leaves are mixed with red pepper, sesame and çökelek (a special cheese in the region) and is heavily used in sürk (a dish made with çökelek) and pastries. This study aims to determine the effects of various drying methods on the essential oil content of thymbra spicata, a naturally growing plat in Hatay. The plant material was harvested from Hatay's flora on May 4, 2015 and dried in the shade, in an oven at 30°C. The essential oil was obtained with vapor distillation, and its contents were determined using GC/MS. The study determined that the ratio of carvacrol, the main component off *Thymbra spicata* essential oil changes between %51.14 and 72.54.

Keywords: *Thymbra*, Drying, GC-MS

INDRODUCTION

Zahter (*Thymbra spicata*) is a member of the *Labiatae* family and is spread around the Eastern Mediterranean countries. In our country, it is widely found in the Thracian, Aegean, Mediterranean coasts and the South-Eastern Anatolia region. *Thymbra spicata* is an evergreen shrub that is approximately 50 cm high (Kızıl *et al*., 2009). Its essential oil content is reported to vary between 4.016 and 4.700% (Mert and Türkmen, 2015). 70-80% of *T. spicata* essential oil consists of Carvacrol (Kızıl *et al*., 2009). Zahteri (*Thymbra spicata*) has an economic value in Antakya region. About 10-12 tons of thyme is sold every year (Dağıstan and Sarıhan, 2000). Thyme is one of the famous spices in Hatay cuisine. Fresh *Thymbra spicata* is basically used to make salad. Dry leaves were mixed with pepper, sesame amd cokelek to wake surk and bakery products. Thyme tea is popular drunk in houses and cafes (Mert and Ark, 2007).

This study aims to determine the effects of various drying methods on the essential oil content of *Thymbra spicata*, a naturally growing plat in Hatay.

MATERIALS AND METHODS

Materials

The plant materials were collected from Hatay floara on May 4, 2015.

Methods

Thyme leaves treated with different drying methods which is given below.
Treatments:

- At shade
- At sunshine
- At 30°C temperature in the oven

(Dry leaves were used for extrection of essential oil of thyme leaves with water steam distilation (Neo-Clevenger) in each treatment.)

Gas Chromatography - Mass Spectrometry (GC-MS)

The components of the *Thymbra spicata* essential oils used in the study were determined using gas chromatography-mass spectrometry in the Medical and Aromatic Plants Analysis Laboratory of the Mustafa Kemal University Faculty of Agriculture Department of Field Crops. The identification of essential oil components was made using a Thermo Scientific ISQ Single Quadrupole gas chromatography device; using TG-Wax WS model (5% Phenyl Polysilphenylene-siloxane, 0,25 mm internal diameter * 30 m length, 0.25 µm film thickness) column. The ionization energy was set to 70 eV and the mass interval m/z was set to 1.2-1200. Scan mode was used for data collection. MS transfer line temperature was 250 °C, MS ionization temperature was 220 °C, column temperature was initially 50 °C and increased to 220 °C with 3 °C/minute increments. The structure of ech compound was identified by the Xcalibur software using mass spectra (Wiley 9).

RESULTS AND DISCUSSION

The components and component ratios of the essential oil obtained from the *Thymbra spicata* plant naturally growing in Hatay are presented in Table 1. As evidenced in Table 1, the main component, Carvacrol constitutes 72.54% of the essential oil, and is followed by γ-Terpinene with 17.18% and o-Cymene with % 4.90.

Table 1. The components and component ratios of the essential oil obtained from the *Thymbra spicata* plant naturally growing in Hatay

RT	Compound Name	Cas #	Area %
3,43	α-Pinene	80-56-8	0.10
3,48	α-Thujene	99-83-2	0.15
5,07	β-Phellandrene	555-10-2	0.06
6,05	β-Myrcene	123-35-3	0.35
6,4	α-Terpinene	99-86-5	0.60
6,88	l-Limonene	5989-54-8	0.09
7,08	Eucalyptol	470-82-6	0.34
8,22	γ-Terpinene	99-85-4	17.18
9,01	o-Cymene	527-84-4	4.90
15,43	1-Octen-3-ol	3391-86-4	0.10
15,82	trans Sabinene hydrate	17699-16-0	0.08
17,46	cis-Limonene oxide	13837-75-7	0.12
20,39	trans-Caryophyllene	87-44-5	0.72
20,9	Terpinen-4-ol	562-74-3	0.14
24,47	Isoborneol	124-76-5	0.11
33,48	(-)-Caryophyllene oxide	1139-30-6	0.12
34,38	α-Sinensal	17909-77-2	0.06
38,55	(+) spathulenol	77171-55-2	0.29
40,45	Thymol	89-83-8	0.09
41,7	Carvacrol	499-75-2	72.54
50,56	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	5293-90-3	0.16
51,12	3,5-Heptadienal, 2-ethylidene-6-methyl-	99172-18-6	0.09

The components and component ratios of the essential oil obtained from the shade-dried leaves of Thymbra spicata that grows naturally in Hatay are presented in table 2. As evidenced in table 2, the main component Carvacrol is present at 58.32% and is followed by γ-Terpinene at 28.53% and 5.20% with o-Cymene.

Table 2. The components and component ratios (%) of the essential oil obtained from the shade-dried leaves of the *Thymbra spicata* plant naturally growing in Hatay

RT	Compound Name	Cas #	Area %
3,42	α-Pinene	80-56-8	0.30
3,48	α-Thujene	2867-05-2	1.05
4,79	β-Pinene	127-91-3	0.08
5,07	Sabinene	3387-41-5	0.11
6,05	β-Myrcene	123-35-3	0.96
6,4	α-Terpinene	99-86-5	2.03
6,88	Limonene	5989-54-8	0.14
7,07	Eucalyptol	470-82-6	0.06
7,13	β-Phellandrene	555-10-2	0.11
8,23	γ-Terpinene	99-85-4	28.53
9,01	o-Cymene	527-84-4	5.20
18,91	trans Sabinene hydrate	17699-16-0	0.06
20,39	trans-Caryophyllene	87-44-5	1.26
20,9	Terpinen-4-ol	562-74-3	0.11
24,46	L-α-Terpineol	10482-56-1	0.08
33,75	Caryophyllene oxide	1139-30-6	0.23
38,54	(+) spathulenol	77171-55-2	0.23
40,92	Thymol	89-83-8	0.12
41,7	Carvacrol	89-83-8	58.32
50,56	3,5-Heptadienal, 2-ethylidene-6-methyl-	99172-18-6	0.08

The components and component ratios of the essential oil obtained from the sun-dried leaves of *Thymbra spicata* plant naturally growing in Hatay are presented in Table 3. As evidenced in Table 3, the main component, Carvacrol constitutes 51.14% of the essential oil, and is followed by γ-Terpinene with 23.38%, α-Terpinyl at 7.45% and o-Cymene at % 3.86.

Table 3. The components and component ratios (%) of the essential oil obtained from the sun-dried leaves of the *Thymbra spicata* plant naturally growing in Hatay

RT	Compound Name	Cas #	Area %
3,41	α-Pinene	80-56-8	0.20
3,47	α-Thujene	2867-05-2	0.67
4,04	Camphene	79-92-5	0.07
4,76	β-Pinene	127-91-3	0.07
5,04	Sabinene	3387-41-5	0.08
6,01	β-Myrcene	123-35-3	0.68
6,36	α-Terpinene	99-86-5	1.50
6,85	Limonene	5989-54-8	0.11
7,04	Eucalyptol	470-82-6	0.11
7,1	β-Phellandrene	555-10-2	0.07
8,19	γ-Terpinene	99-85-4	23.38
8,97	o-Cymene	527-84-4	3.86
14,03	Thujone	546-80-5	0.06
14,12	cis Sabinene hydrate	17699-16-0	0.16
15,41	1-Octen-3-ol	3391-86-4	0.07
17,38	Camphor	76-22-2	0.12
17,89	trans Sabinene hydrate	17699-16-0	0.18
18,28	Linalool	78-70-6	0.34
18,51	1-Terpinenol	586-82-3	0.07
20,14	Terpinen-4-ol	562-74-3	1.61
20,38	trans-Caryophyllene	87-44-5	1.03
22,2	trans-Pinocarveol	547-61-5	0.15
23,06	α-Terpineol	98-55-5	0.28
23,85	α-Terpinyl acetate	80-26-2	7.45
24,07	L-α-Terpineol	10482-56-1	1.66
24,47	Borneol, acetate	5655-61-8	0.08
25,43	Limonene oxide	1195-92-2	0.12
27,57	Myrtenol	515-00-4	0.09
27,82	p-Mentha-1(7),8-dien-2-ol	35907-10-9	0.07
33,65	(-)-Caryophyllene oxide	1139-30-6	0.37
35,4	Anisylacetone	104-20-1	1.09
38,53	(-)-Spathulenol	77171-55-2	0.39
39,55	Acetic acid, cinnamyl ester	103-54-8	0.08
40,1	Eugenol	97-53-0	0.22
40,92	Thymol	89-83-8	0.13
41,41	Junipene	475-20-7	0.07
41,71	Carvacrol	499-75-2	51.14
45,12	Alloaromadendrenoxıd-(1)	85710-39-0	0.06

The components and component ratios of the essential oil obtained from the leaves dried in a 30 ^{o}C oven of *Thymbra spicata* plant naturally growing in Hatay are presented in Table 4. As evidenced in Table 4, the main component, Carvacrol constitutes 60.33% of the essential oil, and is followed by γ-Terpinene with 27.50% and o-Cymene with % 4.98.

Table 4. The components and component ratios (%) of the essential oil obtained from the leaves dried in a 30°C oven of the *Thymbra spicata* plant naturally growing in Hatay

RT	Compound Name	Cas #	Area %
3,42	α-Pinene	80-56-8	0.27
3,48	α-Thujene	2867-05-2	0.89
4,78	β-Pinene	127-91-3	0.07
5,06	Sabinene	3387-41-5	0.10
6,04	β-Myrcene	123-35-3	0.86
6,39	α-Terpinene	99-86-5	1.77
6,88	Limonene	5989-54-8	0.13
7,12	β-Phellandrene	555-10-2	0.15
8,22	γ-Terpinene	99-85-4	27.50
9	o-Cymene	527-84-4	4.98
15,42	1-Octen-3-ol	3391-86-4	0.08
18,9	trans Sabinene hydrate	17699-16-0	0.07
20,39	trans-Caryophyllene	87-44-5	1.09
20,89	Terpinen-4-ol	562-74-3	0.11
24,47	Isoborneol	124-76-5	0.08
33,75	Caryophyllene oxide	1139-30-6	0.20
38,54	(-)-Spathulenol	77171-55-2	0.21
40,91	Thymol	89-83-8	0.12
41,7	Carvacrol	499-75-2	60.33
50,55	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	5293-90-3	0.08

CONCLUSION

The results of the study suggest that the highest ratio of carvacrol, the main component of *Thymbra spicata* essential oil is obtained from the fresh sample that was not dried (72.54%). The highest carvacrol ratio was obtained in essential obtained from the leaves dried in a 30°C oven (%60.33), this was followed by the essential obtained from shade-dried leaves (58.32%) and sun-dried leaves (51.14%). *Thymbra spicata* var. *spicata* both grows naturally and is cultivated in Hatay's flora and a study conducted to determine its essential oil components (Mert *et al.*, 2007), it was found that the carvacrol ratio in the shade-dried leaves was 79.5% in samples harvested from the natural flora, and 80.6% in cultivated samples, and these walues vere higher than the carvacrol values that we obtained in shade-dried (58.32%), fresn (72.54%), sun-dried (51.14%) and leaves dried in a 30°C oven.

REFERENCES

Dağıstan E. and Sarıhan, E.O. (2009), Hatay İline Özgü Bir Lezzet; Zahter. Türkiye VIII. Tarla Bitkileri Kongresi Poster Bildirileri, Hatay 269 – 273.

Kızıl, S., Özgüven, M. and Yamanoğlu, O. (2009), Önemli Bir Kekik Türü: Zahter. (*Thymbra spicata* L.var. spicata) Türkiye VIII. Tarla Bitkileri Kongresi. 19-22 Ekim 2009.Hatay, Cilt 1. Sunulu Bildiriler.s. 86-90.

Mert, A., Arslan, M. and Ayanoğlu, F. (2007), Hatay Florasında Doğal Olarak Yetişen Zahter (Thymbra spicata var. spicata) Uçucu Yağ İçeriği, Yağ Bileşenleri ve Geleneksel Kullanımı. Türkiye VII. Tarla Bitkileri Kongresi 25-27 Haziran 2007. Erzurum (Poster Bildiri) 732 – 735.

Mert, A. and Türkmen, M. (2015), “The Effects of Drying Methods on the Essential Oil Content of Naturally Grown Thyme (Thymbra spicata) in Hatay Province”, *The 3rd International Symposium on Traditional Foods from Adriatic to Caucasus*, 01-04 October 2015, Sarajevo/Bosna and Herzegovina Abstract Book, 335.

COLLAGEN-INDOMETHACIN-HYDROXYAPATITE SPONGIOUS FORMS FOR BONE RECONSTRUCTION TREATMENT

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The aim of this study was to prepare and characterize collagen spongious forms for bone tissue engineering. The ideal scaffold has almost the same structural and biological functions as a naturally occurring extracellular matrix in terms of physical structure and chemical composition Having about 30% collagen and 70% hydroxyapatite, to mimic bone composition, the obtained matrices were characterized by water up-take, enzymatic degradation, scanning electron microscopy and indomethacin release from supports. The results showed that the composites based on collagen and hydroxyapatite absorbed less water than the ones with indomethacin. The degradation process showed that indomethacin inhibits collagenase solution, degradation taking place in more than one week. The pore sizes are proper for bone tissue engineering and the release of drug is fast in first 4 hours and slowly next hours. The results showed us that the combination between collagen, indomethacin and hydroxyapatite is a promising spongious form, slowly degraded in vitro, with specific absorbance properties, with proper pore sizes and porosity and which can release the drug rapidly, ensuring a diminution of the inflammation and of the associated pain; this formulation could be successfully used in bone regeneration.

Keywords: collagen, indomethacin, hydroxyapatite.

INTRODUCTION

The scaffolds for bone tissue engineering should possess several properties such as biocompatibility, mechanical similarities with the target tissue and high porosity for cell adhesion, proliferation, and tissue development. The ideal scaffold has almost the same structural and biological functions as a naturally occurring extracellular matrix in terms of physical structure and chemical composition (Mederle *et al*., 2016).

Collagen is an important constituent of the natural extracellular matrix (ECM) and matrices made from it are used as scaffolds for tissue engineering as well as systems for drug delivery. Collagen is also one of the main components of the bone and that's why most of the materials designed for bone grafting and repair are based on collagen and hydroxyapatite. Collagen matrices have porous structures which influence hydrophilicity, drug diffusion through the network, the degradation properties and interaction with and within cells (Ficai *et al*., 2010).

Hydroxyapatite is a naturally occurring substance that is found in teeth, bones, and tendons. The hydroxyapatite is the one main inorganic component of bone and it has a variety of uses in bone applications such as fillers and replacements due to its excellent biocompatibility, osteoconductivity and biointegration (Rusu *et al*., 2015; Popa *et al*., 2016).

Indomethacin (IND) is a nonsteroidal anti-inflammatory drug commonly used as a prescription medication to reduce fever, for pain, stiffness, and swelling from inflammation. It works by inhibiting the production of prostaglandins, molecules known to cause these symptoms and ease the pain and discomfort (Cabezas, 2012).

The aim of this work was to obtain collagen-hydroxyapatite-indomethacin matrices, uncross and cross-linked with glutaraldehyde with variable pore sizes for hard tissue engineering.

MATERIALS AND METHODS

Collagen Matrices Preparation

Collagen (Col) gel was obtained from bovine skin using the protocol that has been previously described (Albu, 2009). An amount of 0.2% indomethacin (IND), and 70% hydroxyapatite (HA) was embedded under mechanical stirring into collagen gel having the concentration 1% and pH 7.2-7.4. The gels with compositions according to Table 1 were then cross-linked with 0.025% glutaraldehyde (GA) and put in Petri dishes of 3 cm diameter. The collagen gels were freeze-dried using the Delta LSC 2-24 (Martin Christ, Germania) freeze-dryer for 48 hours and the corresponding matrices were obtained. Compositions and codes of the sample are given in Table 1.

Table 1. Compositions and name of collagen gels

Code of gels	Code of matrices	Col*, %	HA**, %	GA**, %	IND*, %
Col	B1	1	0	0.025	0
Col-IND	B2	1	0	0.025	0.2
Col-HA	B3	1	70	0.025	0
Col-HA-IND	B4	1	70	0.025	0.2

* reported to collagen gel volume

** reported to collagen dry substance

Water Up-take Capacity and Enzymatic Degradation

The water uptake capacity and enzymatic degradation were performed using the protocol as we previously described (Albu, 2012) on the obtained matrices with compositions according with Table 1.

Scanning Electron Microscopy

The scanning electron microscopy (SEM) images were registered using FEI Quanta 200. Matrix morphology and pore shape and sizes were determined with SEM.

In vitro Release of Indomethacin

The *in vitro* kinetics release of indomethacin from the obtained matrices was determined using a “sandwich” device adapted to a paddle dissolution equipment (Essa Dissolver), as detailed in our previous studies (Ghica *et al*., 2014). The amount of indomethacin released of different time intervals in the receiving medium (phosphate buffer pH 7.4) was spectrophotometrically evaluated at λ=268 nm (Perkin-Elmer UV-

Vis Spectrophotometer), using the calibration curve ($A_{1\%}^{1cm} = 454$, $R^2 = 0.9990$) (Figure 1).

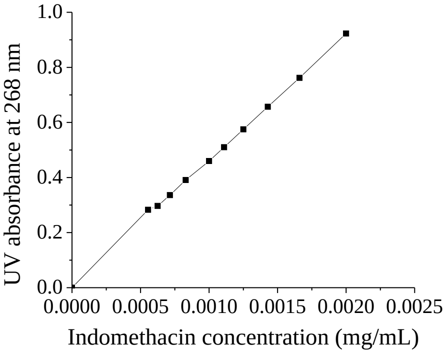

Figure 1. Calibration curve of indomethacin in phosphate buffer (pH 7.4)

The experimental kinetic data were analyzed with the Power law model (eq. 2).

$$\frac{m_t}{m_\infty} = k \cdot t^n \qquad (2)$$

where, m_t/m_∞represents the fraction of drug released at time t, k - the kinetic constant, n - the release exponent characteristic for the drug release mechanism.

RESULTS AND DISCUSSIONS

After lyophilization, sponges based on collagen, hydroxyapatite and indomethacin were obtained. Water absorption capacity for the B1÷B4 matrices is showed in Figure 2.

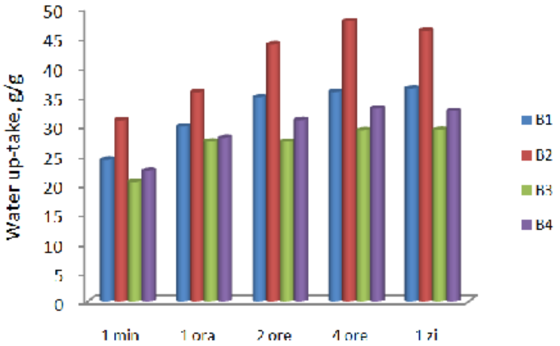

Figure 2. Water absorption of the matrices

The prepared matrices absorbed between 29 and 46% water. The samples are very stable in water, the values at 4 hours being similar with values for 24 hours. Although the indomethacin is an hydrophobic drug, the sample which contain it are more hydrophilic then the one without: after one day B1 absorbed 36 g/g and B2 - 46 g/g water and B3 absorbed 29 g/g and B4 – 32 g/g. The samples with hydroxyapatite content absorbed less about of water then the other without.

Enzymatic degradation of matrices are presented in Figures 3 and 4.

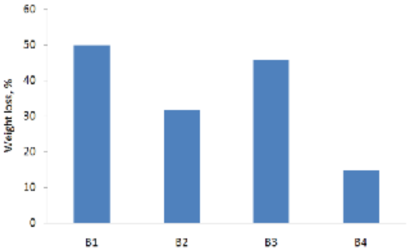

Figure 3. Matrices degradation during 24 hours

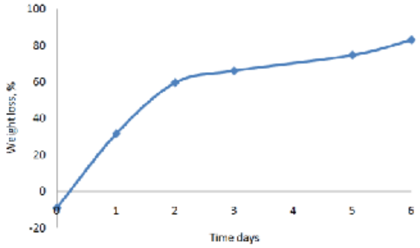

Figure 4. Kinetics of degradation for sample B2, based on Coll and IND

During 24 hours the matrices collagen reference (B1) degraded about 50% and collagen with hydroxyapatite 45%. The indomethacin inhibits collagenase and matrices B2 and B4 degraded in 24 hours 31.6% and 14.7% respectively. This demonstrate that the combination between collagen, indomethacin and hydroxyapatite provide the best stability in simulated environment. The results are in correlation with water up-take.

SEM microscopy shows characteristic pore structure that has a large variation in average pore diameter, for each matrix (Figures 5a, b and c).

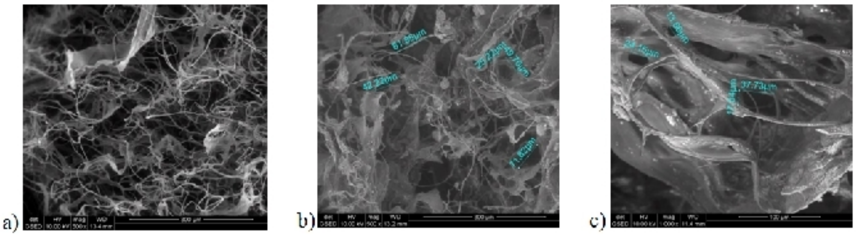

Figure 5. SEM images of matrices: a) B1 (x500); b) B4 (x500); c) B4 (x1000) – transversal section

Figure 5 presents some example of SEM images of matrices. The collagen reference sample, B1 (figure 5a) presents specific interconnected pores of about 100 – 200 µm. In Figures 5b and c we can clear see the hydroxyapatite deposited on collagen fibrils and pores with smaller sizes (between 13 and 81 µm) which give more compact structure for sample B4. These results are in accordance with water up-take and enzymatic degradation.

The influence of the formulation factors on indomethacin release patterns from collagen matrices B2 and B4 is illustrated in Figure 6.

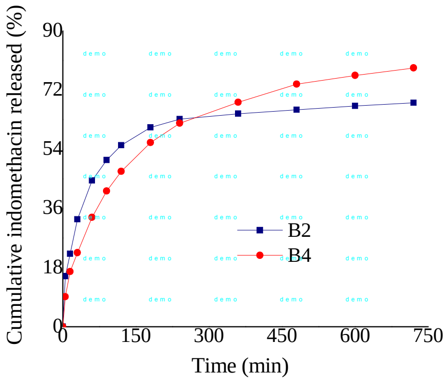

Figure 6. Cumulative release profiles of indomethacin from collagen matrices as a function of time

As can be noticed from Figure 6, the release of indomethacin from collagen matrices tested during 12 hours of experiments recorded two stages. For both samples, in the first two hours, the drug was rapidly release ensuring a diminution of the inflammation and of the associated pain, followed by a gradually and slower drug release during the next hours.

For the matrix with hydroxyapatite, the release of indomethacin was slower in the first 4 hours in comparison with the sample without HA, but after 12 hours of experiments the drug released percentage for this matrix was higher (about 1.16 times) (Table 2). It seems that the presence of bioactive ceramic induced an increase of drug released percentage, this results being in accordance with our previous studies (Ghica *et al.*, 2015).

Table 2. Values for correlation coefficients and kinetic parameters specific to Power law model, and drug released percentage after 12 hours

Collagen matrices	Kinetic constant (1/min^n)	Release exponent	Correlation coefficient	Released percentage (%)
B2	0.159	0.234	0.9713	68.11
B4	0.078	0.360	0.9902	78.69

Applying the Power law model to the release data, the kinetic parameters were determined (Table 2). The values obtained for the release exponent (smaller than 0.5) indicated an anomalous drug transport mechanism.

CONCLUSIONS

Composites matrices based on collagen, nano-hydroxyapatite and indomethacin as anti-inflammatory were obtained by freeze-drying in spongious forms. They were characterized by water up-take and enzymatic degradation. The results showed that because of compact structures, the composites based on collagen and hydroxyapatite absorbed less water, meanwhile the indomethacin induces hydrophilicity to matrices. The degradation process showed that collagen reference crosslinked with glutaraldehyde is degraded about 50% in one day, meanwhile, the collagen with indomethacin, after 6 days degraded in proportion of 83%. The pore sizes of samples with collagen are between 100 and 200 µm and the one with collagen, hydroxyapatite and indomethacin has smaller pore sizes, up to 80 µm. For the matrix with hydroxyapatite, the release of indomethacin was slower in the first 4 hours in comparison with the sample without HA, but after 12 hours of experiments the drug released percentage for this matrix was higher (about 1.16 times). The results showed us that the combination between collagen, indomethacin and hydroxyapatite is a promising spongious form, slowly degraded in vitro, with specific absorbance properties, with proper pore sizes and porosity and which can release the drug rapidly, ensuring a diminution of the inflammation and of the associated pain; this formulation could be successfully used in bone regeneration.

Acknowledgements

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REFERENCES

Albu, M.G., Ferdes, M., Kaya, D.A., Ghica, M.V., Titorencu, I., Popa, L., Albu, L. (2012), "Collagen wound dressings with anti-inflamatory activity", *Molecular Crystal and Liquid Crystal*, 555(1), 271-279.

Cabezas, L.I., Fernandez, V., Mazarro, R., Garcia, I., de Lucas, A., Rodriguez, J.F. (2012), "Production of biodegradable porous scaffolds impregnated with indomethacin in supercritical CO_2", *Journal of Supercritical Fluids,* 63, 155-160.

Ficai, A., Andronescu, E., Voicu, G., Albu, M.G., Ilie, A. (2010). "Biomimetically synthesis of collagen / hydroxyapatite composite materials", *Materiale Plastice*, 47, 205-208.

Ghica, M.V., Ficai, A., Marin, S., Marin, M.M., Ene, A.M., Pătraşcu, J.M. (2015), "Collagen / bioactive glass ceramic / doxycycline composites for bone defects", *Revista Română de Materiale / Romanian Journal of Materials*, 45(4), 307-314.

Ghica, M.V., Kaya, D.A., Albu, M.G., Popa, L., Dinu-Pîrvu, C.E., Cristescu, I., Udeanu, D.I. (2014), "Ibuprofen-collagen sponges for wound healing", *Proceedings of the 5th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, October 23-25, 213-218.

Mederle, N., Marin, S., Marin, M.M., Danila, E., Mederle, O., Albu Kaya, M.G., Ghica, M.V. (2016), "Innovative biomaterials based on collagen-hydroxyapatite and doxycycline for bone regeneration", *Advances in Materials Science and Engineering*, Volume 2016, 5 pages.

Popa, C.L., Albu, M.G., Bartha, C., Costescu, A., Luculescu, C., Trusca, R., Antohe, S. (2016), "Structural characterization and optical properties of hydroxyapatite/collagen matrix", *Romanian Reports in Physics*, 68, 3.

Rusu, L.C., Nedelcu, I.A., Albu, M.G., Sonmez, M., Voicu, G., Radulescu, M., Ficai, D., Ficai, A., Negrutiu, M.L., Sinescu, C. (2015), "Tetracycline loaded collagen/hydroxyapatite composite materials for biomedical applications", *Journal of Nanomaterials*, Volume 2015, 5 pages.

Zamfir, A.V., Voicu, G., Busuioc, C., Jinga, S.I., Albu, M.G., Iordache, F. (2016), "New Coll-HA/BT composite materials for hard tissue engineering", *Materials Science and Engineering C*, 62, 795-805.

INVESTIGATION OF DIALDEHYDE CORN STARCH AS CROSSLINKING AGENT IN COLLAGEN-BASED WOUND DRESSING MATERIALS

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Collagen is the most commonly used tissue-derived natural polymer. Collagen gels are widely used in wound dressing materials due to their good degradability, poor antigenicity, excellent biocompatibility and unique fibril forming properties. But, poor mechanical properties of physically formed collagen gels limit their usage in biomedical fields. However, collagen gels can be stabilized by using cross-linkers. In recent years, use of crosslinking agents obtained from biodegradable, low toxic, abundant, inexpensive and renewable sources in wound dressing materials for stabilization of collagen gels has become important. In the present study use of starch as a crosslinking agent in stabilization of collagen gels was investigated. For this purpose native starch was modified via oxidation of corn starch by NaIO4 in order to obtain dialdehyde starch. Then, the degree of substitution and swelling index of oxidized starches were determined and the starch samples were characterized by Fourier transforms infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM). Collagen gels were crosslinked with dialdehyde starch samples for preparing wound dressing materials. The collagen gel composites were lyophilized and sponge forms were obtained. They were characterized by water uptake capacity and enzymatic degradation. The results revealed that, dialdehyde corn starch can be used successively in collagen-based wound dressing materials as crosslinking agent.

Keywords: dialdehyde starch, collagen, wound-dressing material.

INTRODUCTION

Collagen protein is one of the major components of connective and bone tissues (Cuneo *et al.*, 2010) being one of the mostly used biomaterials due to its excellent biocompatibility, biodegradability and weak antigenicity, well established structure, biologic characteristics and to the way it interacts with the body, being known by the body as one of its constituent and not as an unknown material (Albu *et al.*, 2011; Ficai *et al.*, 2013).

Collagen dressings encourage the deposition and organization of newly formed collagen, creating an environment that fosters healing because of the dressings' chemotactic properties on wound fibroblasts. Collagen-based biomaterials stimulate and recruit specific cells, such as macrophages and fibroblasts, along the healing cascade in order to enhance and influence wound healing (Fleck and Simman, 2010). However, physically formed collagen gel exhibits poor mechanical properties, and low thermal stability, which limit its applications as wound dressings (Mu *et al*., 2011; Li *et al*., 2016). Therefore, extensive research has focused on modifying collagen with some physical, chemical, and biological cross-linkers to strengthen the intramolecular or intermolecular structures (Tan *et al*., 2015). They are often cross-linked by UV light irradiation, multivalent metal ions, formaldehyde, or glutaraldehyde (Gayatri *et al*., 2001; Sheu *et al*., 2001; Mu *et al*., 2011). The use of chemical cross-linkers may lead to the presence of unreacted molecules in the collagen matrix, which can result in cytotoxic reaction. Currently, natural or bio-based cross-linking agents such as genipin, proanthocyanidin, citric acid, malic acid, ferulic acid, tannic acid are being explored and investigated (Cheirmadurai *et al*., 2016). Note that oxidized polysaccharides have received an increasing attention as ideal crosslinking reagents of protein in recent years (Li *et al*., 2016). Polysaccharides are abundant, inexpensive, biodegradable, environmentally friendly and renewable biopolymers. Two aldehyde groups can be introduced in structure of polysaccharides such as starch and cellulose by periodate oxidation. These highly reactive dialdehyde groups can be used as crosslinking agents.

In the present study, native corn starch was modified by periodate oxidation in different molar ratios and dialdehyde starch (DAS) samples were obtained. Then, they were used as a crosslinking agent for obtaining collagen wound dressing materials.

MATERIALS AND METHODS

Materials

Native corn starch was supplied from Hasal Starch Company in Izmir/Turkey. Sodium metaperiodate ($NaIO_4$) were purchased from Sigma Co. Ltd. Type I collagen having a concentration of 2.4% (w/v) was obtain by own technology in ICPI - Collagen Department and type I collagenase from *Clostridium histolyticum* was purchased from Sigma-Aldrich. All other chemicals used in analysis were analytical grade.

Sodium Metaperiodate Oxidation of Corn Starch

The oxidation of the corn starch by $NaIO_4$ was carried out based on the procedure of Zhang et al. (2011) with slight modifications. The molar ratios of $NaIO_4$ to starch were 1:0.2, 1:0.4, 1:0.6, 1:0.8 and 1:1.0. The pH was adjusted to 3.0 with 2% (w/v) hydrochloric acid and the mixture was rigorously stirred with magnetic stirrer at 35 °C for 4 h in dark ambient. After oxidation process, the mixture was filtered and washed 10 times (10x100 mL) with distilled water and then the product was washed with 50 mL acetone. After washing process, it was dried in a hot-air oven at 50°C for 48 h until constant weight. The dried products were milled and sieved.

Determination of Aldehyde Contents of Oxidized Starches by $NaIO_4$

The aldehyde contents of the oxidized samples were determined using the rapid quantitative alkali consumption method described by Hofreiter *et al.* (1955).

$$Da\% = \frac{C_1 \cdot V_1 - 2 \cdot C_2 \cdot V_2}{W/161 \times 1000} \times 100\% \tag{1}$$

where C_1=NaOH normality (mol/L), C_2=H_2SO_4 normality (mol/L), V_1=total volume of NaOH (mL), V_2=total volume of H_2SO_4 (mL), W=dry weight of the dialdehyde sample, 161=average molecular weight of the repeat unit in DAS.

Determination of the Swelling Index of Native and Oxidized Starches

The swelling index of native corn starch and oxidized starches was determined according to method of Willpiszewska and Spychaj (2007).

Fourier Transform Infrared (FT-IR) Spectroscopy

The infrared spectra of native and dialdehyde starches were done by JASCO FT/IR-4200 spectrophotometer in the range of 4000-600 cm^{-1}.

Scanning Electron Microscopy (SEM)

The morphological features of native and dialdehyde starches were observed by scanning electron microscope (SEM- Quanta 200-FEI/Holland). The dried samples were mounted on a metal stub and the images were taken at an accelerating voltage of 10 kV. Micrographs of dialdehyde starches were recorded at 4000x magnifications.

Crosslinking of Collagen with Dialdehyde Starch

Type I collagen gel was crosslinked with different ratio of oxidized starch as Table 1 presents and their pH were adjusted at 7.2-7.4 with 1 M NaOH, to mimic the human body pH.

Table 1. The formulations of prepared samples

Sample Name	Collagen gel (g)	Oxidized starch (g)
C – O	41.68	-
C – 1:0.2	41.68	1.0
C – 1:0.4	41.68	1.0
C – 1:0.6	41.68	1.0
C – 1:0.8	41.68	1.0
C – 1:1.0	41.68	1.0

All the gels were lyophilized using Martin Christ freeze-dryer according to the previously described method (Albu *et al.*, 2011) and collagen-starch composites were obtained.

Determination of Water Uptake Capacity of Collagen-Starch Composites

Three pieces were cut from each lyophilized sample and were weighted (W_d) sensitively. Then 2.5 mL distilled water was added for each weighed sample. At the established time intervals they were reweighed (W_w) to determine their water uptake capacity. The water uptake capacity of samples was calculated according to the following equation:

$$Water\ up\text{-}take\ =\ (W_w - W_d)/W_d\ g/g \tag{2}$$

where W_w represents the weight of wet matrices at immersion time, W_d represents the weight of dried one.

Enzymatic Degradation of Collagen-Starch Composites

Enzymatic degradation of prepared collagen-starch composites was investigated by monitoring the weight loss depending on exposure time to collagenase solution. 0.5 mL collagenase solution was added to previously weighed composites. At regular time intervals, the swollen composites were removed from degradation solution and then weighed. The percentage of matrices degradation was determined by the following equation:

$$\%\ weight\ loss\ =\ [(W_i - W_t)/W_i] \cdot 100 \tag{3}$$

where W_i represents the initial weight, W_t represents the last weight.

RESULTS AND DISCUSSION

Aldehyde group contents of the oxidized starches by $NaIO_4$ are showed in Table 2.

Table 2. The aldehyde contents of oxidized starches

Samples	Aldehyde Content %
1:0.2	16.7
1:0.4	28.9
1:0.6	45.9
1:0.8	67.7
1:1.0	80.2

From the results, it was seen that increasing periodate molar ratio caused significant increase in aldehyde content in starch molecule. It is proved that periodate is a selective oxidant and can cleave the C2, C3 bond of anhydroglucose units with the formation of dialdehyde groups.

Swelling index of native and dialdehyde starches are presented in Table 3.

Table 3. Swelling index of starches

Samples	Swelling Index (cm^3/g)
Native starch	0
1:0.2	1
1:0.4	1.6
1:0.6	0
1:0.8	0.8
1:1.0	2

The swelling indices in water of native and dialdehyde starches were compared and the sample with 1:0.6 presents same swelling index as native starch, 0. The highest swelling index was presented by sample 1:1.0, to be 2.

Characterization of Native and Dialdehyde Starches by FTIR and SEM

The band at 1723.92 cm^{-1} is the most characteristic band of C=O vibration in aldehyde group. The spectra of the obtained starches are presented in Figure 1.

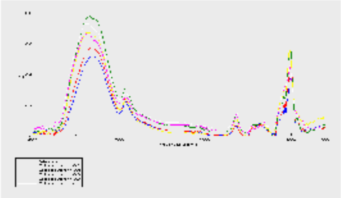

Figure 1. FTIR spectra of native and dialdehyde starches

It can be seen clearly, native corn starch could be oxidized successfully by periodate oxidation method. SEM images were recorded for native and dialdehyde starches at 4000x magnification as Figure 2 presented.

It can be seen that the molecules of starch cluster by increasing oxidation degree; the reason could be that introduction of increasing aldehyde groups into starch molecules provoked formation of intermolecular cross-links.

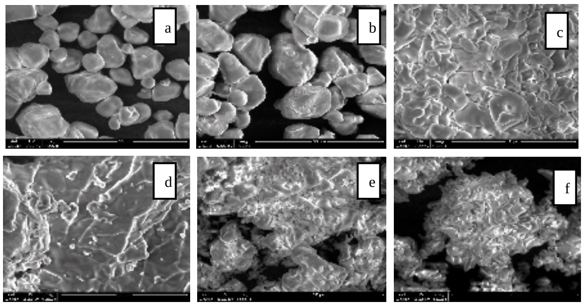

Figure 2. SEM images of native and dialdehyde starches:
a) native starch, b) 1:0.2, c) 1:0.4, d) 1:0.6, e) 1:0.8, f) 1:1.0

Determination of Water Uptake Capacity of Collagen-Starch Composites

The water up-take capacity of collagen-starch samples are presented in Figure 3 starting with first minute to 24 hours at different intervals of time. All the samples absorbed the most amount of water in 24 hours.

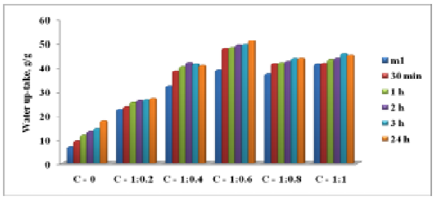

Figure 3. Water uptake capacity (g/g) of collagen-oxidized starch composites in time

As Figure 3 shows the collagen as such absorbed the smallest amount of water, the starch inducing the hydrophilicity to the samples and the most absorbent one is C-1:0.6.

Enzymatic Degradation of Collagen-Starch Composites

The degradation of collagen-starch composites showed their stability in a simulated environment which mimics the body. Weight loss values of matrices during degradation are presented in Figure 4.

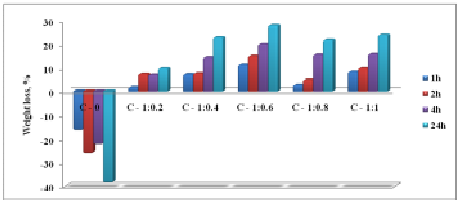

Figure 4. The weight loss (%) of collagen-oxidized starch composites in time

The most resistant sample against degradation was collagen reference during 24 hours. From the figure it was also seen that the degree of degradation is in correlation with hydrophilic degree: increase in absorbent capacity resulted with higher degree of degradation (noticeable till 1:0.6 oxidation degree). While the sample C-1:0.2, which was treated with slightly oxidized starch, was degraded only 9.8%, the sample C-1:0.6, having the highest hydrophilic nature, degraded 28% in 24 hours.

CONCLUSION

New crosslinking agent as dialdehyde starches were obtained with different degree of oxidation and characterized by aldehyde contents, swelling index, FT-IR and SEM images. Successively introduction of aldehyde groups in to the starch structure by $NaIO_4$ oxidation was proved by determining the most characteristic band of C=O vibration specific for aldehyde at 1723.92 cm^{-1} by FT-IR. Dialdehyde starch samples which were oxidized in different degrees were used as crosslinking agents to prepare new collagen-starch composites. Their absorption and degradation capacities showed that the sample (C-1:0.2), having the lowest water uptake capacity (27 g/g), is the most resistant sample to degradation while the sample (C-1:0.6), having the highest water uptake capacity, is the most easily degradable sample (loss 28% in weight in 24 hours).

Since it was seen that absorption and degradability properties of collagen-dialdehyde starch composites can be controlled by crosslinking degree which is in fact related by oxidation degree of the starch it was thought that collagen-diladehyde starch composites are promising for using as wound dressing.

REFERENCES

Albu, M.G., Titorencu, I. and Ghica, M.V. (2011), *Biomaterials Applications for Nanomedicine,* in: P. Rosario (Ed.), InTech: Rijeka, Croatia.

Cheirmadurai, K., Thanikaivelan, P. and Murali, R. (2016), “Highly bipcompatible collagen-Delonix regia seed polysaccharide hybrid scaffolds for antimicrobial wound dressing”, *Carbohydrate Polymers*, 137, 584-593.

Cuneo, F., Costa-Paiva, L., Pinto-Neto, A.M., Morais, S.S. and Amaya-Farfan, J. (2010), “Effect of dietary supplementation with collagen hydrolysates on bone metabolism of postmenopausal women with low mineral density”, *Maturitas*, 65(3), 253–257.

Ficai, A., Albu, M.G., Birsan, M., Sonmez, M., Ficai, D., Trandafir, V. and Andronescu, E. (2013), “Collagen hydrolysate based collagen/hydroxyapatite composite materials”, *Journal of Molecular Structure*, 1037, 154-159.

Fleck, C.A. and Simman, R. (2010), “Modern Collagen Wound Dressings: Function and Purpose”, *Journal of the American College of Certified Wound Specialists*, 2, 50–54.

Gayatri, R., Sharma, A.K., Rajaram, R. and Ramasami, T. (2001), “Chromium(III)-induced structural changes and self-assembly of collagen”, *Biochemical and Biophysical Research Communications*, 283, 229.

Hofreiter, B.T., Alexander, B.H. and Wolff, I.A. (1955), “Rapid Estimation of Dialdehyde Content of Periodate Oxystarch through Quantitative Alkali Consumption”, *Journal of Analytical Chemistry*, 27, 1930-1931.

Li, D., Ye, Y., Li, D., Li, X. and Mu, C. (2016), “Biological properties of dialdehyde carboxymethyl cellulose crosslinked gelatin–PEG composite hydrogel fibers for wound dressings”, *Carbohydrate Polymers*, 137, 508–514.

Mu, C., Liu, F., Cheng, Q., Li, H., Wu, B., Zhang, G. and Lin, W. (2010), “Collagen Cryogel Cross-Linked by Dialdehyde Starch”, *Macromolecular Materials and Engineering*, 295, 100–107.

Sheu, M.T., Huang, J.C., Yeh, G.C. and Ho, H.O. (2001), “Characterization of collagen gel solutions and collagen matrices for cell culture”, *Biomaterials*, 22, 1713.

Tan, H., Wu, B., Li, C., Mu, C., Li, H. and Lin, W. (2015), “Collagen cryogel cross-linked by naturally derived dialdehydecarboxymethyl cellulose”, *Carbohydrate Polymers*, 129, 17–24.

Wilpiszewska, K. and Spychaj, T. (2007), “Chemical modification of starch with hexamethylene diisocyanate derivatives”, *Carbohydrate Polymers*, 70, 334-340.

Zhang, L., Liu, P., Wang, Y. and Gao, W. (2011), “Study on physico-chemical properties of dialdehyde yam starch with different aldehyde group contents”, *Thermochimica Acta*, 512, 196-201.

ESSENTIAL OIL COMPONENTS OF FRESH CORIANDER (*CORIANDRUM SATIVUM* L.) HERBS FROM DIFFERENT LOCATIONS IN TURKEY

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Five different Coriander (*Coriandrum sativum* L.) obtained from different sources were cultivated under Hatay ecological conditions used as a plant material. To determine essential oil rate fresh herbs of coriander were analysed with Neo-Clevenger apparatus by the method steam distillation. Essential oil components were determined by gas chromatography-mass spectrometry (GC-MS) apparatus. Decanal (9.95-16.53), 2-Decanal (3.48-11.86), 1-Decanol (7.67-17.13), Cyclodecanol (4.46-13.78), 2-Dodecenal (5.42-12.65), Cyclododecanal (5.23-7.86) and 13-Tetradecanal (4.87-11.19) were found main components.

Keywords: Coriandrum, GC-MS, Essential oil

INTRODUCTION

Coriandrum sativum L. (Coriander) is a annual, medicinal plant from the Umbelliferae/Apiaceae family. Coriander known as “aşotu”, “kişniş” or “kizbara” in Turkish language. It has long known and used for food preservation (Potter, 1996; Gil *et al*., 2002), flavouring agent and alternative medicine. Despite coriander naturally grown in the Mediterranean region plant widely produced in India, Morocco, Bangladesh, Canada, USA and Mexico, in India and Bangladesh mostly consume in local markets (Small, 1997). World total annual production of coriander (including anise, badian and fennel) leaves and seeds in 2013 was 940.587 ton (FAO stat, 2013). Coriander exports of Turkey in 2013 were 229 tons for USD 566.088.

Coriander become more and more economically important because of biological active compounds by powdered seeds, dry extract, tea, tincture and decoction. The main biological compound is EO which posses antimicrobial (Delaquis *et al*., 2002), antibacterial (Bown, 1995), antioxidant (Rajeshwari *et al*., 2012), antidiabetic (Aissaoui *et al*., 2011) activities.

Coriander essential oil yields and componenet wide range by age, origin, environmetal factors and ontogenesis (Carrubba and Torre, 2002; Msaada *et al*., 2007; Kiralan *et al*., 2009; Kosar *et al*., 2005; Yildirim and Gok, 2012; Baser and Kirimer, 2014).

The main component of essential oil is vary from the different parts of plant. In seed main component is linalool while in flowers Benzofuran,2,3- dihydro and in leaves Decanal, Dodecanal found as main component (Msaada *et al*., 2007; Mandal and Mandal, 2015).

In this study, 5 coriander (*Coriandrum sativum* L.) seeds, from different sources, cultivated in Hatay conditions, fresh herb were obtained for essential oil components.

MATERIALS AND METHODS

Plant Material

Coriander seeds from 5 different sources of Turkey (1.Denizli, 2.Hatay/Altınözü, 3.Ankara, 4.Burdur, 5.Hatay/Samandağ) were cultivated during season 2015-2016, Mustafa Kemal University field area in Hatay.

Essential Oil Isolation

Air-dried plant samples (200 g) were placed in a 1 l round-bottom distillation flask and 300 ml distilled water added. The essential oils were obtained by steam distillation for 3 h with Clevenger's apparatus, according to European Pharmacopoeia method (Council of Europe, 1997). The oils were separated, dried over anhydrous sodium sulphate and stored in an amber bottle at 4 °C until used.

GC-MS Analysis of Essential Oil

Analysis of the essential oils used in these experiments was performed using a Hewlett–Packard 6890 gas chromatography (GC) linked to a Hewlett–Packard 5973 mass selective detector equipped with a HP-5 MS (Crosslinked 5 % Phenyl Methyl Siloxane) capillary column (30 m 0.25 mm i.d., 0.25 mm film thickness). The carrier gas was helium, at a rate of 1.3 ml/min. The oven temperature was initially 45 °C, then increased at 2 °C/min to 130 C, 3 C/min to 170 °C, then to 10 °C/min to 220 °C and finally isothermal for 5 min. The ionization energy was 70 eV. The interface temperature was 250 °C. The essential oil components were tentatively identified by comparing their relative retention times and mass spectra with those of Wiley Registry of Mass Spectral Data (McLafferty, 1994) and publication of Adams (Adams, 1995).

RESULTS AND DISCUSSION

Chemical composition of essential oil of Corianders showed smilar profiles and differed quantitatively (Table 1).

In our study main components of essential oils of sample 1 (seeds supplied from Denizli location) were found Decenal 11.26 %, 2-Decenal 11.86 %, 2-Dodecenal 12.65 %. In sample 2 (seeds supplied from Altinozu/Hatay-202 m), main components were found Decenal 10.81 %, 2-Decenal 10.02 %, 1-Decanol 10.39 %, Cyclodecanol 10.24 % and 2-Dodecenal 10.56 %. Essential oil components of Sample (seeds supplied from Ankara) 3 were found as, Decenal 16.53 %, 1-Decanol 13.21 %, 2-Dodecenal 10.40 %, 13-Tetradecenal 11.19 %. Essential oil components of Sample 4 (seeds supplied from Burdur) were found 1-Decanol 16.16 %, 13-Tetradecenal 10.11 % and Decenal 9.95 %. In Sample 5 (seeds supplied from Samandag/Hatay- 15 m) essential oil composition vary as Decenal 11.84 %, 2-Decenal 10.30 %, 1-Decanol 13.16 % and Cyclodecanol 13.78 %. The results show that even in the same city but different altitutes can effect on essential oil components and components quantity. Potter and Fagerson (1990) were studied coriander fresh leaves from Massachusetts to obtain essential oil composition. In the study, 2-decenal 46.1 %, 2-undodecenal 10.3 %, 2-undecenal 5.6 % and decenal 4.4 % were found as main components. Freires et al. (2014) were investigated coriander essential oil cultivated in Brazil (University of Campinas). In the study decenal (19.09 %), trans-2-decenal (17.54 %), 2-decen-1ol (12.33 %) and cyclodecane (12.15 %) found as major componenets of coriander fresh leaves essential oil. Matasyoh *et al*. (2008) study coriandrum fresh leaves from Kenya. The researchers reported that coriandrum essential oils main components are 2E-decenal (15.9 %), decanal (14.3%), 2E-decen-1-ol (14.2%) and n-decanol (13.6%). Although our results vary according to the study conducted by Potter and Fagerson (1990), it was similar to the results of the studies conducted by Freires *et al*. (2014), Matasyoh *et al*. (2008).

Table 1. Essential oil composition (%) of coriander fresh herb

RT	Cas #	Compound Name	1-Denizli	2-Altınozu	3-Ankara	4-Burdur	5-Samandag
17.1	112-31-2	Decanal	11.26	10.81	16.53	9.95	11.94
21.02	112-44-7	Undecanal	3.37	3.73	3.70	2.80	3.77
22.41	3913-81-3	2-Decenal, (E)-	11.86	10.02	3.48	4.61	10.30
24.89	112-54-9	Dodecanal	4.50	4.11	7.55	5.67	4.56
26.31	2463-77-6	2-Undecenal	5.46	4.74	1.67	1.77	3.41
26.88	112-30-1	1-Decanol	8.29	10.39	13.21	16.16	13.16
28.64	10486-19-8	Tridecanal	-	-	1.53	-	-
28.81	1502-05-2	Cyclodecanol	8.71	10.24	4.46	8.64	13.78
30.1	4826-62-4	2-Dodecenal	12.65	10.56	10.40	6.46	7.03
30.43	112-42-5	1-Undecanol	1.90	2.70	1.61	2.56	2.80
32.29	31502-14-4	2-Nonen-1-ol, (E)-	4.11	4.70	-	3.31	4.14
32.27	124-25-4	Tetradecanal	-	-	2.94	-	-
32.52	504-96-1	Neophytadiene	-	-	1.00	1.20	-
33.86	112-53-8	1-Dodecanol	-	1.07	1.19	1.90	1.17
35.67	1724-39-6	Cyclododecanol	7.26	7.86	5.14	5.73	5.35
37.2	85896-31-7	13-Tetradecenal	6.64	6.12	11.19	10.11	4.87
40.52	2423-10-1	Olealdehyde	1.38	1.45	3.63	2.77	1.05
42.02	69064-37-5	trans-2-Dodecen-1-ol	1.69	2.20	2.81	5.39	2.06
52.57	150-86-7	Phytol	1.12	-	1.98	3.34	1.04

CONCLUSION

The results reported of variation on coriander fresh herb essential oil seed supplied from five different sources of Turkey and cultivated in same ecological conditions. This study illustrates the impact of the genotype on components of volatile oil. In our study Decanal (9.95-16.53 %), 2-Decanal (3.48-11.86 %), 1-Decanol (7.67-17.13 %), Cyclodecanol (4.46-13.78 %), 2-Dodecenal (5.42-12.65 %), Cyclododecanal (5.23-7.86 %) and 13-Tetradecanal (4.87-11.19 %) were found as main componenets.

REFERENCES

Adams, R.P. (1995), *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, Carol Stream, IL: Allured Publishing.

Aissaoui, A., Zizi, S., Israili, Z.H. and Lyoussi, B. (2011), “Hypoglycemic and hypolipidemic effects of Coriandrum sativum L. in Meriones shawi rats”, *Journal of Ethnopharmacology*, 137(1), 652–661.

Baser, K.H.C. and Kirimer, N. (2014), “Essential oils of Anatolian Apiaceae - A profile”, Natural Volatiles and Essential Oils, 1(1), 150.

Bown, D. (1995), *The Royal Horticultural Society Encyclopedia of Herbs and Their Uses*, Dorling Kindersley Ltd. London, p. 424.

Carrubba, A., Torre, R., Di Prima, A. *et al*. (2002), "Statistical analyses on the essential oil of Italian coriander (*Coriandrum sativum* L.) fruits of different ages and origins", *Journal of Essential Oil Research*, 14, 389-396.

Council of Europe (1997), *European Pharmacopoeia*, 3rd edn. Strausbourg, 121–122.

Delaquis, P.J., Stanich, K., Girard, B. and Mazza, G. (2002), "Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils", *International Journal of Food Microbiology*, 74(1–2), 101–109.

FAO stat (2013), http://faostat.fao.org/site/291/default.aspx.

Freires, I.A., Murata, R.M., Furletti, V.F. *et al*. (2014), "*Coriandrum sativum* L. (Coriander) Essential Oil: Antifungal Activity and Mode of Action on Candida spp., and Molecular Targets Affected in Human Whole-Genome Expression", *PLoS ONE*, 9(6): e99086. doi:10.1371/journal.pone.0099086.

Kiralan, M. (2006), Ayçiçek Yağının Oksidadtif Stabilitesi Üzerine Isırgan (*Urtica diocia* L.), Keten (*Linum usitassium* L.), Kişniş (*Coriandrum sativum* L.) ve Çörekotu (*Nigella sativa* L.) Tohum Ekstraktlarının Etkileri. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi (Danışman: Prof. Dr. Ali Bayrak), Ankara.

Kosar, M., Ozek, T., Goger, F., Kurkcuoglu, M. and Baser, K.H.C. (2005), "Comparison of microwaveassisted hydrodistillation and hydrodistillation methods for the analysis of volatile secondary metabolites", *Pharmaceutical Biology*, 43(6), 491-495.

Mandal, S. and Mandal, M. (2015), "Coriander (*Coriandrum sativum* L.) essential oil: chemistry and biological activity", *Asian Pacific Journal of Tropical Biomedicine*, 5(6), 421-428.

Matasyoh, J.C., Maiyo, Z.C., Ngure, R.M. and Chepkorir, R. (2009), "Chemical composition and antimicrobial activity of the essential oil of Coriandrum sativum", *Food Chemistry*, 113, 526–529.

McLafferty, F.W. (1994), *Wiley Registry of Mass Spectral Data*, New York.

Msaada, K., Hosni, K., Taarit, B.M., Chahed, T., Kchouk, E.M. and Marzouk, B. (2007), "Changes on essential oil composition of Coriander (*Coriandrum sativum* L.) fruits during three stage of maturity", *Food Chemistry*, 102, 1131-1134.

Potter, T.L. and Fagerson, I.S. (1990), "Composition of Coriander Leaf Volatiles", *Journal of Agricultural and Food Chemistry*, 38: 2054-2056

Potter, T.L. (1996), "Essential oil composition of cilantro", *Journal of Agricultural and Food Chemistry*, 44, 1824–1826.

Rajeshwari, C.U., Siri, S. and Andallu, B. (2012), "Antioxidant and antiarthritic potential of coriander (*Coriandrum sativum* L.) leaves", *e-SPEN Journal*, 7(6), e223–e228.

Small, E. (1997), *Culinary herbs*, Ottawa, NRC Research Press, pp 219-25.

Yildirim, B. and Gok, N. (2012), "Effect of Sowing Date and Varieties on Essential Oil Ratio and Essential Oil Components of Coriander (*Coriandrum sativum* L.) in Van Ecological Condition", *Journal of Animal and Veterinary Advances*, 11(11), 1925-1929.

POTENTIAL TOXICOLOGICAL RISKS OF INDUSTRIAL HEAVY METALS ON WILDLIFE ECOLOGY: A REVIEW TO DRAW ATTENTION TO AN IMPORTANT ECOLOGICAL REGION

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Many pollutants like heavy metals that are created as a result of increasing population and industrial production in parallel with the increasing population effects the ecological balance adversary with each passing day. The reflections of these developments on the wild life is higher because of their effects of life time, feeding and physiological properties. Hatay region comprises the Samandağ beach and Gulf of İskenderun which hosts the endangered Loggerhead and Green Sea Turtles and Mediterranean seals, Amanos Mountains which are located on the migration route of many migratory birds, Amik plain which hosts Mountain gazelle and Striped hyena and many other endemic species. Because of this ecological importance, the region must be monitored periodically for the presence of pollutants beginning with industrial heavy metals and their effects on living systems and the potential toxicological risks and stress that these pollutants caused on the living systems must be determined in advance and regional protection policies must be developed. For that purpose, the regional stations must be established in the jurisdictions of the general and local governments where take the responsibility mutually that can manage the processes according to local parameters with the information and technology communion at first for information and technology communion.

Keywords: ecotoxicological risk, environmental pollution, heavy metal, wildlife, Hatay

INTRODUCTION

In recent years, one of the most underlined subjects by countries is the protection of the environment by utilization of natural resources in an efficient and sustainable way. For that purpose the prevention and control of harmful effects of chemicals by investigating their toxic effect potentials and assessing the risk of these chemicals has become one of the principal objectives of the developed countries. Despite the increasing international and national policies, the natural resources is continued to be effected adversely and the environment become receded from a livable ideal environment. The effects of environmental pollution are appeared in a wide dimensional way that blankets the ecosystem beyond personal effects (Kaya *et al*., 2002; Republic of Turkey Ministry of Environment and Urbanization, 2011; Yarsan *et al*., 2014; Yipel and Yarsan, 2014; Yipel *et al*., 2015; Yipel *et al*., 2016).

The industrial, urban and agricultural activities that emerge with the increasing population in the Turkey which ranks along with the developing countries causes the undesired but constantly growing environmental problems and cause the natural balance become unbalanced. When evaluated by sectors, it is determined that 60 % of the waste water discharged to the recipient environments are mainly originated from fossil-fuel based thermal power plants and industry (Republic of Turkey Ministry of Environment and Urbanization, 2012).

While the global chemical production was increased from 7 million tones at 1950 to 63 million tones at 1970 and 250 million tones at 1985. Today it is estimated that this production has reached 400 million tones (Yarsan and Yipel, 2013; Yipel *et al*., 2015; Yipel *et al*., 2016).

Metals are the elements that found naturally on the earth crest. 59 of the 90 element characterized as "Heavy Metals" in the periodic table. This term is generally is used as a group name for metals that are connected with contamination and potential toxicity or eco-toxicity and half-metals (metalloids). These can be classified as essential ones for the execution of biochemical processes completely (Cu, Co, Cr, Fe Mn, Mo, Se, Zn), ones which are not essential and beneficial but when exposed to minute quantities that can be beneficial (B, Ni, Si, V) and the ones which are important environmental pollutants an don't have any determined biological function and don't undergo the disintegration process that diminish their their concentration and toxicity (Ag, Al, As, Ba, Be, Cd, Hg, Pb, Sb, Sr, Tl) (Duffus, 2002; Yipel, 2014; Yipel *et al*., 2015; Yipel *et al*., 2016).

Not only humans but also plants and animals are suffered from the pollutants. Thus in our day where the pollution is increasingly continues primarily because of heavy metals and natural resources are continuously dwindles, the understanding how the wild animals affected from pollution become an obligation. In this point the potential adverse effects that form over the ecosystem as a result of fast industrialization are disclosed by Ecological risk assessment methods. Ecological risk assessment means that the determination of potential negative affects aroused when aquatic animals like fish and invertebrates, terrestrial animals like birds and wild mammals and other out of target organism like plants and insects exposed to more than one pollutant (USEPA, 1992). Among the ecological risk Assessment Methods that are employed currently, "Regional Risk Model" is one among the one of the most preferred models (Kanwar *et al*., 2015).

WILDLIFE AND ECOLOGICAL IMPORTANCE OF AREA

Wildlife term is used to refer all non-domestic animals (birds, mammals, fish amphibians and reptiles) that live on their natural habitat and their habitats mutually. Only 1.5 million of the species are defined among the total number of species predicted to be between 10 and 30 million (Ünal, 2011). Turkey has a very important potential in terms of wild life resources and species diversity and carrying capacity of its habitat but these potentials is affected adversary as a result of various reasons (deterioration, destruction, disintegration of habitats, over consumption, environmental pollution, foreign species, global climatic changes, industrial architecture, industrial forestry, hunting) (Şafak, 2006; Ünal, 2011). Ecosystem is especially polluted by agricultural pesticides and industrial wastes and this pollution harms the wild animals (Şafak, 2006).

Turkey is among the rare countries that are able to protect the majority of its wild life and wild species. Many wild that survive with special measures and artificial techniques are surviving and live naturally in our land. The location of Turkey between the Asia, Europe and Africa continents leads the diversification of the ecological conditions, diversity of the geological structure, formation of the different ecological conditions and thus emergence of a very rich biodiversity. Turkey is one of the most richest countries in the world in terms of biodiversity when compared with its land area (Ünal, 2011).

Besides many bird species that some of them are migratory, 7 of the 21 varieties of the salamander varieties which are known as urodela and anura that live in Turkey, 6 of the 9 turtle species, 14 of the 54 lizard species, 16 of the 44 snake species lives in the Hatay and Amanos region (Tok, 2008). Besides it is known that wild species like

Roedeer *(Capreolus capreolus),* Hatay Mountain Gazelle *(Gazella gazella),* Wild goat *(Capra aegrus),* Amanos snake *(Rhynchocalamus),* Striped hyena *(Hyeana hyuna),* Bighead Turtle *(Caretta caretta)* and and Green Sea Turtle *(Chelonia mydas)* that are classified as "Endangered (EN)" by The World Conservation Union, IUCN (Ergün, 2006, IUCN, 2014), Wild Cat, Coyote, Red Deer, Rock Marten, Pine marten, Marbled Polecat, badger, Crested porcupine, Meer cat, Weasel, Wolf, Sea Otter, Jerboa, Puss, Hare, Lynx, Wild Boar, Mole, Fruit Bat lives in the region. There are 3 natural conservation area (Tekkoz, Kengerlidüz, Habibineccar Mountain) and 2 natural life development areas (İskenderun-Arsuz, Altınözü) are present in Hatay. In the borders of the province there are 150 endemic flora, 3 endemic fauna are observed. Species of Hatay Mountain Gazelle which have the population of 150 according to the latest inventory study at the vicinity of Kırıkhan district İncirli village is endangered. Samandağ beach is 14 km long and is a sea turtle *(Caretta caretta, Chelonia mydas)* nesting. Besides the populations of wild goat, roe deer, squirrel, wild boar, fox, coyote, wolf, sea otter, crested porcupine and stripped hyena which is an important member of the wild life is identified. The properties of the ecosystem are significantly affected from the industry and agriculture based pollution and domestic wastes (Republic of Turkey Ministry of Environment and Urbanization, 2011; Republic of Turkey Ministry of Environment and Urbanization, 2013).

POTENTIAL TOXICOLOGICAL RISKS OF INDUSTRIAL IN AREA AND HEAVY METALS

The fast developments in the area of industry and technology while increasing the life quality of the people, on the other hand lead the disruption of the natural balance and environmental pollutants that threaten all creatures. When considering the amount of dangerous wastes in Turkey, Hatay ranked 4th after Izmir, Kocaeli and Manisa. Hatay province is an important center of the iron and steel industry and thus majority of the dangerous wastes formed in the province is iron-steel industry based. The declared dangerous waster are principally wastes that formed from iron-steel industry, solid wastes formed as a result of treatment of gases that include dangerous substances, wastes formed from physical and mechanical surface processes and shaping of the metals and plastics, metallic slushes that include oil. The >98% of produced dangerous wastes in Hatay can be disposed by recycling-burning (Taşkan, 2013). Constant increase of the requirement of increment in the production and necessity of heavy metals in developing and developed countries increases their scattering probability around. This situation shows that the urban and industrial activities are increasing the heavy metal cycles all over the world. The determination of the heavy metal concentration of an area is determined by determination of dangerous chemical content in organisms in that area (Yarsan and Yipel, 2013; Yipel *et al*., 2015; Yipel *et al*.; 2016).

Air pollution is the first priority problem in Hatay (Republic of Turkey Ministry of Environment and Urbanization, 2014). The emissions of industrial facilities in the area (cement plants, refineries, fossil-fuel based thermal power plants etc.) create adverse effects by being carried by the wind, clouds and rains and mixing with in the agricultural land, soil and water (Republic of Turkey Ministry of Environment and Urbanization, 2011). The principal stress factors for the water resources are domestic, agricultural, industrial, mining based, sewage wastes and lack of infrastructure. While there are Antakya, İskenderun and Payas OIS (Organized Industrial site) in the Hatay water basin, only the Iskenderun OIS has the water treatment facility. İt is estimated that 100 m^3 of untreated water is discharged daily into the Asi River where the cities and Hamah and Humus cities of Syria which are concentrated on the industries of fertilizer, iron, plastic, petroleum and energy production are located. Especially the industrialization, population increase, agricultural activities, land and sea transportation, erosion, pesticides, inadequate sewer system, water infrastructure systems, mining and tourism in the Adana Osmaniye and Hatay which are neighboring the same Gulf forms the principles stresses of Asi River and the Gulf (Republic of Turkey Ministry of Environment and Urbanization, 2011). There haven't been any study performed on the properties, amount, types and disposal systems of the dangerous and harmful for the industrial facilities in the province. Even a waste water treatment facilities are present in a small number of industrial facilities it has been determined that these facilities are not operated in some industrial facilities (Republic of Turkey Ministry of Environment and Urbanization, 2011).

HEAVY METALS AND THEIR ACCUMULATION IN WILD ANIMALS

Metals which are resistant to the physical, chemical and biological degradations cannot be turned into benign forms. Thus they can reach high levels that can be harmful for living organisms by accumulating into the organs and tissues of organisms (bioaccumulation) that establishes a food chain or mild exposure from the living environment. More than one metal is present in the environmental mediums. Thus living creatures are exposed to physical-chemical forms of more than one metal. Metals can be utilized for principle biochemical objective after undergoing a series of biochemical process after taken into the body in trace amounts, stored in the body and disposed from the body. But the remaining unutilized portion of the metal can act as toxic to the body immediately or accumulate in the body and form a toxic effect. Wild animal's intake the heavy metals via foods, drinking water or breathing and through their skin into their bodies. Toxic properties of the metal vary for every metal and property of the tissue. But generally more than one organ or system is affected. Thus a single enzyme system or single biochemical process is not affected alone (Yarsan and Yipel, 2013; Yarsan *et al*., 2014; Yipel *et al*., 2015; Yipel *et al*., 2016).

CONCLUSIONS

Investigation of wild animals and their habitat that are exposed to environmental pollution in terms of heavy metals which is one of the important parameter of pollution is very important both for disclosure of dimensions of environmental pollution and exposure level of the species from this pollution. Components of wild life are under according to the National and ınternational Agreements. Thus ecotoxicology and protection of wild life in terms of animal health from chemical pollution is a legal obligation. Ecologically important Hatay region is highly exposed to environmental problems as a result of intense industrial activities. Thus the solution proposals of the regions environmental problems must be revealed. Thus, establishment of "Regional Pollution Monitoring Station" where the effects of the primarily chemical, biologic and physical pollution on the environment can be monitored periodically with information and data communion and the e risks and solutions with the reasons and dimensions of the pollution can be revealed in important ecological regions with intense industrial activity by taking the mutual responsibility in the frame of local and national authorities jurisdictions will be a positive development in short and medium term.

REFERENCES

Duffus, J.H. (2002), "*Heavy metals* a meaningless term? (IUPAC Technical Report)". *Pure and applied chemistry*, 74(5), 793-807.

Ergün, Y. (2006), Hatay'da on sıcak gün. Mustafa Kemal University Press., No: 19, Hatay.

IUCN The World Conservation Union) (2012), *Red list of threatened species*, Version 2012.1. International Union for Conservation of Nature, http://www.iucnredlist.org.

Kanwar, P., Bowden, W.B. and Greenhalgh, S. (2015), "A regional ecological risk assessment of the Kaipara harbour, New Zealand, using a relative risk model", *Human and Ecological Risk Assessment: An International Journal*, 21(4), 1123-1146.

Kaya, S., Pirinçci, İ. and Bilgili, A. (1998), *Environmental Science and Environmental Toxicology*, 2nd ed., Medisan Press., Ankara. p.: 12-78.

Kaya, S., Pirinçci, İ. and Bilgili, A. (2002), *Toxicology in Veterinary Medicine*, 2nd ed., Medisan Press., Ankara. p.: 203-842.

Republic of Turkey Ministry of Environment and Urbanization (2011), *Directorate General of Environmental Impact Assessment, Permit and Inspection. Environmental Status Report of Turkey*.

Republic of Turkey Ministry of Environment and Urbanization (2013), *Directorate General of Environmental Impact Assessment, Permit and Inspection. Hatay Provincial Environmental Status Report.*

Republic of Turkey Ministry of Environment and Urbanization (2014), *Directorate General of Environmental Impact Assessment, Permit and Inspection. Turkey Environmental Problems and Priorities Assessment Report.*

Şafak, İ. (2006), "Problems and Solutions in Game Management in Aegean Region", *1st International Symposium on Non-Wood Forest Products* (1-4 November), KTU Faculty of Forestry, pp:66-73, Trabzon.

Taşkan, Ş. (2013), "The Formation Of Hazardous Waste And Disposal In Turkey", Marmara University, Institute of Science and Technology. Master's thesis. Istanbul.

Tok, V. (2008), Hatay bölgesinde yaşayan iki yaşamlılar (Ampphibia) ve sürüngenler (Reptilia). Ekolojik Okur Yazarlık. Ed: Ergün, Y., Özdilek, Ş., Pamir, H. Mustafa Kemal University Press., No: 20. Hatay.

Turkish Statistical Institute (TSI) (2012), *Environmental Statistics, 2010*. Newsletter, Issue: 13134.

U.S. Environmental Protection Agency (USEPA) (1992), *Framework for ecological risk assessment*, EPA/630/R92/001. Risk Assessment Forum, Washington, DC.

Ünal, Y. (2011), "Game and wildlife inventory in Isparta - Yazilikaya", Süleyman Demirel University, Institute of Science and Technology. PhD thesis. Isparta.

Yalçin-Özdilek, Ş. (2008), Asi nehri ve Amanos derelerine ekolojik bakış. Ekolojik Okur Yazarlık. Ed: Ergün, Y., Yalçin-Özdilek, Ş., Pamir, H. Mustafa Kemal University Press., No: 20. Hatay.

Yarsan, E. and Yipel, M. (2013), "The Important Terms of Marine Pollution 'Biomarkers and Biomonitoring, Bioaccumulation, Bioconcentration, Biomagnification'", *Journal of Molecular Biomarkers & Diagnosis*, S1:003.

Yarsan, E., Yipel, M., Dikmen, B., Altintaş, L., Ekici, H. and Köksal, A. (2014), "Concentrations of essential and non-essential toxic trace elements in wild boar (Sus Scrofa L., 1758) tissues from southern Turkey", *Bulletin of Environmental Contamination and Toxicology*, 92(1), 10-14.

Yipel, M. and Yarsan, E. (2014), "A risk assessment of heavy metal concentrations in fish and an invertebrate from the Gulf of Antalya", *Bulletin of Environmental Contamination and Toxicology*, 93(5), 542-548.

Yipel, M., Oğuz, H. and Tekeli, İ.O. (2015), "An Overview of Hatay Environmental Pollution", *Hatay Environmental Problems and Solutions Symposium*, p.:27, 28-30 May, Hatay, Turkey.

Yipel, M., Tekeli, İ.O., Dikmen, B. and Yarsan, E. (2016), "Distribution and Ecotoxicological Risk Assessment of Heavy Metals: A Case Study in Streams of Amanus Mountains from Southern Turkey", *2nd International Congress Of Forensic Toxicology. Industrial and Environmental Toxicology*, p:43, 26-30 May, Ankara, Turkey.

III.
INNOVATIVE TECHNOLOGIES

INK DYES FOR WASHABLE LEATHERS

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Inks, contain pigments or dyes in liquid or paste form, are used to color an image, text, or design. Although some patents describing the ink jet printing methods for textile and leather were found, no report was found addressing the application of ink dyes in leather dying process and determination of washing and other fastness properties. In this study, the potential application of ink dyes in leather industry was investigated as an alternative to conventional dyes for the production of washable leathers in dying process. For this purpose, leather-dyeing recipes were developed by using two different ink dyes and applied at dying process of metis type crust leathers in order to produce washable leathers. The quality performance of dyed leathers were investigated in terms of color measurements, to-and-fro rubbing fastness, crockmeter rubbing fastness, and light fastness characteristics before and after washing leathers in a washing machine under specified conditions according to ISO 15702 standard. The color measurements prior and subsequent to washing process were determined with Minolta CM-3600A spectrophotometer. To-and-fro rubbing, crockmeter and light fastness properties were examined in accordance with ISO 11640, ASTM D5053 and ISO 105-B02 standards respectively. The results of the study showed that ink dyes could be used as a newly adapted dye group and applied successfully in leather dyeing process for the production of washable leather goods.

Keywords: washable leathers, ink dyes, color fastness, leather dyeing

INTRODUCTION

Dyes and pigments offer significant potential for functional and aesthetic design of leather materials. Search for viable alternative leather colorants, which have the potential to develop new creative designs and provide functionality are presently in focus. In this context application of alternative textile dye substances that can offer special effects to leather have been investigated (Şeren, Adıgüzel Zengin, & Bitlisli, 2013).

During the last decade ink jet printing has made significant progress and expanded their fields of applications. Inks, used in these applications contain pigments or dyes in liquid or paste form, are used to color an image, text, or design. Although some patents describing the ink jet printing methods for textile and leather were found, no report was found addressing the application of ink dyes in leather dying process. In order to ensure the applicability of ink dyes in leather dyeing processes, to develop a leather dyeing recipe suitable for ink dyes, the initial treatment of leather samples with dye and subsequent determination of brightness, colour as well as testing the colour fastness performances are of critical importance.

In this study three types of ink dyes such as dye, pigment and sublimation based ink dyes were applied to crust leathers through newly developed dyeing recipes to investigate whether they can be considered as a new class of dye substance for the leather industry. The qualitative properties and fastness performance of ink-dyed leather were analyzed before and after washing trials.

MATERIAL AND METHOD

Material

In the study, metis type crust leathers were used for the application of the ink dye dispersions and pigments in the dyeing process. Three types of dyes were used in the trials such as pigmented, dye and sublimation based inks. All of them were water based dyes and supplied from Akici Inkjet and Laser Technologies in Istanbul, Turkey.

Experimental

The dyeing process of the crust leathers was given in Table 1. After drying and mechanical operations, the visual displays of the leathers dyed with pigmented, dye and sublimation based ink dyes were shown in Figure 1.

Dyeing Trials

Table 1. Dyeing recipe of crust leathers by three type ink dyes

Process*	%	Material	ºC	Time (min)	pH
Re-wetting	1000	Water	35-40		
	2-3	Ammonium			
	1,5	Wetting agent stabile to electrolytes			5.0-5.5
Dyeing	80	Water	35-40		
	x	Ink dye		45	
	70	Water	50		
	7	Neutral fatliquoring agent with synthetic emulgators			
	5	Synthetic fatliquoring agent		45	
	3	HCOOH		30	3.8

*The dyeing process of the crust leathers were based on dry weight

Figure 1. The leathers dyed with sublimation, pigmented and dye based inks, respectively

Color Measurements

The color measurements prior and subsequent to washing process were determined with Minolta CM-3600A spectrophotometer (Konica, Japan). The measurements were performed according to the Commission Internationale de l'Eclairage (CIE) Lab color system (McLaren, 1983). Color of samples was assessed performing multiple surface color measurements (minimum 10 measurements) from different regions of the sample, and triplicate experiments were performed.

To and Fro Rubbing Fastness

The rubbing fastness properties of leathers were examined by Bally Finish Tester 9029 according to ISO 11640 standard (100 rubs in dry and 25 rubs in wet) (ISO 11640, 2012). Prior and subsequent to the rubbing tests, color measurements were performed and the changes in color were compared.

Crock-meter Rubbing Fastness

The crockmeter rubbing fastness of the leathers was performed in accordance with ASTM D5053 standard (ASTM D5053, 2003). The dry and wet rubbing fastness (10 rubs for each) was tested by Atlas CM5 (SDL ATLAS Company, USA) test device. The fabric used in the test was evaluated according to the standard ISO 105-A03 while the leather samples were evaluated as follows; 1 = Good, 2 = Medium, 3= Poor (ISO 105-A03, 1993).

Light Fastness

The light fastness properties were determined in accordance with the standard of ISO 105-B02 by the Atlas-Xenotest Alpha+ device (Illinois, USA) (ISO 105-B02, 2013). The color change of the leathers was evaluated using gray scale with the standard of ISO 105-A02 (ISO 105-A02, 1993).

Washing Trials

Washing fastness tests were carried out in accordance with ISO 15702 (ISO 15702, 1998) to determine the color fastness of ink-dyed leathers to washings. Dyed samples were exposed to 30 minutes washing process in a detergent medium (4g/L) using a steel laundry machine at 40°C and 40 rpm.

RESULTS AND DISCUSSION

The spectrophotometric color measurements of ink- dyed leathers prior and subsequent to washing are shown in Table 2 and 3. The results were evaluated according to CIE Lab color system and the explanation of the CIE coordinates are as follows: L* = 0 yields black and L* = 100 yields white; negative values of a* indicate green, positive values indicate red; negative b* values indicate blue and positive values indicate yellow (Mutlu *et al*., 2014).

Table 2. Color measurement values of ink dyed leathers before washing

	L	a	b	dL	da	db	dE
Dye based_grain	68.71	-26.43	-13.14	-30.20	-26.32	-12.78	42.07
Dye based_suede	58.80	-29.56	-17.38	-40.11	-29.45	-17.02	52.62
Sublimation dye_grain	57.93	-28.62	-26.71	-40.99	-28.52	-26.35	56.49
Sublimation dye_suede	61.05	-24.13	-14.90	-37.86	-24.02	-14.54	47.50
Pigment based_grain	60.60	28.87	-9.72	-38.31	28.97	-9.36	49.16
Pigment based_suede	44.54	23.99	-4.95	-29.65	24.07	-4.68	41.52

*L, a, b values of white color as a target given as respectively; 98.92, -0.105, -0.36

The color measurement values of ink dyed leathers showed that there was a color difference between the grain and suede side of the leathers (ΔE). Sublimation based dyes resulted similar L values compared to other ink dyed leathers. The values of ‘a’ and ‘b’ indicated that dye based ink dyed leathers had more green color compared to leathers dyed with sublimation based dyes. The leathers dyed with pigmented based ink dyes had positive a values indicating the red color of the leathers. The negative values of b gave the blue color to the leathers dyed with pigmented based ink dyes that resulted purple colored leathers (Table 2).

Table 3. Color measurement values of ink dyed leathers after washing

	L	a	b	dL	da	db	dE
Dye based_grain	77.30	-12.17	-1.92	-21.61	-12.07	-1.56	24.85
Dye based_suede	71.17	-17.81	-5.44	-27.74	-17.71	-5.07	33.34
Sublimation dye_grain	63.01	-28.25	-22.52	-35.90	-28.15	-22.15	50.75
Sublimation dye_suede	65.86	-21.69	-10.92	-33.05	-21.58	-10.56	41.00
Pigment based_grain	64.70	22.41	-6.40	-34.21	22.51	-6.04	42.93
Pigment based_suede	66.68	11.93	-3.38	-32.24	12.04	-3.02	34.61

*L, a, b values of white color as a target given as respectively; 98.92, -0.105, -0.36

After washing, the brightness of the ink dyed leathers was obtained higher than the brightness values of the leathers before washing. The color difference of the grain and suede side of the leathers was still found (ΔE). The less affected leather group from the washing treatment was determined as the leathers dyed with sublimation based dyes. The decrease of the negative ‘a’ and ‘b’ values were the indication of the unfavorable washing effect (Table 3). The to and fro rubbing fastness results of ink dyed leathers are given in Table 4 and 5.

Table 4. To and fro rubbing fastness results of ink dyed leathers before washing

	Dry		Wet	
	Felt	Leather	Felt	Leather
Dye based	4/5	4/5	4/5	4/5
Sublimation dye	4/5	4/5	4/5	4/5
Pigment based	5	4/5	4/5	4/5

All three groups of ink dyed leathers gave the highest wet/dry rubbing fastness results for the felt and leather examination according to gray scale (Table 4) before washing. The only slight difference was observed for the leathers dyed with pigmented based ink dyes for the dry felt examination.

Table 5. To and fro rubbing fastness results of ink dyed leathers after washing

	Dry		Wet	
	Felt	Leather	Felt	Leather
Dye based	5	4/5	4/5	3/4
Sublimation dye	5	5	4	4/5
Pigment based	5	4/5	4	4

No difference was observed from the dry to and fro rubbing fastness test results after washing for the ink dyes. However, lower values were obtained from the ink dyed leathers in terms of wet rubbing fastness of leathers especially for dyed based ink dyes, in addition similar results were obtained from the leathers dyed with sublimation based ink dyes (Table 5).

The crockmeter rubbing fastness results of the ink dyed leathers prior and subsequent to washing are given at Table 6 and 7. The dry crockmeter fastness results of the ink dyed leathers were found quite high. Although, wet crockmeter fastness results of the leathers were found lower than the dry crockmeter results, satisfactory fastness results were obtained before and after washing of the leathers.

Table 6. Crockmeter fastness results of ink dyed leathers before washing

	Dry		Wet	
	Fabric	Leather	Fabric	Leather
Dye based	4/5	1	3	2
Sublimation dye	4	1	4	2
Pigment based	4/5	1	3/4	2

The evaluation of the leather for crockmeter; 1 good; 2 medium; 3 poor

Table 7. Crockmeter fastness results of ink dyed leathers after washing

	Dry		Wet	
	Fabric	Leather	Fabric	Leather
Dye based	5	1	3/4	2
Sublimation dye	4/5	1	3/4	1
Pigment based	4/5	1	4	1

The evaluation of the leather for crockmeter; 1 good; 2 medium; 3 poor

The color fastness to machine-washing results of the ink-dyed leathers, are shown in Table 8. Washing fastness results were indicated that the leathers dyed with sublimation based ink dyes had the highest results, however the dye of the leathers dyed with dye based ink dyes was removed. Also, pigmented and sublimation based dyes discolored the fabric.

Table 8. Washing fastness results of ink dyed leathers

	Fabric	Grain Side	Suede Side
Dye based	1	1	1
Sublimation dye	1	4/5	4/5
Pigment based	1	4	4

The lightfastness properties of sublimation and pigmented based ink dyes were found quite high (4/5). This property was also kept after the washing treatment that provides a good opportunity for the production of washable leathers (Table 9). The washing and light fastness properties were correlated for the leathers dyed with dyed based inks. The dye based ink dye was almost removed after the washing process that also affected the lightfastness results.

Table 9. Light fastness properties of ink dyed leathers

	Before Washing	After Washing
Dye based	2	1
Sublimation dye	4/5	4/5
Pigment based	4/5	4/5

CONCLUSION

In this study, the application of ink based pigments/dyes as an alternative dye substance for leather industry was investigated. Three ink dyes such as pigment, sublimation and dye based ink dyes were evaluated within this scope and the results of the study showed that ink dyes especially sublimation based dyes could be used as a newly adapted dye group and applied successfully in leather dyeing process for the production of washable leather goods.

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REFERENCES

ASTM D5053. (2003). Standard test method for color fastness of crocking leather.

ISO 105-A02. (1993). Textiles-Tests for color fastness-Part A02: Grey scale for assessing change in color.

ISO 105-A03. (1993). Textiles-Tests for color fastness - Part A03: Grey scale for assessing staining.

ISO 105-B02. (2013). Textiles-Tests for color fastness - Part B02: Color fastness to artificial light: Xenon arc fading lamp test.

ISO 11640. (2012). Leather - Tests for colour fastness -Colour fastness to cycles of to-and-fro rubbing.

ISO 15702. (1998). Leather -- Tests for colour fastness -- Colour fastness to machine washing, International Organization for Standardization (ISO).

McLaren, K. (1983), *The color science of dyes and pigments*, Bristol, UK: Adam Hilger Ltd.

Mutlu, M.M., Crudu, M., Maier, S.S., Deselnicu, D., Albu, L., Gulumser, G. *et al*. (2014), “Eco-Leather: Properties of Chromium-Free Leathers Produced with Titanium Tanning Materials Obtained from the Wastes of the Metal Industry”, *Ekoloji, 23*(91), 83-90.

Şeren, A., Adıgüzel Zengin, A.C., and Bitlisli, B.O. (2013), “Thermo-chromic Pigments in Leather Finishing”, Paper presented at the XXXII. Congress of IULTCS.

PECULIARITIES OF NEUTRALIZATION OF LIME-FREE PELT OBTAINED USING SODIUM ALUMINATE

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Sodium aluminate can be used as an alternative to calcium hydroxide for qualitative unhairing of hide. Due to the absence of calcium compounds in the derma of the unhaired pelt, less-ammonia and non-ammonia methods of pelt neutralizations were tested. The experiments have shown the possibility to use almost two times less ammonia sulphate than conventionally or boric acid for qualitative neutralization of the pelt obtained using sodium aluminate. The pelts neutralized by both methods can be pickled and chromed conventionally, and properties of these pelts after chroming are close to the chromed leather produces by control method.

.Keywords: unhairing, sodium aluminate, neutralization.

INTRODUCTION

A major contributor to the pollution from the tanning industry is conventional unhairing using sodium sulphide and lime. Huge amounts of lime sludge and total solids formation are the main drawbacks of lime (Thanikaivelan *et al*., 2001). Herewith, the cleaning of unhairing solutions, polluted with lime, sulphides and products of protein degradation remains as very difficult and expensive. For this reason, the development of new and cleaner liming methods is perhaps the most urgent, compared with the perfection of other leather producing processes.

The use of lime-sulphide process remains as mostly applied unhairing-opening up of derma method in leather industry (Fennen *et al*., 2014). On the other hand, such process can be improveed as more clean and environment friendly as well.

The way to avoid the formation of solid lime sludge polluted by sulphides, protein degradation products, detergents etc. is replacement of lime by other soluble in water strong alkalis. Lime can be successfully replaced by sodium hydroxide. The developed lime-free method of unhairing-dermal opening up allows diminishing of consumption of sulphides and reaching of properties of chromium tanned leather not worse than those of leather produced using conventional method (Valeika *et al*., 2000; Kazlauskaite *et al*., 2002).

Thanikaivelan *et al*. (2001) proposed the method of hide unhairing-dermal opening up method which employs enzyme, low amount of sodium sulphide and sodium hydroxide. Later, a new hair saving unhairing method using a system containing enzyme preparation, sodium hydroxide, sodium sulphide, and disodium hydrophosphate was developed (Valeika *et al*., 2009).

Munz and Sonleitner (2005) report about developed methods of unhairing applying sodium silicate as lime substitute. They state that lime in the unhairing process can be substituted by sodium silicate without problems. As a result, effluent loading is reduced and so also sludge volumes from effluent treatment decreases.

Saravanabhavan *et al*. (2005) investigated a lime and sulphide-free unhairing process using a commercial enzyme with the activation using sodium metasilicate. The process led to significant reduction in chemical oxygen demand and total solids by 53 and 26%, respectively. Liu et al. (2009) developed novel lime-free unhairing method based on system containing sodium silicate, enzyme, surfactant and urea. The environmental factors were all superior to the conventional liming process.

There is one more interesting material having strongly alkaline properties, good solubility in water and, comparatively, low price. This material is sodium aluminate, which is an important commercial inorganic chemical for various industrial technical applications (Sirvaityte *et al*., 2015).

It is very important that avoiding of the lime allows an improvement of deliming (or just neutralization) because there is not lime in the unhaired pelt. Since Helsinki convention (Helcom Recommendation, 1995) recommends refusing of ammonium compounds for the deliming, the serious attempts to replace the ammonia salts by other materials are done during these two decades.

Firstly, the use of carbon dioxide for the deliming could be mentioned. The method is investigated enough widely, and it is adopted for the practical use (Deng *et al*., 2015). Carbon dioxide can be used in solid state (dry-ice) as well. The option of using dry ice for deliming would be effective for cleaner leather manufacture (Sathish *et al*., 2013).

Zeng *et al*. (2011) report about deliming with a composite of sodium hexametaphosphate and boric acid. An analysis indicated that the deliming by sodium hexametaphosphate and boric acid produced clearer grain with less calcium salts.

Crudu *et al*. (2012) investigated deliming process based on a use of new multifunctional products based on maleic copolymers. The significant reduction in ammonium salts (up to 66%) and sulphates (78%) was reached in such case.

Weak acids such as lactic, boric, formic, citric and acetic acids or esters of organic acids can be used to substitute for ammonium salts. The investigations have shown that leather quality specifications obtained using different acids and ammonium sulphate were close to each other (Colak and Kilic, 2008). Peracetic acid acts as qualitative deliming agent as well (Sirvaityte *et al*., 2007; Sirvaityte *et al*., 2009). The treatment of limed pelt by peracetic acid allows removing not less calcium compounds than while using ammonium sulphate, qualitative deswelling of pelt. The use of peracetic acid entirely eliminates the emission of H_2S.

Therefore, the literature data suggest application of various materials instead of ammonia compounds for deliming process.

The main aim of the present research was to investigate neutralization of hide unhaired using sodium aluminate reaching to reduce ammonia compounds use or to replace them by other more environmentally friendly materials.

EXPERIMENTAL

Salted cowhide was used as raw material for this study. The soaked and washed hide was cut into pieces 5x10 cm and experimental series were prepared from these pieces. An unhairing-opening (experimental) up of derma structure of samples was carried out as follows: H_2O - 100%, temperature 25°C, $NaAlO_2$ 2%, Na_2S (100%) 1.5%, 2 hours run continuously, NaOH 0.5%, 2 hours run continuously, later 5 minutes every 4 hours, total duration 24 hours (analytical grade sodium aluminate ($NaAlO_2$) containing Na_2O 40-45% and Al_2O_3 50-56% was used in this study). The liming-unhairing using lime and sodium sulphide was used as control method. The pickling and chroming of neutralised-bated samples were carried out accordingly to conventional technology.

The enzyme preparation (EP) *OROPON ON2* „TFL“ (Switzerland) was employed for the bating process.

The amount of collagen protein was estimated from the amount of hydroxyproline in the solution, and the amount of hydroxyproline was determined using a photo colorimetric method (Zaides *et al*., 1964).

The shrinkage temperature of hide samples, the pH of pelt and chromed leather, and the amount of chrome compounds in leather were determined according to standards (Standard ISO, 2002; Standard ISO, 1977; Standard ISO, 2009). Shrinkage temperature of chromed leather samples (wet-blue) was determined as described in the literature using special equipment and replacing the distilled water with glycerol (Golovteeva *et al*., 1982). The concentration of chromium in solution was determined according to the method described in the literature (Golovteeva *et al*., 1982).

RESULTS AND DISCUSSION

Two directions of the unhaired hide neutralization have been chosen: a) neutralization with decreased amounts of ammonium compounds, and b) neutralization with non-ammonia compounds. As non-ammonia compounds were tested lactic acid, boric acid and buffer containing acetic acid with sodium acetate. After preliminary investigation the most auspicious methods were found: a use of almost two times decreased amount of ammonia sulphate comparing as conventionally, and a use of boric acid.

Results of the neutralization are presented in Table 1.

Table 1. Methods of pelt neutralization-bating and process run quality indexes

Indexes	Pelt obtained by variant			
	1	2	3	4
	Unhairing-derma opening up method			
	Control	Experimental		
	Neutralization			
	H_2O – 40%, temperature 34-36°C, a) $(NH_4)_2SO_4$ – 2.2%, 30 min.; b) $(NH_4)_2SO_4$ –1.5%, 30 min.		H_2O – 40%, temperature 34-36°C, $(NH_4)_2SO_4$ – 2%, 1 hour	H_2O – 40%, temperature 34-36°C, a) boric acid 1.5%, 30 min.; b) boric acid 1.5%, 30 min.
	Bating (in neutralization solution) H_2O – 100%, EP *OROPON ON2* – 0.15%, 1 hour			
Solution pH after process	8.97	8.76	9.04	8.10
pH of pelt	8.98	8.96	9.12	8.58
Shrinkage temperature, °C	64.0	65.3	65.2	66.0
Removed collagen proteins, g/kg of hide	0.42	0.34	0.33	0.41

Note. Shrinkage temperature of pelt after unhairing: control 56.3°C; experimental 57.3°C.

After the neutralization adding almost two times less amount of ammonia sulphate (variant 3), about 10-15% of thickness of the pelts’ cross-section was coloured into slight pink when phenolphthalein had been dropped onto it. The pelt neutralized by boric acid after phenolphthalein test was very similar to the previous mentioned one. The control pelt (1 variant) and the pelt obtained by the use of sodium aluminate but neutralized conventionally (2 variant) had no pink colour after phenolphthalein test.

Other qualitative indexes after neutralization were close for the pelt obtained following all four variants. On the other hand, the less effect on collagenous proteins during the neutralization of the experimental pelt by the use of ammonia sulphate was observed.

The neutralized and bated pelts were washed, pickled and chromed. The indexes showing quality of chroming process and of chromed leather are presented in Table 2.

Table 2. Indexes of chroming and chromed leather

Indexes	Pelt obtained by variant			
	1	2	3	4
Solution pH after process	3.91	3.94	3.78	3.84
pH of leather	3.61	3.78	3.89	4.08
Shrinkage temperature, °C	109.3	111.0	110.6	109.6
Cr_2O_3 content in leather, %	4.91	4.23	4.18	3.87
Exhaustion of Cr_2O_3, %	80.3	79.0	78.7	75.4

The analysis of the chromed leather allows supposition that leather treated with sodium aluminate bounds less chromium compounds comparing with conventional one. This index slightly depends on the neutralization method as well. The exhaustion of the chromium compounds has similar trend of course: more chromium in a leather means less amount in the used chroming solution. The most interesting thing in this situation is that chromium content in the leather had not direct influence on the shrinkage temperature of it. All samples had almost the same shrinkage temperature. It can be supposed that in control sample not all chromium compounds are bound by two links. Therefore, they are joined in the leathers' structure but do not increase the thermostability of the leather.

CONCLUSSIONS

The pelt unhaired and opened up using sodium aluminate, sodium sulphide and sodium alkali due to the absence of calcium compounds in the derma can be neutralized using 2% of ammonia sulphate or 3% of boric acid. The pelt after neutralization-bating, and had similar properties as conventionally neutralised one. Accordingly, after chroming these pelts were also very close in their properties. On the other hand, they characterised by less chromium content comparing with control leather.

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REFERENCES

Colak, S.M. and Kilic, E. (2008), "Deliming with weak acids: Effects on leather quality and effluent", *Journal of the Society of Leather Technologists and Chemists*, 92, 120-123.

Crudu, M., Gaidau, C., Crudu, A., Popescu, M. and Ionescu, M. (2012) "Innovative material-technology system for reduction of environmental pollution with salts", *Polymer chemistry and technology: proceedings of scientific conference Chemistry and Chemical Technology*, Kaunas, Lithuania.

Deng, W.J., Chen, D.H., Huang, M.H., Hu, J. and Chen, L. (2015), "Carbon dioxide deliming in leather production: a literature review", *Journal of Cleaner Production*, 87, 26-38.

Fennen, J., Herta, D., Pelckmans, J.T. and Christner, J. (2014), "Reliable and environmentally friendly enzymatic unhairing with low amounts of sulphide", *Sustainability strategy of leather industry: proceedings of the 10th Asian International Conference on Leather Science and Technology Workshop on Environmental Technologies*, November 23-26, Okayama, Japan.

Golovteeva, A.A., Kutsidi, D.A. and Sankin, L.B. (1982), Laboratornyj praktikum po khimiyi i tekhnologiyi kozhy i mekha, Legkaiya i Pischevaiya Prom. Moscow (In Russian).

Helcom Recommendation (2015), Adopted 15 March 1995 of the Helsinki Convention.

Kazlauskaite, E., Balciuniene, J., Zaliauskiene, A., Beleska, K., Valeika, V. and Valeikiene, V. (2002), "Influence of unhairing method on dermatan sulphate and microscopical investigation of pelt and leather", *Journal of the Society of Leather Technologists and Chemists*, 86, 59-64.

Liu, Y. S., Yi C., Fan, H. J., Hu, Z., Luo, Z.Y. and Bi, S. (2009), "Environment friendly lime-free liming", *Journal of the Society of Leather Technologists and Chemists*, 93, 56-60.

Munz, K.H. and Sonnleitner, R. (2005), "Application of soluble silicates in leather production", *Journal of the American Leather Chemists Association*, 100, 66-75.

Saravanabhavan, S., Thanikaivelan, P., Rao, J.R. and Nair, B.U. (2005), "Silicate enhanced enzymatic dehairing: A new lime-sulfide-free process for cowhides", *Environment Science & Technology*, 39, 3776-3783.

Sathish, M., Madhan, B., Saravanan, P., Rao, J.R. and Nair, B.U. (2013), "Dry ice - an eco-friendly alternative for ammonium reduction in leather manufacturing", *Journal of Cleaner Production*, 54, 289-295.

Sirvaitytė, J., Beleška, K., Alaburdaitė, R., Valeikienė, V. and Valeika V. (2015), "Lime-free unhairing: sodium aluminate as a cleaner alternative to lime", in: Plavan, V.P., Barsukov, V.Z and Rezanova, O.P., *Advanced polymer materials and technologies*, КНУТД, Kiev, 422-427.

Sirvaitytė, J., Valeika, V., Beleska, K. and Valeikiene, V. (2007), "Action of peracetic acid on calcium in limed pelt", *Journal of the Society of Leather Technologists and Chemists*, 91, 123-127.

Sirvaitytė, J., Valeika, V. and Plavan, V. (2009), "Peracetic acid deliming. Part II: Pickle-free and low-pickle processing of peracetic acid delimed pelt", *Journal of the Society of Leather Technologists and Chemists*, 93, 40-45.

Standard ISO 3380: 2002. Leather – Physical and mechanical tests – Determination of shrinkage temperature up to 100 degrees C.

Standard ISO 4045:1977. Determination of pH

Standard ISO 5398-2: 2009. Leather - Chemical determination of chromic oxide content - Part 2: Quantification by colorimetric determination.

Thanikaivelan, P., Rao, J.R., Nair, B U. and Ramasami, T. (2001), "Approach towards zero discharge tanning: Exploration of NaOH based opening up method", *Journal of the American Leather Chemists Association*, 96, 222-233.

Valeika, V., Balciuniene, J., Beleska, K. and Valeikiene, V. (2000), "Lime-free unhairing: Part 3 Influence of unhairing method on chemical, physical and mechanical properties of pelt and leather", *Journal of the Society of Leather Technologists and Chemists*, 84, 165-169.

Valeika, V., Beleska, K., Valeikiene V. and Kolodzeiskis V. (2009), "An approach to cleaner production: from hair burning to hair saving using a lime-free unhairing system", *Journal of Cleaner Production*, 17, 214-221.

Zaides, A., Mikhailov, A. and Pushenko, O. (1964), "Modiphitsirovanyi method opredeleniya oksiprolina", *Biokhimiya*, 1, 5-6 (In Russian).

Zeng, Y.H., Lu, J.H., Liao, X.P., He, Q. and Shi, B. (2011), "Non-ammonia deliming using sodium hexametaphosphate and boric acid", *Journal of the American Leather Chemists Association*, 106, 257-263.

ECOFRIENDLY DYEING PROCESS WITH ENZYMES

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Biological degradation of many dyes is difficult. Therefore dyeing baths give high pollution load to waste water. In this work, the use of enzyme in the dyeing of leather has been studied with the aim to improve the exhaustion of dye and to reduce pollution load of dyeing waste bath. For this purpose, chromium-tanned leather were treated with different ratio enzyme after the neutralization process. Then dyeing has been carried out. The effect of bacterial protease on colour properties of leathers was investigated. Also the change of the amount of COD load in the waste dye baths and leather physical properties were investigated. The obtained test and analysis results show that when the enzyme was used in the dyeing process, the COD load of waste dye baths was reduced.

Keywords: leather, ecofriendly dyeing, enzyme

INTRODUCTION

Tanning process involves conversion of putrefiable skin or hides to a nonputrescible material. Leather making involves operations like soaking (rehydration), liming, deliming, pickling, tanning, post-tanning and finishing processes (Kanagaraj *et al.*, 2015).

Dyeing is one of the inevitable steps of imparting color to the leather carried out in post-tanning operations. Synthetic dyes hold a major share in the dyeing of leather. In general, about 70% and 20% dyestuffs used today belong to acid and direct class (Hunger, 2002; Zengin *et al.*, 2012). These dyes have poor biodegradability due to higher biological and chemical oxygen demands. The conventional leather dyeing process is also very cumbersome and employs numerous chemicals and auxiliaries. Due to the number of pollutants involved in the wet processing of leather, this industry is striving to find natural and eco-friendly dyestuffs, auxiliaries and methods to reduce the environmental pollution (Dave, 2015). The total worldwide consumption of dye in several industries like textile, paper, pulp, leather, plastics is in excess of 104 tons/year. It is estimated that about 10% of unexhausted dyes are discharged into the waste streams irrespective of the substrate involved in dyeing. Hence, the generated effluent contributes to very high biological oxygen demand, chemical oxygen demand, color and suspended solids. The biotreatability of many of these synthetic dyes is normally poor and therefore, the treated waste water retains residual color, leading to the constant criticism from the civic community. In addition, the colored dye effluents become toxic to aquatic biota and thus affect the symbiotic process by disturbing the natural equilibrium thereby reducing the photosynthetic activity and primary production (Kanth *et al.*, 2009). In recent years, nearly 30% of the leather industries have been shut down due to environmental concern which urges the industries to adopt cleaner processing methods. Several greener approaches were proposed in this context, among them enzyme based processes for pre-tanning and pigment from natural sources (plants, microbes, insects/animals and minerals) for dyeing had been considered as effective, safer and a cleaner technology for making leather (Haddar *et al.*, 2014).

Different types of enzymes are commonly used in various stages of leather processing for modification of physical and chemical properties of leather. But the information available on the use of enzymes in dyeing process of post-tanning operations in leather manufacture is scanty.

In this work, the influence of bacterial protease enzyme on leather properties and wastewater pollution load in dyeing process was investigated.

MATERIALS AND METHODS

Enzyme-aided Dyeing Process

The chrome tanned leather samples were neutralized, dyed and fatliquored according to the recipe given in Table 1. Enzyme concentrations 0,05 and 0,1% (based on weight of shaved chrome tanned leathers) have been used. The leathers have been further treated with fatliquors as per a standard leather processing recipe for garment leathers.

Control Dyeing Process

The control dyeing processes have been carried out without enzyme treatment. Fatliquoring and dyeing processes were done the same way in all leather groups (Table 1).

The process liquors from all the experimental and control trials have been analyzed for the uptake of dye and load of COD. The leathers have been washed, set, hooked to dry and stored at room temperature.

The unspent dyestuff in the exhausted process liquor was analyzed using a Shimadzu UV–Visible 1601 spectrophotometer. The percentage of dyestuff exhaustion (DE) was calculated using the following equation:

$$\% \text{ DE} = [(C_r - C_t)/ C_r] \times 100 \qquad (1)$$

where C_r and C_t represent the amount of dyes at the end of the dyeing procedure for the reference and treated samples respectively.

Table 1. The dyeing recipes of study

Cr Retannage Neutralisation	150% Water 40 C 3% Electrostable fatliquoring agent 4% Basic chrome sulphate 45’ 1% Na -Formiate 30’ 1% $NaHCO_3$ pH: 6.5
Enzyme treatment	100% Water 40 C X% Bacterial protease 30’ (X= 0.05% and 0.1%)
Dyeing	100% Water 40C 1 % Dyeing auxiliary agent 10’ 2 % Acid dye 1 % formic acid 30’
Fatliquoring	150% Water 60 C 12 % Combine fatliquoring agent 2 % Electrostable fatliquoring agent 2 % Syntetic fatliquoring agent 60’ 1% Formic acid 30’ pH: 3.8 Washing

Chemical Oxygen Demand (COD) in the exhausted retanning process liquor was using Merck Cell Test kit Merck Spectroquant Move 100 spectrophotometer.

The leather samples were prepared in accordance with “sampling location” and conditioned according to “sample preparation and conditioning” standards TS EN ISO 2418 and TS EN ISO 2419 respectively prior to analysis (EN ISO 2419/2006).

In order to identify the color differences between research and control leathers , lab type spectrometer which has 4 mm width measuring range, Konica Minolta CM- 3600A brand was used. Measurements were performed according to CIELAB color coordination, under the conditions of CIE 100 standard observer angle and CIE standard D65 light source (Zengin *et al.*, 2012).

Wet and dry rub were carried out using standard procedures (EN ISO 11640/2001). Rubbing fastness was carried out by using the device "Otto Specht Bally Finish Tester". Significance of differences between fastness values were evaluated to a grey scale as shown in Table 2.

Determination of tensile strength and elongation was performed according to the ISO 3376:2011 standard method. The tensile strength of the dyed leathers were measured using a Shimadzu AG-IS Test Apparatus.

Scanning electron micrographs of the cross section of the leathers was taken used by HITACHI TM-1000 tabletop microscope with x250 magnification.

RESULTS AND DISCUSSION

Dyeing is an important process in the leather industry. Many dyes suffer from incomplete exhaustion and this causes concern, as the biotreatability of the unexhausted dyes in effluent is normally difficult. In the present study, an attempt has been made to improve the exhaustion of dyes by using enzymes. The effect on leather properties and waste dyeing baths of enzymatic treatment have been studied.

Table 2. Evaluation of fastness values with grey scale

Fastness value	Evaluation
1	Very poor
2	Poor
3	Average
4	Good
5	Very good

Table 3. The results of test and analyses of control and enzyme aided dyed leathers

	Exhaustion %	Tensile strength (N/mm^2)	Elongation (%)	COD (mg/l)
Control	36.7	12,68	72,57	5580
0.05% Enzyme	78.5	17.48	59,27	1064
0.1% Enzyme	85.5	15.08	62.73	638

The results of dyestuff exhaustion for control and enzyme-aided dyed leathers are given in Table 3. In the study, dye consumption value analyzed, in control group exhaustion of dye was found to be 36.7%. When the results are evaluated, enzyme treated groups have been found rather good than control group. 0,1% enzyme treated group leather samples had the best consumption values when compared to other enzyme group in the study (Table 3).

The examination of Table 2 indicates that with the increasing ratio of the enzyme used from 0,05% to 0,1%, the exhaustion in the dye bath gradually increases from 78,5% to 88,5%. When the control group and enzyme aided leathers are compared to each other, it is clearly observed that the dyestuff exhaustion of the enzyme aided leathers are higher than that of the control groups. It was found that there was a significant increase in dye uptake after enzymatic treatment.

One of the most important rapid parameters in determining the pollution load of leather effluent is the COD test. The COD values of the dye liquors of leathers consumed in our study are shown in Table 2. When dyeing waste water’s pollution load was examined in the study, it was seen that 0.1% enzyme treated group leather samples had the lowest COD values when compared to other trials. The COD values of 0.1% group were detected as 638 mg/l. COD value of the 0.05% enzyme treated group was found 1064mg/l. In order to identify the pollution load and leather properties, no enzyme was used in control group. The COD value of control group was obtained as 5580 mg/l. When control group and enzyme treated groups were compared, it was seen that dyeing process had substantially increasing effect on waste water pollution load. Waste dyebath COD values are seen in Table 3. The results show that there is a significant improvement in the pollution load of dye waste bath due to the presence of enzyme, when compared to dyeing in absence of enzyme.

The tensile strength and elongation test results of the leather samples in the study are given in Table 3. Table 3 shows that strength characteristics of leather dyed in presence of bacterial protease are comparable to those of control leathers, which implies that strength properties were increased use of enzyme during the dyeing process. The tensile strength test results show that the leathers treated with 0.1% and 0.05% enzyme demonstrated the best values of 17.48 N/mm and 15,08 N/mm respectively. The lowest values in the tear loading test were obtained in control groups leathers. According the research results, the strength properties are improved but the elongation of leather decreased by use of the bacterial protease.

All dyeing combinations of control and enzyme-aided leathers were analyzed spectrophotometrically and the color values of leathers are provided in Table 4.

Table 4. Values of the colors obtained with and without enzyme

	L*(D65)	a*(D65)	b*(D65)	ΔE(D65)
Control	28,60	6,02	4,57	70,77
0.05% Bacterial protease	33,45	6,74	5,64	66,10
0.1% Bacterial protease	33,87	7,27	5,99	65,77

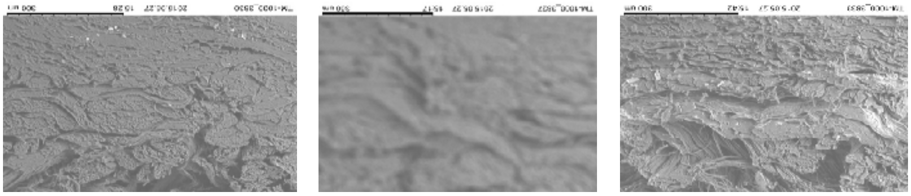
When ΔE values are examined after enzyme implementations that have different concentrations, it has been observed that there is color difference between the leathers that are treated with enzyme-aided dyeing and control group's samples. When control group leather samples and enzyme group samples are compared, it has been detected that there is color difference. Colors that are closest to black are acquired from 0.1% enzyme applied leathers. It has been observed that black color of leather samples in enzyme-aided dyeing is lighter (brighter) than control group's leather. When enzyme implementations are compared among each other, it has been detected that closest color to black is acquired from 0.1% enzyme implementation and 0.1–0.05% enzyme implementations show very similar black color rates.

Besides, when a* rates are examined, it has been detected that color of control leather samples and leathers that were treated with enzyme implementations come from red. Also, there are differences in redness value of leathers that are acquired through control and enzyme implementation. Same effect is remarkable in enzyme implementations too. When "b*" rate, which shows yellowness and blueness values, is examined, it has been seen that same situation in "a*" rate is same for this rate too. It has been detected that control sample leathers that were processed with enzyme implementation has yellowness in their color. Also, in the yellowness rates of leathers that are acquired through control group and enzyme implementations has differences. It has been detected that same effect is also present in enzyme implementations.

Wet and dry color fastnesses of leathers analyzed on the grain sides to rubbing are comparatively shown in Table 2.

The colour fastness properties of dyed leathers were tested according to the standards and compared with the greyscale. The colour fastness properties of the enzyme treatment and control groups leather results are given in Table 4. The results of colouristic evaluation show that the wet fastness properties of control leathers and 0.1% enzyme treated leathers are not different, but dry fastness of control leathers were found lower than 0.1% enzyme treated leathers.

The Table shows that the dry and wet rubbing fastness of 0.1% enzyme treated leathers were better than the control leathers and 0.05% enzyme treated leathers. Although wet rubbing fastness of 0.5% enzyme treated leathers were lower than the control leathers and 0.1% enzyme treated leathers, dry rubbing fastness of 0.5% enzyme treated leathers were proved higher than control leathers. This implies that colour of the 0.1% enzyme treated leathers can withstand dry and wet conditions in a better way.



Control group 0.05% Enzyme 0.1% Enzyme

Figure 1. Scanning electron micrographs (250× magnification) showing the cross-section view of leathers

Scanning electron microscopy analysis showed a well opened-up fibre matrix for the enyzme treated leather.

CONCLUSION

This study showed that enzyme treatment improved the performance characteristics of leather that it provided some environmental protection. The best values of dyestuff consumption, wet-dry rubbing fastness, tensile strength and COD were found with the 0.1% bacterial protease treatment. The CF treated TTC leathers gave the best results in all the tests and analyses. The results suggest that bacterial protease can be used in leather processing. It was found that there is a significant increase in dye uptake after enzymatic treatment. The results show that there is a significant improvement in the exhaustion of dye due to the presence of enzyme, when compared to dyeing without enzyme. From the ecological perspective, it could further be said that using bacterial protease is of extreme importance in terms of reducing the environmental pollution load and providing a sustainable solution to the leather industry.

REFERENCES

Dave, H., Ledwani, L. and Nema, S.K. (2015), “Surface modification by atmospheric pressure air plasma treatment to improve dyeing with natural dyes: an environment friendly approach for leather processing”, Plasma Chemistry and Plasma Processing, 36(2), 599-613, http://dx.doi.org/10.1007/s11090-015-9687-9

Haddar, W., Ticha, M.B., Guesmi, A., Khoffi, F. and Durand, B. (2014), “A novel approach for a natural dyeing process of cotton fabric with Hibiscus mutabilis (Gulzuba): process development and optimization using statistical analysis”, Journal of Cleaner Production, 68, 114-120.

Hunger, K. (2002), Industrial Dyes: Chemistry, Properties, Applications, first ed. Wiley-VCH, Weinheim.

ISO 3376: 2011 (2013), Determination of tensile strength and percentage extension. International Organization for Standardization, Geneva.

Kanagaraj, J., Senthilvelan, T., Panda. R.C. and Kavitha, S. (2015), “Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: a comprehensive review”, Journal of Cleaner Production, 89, 1-17.

Kanth, S.V., Venba, R., Jayakumar, G.C. and Chandrababu, N.K. (2009), “Kinetics of leather dyeing pretreated with enzymes: Role of acid protease”, Bioresource Technology, 100, 2430–2435.

TS EN ISO 11640 (2001), Leather - Tests for colour fastness - Colour fastness to cycles of to-and-fro rubbing, Turkish Standards Institution, Ankara.

TS EN ISO 2419 (2006), Physical and mechanical tests-Sample preparation and conditioning. Turkish Standard Institute (TSE), Ankara, Turkey.

Zengin, A.C.A., Crudu, M., Maier, S.S., Deselnicu. V., Albu. L., Gulumser. G., Bitlisli. B.O., Basaran. B. and Mutlu. M.M. (2012), “Eco-leather: Chromium-free Leather Production using Titanium, Oligomeric Melamine Formaldehyde Resin, and Resorcinol Tanning Agents and the Properties of the Resulting Leathers”, Ekoloji, 21(82), 17-25.

Zengin, G., Ozgunay, H., Ayan, E.M. and Mutlu, M.M. (2012). “Determination of dyestuffs remaining in dyeing processes of vegetable-tanned leathers and their removal using shavings”, Polish Journal of Environmental Studies, 21, 499-508, retrieved from: http://www.pjoes.com/pdf/21.2/Pol.J.Environ.Stud.Vol.21.No.2.499-508.

THE RELATIONSHIP BETWEEN BODY MASS INDEX AND PLANTAR PRESSURES OF THE ELDERLY

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This paper approaches the relationship between obesity and foot disorders, an important topic in designing and producing preventive or palliative strategies to improve the quality of life for the elders. In spite the growth of old people, they are constrained to accept the footwear that the market is offering, which doesn't meet their special requirements in case of feet parameters. The analyzed sample consisted in 67 women, aged between 52-84 years old. By comparing the values of body mass index for the analyzed group it is demonstrated that with age, the weight increases for a significant number of women, going from overweight to obese. The authors performed as well, a descriptive statistics and a Pearson correlation between the body mass index and several plantar footprints parameters obtained in a previous research. As an important instrument, the body mass index can be used by footwear producers to modify and customize the last and the elderly shoes. The results of this study are important in various stages of producing footwear, highly adapted to the needs of different pathologies for the elderly consumers.

Keywords: footwear, customization, plantar parameters correlation

INTRODUCTION

The loss of the ability to walk due to foot problem, not only produces physical limitations, but also has a significant impact on the persons's mental, social, and economic status (Helfand, 2015; Herghiligiu *et al*., 2016; Sarghie *et al*., 2016; Ionesi *et al*., 2014; Deselnicu *et al*., 2015). The world population is heading toward 10 billion people (Walker and Mesnard, 2009; Bougourd, 2015). While global population growth will bring its own challenges, aging is also unprecedented. Reports published by the United Nations (UN, 2009), Population Division suggests that in 1950, 200 million people were aged over 60 years; by 2000 they increased to 600 million; and predicts that by 2050 there will be 2 billion. During this period the median age increased in all of the EU Member States, the highest rising, by 5.4 years, being in Romania (*, **). According to national statistics, at the present in Romania are 3.4 million elderly, representing over 15% of the population (***).

In a study by Thaı's Rabiatti Aurichio and others (Aurichio *et al*., 2011) was demonstrated that as the weight increases, the foot flattens. In case of that particular study, the mechanism of evaluating the foot high arch index was possible using plantar footprints. In addition to structural changes due to aging, obesity is an important factor to be taken into account when assessing morphological changes of elders' feet. Tavares and Anjos (1999), Barreto *et al*. (2003), Santos and Sichieri (2005) have demonstrated that women are more prone to obesity than men.

METHOD

In a previous study (Mihai and Păştină, 2012; Păştină *et al*., 2012), it was determined the foot types, based on plantar footprints taken from 67 women, aged between 52-84 years old and weighing 45-70 kg, figure 1. The subjects were divided in three categories, group 1: 52-59 years old, group 2: 60-64 years old and group 3: 65-84 years old. All subjects read and signed an informed consent before testing.

Figure 1. Taking plantar footprints and static plantar pressures using RSscan pressure plate

RESULTS

Body Mass Index

The body mass index is weight divided by height squared an individual thereof (kg / m^2), and it is a useful tool in the assessment of excess fat (Pinheiro *et al*., 2004). According to World Health Organization, normal BMI values are between 18.5 and 24.9; values below 18.5 represent underweight, and over 30, obese (WHO, 1997).

For all 67 women the BMI was calculated before taking plantar footprints and static and dynamic measurements. The results can be observed in table 1.

Table 1. Body mass index

BMI /Age	Group 1, 52-59 years old	Group 2, 60-64 years old	Group 3, 65-84 years old
Underweight (BMI<18.5)	2	0	2
Normal (BMI 18.5-25)	13	5	3
Overweight (BMI 25-30)	12	9	9
Obese (BMI >30)	2	4	5

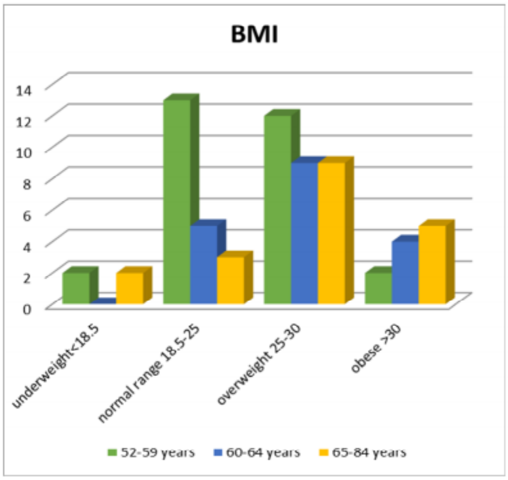

Figure 2. Body Mass Index, BMI

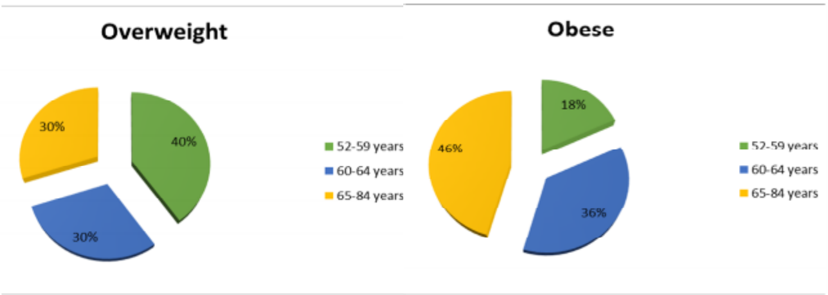

Figure 3. BMI grouped by age

By comparing the values of BMI for all three groups, it can be observed that the overweight percentage is higher for group 1, and obesity for groups 2 and 3. These values demonstrate that with age, the weight increases for a significant number of women, going from overweight to obese. The results are closely related to foot pain and disorders, which occur while ageing.

DISCUSSIONS

Based on plantar footprints from the previous study, the following parameters were obtained: Heel Width (HW), Minimum Width of Plantar Footprint (MWF), Toe Width (TW), Hallux-Valgus Angle (HVA), Chippaux-Simark Index (CSI).

Using the previous obtained data, BMI results and SPSS software, a descriptive statistics is performed, table 2.

Table 2. Descriptive statistics of foot parameters

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
BMI	67	19.67	17.63	37.30	26.3672	4.29880	18.480	-.031	.578
HW	67	18.00	45.50	63.50	53.8806	3.69796	13.675	-.250	.578
MWF	67	46.00	.00	46.00	25.1269	12.61173	159.056	-.333	.578
TW	67	27.50	72.50	100.00	88.9030	5.84214	34.131	-.420	.578
HVA	67	54.50	-19.00	35.50	6.3881	11.38041	129.514	-.001	.578
CSI	67	48.70	.00	48.70	27.9746	13.85177	191.872	-.297	.578
Valid N	67								

The statistically significant correlations between parameters were studied using Pearson correlation which is commonly used as a measure of the degree of linear dependence between two variables****. The results are available in table 3.

Table 3. Pearson Correlation of foot parameters

		BMI	HW	MWF	TW	HVA	CSI
BMI	Pearson Correlation	1	0.257*	0.293*	0.245*	-0.177	0.278*
	Sig. (2-tailed)		0.036	0.016	0.046	0.152	0.023
	Sum of Squares and Cross-products	1219.660	269.562	1048.924	406.207	-571.671	1090.822
	Covariance	18.480	4.084	15.893	6.155	-8.662	16.528
	N	67	67	67	67	67	67
HW	Pearson Correlation	0.257*	1	0.373**	0.399**	-0.014	0.343**
	Sig. (2-tailed)	0.036		0.002	0.001	0.912	0.004
	Sum of Squares and Cross-products	269.562	902.545	1148.015	568.724	-38.146	1160.197
	Covariance	4.084	13.675	17.394	8.617	-.578	17.579
	N	67	67	67	67	67	67
MWF	Pearson Correlation	0.293*	0.373**	1	0.399**	0.000	0.993**
	Sig. (2-tailed)	0.016	0.002		0.001	0.998	0.000
	Sum of Squares and Cross-products	1048.924	1148.015	10497.672	1938.075	-3.049	11444.616
	Covariance	15.893	17.394	159.056	29.365	-.046	173.403
	N	67	67	67	67	67	67
TW	Pearson Correlation	0.245*	0.399**	0.399**	1	0.214	0.304*
	Sig. (2-tailed)	0.046	0.001	0.001		0.082	0.012
	Sum of Squares and Cross-products	406.207	568.724	1938.075	2252.619	937.772	1623.535
	Covariance	6.155	8.617	29.365	34.131	14.209	24.599
	N	67	67	67	67	67	67
HVA	Pearson Correlation	-0.177	-0.014	0.000	0.214	1	-0.031
	Sig. (2-tailed)	0.152	0.912	0.998	0.082		0.803
	Sum of Squares and Cross-products	-571.671	-38.146	-3.049	937.772	8547.910	-323.690
	Covariance	-8.662	-0.578	-0.046	14.209	129.514	-4.904
	N	67	67	67	67	67	67
CSI	Pearson Correlation	0.278*	0.343**	0.993**	0.304*	-0.031	1

	BMI	HW	MWF	TW	HVA	CSI
Sig. (2-tailed)	0.023	0.004	0.000	0.012	0.803	
Sum of Squares and Cross-products	1090.822	1160.197	11444.616	1623.535	-323.690	12663.527
Covariance	16.528	17.579	173.403	24.599	-4.904	191.872
N	67	67	67	67	67	67

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Positive correlations are found between BMI and HW, MWF, TW and CSI and a negative correlation between BMI and HVA. The highest correlations are found to be between MWF and CSI (0.993) confirming a strong relation of those two parameters, the lower the plantar width, the higher arched foot.

CONCLUSIONS

Demonstrating the relationship between obesity and foot disorders in case of older people is important in designing and producing preventive or palliative strategies to improve the quality of the elders. The old people are constrained to accept the footwear that the market is offering, which doesn’t meet their special requirements in case of feet parameters.

By comparing the values of BMI for the analyzed group it is demonstrated that with age, the weight increases for a significant number of women, going from overweight to obese. A good fit of the shoe on the foot involves customizing the last, but this can only be achieved under certain standards. As an important instrument, the body mass index can be used by footwear producers to modify and customize the last and the elderly shoes. The results of this study are important in various stages of producing footwear adapted to the needs of different pathologies of elderly consumer.

Acknowledgement

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REFERENCES

Barreto, S.M., Passos, V.M.A. and Lima-Costa, M.F.F. (2003), “Obesity and underweight among Brazilian elderly: the Bambuı Health and Aging Study”, *Cadernos de Saude Publica*, 19, 605–612

Bougourd, J. (2015), “Ageing populations: 3D scanning for apparel size and shape”, *Textile-led Design for the Active Ageing Population*, http://dx.doi.org/10.1016/B978-0-85709-538-1.00010-9.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A. and Militaru, G. (2016), “New products development through customized design based on customers’ needs. Part 2: Foot Pathology Manufacturing Parameters”, *The 9th International Conference Interdisciplinarity in Engineering, INTER-ENG 2015, Procedia Technology* 22, pages 1059 – 1065, available at www.sciencedirect.com.

Helfand, A.E. (2013), “Design issues in geriatric footwear, Handbook of Footwear Design and Manufacture”, *A volume in Woodhead Publishing Series in Textiles*, 372–399.

Herghiligiu, I.V., Mihai, A., Sarghie, B., Souto Bizarro, R. and Arias, C. (2016), “Framework of the e-learning training program on corporate social responsibility”, *The 12th International Scientific Conference eLearning and Software for Education*, Bucharest, April 21-22, 10.12753/2066-026X-16-255, page 526.

Ionesi, D., Ciobanu, L. and Sarghie, B. (2014), “E-Learning Application for a better understanding of shoes 3D modeling”, *10th International Scientific Conference ELSE*, ISSN 2360-2198, 10.12753/2066 026X 14 285, page 196.

Mihai, A. and Pastina, M. (2012), “Classification of foot types, based on plantar footprint”, *Proceedings of “The 4th International Conference on Advanced Materials and Systems”, ICAMS 2012*, Bucharest, ISSN 2068-0783, 347-352.

Păştină, M., Mihai, A. and Bilalis, N. (2012), “Finite element analysis for insole-sole prototypes”, *Proceedings of “The 4th International Conference on Advanced Materials and Systems”, ICAMS 2012*, Bucharest, ISSN 2068-0783, 359-364.

Pinheiro, A.R.O., Freitas, S.F.T. and Corso, A.C.T. (2004), “An epidemiological approach to obesity”, *Revista de Nutrição*, 17, 523–533.

Santos, D.M. and Sichieri, R. (2005), “Body mass index and measures of adiposity among elderly adults”, *Revista de Saude Publica*, 39, pages 163–168.

Sarghie, B., Mihai, A. and Herghiligiu, I.V. (2016), “E-learning application for 3D modelling of custom shoe lasts using templates”, *The 12th International Scientific Conference eLearning and Software for Education, Bucharest*, April 21-22, 10.12753/2066-026X-16-260, page 553.

Tavares, E.L. and Anjos, L.A. (1999), “Anthropometric profile of the elderly population: results from the National Health and Nutrition”, *Cadernos de Saude Publica* 15, 759–768.

Aurichio, T.R., Rebelatto, J.R. and de Castro, A.P. (2011), “The relationship between the body mass index (BMI) and foot posture in elderly people”, *Archives of Gerontology and Geriatrics*, 52, e89–e92, www.elsevier.com/locate/archger.

UN (2009), *World population ageing*, New York: United Nations Department of Economic and Social Affairs/Population Division.

Walker, M. and Mesnard, X. (2009), *What do mature consumers want?*, Published by the corporation’s Global Business Policy Council in conjunction with the A.T. Kearney Global Consumer Institute.

WHO (World Health Organization) (1997), *Obesity: Preventing And Managing the Global Epidemic (Report of aWHOConsultation on Obesity)*, WHO, Geneva, page 98.

*http://ec.europa.eu/eurostat/statistics-explained/index.php/Population_statistics_at_regional_level/ro

**Eurostat,http://ec.europa.eu/eurostat/statistics-explained/index.php/Population_structure_and_ageing

***Romanian National Institute of Statistics. INSSE Statistical DB Tempo. Romania. Available from: https://statistici.insse.ro/shop/index.jsp?page=tempo2&lang=en&context=21

****https://en.wikipedia.org/wiki/Pearson_product-moment_correlation_coefficient

ANALYSIS OF BIOMECHANICAL PARAMETERS VARIANCE BY WEIGHT FOR ELDERLY WOMEN IN ROMANIA

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The paper presents the results of a fieldwork study conducted in order to analyze the variation of different biomechanical parameters of elderly women in Romania. The study has an exploratory and descriptive nature and uses quantitative methodology. The sample consisted of 100 elderly women from Romania, ranging from 55 to over 75 years of age. The collected data was analyzed using a statistic analysis software program. The analysis of variance demonstrated significant differences across weight groups in terms of biomechanical parameters such as toe off phase and support phase in the case of elderly women.

Keywords: elderly, biomechanical parameters, force platform.

INTRODUCTION

Despite their specific needs, old people are currently constrained to accept the mass footwear that they are offered. Because of their foot deformities, their biomechanical characteristics are different than the rest of the population (Deselnicu *et al*., 2016a) and constitute important indicators for a better understanding and analysis of their particular footwear necessities (Deselnicu *et al*., 2016b; Deselnicu *et al*., 2008). The elderly people, with a lot of illnesses caused by age, are mainly disposed to osteopathy (Galasso *et al*., 2009). In the World Health Organization list of preventive activities, the major place is taken by the relevant orthopedic means, among them choosing and processing the correcting means for the normalization of the deformations of footwear and foot pathologies.

From a mechanical point of view, the human locomotor apparatus represents a complex system of levers acted upon by a force field. The biomechanics studies reveal the nature of these forces, their actions, as well as the loads acting upon the foot and, through it, on the footwear components (Vasilescu *et al*., 2010). These studies are used for offering information for the rational footwear manufacturing (Deselnicu *et al*., 2014), in particular, the shape of the insole, sole or heel. Since footwear represents a necessity nowadays (Zainescu *et al*., 2014), the constructive parameters of the pattern of the footwear product are very important (Mihai *et al*., 2005).

RESEARCH METHODOLOGY

The study used the quantitative methodology and an exploratory and descriptive approach. The research methods which best fit the objective of the study were the biomechanical gait measurement for ground reaction force, the questionnaire-based survey, and observation.

Data Collection

The data was collected in Bucharest over a period of 3 weeks in November 2015. The ground reaction force was measured using AMTI´s AccuGait System (www.amti.biz), with NetForce and NetForce/ BioAnalysis components. The respondents were also applied a short questionnaire consisting of questions referring to anthropometric and demographic data, enquiring about the respondents' age, height, weight and medical conditions.

Research Sample

The sample was composed of 100 elderly women. The main statistic indicators characterizing the sample are presented in Table 1:

Table 1. Statistic indicators for the main demographic and anthropometric parameters

		Height (cm)	Weight (kg)	Age (years)
N	Valid	100	100	100
	Missing	0	0	0
Mean		161.75	72.96	67.05
Median		163.00	71.00	64.00
Mode		165	80	59
Std. Deviation		6.663	11.414	8.916
Minimum		148	50	55
Maximum		184	112	87

Source: SPSS software

The age of the participants varied between 55 to 87 years, with the eldest female subjects (over 71 years) making the most of the sample (34%), followed by the subjects of the 60 – 64 years (27%) and 55 – 59 years (26%) age groups, respectively. The average height of the subjects of the studied sample was 161,75 cm, the average weight was 73 kg and the average age was 67 years.

DATA ANALYSIS AND INTERPRETATION OF RESEARCH RESULTS

Statistical processing of collected data used the statistical software SPSS for Windows, version 19.0, and descriptive and inferential statistical analysis was conducted in the following stages: the statistic summary of relevant information and the analysis of variance. In order to investigate the significant variance of the investigated variables, the participants have been divided into three weight groups, as presented in Figure 1:

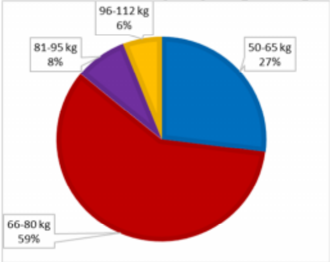

Figure 1. Sample structure by weight

The Analysis of Variance

The authors investigated the variance of the main biomechanical parameters of elderly women across the three weight groups. The most appropriate testing method was considered the One-Way ANOVA, which was performed using the SPSS software. Four working hypotheses were formulated as follows:

H_1: There are statistically significant differences between the three weight groups in terms of the travel speed.
H_2: There are statistically significant differences between the three weight groups in terms of the heel strike.
H_3: There are statistically significant differences between the three weight groups in terms of the toe – off phase.
H_4: There are statistically significant differences between the three weight groups in terms of the support phase.

The null hypotheses stated that there are no differences between the three weight groups in terms of these biomechanical parameters. The results can be consulted in Table 2:

Table 2. One-Way ANOVA test results for the variance of biomechanical parameters across weight groups

		Sum of Squares	df	Mean Square	F	Sig.
Travel speed	Between Groups	,191	3	,064	1,986	,121
	Within Groups	3,078	96	,032		
	Total	3,269	99			
Heel strike	Between Groups	,000	3	,000	,592	,622
	Within Groups	,002	96	,000		
	Total	,002	99			
Toe off phase	Between Groups	,867	3	,289	4,109	,009
	Within Groups	6,750	96	,070		
	Total	7,616	99			
Support phase	Between Groups	,861	3	,287	4,071	,009

Source: SPSS software

As the significance level is 0,121 (p= 0,121) which is greater than the significance level of 0,05 established for this analysis, hypothesis H_1 was rejected. Therefore, the null hypothesis was accepted, stating that there are no statistically significant differences between the three weight groups of elderly women in the investigated sample in terms of the travel speed.

The same decision applies for H_2 also (p= 0,622), resulting that there are no statistically significant differences between the three weight groups of women in terms of the heel strike.

The significance level for hypothesis H_3 is 0,009 (p= 0,009), which is smaller than the significance level of 0,05 established for the ANOVA analysis. Hypothesis H_3 was therefore accepted, confirming that there are statistically significant differences between the three weight groups in terms of the toe off phase. Consequently, the null hypothesis was rejected.

The significance level for hypothesis H_4 is also 0,009 (p= 0,009) smaller than the significance level of 0,05 established for the analysis. This recommends that the working hypothesis H_3 should be accepted, confirming that there are statistically significant differences between the three weight groups of elderly women in the investigated sample in terms of the support phase.

As expected, weight is one of the most important factors that causes variations to the biomechanical parameters of elderly women.

CONCLUSIONS

In this research, the authors conducted a study in order to investigate the variance of various biomechanical parameters of elderly women across three weight groups. Four working hypotheses were formulated and tested using thee One – Way ANOVA test.

Following the testing, the first hypothesis H_1 was rejected, determining that there are no statistically significant differences between the three weight groups in terms of the travel speed.

Hypothesis H_2 was also rejected, showing that there are no significant differences between the three weight groups of elderly women in the investigated sample in terms of heel strike.

The working hypothesis H_3 was accepted, confirming that there are statistically significant differences between the three weight groups of elderly women in the investigated sample in terms of the toe-off phase.

Hypothesis H_4 was also accepted, confirming that there are statistically significant differences between the three weight groups of elderly women in the investigated sample in terms of the support phase.

Therefore, weight proved to be an important factor that influences important biomechanical parameters in the case of elderly women. Such biomechanical indicators include the toe off phase and support phase, while the travel speed and heel strike are not affected by the variation of subjects’ weight.

Acknowledgements

This work was supported by UEFISCDI Bucharest under the Partnership Programme project “Preventing gait deficiencies and improving biomechanical parameters for the elderly population by designing and developing customized footwear”, acronym MOBILITY, code PN-II-PT-PCCA 2013-4, Contract no. 122/2014.

REFERENCES

AMTI, Accugait Walkway [Internet], [cited 10th May 2016], available from: www.amti.biz.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A. and Militaru, G. (2016a), “New Products Development through Customized Design Based on Customers’ Needs. Part 1: Footwear Comfort Parameters”, *Procedia Technology,* 22, 1043-1050.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A. and Militaru, G. (2016b), “New Products Development through Customized Design Based on Customers’ Needs. Part 2: Foot Pathology Manufacturing Parameters”, *Procedia Technology,* 22, 1059-1065.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), "Sustainable Consumption and Production in the Footwear Sector", *Leather and Footwear Journal*, 14(3), 159-180.

Deselnicu, V., Arca, E., Badea, N., Maier, S.S. and Deselnicu, D.C. (2008), "Alternative process for tanning leather", *Leather and Footwear Journal*, 8(4), 25-34.

Galasso, V., Gatti, R. and Profeta, P. (2009), "Lessons of an aging society; The Political Sustainability of Social Security", *Economic Policy,* 19, 63-115.

Mihai, A., Curteza, A., Harnagea, F. and Malureanu, G. (2005), "The role of the biomechanics studies in conceiving the performing footwear", *Proceedings of the 4th International Conference on the Management of Technological Changes,* 281-286.

Vasilescu, A.M., Berijan, G. and Micu, A. (2010), "Analysis of forces causing or restricting movements of the human body", *Proceedings of the 3rd International Conference on Advanced Materials and Systems*, 331-336.

Zainescu, G., Albu, L., Deselnicu, D.C., Constantinescu, R.R. and Vasilescu, A.M. (2014), "A new concept of complex valorization of leather wastes", *Materiale Plastice*, 51, 90-93.

SHOE MAKING TECHNOLOGY

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Footwear production industry is facing a period of change and, in the next few years, will be front a significant process of innovation of systems, machines and equipment. The evolution starts and makes its way through the opinion that new approaches to electronics, software and networking can express a common denominator that allows all the players involved to manage together and organically all phases of design, prototyping and manufacturing of shoes and leather products. The shoe, treated in a scientific way and enriched with technological values, is the result of a series of treatments of data related to the foot, the last and the materials used in its manufacture. The main innovative aspects of the footwear industry are summarized by department.

Keywords: Evolution, Technology, Machinery.

PATTERN CAD

It represents the possibility to scan the foot, rebuild the complete image virtually through a software, import data from a 3D CAD shoe, from which to carry out the process.

They are connected systems and machines for an accurate construction of the last and its standardization.

Parametrics, modularity, codesing, concurrent engineering are the benefits of great importance that are obtained by 3D footwear CAD designing and by productive CAM systems. The rapid prototyping contracts the "time-to-market" and becomes essential to compete in the world market.

About "software", the ability to import and/or export files of last, upper, shoe and components is required to make them readable by most external interfaces dedicated to the individual interventions.

The CAD approach makes the creation of the shoe easy and quick, exploiting the potential of the "object oriented programming" with friendly and logical operating interface.

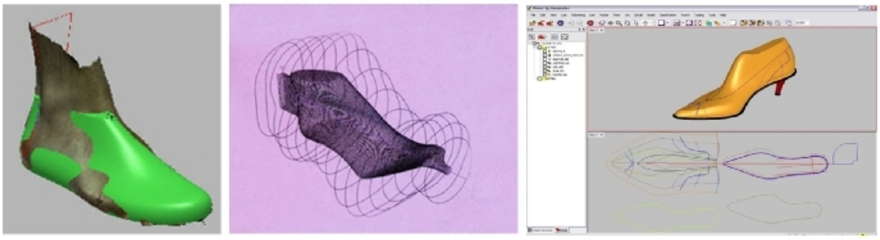

Figure 1. Rapid prototyping, last and shoe CAD

CUTTING

Significant technological change has occurred in this department through the use of cutting systems in continuous and without the die.

Cutting table equipped with oscillating blade, vision system and model templates projection represents the first building block of the future shoe factory.

The system is characterized by high cutting, punching and scoring speed, and operating costs optimized to energy saving.

The technology suppliers propose and continue to study versions of machine depending on the area of skin machines, on the production requirements and on the area available for installation. There are also present cutting, engraving and leather processing tables with laser technology.

Cutting technological offer is completed by dedicated nesting tables (automatic, semiautomatic, manual) to combine the skill of senior leather expert and the cutting process for large productions.

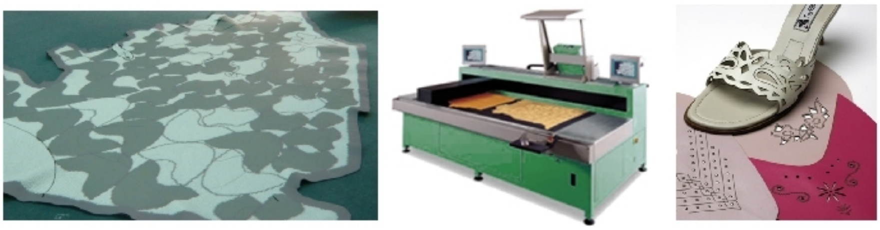

Figure 2. Cutting and nesting

UPPER PREPARATION AND CLOSING

Upper preparation interventions can relate to the drilling, engraving, plating, printing, edge treatments, cementing and closing. Machines and equipment characterized by operating and configuration parameters that cover every need. Thousands versions realized and sometimes focused to maximize and standardize production and quality sometimes to get a specific fashion effect.

Computerized perforating machine with one equipment is able to realize different ornamental designs based on simple on-screen programmability and allows the personalization of products.

Integrated machine can decorate leather/textile upper with nailheads, rivets, eyelets and laser process.

Automatic gluing plotters permit to automate and speed up the selective gluing operation made on flat patterns.

About automatic edge folding of upper parts, the features present in the machines relate to the programming of operating speed, the high flexibility, the correct deposit of glue along the path to fold up and the precise control of the cuts on the internal curves.

The wide range of sewing machines covers various aspects and needs. Fast machines, accurate in managing the length of the stitch and keeping it constant in time, capable of regular holes remarking and able to switch to a differential transport without interrupting the action of sewing.

Automatic sewing systems have a wide range of uses often strongly dedicated to specific interventions. They give the possibility to program one or more seams, bartacks and to make changes and corrections in a simple and fast way.

Figure 3. Upper pattern template, stitching operation

COMPONENTS PREPARATION

Systems and machines for producing and manufacturing the components used in the footwear production cycle (shape, die) and the components that are part of the shoe (toe puff, stiffener, insole, heel, sole, welt, strips, small metal parts, etc.)

In this area more and more solutions are present driven by software and operating through multi-axis and multi operating CNC.

It works on molds, soles, heels, insoles and inserts of varying shape and material, always in a precise, rapid and optimal production quality.

Stylists and fashion designers pushing the technological research to the realization of equipment suited to specific tasks and achieved in a short time.

SHOE LASTING, BOTTOM MAKING

The most important phases of this department are lasting machines: pulling-over, side and heel seat. They have been made flexible by programmable functional modules for the automatic adjustment of the machine to different shoe styles.

Characteristics: mechanical items that incorporate cement tracers and/or helicoidal rollers, controllable hydraulic pressures, perform with precision paths and speeds, for any type of upper and/or softness of leathers, upper fitting to the last during the lasting phase, high versatility in work changes, console touch screen panel control, remote control for assistance.

Programmable and automatic shoe lasted bottom and sole cementing systems, versions with the "vision" or programmable axes. Technical solutions with spray nozzle or rotary brush.

It is available an integrated machine for roughing and cementing the side wall sole shoe, 3/5 axes interpolation functionality.

A particular solution is represented by the “Reverse” shoe assembly seam, useful for a specific type of light footwear or to develop easily the first footwear mechanization in specific geographical areas.

The lasted shoe requires special and differentiated treatment of air conditioning: circulated air oven at high speed, oven-drying glue and reactivation, cooling station.

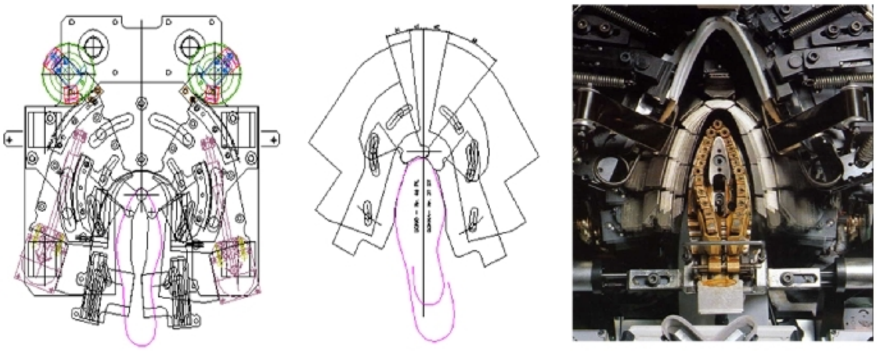

Figure 4. Flexible and programmable lasting

CONCLUSION

Technological development has accelerated in recent decades, so changes come faster and in more areas. Computers and robots will take on increasingly complex assignments, and Internet will be a breeding ground for completely new, virtual industries. The footwear industry must adapt to this mega-trend and take benefit of it. There are aspects that the footwear industry should align itself with the help of the technology: machines networking, design and implementation of intelligent machines and processes, conception of energy efficient machines and processes, direct communication channels between machines and specialists.

The challenge to the shoe factory of the future is launched. Technology is renewed and offers more and more targeted solutions according flexibility and connectivity. The appointment to stay updated and informed is represented by the international exhibitions (first fair in Milan in February), a meeting place for experts and technologists belonging to leading companies in the industry.

REFERENCES

AAVV (2002-2004), “Assomac Innovation Notebooks SHOEMAKING Industry”.
Assomac (1996-2016), “Technological News, Review of the innovations exposed in SIMAC fair”.

PERSONAL DESIGN, THE NEW FASHION TREND BASED ON INNOVATIVE TECHNOLOGY

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Fashion design expresses not only a new idea in the creation of a product but also its identification with the person wearing it. Thus arises the notion of personal design, which will be increasingly used in the coming years. Everything will become a cultural communication through the message that the product idea sends, a technological communication through technical drawings of the product, a commercial communication through the collection and advertising by fashion illustration. These clues will express the wearer's personality, will emphasize certain features and blur others, and will enable them to recompose the specific elements of the product. The concept of personal design has rapidly evolved and is complementary to mass production and even a competitor for haute couture. Elements of innovation and performance are not only a set of new knowledge in the field but also a real tool to correctly assess the creative opportunities of footwear companies and develop product strategies. The idea or concept sketches are a graphic illustration of the product, a mandatory work instrument in all phases of design and in all its manifestations. New technologies and the growing involvement of the "fashion customer" lead to the emergence of new architectures in the aesthetic and emotional expression of the wearer. Some topics will reveal each form of characters' identification with the product through concept drawings.

Keywords: personal design, fashion, trend

INTRODUCTION

Fashion design expresses not only a new idea in the creation of a product but also its identification with the person wearing it. Thus arises the notion of personal design, which will be increasingly used in the coming years.

Everything will become a cultural communication through the message that the product idea sends, a technological communication through technical drawings of the product, a commercial communication through the collection and advertising by fashion illustration. These clues will express the wearer's personality, will emphasize certain features and blur others, and will enable them to recompose the specific elements of the product.

The concept of personal design has rapidly evolved and is complementary to mass production and even a competitor for haute couture.

Fashion and technology are two words that go together very well. And this is only the beginning, because high-tech will continue to transform what we wear, in many ways, in the years to come. 3D printing technology is still in its infancy, but has already made its way into the fashion world.

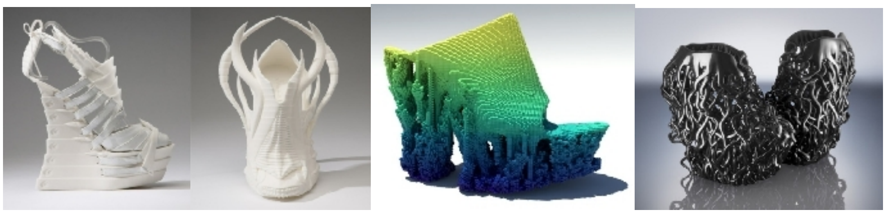

Figure 1. Footwear designed using 3D technology

In the future we will see increasingly more shoes with a unique design. Product development and innovation are an important part of the design process. Following the stages in the process design, after having defined the new product concept, it seems necessary to diversify (elaborate) this product idea.

Diversification techniques (product development) aim to obtain a sufficient number of models within the same family (concept), models that undergo an analysis that aims to define the best solutions both for the consumer and for the producer.

EXPERIMENTAL

Elements of innovation and performance are not only a set of new knowledge in the field but also a real tool to correctly assess the creative opportunities of footwear companies and develop product strategies. The idea or concept sketches are a graphic illustration of the product, a mandatory work instrument in all phases of design and in all its manifestations. New technologies and the growing involvement of the "fashion customer" lead to the emergence of new architectures in the aesthetic and emotional expression of the wearer. Some topics will reveal each form of characters' identification with the product through concept drawings (Figures 2-11).

Figure 2. Lacework and perforations made using laser cutting provide elegance and preciousness to the proposed models

Figure 3. Innovative technologies allow carbon models with a futuristic and sophisticated look

Figure 4. Creating high-tech hybrid materials with sophisticated effects heading to the sports style area

Figure 5. Returning to nature and environmentalism are desiderata with a great involvement on the part of designers who create spectacular shapes out of wood. "Wood Effect" is part of personal design.

Figure 6. Hand painted shoes with metallic and matte, shiny and opaque effects are a particular area of the exclusive market

Figure 7. The elegance and angular brightness transparencies undoubtedly spark the joy of wearing a shoe with personality

Figure 8. High class technologies are increasingly prominent by producing protective materials that stand out by applying films (elastomer) that give a particular and nonconformist look

Figure 9. The perceptible space is an area of excellence for designers who have created a performance of footwear creations using 3D technology

Figure 10. Inspired by the surrounding space with analytical geometry, discovering the essence, architectural forms that defy conventionality are recomposed

Figure 11. Creativity and passion, terms that define designer footwear with a great cultural impact, turning into real items of exhibition

DISCUSSIONS

The impact 3D printers will have on society is difficult to predict, however, several important effects have already begun to take shape. Thus, at the moment most of the products are made in countries with the lowest production costs, and are then transported all over the world. Thanks to 3D printers, the products will be created where the customers are, and the ability to customize designs depending on the customer's taste will, in some cases, eliminate the need for mass production.

Producers' optimism is justified by the huge potential this technology offers and its anticipated accelerated development. Although implementation is running slow, we already see how 3D printing influences all areas.

The pragmatic approach to the relationship of design, technology, and image becomes a scientific instrument for the production of ideas that define the personality of a category of products, its demand on the market and the chances of success compared to other products.

Aesthetic design of a new product involves creative spirit and artistic talent; the designer must find the optimal shape of the new product. This shape must, however, match the improved functionality of the product.

Improving the functionality of the designed product requires implementation of results from various fields of science and technology.

CONCLUSIONS

Design focuses on investigations in many areas and is provided by a multidisciplinary team, directed and coordinated by an expert designer, who conducts the study of all product components in terms of design, manufacture and market promotion.

This process has implications in various fields of science and technology: research, engineering, engineering design, economics, ergonomics, psychology, sociology, economics, marketing, finance, ecology, law, semiotics, public services etc.

The activity of the design industry can be defined as a harmonization of the relationship between people and objects, between the environment and the messages they come in contact with, exerting a positive influence on quality of life.

REFERENCES

*** (2015), Ars Sutoria, no. 392, Milano, Italy.
*** (2015), Ars Sutoria, no. 398, Milano, Italy.
*** (2015), Ars Sutoria, no. 399, Milano, Italy.
*** (2015), Ars Sutoria, no. 401, Milano, Italy.
*** (2015), Ars Sutoria, no. 402 bis, Milano, Italy.
*** (2015), Ars Sutoria, no. 406, Milano, Italy.
Katrantzou, M. (2012), "Technological Innovations in Fashion", *Art Act Magazine*, available at http://artactmagazine.ro/mary-katrantzou-inovatii-tehnologice-in-moda.
Maslow, A.H. (2006), *Motivation and personality*, London.

CHROMIUM REMOVAL FROM THE TANNERY WASTEWATER USING INDIGENOUS ADSORBENT

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Tannery is recognized as high-strength waste-generating industry. In tannery, leather processing involves a series of chemical treatments and mechanical operations to attain the prescribed characteristics. Inapt disposal of solid and liquid waste from the tannery cause a serious environmental pollution. Chromium containing wastewater is generated from chrome tanning operation which is the most hazardous pollutants released to the environment. Treatment of high-chromium-containing wastewater is a major concern in leather processing. In this study, indigenous adsorbent was investigated to remove the metals, especially chromium from the chrome tanning wastewater. The prepared adsorbent was directly mixed with chrome tanning wastewater. Various parameters such as adsorbent dose and contact time were optimized in batch-wise technique. The maximum removal of chromium was attained 99.9%. The use of indigenous adsorbent for the removal of chromium could be better instead of conventional methods.

Keywords: tannery, chrome tanning wastewater, indigenous adsorbent.

INTRODUCTION

Tannery strengthens the economy significantly, though it is facing severe challenges due to its environmental pollution caused by the wastes produced during leather manufacturing. In tannery, leather processing involves a series of chemical treatments and mechanical operations to attain the recommended characteristics. Inapt disposal of wastewater and solid waste from the tannery cause a serious environmental pollution.

During leather processing, huge volume of wastewater is generated. Since soaking to finishing operations, each and every chemical operation generate considerable amount of wastewater. Chromium containing wastewater is generated from chrome tanning operation which is the most hazardous pollutants released to the environment from the tannery. Tanning is the subsequent operation of pickling and is the most common technique in leather processing. In tanning, 90% tanneries use basic chromium sulfate as tanning agent to obtain better quality leather (Avindhan *et al*., 2004). The basic chromium sulfate binds with collagen to make it stabilize against biodegradation (Hashem *et al*., 2015). Averagely, only 60% of the chromium is up taken by the pickled pelt and 40% chromium remains in the solid/liquid wastes, especially as spent chrome liquor (Fabiani *et al*., 1997). In conventional chrome tanning, wastewater contains 1500-3000 mg/L chromium (Suresh *et al*., 2001).

Discharging of high chromium containing tanning wastewater is a major concern in leather processing. It is also a potential pollutant to soil, water, and air under definite conditions. A fraction of discharged spent chrome liquor is directly mixed with the water body, which causes serious environmental pollution; another fraction of chromium is settled in the lagoon or adsorbed by sediment/soil. Chromium has toxicity to humans at high doses. It exists in several oxidation states, with trivalent chromium and hexavalent chromium species being the most common forms (Kotas and Stasicka, 2000). The occupational exposure of chromium has been widespread and it is shown that chromium (III) under certain ligand conditions in environments leads to cell death and structural modification of proteins (Balamurugan *et al*., 2002). Removal of the chromium present in the wastewater is necessary for human and environmental causes.

In the past few decades, many researches have been carried out to recovery or remove chromium from the tannery wastewater using stone cutting solid waste (Al-Jabari *et al*., 2012), bone charcoal (Dahbi *et al*., 2002), natural marl (Jabari *et al*., 2009); but most of the techniques approached by treating mixed tannery wastewater. In this study, an approached was made to remove chromium from the chrome tanning wastewater using natural adsorbent. The use of low cost natural products to remove chromium could be preferable instead of conventional methods.

MATERIALS AND METHODS

Sample Collection

The chromium containing wastewater was collected from the SAF Leather Industries Ltd., Jessore, Bangladesh. Just after the chrome tanning operation, the wastewater was collected in polyethylene container which was pre-washed with diluted nitric acid, and immediately transported to the laboratory for experimentation. Abundantly available plant seed husk was collected from local area.

Materials

The reagents perchloric acid (Merck, India), sulphuric acid (Merck KGaA, Germany), nitric acid (Merck KGaA, Germany), ammonium ferrous sulphate (Merck, India), *N*-phenylanthranilic (Loba Chemie, India), glass bed (Loba Chemie, India), and filter paper (Whatman No. 1) were purchased from a local scientific store.

Characterization of Wastewater

The wastewater was characterized by analysing it for chromium following Society of Leather Technologists and Chemists (1996) official method of analysis (SLC 208). The analysis was done in triplicates, and the mean was used for calculations. pH meter (UPH-314, USA) was used to measure the pH of the wastewater. Before measuring the pH, the meter was calibrated with standard solutions.

Adsorbent Preparation

The plant seed husks were sun dried and stored at room temperature. After drying the samples were burnt at 450-550°C and grinded to make powder using a mortar. After shaking on a sieve, the adsorbent with the required size was obtained.

Adsorption Experiments

The given amount of prepared charcoal was directly mixed with a fixed volume of chrome tanning wastewater. The samples were carefully stirred over a fixed time period, then filtered and the remaining chromium was determined in the solution was determined by following Society of Leather Technologists and Chemists (1996) official method of analysis (SLC 208).

Batch Adsorption Experiments

Various parameters such as adsorbent dose and contact time were optimized in batch-wise technique. The optimized conditions were established by investigating the chromium removal efficiency.

Effect of the Dose of Adsorbent

The chromium removal percentage of the adsorbent is increased as the dose of the adsorbent is increased because of the increase in surface area and metal binding sites. To optimize the dose of adsorbent, varying adsorbent doses were used for each batch 1, 2, 3, 4, 5, 6, and 7 g, where other parameters were left unchanged, such as contact time (15 min) and initial pH (4.0).

Effect of Contact Time

Chromium removal percentage is raised with increasing contact time. When the contact time of the chromium ions and binding sites is extended, the adsorption becomes more effective. To determine optimal contact time, varying stirring time were maintained for each batch 5, 10, 15, 20, 25, and 30 min respectively, while the other parameters remained constant adsorbent (5 g) and initial pH (4.0).

RESULTS AND DISCUSSION

Optimal Adsorbent

The dose of adsorbent is the most important parameter in the treatment process. The effect of adsorbent dose on the removal efficacy of chromium from wastewater is represented in Fig. 1.

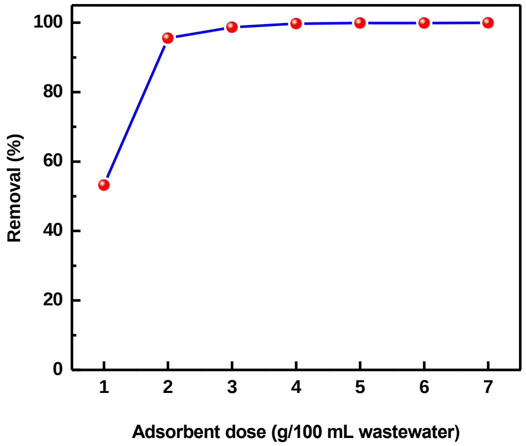

Figure 1. Effect of the dose of adsorbent on the removal of chromium

The figure clearly indicates that the dose of adsorbent has a significant effect on the removal of chromium. It was perceived that with increasing adsorbent dose, the removal percentage of chromium was increased gradually to 5 g per 100 mL wastewater and subsequently with increasing adsorbent dose removal percentage of chromium was increased slightly. The concentration of chromium for the adsorbent dose of 5 g/100 mL wastewater was 1.9 mg/L; for a dose of 7 g/100 mL wastewater was 1.4 mg/L. Therefore, 5 g adsorbent for every 100 mL wastewater was determined to be the optimal adsorbent dose.

It was perceived that the addition of adsorbent dose increased the pH of wastewater and adsorbent mixture. As Fig. 2 shows, with the increase of adsorbent dose, there was a gradual increase of the final pH.

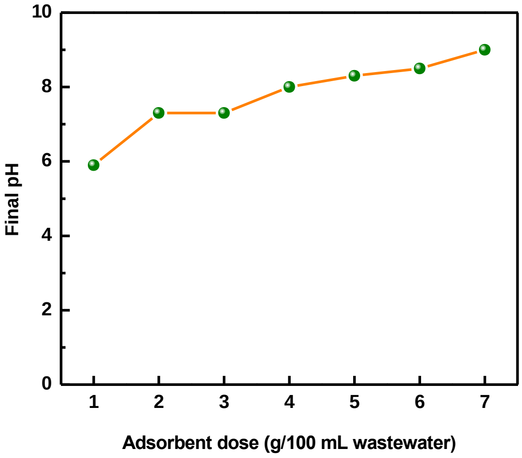

Figure 2. Change of final pH with different adsorbent dose

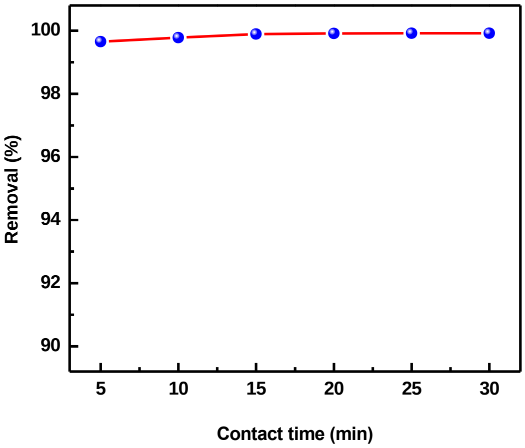

Figure 3. Effect of contact time on the removal of chromium

Optimal Contact Time

Chromium removal efficiency was observed at regular time intervals to determine the optimal contact time. As Fig. 3 shows, with the lapse of time, there was a gradual increase of the chromium removal percentage; at 15 min, the chromium removal efficiency was at its maximum (99.9%), after which the removal percentage of chromium was increased slightly.

Efficiency of the Treatment Process

The results of the treatment process with optimum conditions are represented in Table 1. The optimized treatment conditions were: adsorbent dose 5 g/100 mL wastewater and contact time 15 min. The initial concentration of chrome tanning wastewater was 3581.1 mg/L and after treatment at optimised conditions concentration was 1.9 mg/L. Therefore, it could be concluded that the maximum removal of chromium was attained 99.9%.

Table 1. Removal of chromium from the wastewater and compare with permissible limit

Parameter	Before treatment	After treatment	Permissible limit (MoEF*, 1997)
Chromium (mg/L)	3581.1	1.9	2.0
pH	4.0	8.3	6.0–9.0

*MoEF – Ministry of Environment & Forests

CONCLUSION

In this study, the most hazardous chrome tanning wastewater of tannery was treated with indigenous adsorbent. The results show excellent chrome removal efficiency (99.9%) at optimized conditions. The optimized treatment conditions were: adsorbent dose 5 g/100 mL wastewater and contact time 15 min. Therefore, the use of low cost indigenous adsorbent for the removal of chromium is could be better option.

REFERENCES

Al-Jabari, M., Abdulfailat, M. and Shaleen, S. (2012), “Treating Leather Tanning Wastewater with Stone Cutting Solid Waste”, *Clean – Soil, Air, Water*, 40(2), 206–210.

Avindhan, R., Madhan, B., Rao, J.R., Nair, B.U. and Amasami T. (2004), “Bioaccumulation of chromium from tannery wastewater: an approach for chrome recovery and reuse”, *Environmental Science and Technology*, 38, 300–306.

Balamurugan, K., Rajaram, R., Ramasami, T. and Narayanan, S. (2002), “Chromium(III) induced apoptosis of lymphocytes: death decision by ROS and Src-family tyrosine kinases”, *Free Radical Biology and Medicine*, 33, 1622–1640.

Dahbi, S., Azzi, M., Saib, N., de la Guardia, M., Faure, R. and Durand, R. (2002), “Removal of trivalent chromium from tannery waste waters using bone charcoal”, *Analytical and Bioanalytical Chemistry*, 374, 540–546.

Fabiani, C., Ruscio, F., Spadoni, M. and Pizzichini, M. (1997), “Chromium(III) salts recovery process from tannery wastewaters”, *Desalination*, 108, 183–191.

Hashem, M.A, Islam, A., Mohsin, S. and Nur-A-Tomal, M.S. (2015), “Green environment suffers by discharging of high-chromium containing wastewater from the tanneries at Hazaribagh, Bangladesh”, *Sustainable Water Resources Management*, 1, 343–347.

Jabari, M., Aqra, F., Shahin, S. and Khatib, A. (2009), “The treatment of chromium tanning wastewater using natural marl”, *Chemical Speciation & Bioavailability*, 21(3), 185-191.

Kotas, J. and Stasicka, Z. (2000), “Chromium occurrence in the environment and methods of its speciation”, *Environmental Pollution*, 107, 263–283.

Ministry of Environment and Forests (MoEF) (1997), *Environment Conservation Rules*, Government of the People's Republic of Bangladesh.

Society of Leather Technologist and Chemists (1996), *Official Methods of Analysis*, Northampton, UK.

Suresh, V., Kanthimathi, M., Thanikaivelan, P., Rao, J.R. and Nair, B.U. (2001), "An improved product-process for cleaner chrome tanning in leather processing", *Journal of Cleaner Production*, 9, 483–491.

HEAVY METALS REMOVAL FROM CONTAMINATED WATER USING POLY(ACRYLAMIDE-CO-ACRYLIC ACID)-SODIUM ALGINATE FLOCCULANT OBTAINED BY ELECTRON BEAM IRRADIATION

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The goal of the paper is to present the obtaining, characterization and testing of a new type of polyelectrolyte based on acrylamide, acrylic acid and sodium alginate for flocculation purposes. Polyelectrolytes, also called flocculants, were obtained by electron beam irradiation in atmospheric conditions and at room temperature. They have been characterized using various physical and chemical methods in order to determine conversion coefficient, residual monomer content, intrinsic viscosity and molecular weight. The heavy metals removal (Cu^{2+} and Cr^{6+}) was evaluated at room temperature on synthetic water having the contents of 0.05 wt/vol % $CuSO_4$ and 0.05 wt/vol % $K_2Cr_2O_7$, respectively.

Keywords: flocculants, heavy metals, electron beam

INTRODUCTION

Over the past decades, increasing effort has been concentrated on developing various methods that can effectively remove heavy metal ions from aqueous environments. There are many methods for removing heavy metals that include chemical precipitation adsorbents (Meunier *et al*., 2006), membrane filtration adsorbents (Bessbousse *et al*., 2008), ion exchange adsorbents (Alyuz and Veli, 2009), liquid extraction adsorbents (Sprynskyy, 2009), reverse osmosis adsorbents (Liu *et al*., 2008), activated carbon adsorption adsorbents (Ucer *et al*., 2006) and the use of biopolymer adsorbents (Guclu *et al*., 2003). The removal can be accomplished by gravitation (very slow), by coagulation (dependent on electric charge situation) and by flocculation (not dependent on electric charges and the fastest) (Brostow *et al*. 2009). Coagulation is the phenomenon in which the system consisting of colloidal particles from water is destabilized. Flocculation is the phenomenon in which destabilized colloidal particles join together in larger agglomerations. It is caused by the addition of small quantities of chemicals known as flocculants and the effectiveness is manifested especially in the situations where colloidal particles are already destabilized. Flocculants are of two types, i.e., inorganic and organic. The organic flocculants are essentially polymeric in nature. Both, synthetic and natural water soluble polymers are used as flocculants. The synthetic polymers are mostly linear and water soluble, such as polyacrylamide, polyacrylic acid, poly(diallyl dimethyl ammonium chloride) or poly(styrene sulphonic acid). They are available in all the three forms, i.e., cationic, anionic and non-anionic. High molecular weight synthetic polymers like polyacrylamides are very effective flocculating agents. However these polymers are quite unstable in shear fields and hence lose their flocculation effectiveness. The natural polymers like polysaccharides exhibit good resistance to shear degradation, but they are less effective flocculating agents compared with synthetic polymers and their aqueous solutions are also susceptible towards biodegradation (Tripathy and De, 2007).

The goal of the paper is to present the obtaining, characterization and testing of a new type of flocculant based on acrylamide, acrylic acid and sodium alginate for flocculation purposes. In the present study, the alginate - one of the most nontoxic sulphated polysaccharides - has been chosen as a backbone. The flocculants were obtained by electron beam irradiation (EB) using doses in the range of 2 kGy to 10 kGy in atmospheric conditions and at room temperature. They were characterized by various physical and chemical methods in order to determine conversion coefficient, residual monomer content, intrinsic viscosity, molecular weight and radius of gyration. The heavy metals (Cu^{2+} and Cr^{6+}) removal efficacy was evaluated at room temperature.

EXPERIMENTAL

Materials

In order to obtain the flocculants, the following materials have been used: acrylamide - AMD (molar mass of 71.08 g mol^{-1}; density of 1.13 g/cm^3); acrylic acid - AA (molar mass of 72.06 g mol^{-1}; density of 1.051 g/mL), sodium alginate - SA (molar mass of 216.12 g mol^{-1}) and potassium persulfate (molar mass of 270.322 g mol^{-1}; density of 2.477 g/cm^3) - used as initiator in the copolymerization process. All materials were from E-Merck, Germany.

Preparation and Irradiation of the Samples

Two different types of aqueous solutions based on acrylamide, acrylic acid and sodium alginate for the irradiation experiments were prepared: (a) the first type based on acrylamide (20 wt/vol %, acrylic acid 20 vol/vol % and sodium alginate 2 wt/vol %) noted POL-I and (b) the second type on acrylamide (20 wt/vol %, acrylic acid 20 vol/vol %, sodium alginate 2 wt/vol % and sodium persulphate 0.2 wt/vol %) noted POL-II. Each mixture was stirred at 250 rpm at room temperature for 30 min. 5 ml from each type of solution was then transferred into 50 cm^3 glass tubes and irradiated using electron beam with various doses in atmospheric conditions and at room temperature of 25°C. Electron beam dose rate was fixed at 2 kGy/min in order to accumulate irradiation doses between 2-10 kGy.

Experimental Installation and Sample Irradiation

Experiments were carried out with an experimental installation consisting mainly of the following units: an electron linear accelerator (ALIN-10) of 6.23 MeV energy and 75 mA peak current of the electron beam and an irradiation chamber containing the samples of monomer solution. The optimum values of the EB peak current I_{EB} and EB energy E_{EB} to produce maximum output power P_{EB} for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: E_{EB} = 6.23 MeV, I_{EB} =75 mA, P_{EB} = 164 W (f_{EB} = 100 Hz, τ_{EB} = 3.5 μs). The EB effects are related to the absorbed dose (D) expressed in Gray or J kg^{-1} and absorbed dose rate (D*) expressed in Gy s^{-1} or J kg^{-1} s^{-1}.

Sample Characterization (Physical and Chemical Characteristics)

In order to determine the conversion coefficient (CC) and the residual monomer concentration (M_r), 2 grams of polymer (flocculant) were placed in 200 ml distilled water for 24 hours and then every sample was stirred for 1 hour at 400 rpm to ensure a very well mixing. CC and M_r were determined by titrimetric method. The intrinsic viscosity ($\eta_{intr.}$)

and the Huggin's constant (k_H) were determined by the viscosity measurements, using the Hoppler type BH-2. Hoppler viscometer is a falling ball one. The measured parameter is the time of fall of the ball in a cylindrical tube inclined by 10 deg with respect to the vertical plane and filled with the liquid subjected to investigation. The principle of falling-ball viscosimeters is based on the fact that the viscosity of liquid modifies the speed of the ball falling through this liquid. The time of fall of the ball through the polymeric solution was measured for five different concentrations. As a solvent, the sodium nitrate 1N ($NaNO_3$) was used and the working temperature was of 30°C. Using the time of fall of the ball through the polymeric solution (t) and the time of fall of the ball through the solvent (t_0) the relative viscosity was obtained:

$$\eta_{rel.} = t / t_0 \tag{1}$$

Specific viscosity was calculated from the relation:

$$\eta_{sp.} = (\eta_{rel.} - 1) \tag{2}$$

Reduced viscosity was determined using the relation:

$$\eta_{red.} = \eta_{sp.} / c \tag{3}$$

where c is the polymer concentration (g/dL).

From the graphical representation of the η_{red} as a function of the copolymer concentration, by extrapolation, was obtained the intrinsic viscosity η_{intr} and tgα. Linearity constant was determined from the following relation:

$$k_H = tg\alpha / [\eta_{intr.}]^2 \tag{4}$$

Intrinsic viscosity is a measure of the hydrodynamic volume occupied by the individual polymer molecules in isolation (Richardson and Kasapis, 1998). In dilute solutions, the polymer chains are separate and the [η_{intr}] of a polymer in solution depends only on the dimensions of the polymer chain. From the Mark-Houwink equation, the relationship among the molecular weight and viscosity is given below:

$$[\eta] = K \times M_w^a \tag{5}$$

where [η] is the intrinsic viscosity (dl/g), M_w is viscosity average molecular weight, K and a are constants for a particular polymer – solvent system (K and a are $3.73*10^{-4}$ and 0.66 in 1N aqueous sodium nitrate at 30°C) (Richardson and Kasapis, 1998; Zeynali and Rabbii, 2002).

Flocculation Studies

Water samples containing Cu^{2+} and Cr^{6+} were synthesized using $CuSO_4*5H_2O$ (0.1 mol/l), $K_2Cr_2O_7$ (0.05 wt/vol %), kaoline (0.2 wt/vol %) and distilled water. The jar test apparatus (Velp FC 6S, Italy) consists of 6 stirrer blades connected to a variable speed (0-100 rpm) motor through a gear system. In each beaker, 500-mL of synthetic water (with Cu^{2+} or Cr^{6+}) was taken and placed on the flocculator. Under a slow stirring condition, the polymer solution was added by means of a pipette in order to determine the polymer concentration influence (5-20 ppm). Immediately after the addition of polymer solution, the suspensions were stirred at a constant speed of 60 rpm for 15 min, and than allowed to sediment for 15 min. Clear supernatant was drawn from the top layer (up to depth 1-2 cm) and its absorbance at 275 nm for Cu^{2+} (Wen *et al.*, 2013) and 540 nm for Cr^{6+} (Iva and Zeiner, 2008) was measured using the Cary Bio-100 UV-VIS spectrophotometer. The heavy metals (Cu^{2+} and Cr^{6+}) removal was evaluated from the calibration curves. For dilute solutions, there is a linear relationship between absorbance and concentration.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics

In Figures 1-5 are represented the conversion coefficient (CC), the residual monomer concentration (M_r), the intrinsic viscosity ($\eta_{intr.}$), the linearity constant (k_H) and the molecular weight (M_w) depending on the absorbed dose, for all the 10 samples subjected to electron beam irradiation.

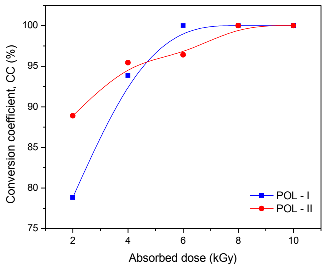


Figure 1. Conversion coefficient of the flocculants versus EB absorbed dose

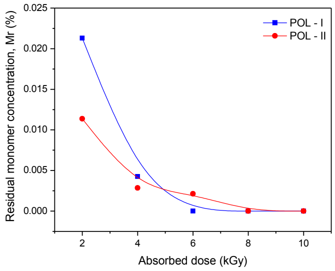


Figure 2. Residual monomer concentration of the flocculants versus EB absorbed dose

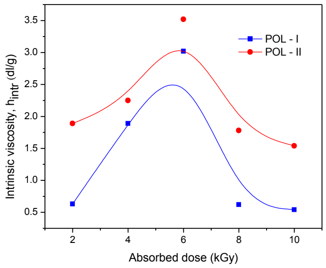


Figure 3. Intrinsic viscosity of the flocculants versus EB absorbed dose

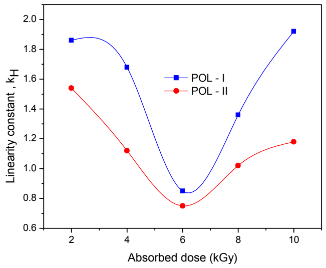


Figure 4. Linearity constant of the flocculants versus EB absorbed dose

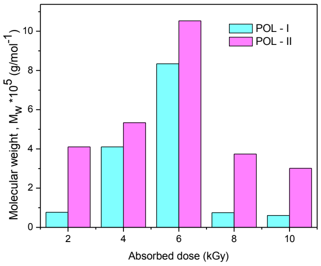
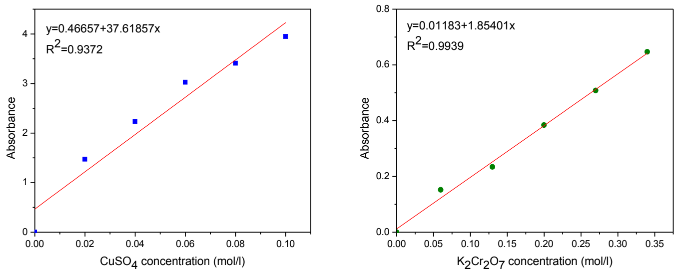


Figure 5. Molecular weight of the flocculants versus EB absorbed dose

The solutions subjected to EB irradiation have lead to the obtaining of polymers having relatively high conversion coefficients and molecular weights, especially at the upper limit of the used dose range (2 kGy - 10 kGy). Also, all the samples have presented low residual monomer in the polymer (<0.05%, which is the limit imposed by the standards of use of AMD as a flocculant) and a good linearity. It was found that the CC has increased with increasing of absorbed dose. The probability of molecular contact became higher with increasing of the absorbed dose, more polymer molecules react, resulting in the propagation of the active chain and the continuously CC increasing. Intrinsic viscosity of a polymer is indicative of its hydrodynamic volume in solution, which depends on its molecular weight, structure and nature of the solvent as well as the temperature of the medium. For two polymers with approximately similar molecular weights, the branched polymer has lower hydrodynamic volume compared to its linear counterpart and thus has lower intrinsic viscosity value. Furthermore, long branches determine higher intrinsic viscosity and vice versa (Xie *et al.*, 2009).

Flocculation Results

By UV–VIS spectrophotometry it is possible to easily determine the concentration of different heavy metals from water samples. The disadvantage is the relatively low linear working range, as can be observed in Figure 6. Two types of samples have been tested for Cu^{2+} and Cr^{6+} removal: POL – I and POL – II, both obtained at 6 kGy. We chose to use these samples, because all physical properties have maximum values. The results are presented in Figure 7. The Cu^{2+} and Cr^{6+} removal from synthetic water was done by determining the concentration of both metals from water using the spectrophotometric method and calibration curves.



(a) (b)

Figure 6. Calibration curve for (a) Cu^{2+} and (b) Cr^{6+}

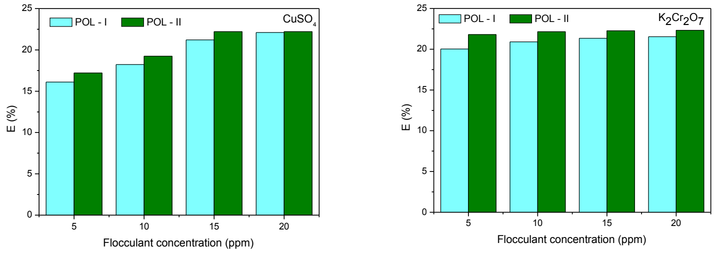


Figure 7. Heavy metals removal efficiency as a function of flocculant concentration

The heavy metals removal performance is presented in terms of “efficiency” calculated as follows:

$$E(\%)=\frac{C_0-C_1}{C_0}\times 100 \tag{6}$$

where C_0 is the initial heavy metals concentration (Cu^{2+} and Cr^{6+}) and C_1 is the heavy metals concentration after flocculation.

Four concentrations from each flocculant were used, obtained from stock solutions of 0.1%, 5, 10, 15, and 20 ppm, respectively. In Figure 7 the heavy metals removal efficiency is represented as a function of the flocculant concentration and it is observed that the flocculation efficiency has increased with the concentration of flocculant.

CONCLUSIONS

This study was carried out to illustrate the synthesis of poly[acrylamide-co-acrylic acid]-sodium alginate flocculants in presence and absence of initiator under the effect of electron beam irradiation. For the samples obtained this way it was found that the conversion coefficient (CC) and residual monomer concentration M_r shows a linear dependence on radiation dose, while the intrinsic viscosity ($\eta_{intr.}$), the linearity constant (k_H) and the molecular weight (M_w) shows good values only in the middle of dose range. From the flocculation studies, in order to determine the heavy metals removal efficiency, it is observed that the flocculation efficiency has increased with the concentration of flocculant and also that the best results correspond with the use of flocculants obtained in the presence of initiators.

REFERENCES

Alyuz, B. and Veli, S. (2009), “Kinetics and equilibrium studies for the removal of nickel and zinc from aqueous solutions by ion exchange resins”, *Journal of Hazardous Materials*, 167(1-3), 482-488.

Bessbousse, H., Rhlalou, T., Verchere, J.F. and Lebrun, L. (2008), “Removal of heavy metal ions from aqueous solutions by filtration with a novel complexing membrane containing poly(ethyleneimine) in a poly(vinyl alcohol) matrix”, *Journal of Membrane Science*, 307(2), 249-259.

Brostow, W., Lobland, E.H.E., Pal, S. and Singh, R.P. (2009), “Polymeric flocculants for wastewater and industrial effluent treatment”, *Journal of Materials Education*, 31, 157-166.

Guclu, G., Gurdag, G. and Ozgumus, S. (2003), “Competitive removal of heavy metal ions by cellulose graft copolymers”, *Journal of Applied Polymer Science*, 90(8), 2034-2039.

Iva, R. and Zeiner, M. (2008), “Determination of extractable chromium from leather”, *Monatshefte fuer Chemie*, DOI 10.1007/s00706-008-0026-1.

Liu, F.N., Zhang, G.L., Meng, Q. and Zhang, H.Z. (2008), “Performance of nanofiltration and reverse osmosis membranes in metal effluent treatment”, *Chinese Journal of Chemical Engineering*, 16(3), 441-445.

Meunier, N., Drogui, P., Montane, C., Hausler, R., Mercier, G. and Blais, J.F. (2006), “Comparison between electrocoagulation and chemical precipitation for metals removal from acidic soil leachate”, *Journal of Hazardous Materials*, 137(1), 581-590.

Richardson, R.K. and Kasapis, S. (1998), “Rheological methods in the characterization of food biopolymers”, *Developments in Food Science*, 39, 1-48.

Shaoping, Y., Shiyu, F., Hao, L., Yiming, Z. and Xueyun, L. (2011), “Hydrogel Beads Based on Carboxymethyl Cellulose for Removal Heavy Metal Ions”, *Journal of Applied Polymer Science*, 119, 1204–1210.

Sprynskyy, M. (2009), “Solid-liquid-solid extraction of heavy metals (Cr, Cu, Cd, Ni and Pb) in aqueous systems of zeolite-sewage sludge”, *Journal of Hazardous Materials*, 167(2-3), 1377-1383.

Tripathy, T. and De, B.R. (2007), “Biodegradable Flocculants Based on Partially Hydrolysed Starch-g-Polyacrylamide”, *Journal of Physical Sciences*, 11, 139-146.

Ucer, A., Uyanik, A. and Aygun, S.F. (2006), “Adsorption of Cu(II), Cd(II), Zn(II), Mn(II) and Fe(III) ions by tannic acid immobilised activated carbon”, *Separation and Purification Technology*, 47(3), 113-118.

Xie, C., Feng, Y., Cao, W., Teng, H., Li, J. and Lu, Z. (2009), “Novel Biodegradable Flocculating Agents Prepared by Grafting Polyacrylamide to Konjac”, *Journal of Applied Polymer Science*, 111, 2527-2536.

Zeynali, M.E. and Rabbii, A. (2002), “Alkaline Hydrolysis of Polyacrylamide and Study on Poly(acrylamide-co-sodium acrylate) Properties”, *Iranian Polymer Journal*, 11, 269-275.

Wen, T., Qu, F., Li, N.B. and Luo, H.Q. (2013), “A facile, sensitive, and rapid spectrophotometric method for copper(II) ion detection in aqueous media using polyethyleneimine”, *Arabian Journal of Chemistry*, http://dx.doi.org/10.1016/j.arabjc.2013.06.013.

IMPROVING OF LEATHER LIQUID FINISHING THROUGH USAGE OF POLYMERIC COMPOUNDS

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Nowadays, leather industry is suffering from low quality of raw materials, usage of efficient and environment unfriendly materials. This situation may be corrected by upgrading the existing or developing new technologies using efficient chemical materials, such as polymeric compounds. The aim of the study was to improve the leather dyeing processes through usage of water-soluble polymers derivatives of acrylic and maleic acids in the direction of simplification and reduction of processing materials consumption, ensuring high product quality, establishing the mechanism of interaction between these materials and collagen. The reactions in the "collagen-polymeric compounds-dye" system were studied using IR-spectroscopy. The fiber preparation from the untanned leather industry waste was used for the collagen prototype. The data obtained show correlation between interaction type in the system under study as well as leather properties and processing conditions. We have established a positive effect of investigated polymers on the physical, mechanical, and hygienic leather properties. It can be explained through their ability to be absorbed on the surface of the derma structural elements; to interact with them and other applied reagents (tannins, dyes etc.), there by promoting structure formation and improving product quality.

Keywords: liquid finishing of leather, polymeric compounds, IR-spectroscopy.

INTRODUCTION

While conducting finishing processes, leather is given some elastic-plastic, hygienic, aesthetic qualities as well as colour. A wide range of chemicals is used for this purpose. Thus, in practice, from 50 to 100 dyes of different types are needed in order to dye leather, mostly they are acid, basic, metal complex, active and sulphur-containing ones. The choice of dyes depends on products assortment and fashion trends.

Majority of synthetic dyes, intended for dyeing natural leather, are practically non-biodegradable due to their complex structure and xenobiotic features (Tamil Selvi *et al*., 2013). As a result, there is a need for leather dyeing technology modernization. This new technology must ensure better dyes absorption from work solutions, which means decrease of their quantity in factories sewages. This can be achieved either by creating new types of dyes of biological origin, which are capable of quick biodegradation, or by improvement of existing technologies, using effective chemical materials. For example, the paper by Tamil Selvi *et al*. (2013) is dedicated to research on the possibility of applying the dye, extracted from the seeds of *Bixa orellana*, to the leather dyeing process. In the paper by Sudha *et al*. (2016) the dye of microbiological origin with *Penicillium minioluteum* has been studied. This dye has been used for dyeing a wet-blue semi-finished product made of goatskin. The authors have discovered that at the high level of abrasion resistance and sweat finished leather has low resistance to light. We should mention that synthesis and industrial implementation of new types of dyes mean considerable money, time and material investment. For this reason researches (Haroun and Mansour, 2008; Danylkovych *et al*., 2016) are being done, dealing with the use of chemicals which can influence the colour and aesthetic qualities of finished leather positively without damaging its consumer properties in liquid finishing processes. To understand the interaction between collagen of derma, materials used and dyes better various analytical methods are used, such as the method of Fourier infrared spectroscopy (Sudha *et al*., 2016; Pellegrini *et al*., 2015; Campos Vidal and Mello, 2011; Sukhodub *et al*., 2004; Kanagaraj *et al*., 2015).

In this paper we have studied the influence of water-soluble polymeric compounds (derivatives of maleic and acrylic acids) on the liquid finishing and finished leather qualities.

EXPERIMENT

Materials Used

In this research we have used chemical materials common for leather industry: *savenol NWP* (6-00-00205601-37-92, Ukraine) – gel-like colourless liquid, surface-active neoionogenic substance, which includes, mass content, %: polyethyleneglycol – 5, water- 45, nylon AF 9-10 – 50, *anionic acid violet C dye* (*D*); *OLEAL 145* – anionic fat material manufactured by CODYECO S.p.A (Italy), which is an orange-brown transparent liquid with 66.0±3% active material content; *quebracho extract* – vegetative tanning extract, which is a red-brown hygroscopic cold water-soluble powder with 85% tannin content. Polymeric compounds based on maleic and acrylic acids, a collagen-containing preparation and a leather semi-finished product were also used. Their properties are described below.

Polymeric Compounds

In the research we have used polymeric compounds based on maleic (Kro) and acrylic (TP, CP) acids (Lukianets and Andreyeva, 2009). These are liquids, whose main properties are stated in table 1.

Table 1. Characteristics and main properties of polymeric compounds (Lukianets and Andreyeva, 2009)

Index	Polymeric compounds		
	*Kro**	*TP*	*CP*
Dry residue, %	21,5	13,5	32,5
Density, g/cm^3	1,078	1,060	1,158
Superficial tension of 10% solution, dyn/cm	54,1	76,6	76,9
Relative viscosity	2,00	13,33	78,00

Note: * good stability to light

Collagen-containing Preparation

A Ukrainian protein preparation has been used as a collagen model for Fourier infrared spectroscopy tests. This preparation is extracted from untanned collagen-containing waste products of leather industry from cattle raw materials. In terms of its properties and amino acid composition it is identical to derma collagen (Tehza and Andreeva, 2011; Andreyeva and Maistrenko, 2016). The following are properties of the collagen preparation, mass content % (in terms of completely dry substance): hide substance – 90.1±0.4; mineral substances – 3.9±0.2; substances, extracted with organic solvents – 1.0±0.3.

Leather Semi-Finished Item (Wet-Blue)

During the experiment we have used a leather semi-finished product of chrome tanning method, obtained from cattle skin (medium cowhide). The properties of this semi-finished product are given in table 2.

Table 2. Original wet-blue properties

Index	Value
Shrinkage temperature, °C	123±1,0
Thickness, mm	2.2±0,1
Mass content, %:	
– humidity	33.90±0.13
– chromium oxide*	5.75±0.12
– hide substance*	91.58±0.30
– mineral substances*	6.54±0.34
– substances, extracted with organic solvents *	0.71±0.12

Note: * the data are given for completely dry substance.

Research Techniques

In the research we have used both traditional for leather industry techniques and more modern and accomplished analytical ones: microscopy, infrared spectroscopy, statistical processing of experimental data.

To get statistically significant results, all measurements were carried out no less than 3-5 times. Control studies were performed simultaneously with the data correction process.

Chemical Analysis and Physico-Mechanical Tests

Chemical and physico-mechanical indices have been identified with the help of basic techniques: moisture content ISO 4684:2005, mineral substances content ISO 5397:1984, hide substance content ISO 5397:1984, chromium oxide content ISO 5398-1:2007, content of substances extracted with organic solvents ISO 4048:2008, shrinkage temperature ISO 3380:2015, determination of tensile strength and percentage extension ISO 3376:2011, strength of surface ISO 3379:2015, vapour permeability ISO 14268:2012, determination of bending force ISO 14087:2011, determination of abrasion resistance ISO 17076-1:2012, test for adhesion of finish ISO 11644:2009, determination of surface coating thickness ISO 17186:2011, determination of water absorption by capillary action (wicking) ISO 19074:2015.

Infrared Spectroscopy Analysis Technique

Infrared spectroscopy analysis technique has been used to identify optical density of seepage zones and chromed collagen preparation active groups before and after processing with polymeric compounds and dye (spectrophotometer TENSOR 37 (Brucer, Germany) (Stuart, 1997; Jie *et al.*, 2014; Sarver and Krueger, 1991; Maistrenko and Andreyeva, 2011). Samples of the collagen preparation (after processing with chrome tanning agent in the quantity of 10%) have been processed with polymeric compounds and dye in glass vessels, using the installation for agitation, under the following conditions: water consumption was 500%, temperature was 20°C, continuation was one hour and a half, polymeric compounds and dye consumption –

10% of the samples mass. Later the original and processed samples of the preparation were fined, pressed into a pill containing potassium bromide and sent to infrared spectroscopy test.

Microscopical Analysis Technique

To identify the depth of derma staining by dye we have used an optical microscope with 40-1000 times magnification Bresser Researcher Bino (Bresser, Germany).

RESULTS AND DISCUSSION

Infrared Spectroscopy Tests of the System "Chromed Collagen-containing Preparation – Polymeric Compounds – Dye"

On the infrared spectrogram of the chromed collagen preparation we can see the bands at the following frequencies the most distinctively: 3394, 1650, 1537, 1236 and 526 cm^{-1}, which correspond to oscillation of amides A, I, II, III, VI with various proportions of constituents; 1448, 1200, 1082, 1033 cm^{-}1 of hydroxyl groups; 602 cm^{-1} of chromium compounds. Presence of different active groups (carboxyl, hydroxyl) in the structure of polymeric compounds shows ability of these materials to interact with active groups of collagen (Maistrenko and Andreyeva, 2011) (Figure 1).

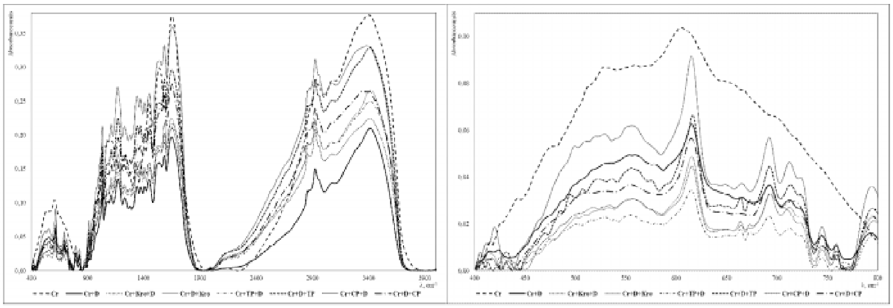

Figure 1. IR-spectrums of collagen-containing preparation after treatment by polymeric compounds and dye

Peaks intensity at the spectrograms of chromed collagen preparation (*Cr*), chromed preparation after processing with dye (*Cr+D*), polymeric compound and dye (*Cr+Kro+D, Cr+TP+D, Cr+CP+D)* or dye and polymeric compound (*Cr+D+Kro, Cr+D+TP, Cr+D+CP*) is caused by interaction between polymeric compounds and nitrogen-containing and hydroxyl collagen groups, as well as chromium complexes. Peaks intensity decrease and change of their pattern in the chromed collagen preparation at low frequencies can probably be explained by interaction between the dye and the polymeric compounds with creation of bonds of different types (hydrogenic, ionic etc.)

Wet-Blue Semi-Finished Product Processing

In order to simplify technological scheme and decrease materials consumption of the liquid finishing processes the use of polymeric compounds at the dyeing stage has been researched. Semi-finished product processing scheme has been chosen with the help of typical chromed leather production of upper of shoes made of cattle raw material: washing – neutralization – washing – dyeing – fatting – retanning with quebracho tannins. Tannins consumption in the control group was 4%, while in groups under study it was half as much as that (2%) due to polymeric compounds use. Polymeric compounds, which constituted 2% of shaved wet-bleu mass (in terms of dry residue), were added at both at the beginning (*b.d.*) and at the end of dyeing process (*a.d.*).

There were no complications during the processing of the groups under test. The leathers obtained were soft to the touch, filled (thickness output at 85-100%), heatproof. Even visually both facing and flesh surface looked bright and saturated.

Polymeric compounds influence on finished leather properties has been tested after coating with acryl. As compared to control group, tensile and surface strength of investigated leathers has increased by 1.1-1.5 times, percentage extension at the 10 MPa has gone up by 1.2-1.8 times, porosity – by 1.4-1.5 times, water absorption by capillary action – by 1.1-1.3 times, adhesion of finish to dry leather – by 1.4-2.2 times (table 3). Indices of bending force and abrasion resistance of dry friction of the coating, as well as those adhesions of finish to the wet leather are mostly the same as corresponding indices of the control group.

Table 3. Chemical and physico-mechanical properties of finished leather products

Index	Group						
	1	2	3	4	5	6	7
Polymer type	Kro b.d.	Kro a.d.	TP b.d.	TP a.d.	CP b.d.	CP a.d.	control
Mass content, %							
- humidity	14.1	14.2	13.4	14.6	16.2	13.3	14.1
- chromium oxide*	71.4	71.6	77.2	79.1	77.4	73.0	71.0
- hide substance*	4.3	4.9	4.1	4.8	4.8	4.1	4.9
- mineral substances*	6.3	5.6	5.8	5.2	5.9	5.9	5.2
- substances, extracted with organic solvents *	6.1	10.7	9.0	9.5	7.5	5.4	9.8
Tensile strength, MPa	19.8	12.7	13.1	18.7	11.1	14.1	11.8
Strength of surface, MPa	17.5	12.0	12.2	14.1	10.9	12.9	11.8
Percentage extension at 10 MPa, %	42.5	52.5	64.5	43.0	80.5	53.5	35.0
Porosity, %	54.3	53.1	54.4	56.0	53.2	56.8	38.5
Water absorption by capillary action (wicking), min	333	290	303	342	253	316	256
Water vapour permeability, $mg/cm^2 \cdot h$	3.3	2.9	3.1	3.3	2.8	4.1	4.0
Bending force, points	4	4	4	4	4	4	4
Abrasion resistance, terns							
- dry friction	>500	>500	>500	>500	365	>500	>500
- wet friction	98	136	82	57	61	100	80
Adhesion of finish, N/m							
- dry leather	574	545	349	360	493	488	257
- wet leather	129	133	130	110	225	127	126
Depth of derma staining by dye, %	50	40	40	40	80	25	40
Colouring uniformity, ΔE	0.0	3.2	7.9	1.3	2.5	2.3	9.2
Surface coating thickness, g/dm^2	0.25	0.25	0.33	0.25	0.20	0.25	0.31
Yield of area, %	95.0	100.0	90.0	110.0	100.0	80.8	85.5

Thus, according to the results of this series of tests, we can note that application of polymeric compounds based on maleic acid before dyeing of chrome leathers is the most advisable. The reason for this is that such processing technique provides the whole range of necessary consumer properties of chrome leather (strength, softness, filling, shaping, bright and even colouring and quality coating, good hygienic properties).

CONCLUSION

In order to simplify the technological scheme of processing and to decrease materials consumption of the liquid finishing processes the use of chrome leather, processed with polymeric compounds, based on maleic and acrylic acids, before and after dyeing process has been researched. We have also identified efficient conditions of liquid finishing, which require a semi-finished product processing with polymeric compound *Kro* in the amount of 2% before dyeing at the temperature of 50 ^{o}C during 1.0-1.5 hours (water consumption 200%). Previous and further processing using typical procedure are also required. It has been proved by experiment that the usage of investigated polymers during liquid finishing improves both usability and cutting properties of chrome leather, such as quality of colouring and coating. At the same time, they allow us to use deficient and expensive material resources, such as leather raw materials and vegetable tanning agents, more effectively.

REFERENCES

Andreyeva, O. and Maistrenko, L. (2016), “Experimental Study of New Collagen-containing Preparations”, *American Chemical Science Journal*, 10(2), 1-6.

Campos Vidal, B. and Mello, M.L.S. (2011), “Collagen type I amide I band infrared spectroscopy”, *Micron*, 42(3), 283-289.

Danylkovych, A., Mokrousova, O. and Zhygotsky, A. (2016), “Improvement of the filling and plasticization processes of forming multifunctional leather materials”, *Eastern-European Journal of Enterprise Technologies*, 76, 23-31

Haroun, A.A. and Mansour, H.F. (2008), “New approaches for the reactive dyeing of the retanned carbohydrate crust leather”, *Dyes and Pigments*, 76(1), 213-219.

Jie, L., Xiaoyu, L., Wei, W., Wu, X., Jun, Z. and Zhu, Z. (2014), “Measurement of protein content in chestnuts using near infrared spectroscopy”, *Journal of Chemical and Pharmaceutical Research*, 6(6), 938-941.

Kanagaraj, J., Panda, R.C., Senthilvelan, T. and Gupta S. (2015), “Cleaner approach in leather dyeing using graft copolymer as high performance auxiliary: related kinetics and mechanism”, *Journal of Cleaner Production*, (112), 4863-4878.

Lukianets, L.A. and Andreyeva, O.A. (2009), “Investigation of characteristics of new polymeric materials for leather and fur production”, *Bulletin of the Khmelnitsky National University*, 4, 208–211. Ukrainian.

Maistrenko, L.A. and Andreyeva, O.A. (2011), “IR-spectroscopic studies of polymeric compounds of the new generation. Message 1”, *Bulletin of the Kherson National Technical University*, 4, 143–147. Ukrainian.

Pellegrini, D., Corsim, M., Bonanni, M., Bianchini, R., D'Ulivo, A. and Bramanti, E. (2015), “Study of the interaction between collagen and naturalized and commercial dyes by Fourier transform infrared spectroscopy and thermogravimetric analysis ”, *Dyes and Pigments*, 116, 65-73.

Sarver, R.W. and Krueger, W.C. (1991), “Protein secondary structure from Fourier transform infrared spectroscopy: A data base analysis”, *Analytical Biochemistry*, 194(1), 89-100.

Stuart, B. (1997), *Biological application of infrared spectroscopy*, University of Grinwich, Grinwich.

Sudha, Gupta, C. and Aggarwal, S. (2016), “Dyeing wet blue goat nappa skin with a microbial colorant obtained from Penicillium minioluteum”, *Journal of Cleaner Production*, 127, 585-590.

Sukhodub, L.F., Moseke, C., Sukhodub L.B., Sulkio-Cleff, B., Maleev, V.Ya., Semenov, M.A., Bereznyak, E.G. and Bolbukh T.V. (2004) “Collagen–hydroxyapatite–water interactions investigated by XRD, piezogravimetry, infrared and Raman spectroscopy”, *Journal of Molecular Structure*, 704, 53-58.

Tamil Selvi, A., Aravindhan, R., Madhan, B. and Raghava Rao, J. (2013), “Studies on the application of natural dye extract from Bixa orellana seeds for dyeing and finishing of leather”, *Industrial Crops and Products*, 43, 84-86.

Tehza, M.M. and Andreeva, O.A. (2011), “Investigation of collagen preparations”, *Bulletin of the Khmelnitsky National University*, 4, 128-132. Ukrainian.

MODIFICATION OF MIMOSA AND QUEBRACHO TANNINS AND THE LIGHTFASTNESS PROPERTIES OF THE PROCESSED LEATHERS

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Vegetable tannins give leathers some properties like natural look, firmness, toughness and characteristic colour of themselves. Besides these favourable properties, some of the tannins as mimosa and quebracho which belong to condensed tannins, have some undesirable properties like colour changing and darkening caused by exposure to light for prolonged times. This research aims to increase the lightfastness properties of mimosa and quebracho tanned leathers, by chemical modification of these tannins. For this aim sulphitation, sulpho methylation and novalac synthesis modifications were applied to mimosa and quebracho tannins. Comparative lightfastness test results showed that leathers tanned with sulpho methylated mimosa and quebracho tannins had better fastness to light. Additionally the physical properties of the leathers tanned with modified tannins were investigated to compare with the leathers tanned with standard tannins.

Keywords: vegetable tanning, mimosa, quebracho, lightfastness, leather

INTRODUCTION

Vegetable tanning materials are preferred in production of sole leathers, shoe uppers, upholstery leathers and leather goods because of the favourable natural character they give to leathers but also they are more or less sensitive to light and they have the disadvantage of colour changing by exposure to light with time.

According to Pizzi *et al*., (2004), the variation of leather colour as a function of ageing time on prolonged irradiation with UV light of the leather produced based on different vegetable tannins was found to be composed of two main effects: The first one of these is the darkening reaction of the leather. This is due to the formation of quinones on the phenolic structure of the vegetable tannin. The second one is the leather-lightening (fading) reaction due to the photo degradation of the system.

For Covington (2009), the ability of phenols to discolour depends on the formation of phenyl radicals by the loss of hydrogen to atmospheric oxygen. The free radical formation causes bond shifts and oxidative coupling, which means polymerisation: if this results in the creation or the linking of chromophore groups then colour is developed. In the case of the hydrolysable tannins, the chromophores, the benzene rings of the ester moieties, are not linkable because they are too far apart in the molecule: in this way they are resistant to reddening, referred to as lightfast. In the case of the condensed tannins, the proximity of the aromatic nuclei in the flavonoid structure means that the free radical oxidative bond rearrangements can take place easily. Therefore, these tannins redden, creating a rapid colour change on the leather surface.

In this research, sulphitation, sulpho methylation and novalac synthesis modifications were applied to mimosa and quebracho tannins to introduce UV stabile groups to their flavonoid structure to avoid free radical oxidative bond rearrangements. The modified tannins were used in leather production. Then their physical and lightfastness properties were measured and compared with the leathers tanned with standard mimosa and quebracho.

EXPERIMENTAL

Material

10 pickled whole domestic hides each weighing approximately 10 kg, at pH 2.5, with their thickness adjusted to 1.4mm were used as the raw material for vegetable tanning. Mimosa and quebracho tannins were used from the condensed tannins group due to their lower light fastness properties considering the pre trials, literature and studies of Ozgunay (2008), Pizzi et al, (2004), Frediani et al. (2008). Phenolsulphonic acid, urea, formaldehyde, sodium sulphide at analytic grade (purchased from Merck) and a commercial UV stabiliser containing Hindered Amine Light Stabilizers (HALS) (purchased from BASF) were used in modification experiments.

Method

Modification Procedure

In this research standard, sulphomethylated, novalac type modified, sulphitated mimosa and quebracho were encoded as M0, K0, M1, K1, M2, K2, M3 and K3 respectively. Modification procedures are given below.

Sulphomethylation

Sulphomethylation reaction occurs by the binding of sulphomethyl group ($-CH_2-SO_3R$), to the amine and amide compounds. This reaction has been widely used in urea and melamine based amino resin synthesis (Kasgoz, 1999).

In this modification 30% mimosa and quebracho tannins were modified with 8% sodium sulphide and 8% formaldehyde addition at 70 °C, based on pelt weight.

Novalac Synthesis

This modification process of mimosa and quebracho tannins was based on the process given by Covington (2009).

In this modification, 30% mimosa and quebracho tannins were modified with 20% phenol sulfonic acid, 1.5% urea and 5.5% formaldehyde based at 75 °C, based on pelt weight. Then the modification was completed by addition of 12.5% sodium sulphide.

Sulphitation

This modification procedure was based on the literature of Frediani et al., (2008). In the modification process the solvent free HALS compound was used. This compound is a dispersion that can be used in water based systems and prevents breaking, powdering and discolouring of coatings by preventing photo oxidation of binders.

In this modification 30% mimosa and quebracho tannins were modified with 12.5% Sodium sulphide based on tannin weight at 70 °C. Then the modification was completed by addition of 5% UVS-HALS.

Tanning Process

Before vegetable tannage the pH value of pickled pelts were adjusted to pH 5.5 by depickling process. The hides were tanned by using the vegetable tanning process illustrated in Table 1. Then the leathers were dried in a dark place and mechanical processes like milling and toggling were carried out.

Table 1. Vegetable Tanning Recipe

Material	Pickled Hide	Temperature	Time
Weighting	Pickle weight + 20%		
Vegetable Tanning	200% Water	30°C	
	2% Lightfast Syntan		30min
	10% Tannin		
	1% Lightfast synthetic fatliquor		20min
	20% Tannin		60min
	60% Water	55°C	10 min
	6% Lightfast synthetic fatliquor		300min
Fixation	1.5% Formic Acid (1/10 Diluted)		240 min
Draining			pH=3.8
Fixation	300% Water	50°C	
	0.3% Formic Acid (1/10 Diluted)		30min
Draining	150% Water	50°C	15min
Rinsing	300% Water	25°C	20 min

Physical and Instrumental Analysis of Tanned Leathers

The resulting leather samples were placed in Alpha+ Xenotest light fastness measuring instrument and light fastness was determined by exposing to light according to standard TS EN ISO 105-B02, 2014. Colour measurements were made by using Minolta 508d colour spectrophotometer.

The tests were performed on leather samples prepared as reported by the TS EN ISO 2418 (2006) and TS EN ISO 2419 (2012) standards. The tests and related standards used in the research are: Determination of the thickness, TS 4117 EN ISO 2589 (2006), Determination of shrinkage temperature, TS 4120 EN ISO 3380 (2005), Determination of the tensile strength and percentage elongation, TS 4119 EN ISO 3376 (2012), Determination of tear load, TS 4118-1 EN ISO 3377-1 (2012), Determination of distension and strength of grain by Ball Burst, TS 4131 (1985), Determination of colour fastness of leather to light: Xenon lamp, TS EN ISO 105-B02 (2014), Determination of colour change, TS 423-5 EN ISO 105-A05 (2001), Determination of free and released formaldehyde in leathers, TS EN ISO 17226-1, (2009).

RESULTS AND DISCUSSION

The results of colour measurements and colour differences (dE) of standard and modified mimosa and quebracho tanned leathers before and after artificial light exposure are illustrated in Table 2 and Table 3 in CIE Lab coordinates.

Table 2. Colour Change Measurements of Leathers Tanned with Non-Modified and Modified Mimosa Tannins

	Before			After			
Leather Sample	L	a	b	L	a	b	dE
M0	69.42	8.28	12.21	55.85	17.18	29.51	23.72
M1	46.24	12.25	18.35	42.47	17.94	25.28	9.72
M2	64.28	12.74	19.51	46.59	20.46	29.90	21.91
M3	50.94	10.39	14.56	53.43	13.19	28.45	14.38

Table 3. Colour Change Measurements of Leathers Tanned with Non-Modified and Modified Quebracho Tannins

	Before			After			
Leather Sample	L	a	b	L	a	b	dE
K0	67.07	12.20	20.59	51.61	18.63	28.99	18.73
K1	45.77	13.53	19.8	43.1	20.7	27.93	11.16
K2	44.03	17.37	23.34	30.06	16.97	12.75	17.54
K3	63.63	12.30	21.71	51.84	19.27	29.76	15.89

According to the results in Table 2 and Table 3, the lowest colour changes are observed from the leathers which were tanned with sulphomethylated mimosa and quebracho tannins (M1 and K1). As a result, sulphometilation modification is the best process for decreasing the colour changing effect of UV light on mimosa and quebracho tannins among other modification trials tested in this study.

Afterwards physical tests were applied to sulphomethylated mimosa and quebracho tanned leathers and the results were compared with standard mimosa and quebracho tanned leathers. And physical test results of leathers tanned with standard and sulphomethylated mimosa and quebracho tannins are illustrated in Table 4.

Table 4. Physical Test Results of Leathers Tanned with Standard and Modified Mimosa and Quebracho Tannins

Leather Sample	Ts (ºC)	Tensile strength (N/mm^2)	Elongation (%)	Tear load (N/mm)	Strength of grain (kgf)
M0	83,77	29,86	30,01	85,55	100
M1	92,23	19,87	31,46	65,78	96,5
K0	79,45	21,83	27,66	58,48	92,5
K1	83,85	20,24	31,48	78,15	100

According to Table 4 it can be seen that, leathers tanned with sulphomethylated mimosa and quebracho tannins show higher hydrothermal stability values than leathers tanned with standard mimosa and quebracho.

UNIDO has advised a minimum of 15N/mm^2 of tensile strength for acceptable quality standards. When tensile strength values of leathers tanned with both modified and standard mimosa and quebracho are evaluated, it is seen that they match with quality standard limits.

No significant difference was found for elongation at break values of leathers tanned with modified mimosa (M1) and quebracho (Q1). It is advised that elongation at break of vachetta for leather goods should be 70% maximum (TS 223, 1965). So the findings are compatible with the references.

As regards Table 4 is examined, it is seen that sulphomethylation modification causes a decrease in tear load values of leathers tanned with modified mimosa and an increase in the values of modified quebracho. And again these tear load values are higher than the suggested figure of UNIDO guidelines for upholstery leathers which is a minimum of 40 N/mm. The tearing load values of modified leathers were also close to the values obtained at the studies of John (1997) and Ozgunay (2005).

Although strength and distension of grain by the ball burst test is an important test usually for shoe upper leathers, it is an important physical-mechanical test that obtains valuable information about the behaviour of grain for all types of leathers. When we look at the Table 4, it is seen that strength of grain by the ball burst test decreases for Mimosa modification and increases for Quebracho modification yet these changes are not found to be statistically significant.

Ecological and toxicological demands are playing an increasingly important role in the marketing of leather for all types of application. Manufacturers and consumers of leather goods have been paying much more attention to the residual monomer content of leather especially over the past two years. Formaldehyde initially came under scrutiny from automobile manufacturers, and shoe and garment manufacturers have followed in their footsteps (Wolf and Huffer, 2002). Because of that, in this research a necessity of determining the formaldehyde content of leathers tanned with modified tannins was considered. The formaldehyde content of leathers tanned with sulpho methylated mimosa was detected as 1.45 ppm and formaldehyde content of leathers tanned with sulpho methylated quebracho was detected as 2.28 ppm. OEKO-TEX (2015), has suggested the limits of formaldehyde for leathers in direct contact with skin, leathers with no direct contact with skin, decoration material as 75 ppm, 300 ppm and 300 ppm respectively. The obtained results from the study were found below the limits.

CONCLUSIONS

Leathers tanned with mimosa and quebracho show darkening and reddening with an increase in red and yellow tones when exposed to light for prolonged times. This is due to formation of phenyl radicals by the loss of hydrogen to atmospheric oxygen. These free radicals can lead to creation or linking of chromophore groups by polymerisation and results colouring.

The random polymerisation of phenyl radicals can be oriented to a controlled modification of vegetable tannins with light stable synthetic tannin monomers. Mimosa and quebracho tannins can be modified by sulphitation, sulpho methylation and novalac synthesis.

Although all the experimented modification types have more or less increasing effect on the lightfastness properties of mimosa and quebracho tanned leathers, sulpho methylation was found to be the most effective one.

Leathers tanned with sulpho methylated mimosa and quebracho have clearly better stability to light than the ordinary mimosa and quebracho tanned leathers.

When physical test results of leathers tanned with sulpho methylated mimosa and quebracho were compared to classic production, some changes in shrinkage temperature, tensile strength and tear load were statistically determined. However all the properties were found compatible with acceptable quality standards.

As a final conclusion, sulpho methylation can be used in modification of mimosa and quebracho tannins to increase their stability to light; and the modified mimosa and quebracho tannins can be used in leather production without quality problems.

REFERENCES

Covington, A.G. (2009), *Tanning Chemistry*, Royal Society of Chemistry Publishing, Northampton.

Frediani, P., Tambi, L. and Frediani M. (2008), “Hide Tanning with Modified Natural Tannins”, *Journal of Applied Polymer Science*, 108,1797–1809.

John, G. (1997), *Possible Defects in Leather Production*, Druck Partner Rübelman GmbH, Hemsbach.

Kaşgöz, H. (1999), “Modifiye Poliakrilamidler ve Uygulamaları”, İstanbul Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, İstanbul.

OEKO-TEX (2015), https://www.oekotex.com/en/manufacturers/test_criteria/ limit_values/limit_values.html, (Visited May 3rd, 2015).

Omur, S., Mutlu, M.M. (2016), “Modification Of Mimosa And Quebracho Tannins and the Lightfastness Properties of the Proccessed Leathers”, *Tekstil ve Konfeksiyon*, 26(2), 230-235.

Ozgunay, H. (2000), “Meşe Palamutu Ekstarktı Valeksin Deri Sanayiinde Kullanılabilirliğinin Artırılması Üzerine Araştırmalar”, Ege Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, İzmir.

Ozgunay, H. (2008), “Light Fastness Properties of Leathers Tanned with Various Vegetable Tannins”, *Journal of the American Leather Chemists Associations*, 103, 345-351.

Pizzi, A., Simon, C., George, B. and Triboulot, M.C. (2004), “Leather Light Stability/Tannins Antioxidant Characteristics for Leather Made with Vegetable Tannins/MUF Resins”, *Journal of the American Leather Chemists Associations*, 99, 1-11.

TS 223 (1965), Vaketa Mamul Deri, Türk Standartları Enstitüsü, Ankara.

TS 4117 EN ISO 2589 (2006), Kalınlık Tayini Türk Standartları Enstitüsü, Ankara.

TS 4120 EN ISO 3380 (2005), Determination of shrinkage temperature, Türk Standartları Enstitüsü, Ankara.

TS 4131 (1985), Sırça Dayanımı ve Gerilebilirlik Tayini- Bilye Patlama Deneyi, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 105-B02 (2014), Yapay ışığa karşı renk haslığının tayini- Ksenon ark soldurma lambası deneyi, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 17226-1 (2009), Fomaldehit İçeriğinin Kimyasal Tayini-Bölüm 1: Yüksek Performans Sıvı Kromotografik Yöntem, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 2418 (2006), Kimyasal, Fiziksel, Mekanik Renk Haslığı Deneyi Numune Alma Bölgesi, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 2419 (2012), Numune hazırlama ve şartlandırma, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 3376 (2012), Çekme Mukavemeti Ve Uzama Yüzdesinin Tayini, Türk Standartları Enstitüsü, Ankara.

TS EN ISO 3377-1 (2012), Yırtılma Yükü Tayini, Bölüm 1: Tek Kenar Yırtığı, Türk Standartları Enstitüsü, Ankara.

UNIDO (1994), *Acceptable Quality Levels in Leathers*, United Nations Publications, New York.

UPAC (1997), *Compendium of Chemical Terminology*, Blackwell Scientific Publications, 2nd ed., Oxford.

Wolf, G. and Hüffer, S. (2002), “Formaldehyde in Leather-A Survey”, *Journal of the American Leather Chemists Associations*, 97, 456-464.

DECOLORIZATION OF CHROMIUM AND DYEING SPOTS ON LEATHER BY BLEACHING AGENTS

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Decolorization was carried out on the leathers with chromium and dyeing spots by different bleaching agents for valorization of defected products. Sodium perborate, sodium percarbonate, hydrogen peroxide, oxalic acid and sodium thiosulfate were used as bleaching agents. Sodium perborate was used by itself and together with other bleaching agents as combined. Color difference on leathers was spectrophotometrically measured by Konica Minolta CM-3600d apparatus. Leathers were re-processed after the bleaching and bleaching process was examined in terms of the strength properties of leather. Sodium perborate provided the best bleach and did not negatively affect the physical properties of leather.

Keywords: leather, defects, bleaching, decolorization, strength.

INTRODUCTION

Chrome stains usually appear as large blue/green spots often in a liquor run mark pattern. Some of the common causes of this type of chrome stains are unneven application of the chrome liquor, patches of uneven pH at the time of tanning and heat damages. Heat degraded collagen will take up more chrome as the breaking of the bonds in the collagen molecule by the heat creates more sites for chrome fixation. Heat damage occurring due to hot liquors or undiluted acids can cause dark run marks to show up after tanning. The most important problem is caused by pooling of chrome liquor. If pools of chrome liquor are allowed to stand on the surface of the skins after unloading from the tanning vessel, the chrome will continue to fix. Wash skins well at the end of tannage and preferably horse up to drain rather than leaving them in skips or tubs for long periods of time. Sometimes chrome stains appear as small spots. This could be due to contamination of the surface with neat chrome powder or contamination with an alkaline powder during tannage causing rapid chrome fixation in the contaminated areas. Occasionally, these intense stains can give rise to the dye resists. Chrome soaps can cause pink stains on wet-blue which can then go on to cause problems in dyeing process. Chrome soaps are caused by the decomposition of natural fats to free fatty acids which react with chrome to give pink stains. Sometimes they can also inhibit the penetration of the tannage and cause raw streaks in the centre of the hide (John, 1996).

Stained appearance in leather products can be also occured because of the defects in dyeing process. Fast fixation, insoluble dyestuffs, inaccurate temperature of the dyeing float and pH adjustment for neutralization can cause the undesirable impurities on leather surface. Thus, homogeneity in color is destroyed. Therefore, bleaching is required for the preparation of fiber based products to remove the colored impurities (Spicka *et al*., 2015; Xu *et al*., 2015).

Oxidative bleaching agents, such as sodium perborate and sodium percarbonate, have been widely used as bleaching components of fabric bleach compositions (Fujiwara *et al*., 1995). Some authors reported that sodium perborate and hydrogen peroxide release oxygen, so their combination should be synergistic and more effective (Valera *et al*., 2009). This study aimed to use different and combined bleaching agents as decolorant for safely reducing of the impurities on wet-blue and dyed leather surfaces.

MATERIALS AND METHODS

Materials

Sheepskins having chromium and dyeing spots on the surface were used as the leather material. Sodium perborate, sodium percarbonate, hydrogen peroxide, oxalic acid and sodium thiosulfate purchased from Sigma-Aldrich were used as bleaching agents.

Methods

Bleaching of Defected Leathers

300% water on the wet leather weight was used at 38^{o}C during 60 min. Sodium perborate and sodium percarbonate with 1% and 3% of solutions were processed by itself at the beginning, and then together with other bleaching agents as combined. All experiments were carried out in triplicate.

Colorimetric Measurements

Konica Minolta spectrophotometer (CM-3600d) was used for measuring the colors of the leather samples which were processed with bleaching agents, and how they bleach and change the leather color was examined compared to the original samples. Spectrophotometric measurements were carried out from five different points on the leather surface and averages were noted. Color differences between the original sample and the leathers processed by bleaching agents were calculated according to CIE Lab-76 color difference formula (CIE, 1976).

$$\Delta E= \sqrt{(\Delta \mathrm{L})^2+(\Delta \mathrm{a})^2+(\Delta \mathrm{b})^2} \tag{1}$$

Re-processing of Leathers after Bleaching

Re-processing recipe of wet-blue leathers after decolorizing by bleaching was given in Table 1. It contains neutralization, washing, dyeing, fatliquoring and retanning processes. Dyeing spotted leathers were just re-dyed and washed after bleaching application. Three leather samples were processed without bleaching as control group.

Table 1. Re-processing recipe after bleaching of wet-blue leathers

Process	%	Substance	Temperature (^{o}C)	Time (min)	Remarks
Washing	100	Water	30		
	0.5	Formic acid		45	Drain
Neutralization	100	Water	35		
	1	Sodium formate		10	
	1	Sodium bicarbonate		3x15+45	pH: 5.0-5.2, drain

Process	%	Substance	Temperature (°C)	Time (min)	Remarks
Washing	300	Water	35	10	Drain
Dyeing-fatliquoring-retanning	100	Water	40		
	5	Dyestuff		60	
	8	Combined natural and synthetic fatliquor		60	
	2	Phenolic syntan		20	
	2	Synthetic tanning agent		20	
	1	Formic acid		3x15+45	Drain
Washing	300	Water	20	10	Drain

Strength Analyses of Final Products

Prior to the analyses all finished leathers were conditioned according to the standard of EN ISO 2419 and sampling was done in accordance with the standard of EN ISO 2418. The final products were subjected to the tests of tensile and tear strength. Shimadzu AG-IS testing device was used for all analyses. Thickness measurement of the samples was performed in accordance with EN ISO 2589, tensile strength with EN ISO 3376 and tear load with EN ISO 3377-2.

Statistical Analyses

The results were evaluated statistically by using One-Way ANOVA, descriptive statistical and Duncan tests at SPSS 15.0 statistical software package. All data were represented as mean for three independent measurements. Comparison of means was analyzed by Duncan test and differences were considered as significant when $p<0.05$.

RESULTS AND DISCUSSIONS

Bleaching of the defected surfaces was firstly applied with 1% and 3% sodium perborate and percarbonate agents. Four runs were carried out on the dyed leathers to optimize the proportions as beginning. Parameters were 300% water, 38°C and 60 min for all combinations of the process. Table 2 shows the effectiveness of sodium perborate and percarbonate bleaching with two different percentages.

Table 2. Sodium perborate and percarbonate bleaching effects on dyed surfaces

Runs	Parameters	Bleaching agents	ΔE
R1	300% water 38°C 60 min	1% sodium perborate	2.39±0.41
R2		3% sodium perborate	5.44±0.52
R3		1% sodium percarbonate	1.52±0.33
R4		3% sodium percarbonate	4.00±0.48

Bleaching effect was increased with the increased proportions of both sodium perborate and percarbonate. The most effective bleaching was carried out with 3% of sodium perborate with R2 as seen in Table 2. Color difference of the bleached surface and control sample was 5.44±0.52.

After determining the better effect of sodium perborate than sodium percarbonate, combined bleaching processes were tried with sodium perborate. Table 3 shows the combined bleaching processes with 3% sodium perborate. Combination agents were hydrogen peroxide, oxalic acid and sodium thiosulfate.

Table 3. Combined bleaching processes with sodium perborate agent

Runs	Bleaching agents	ΔE
R5	3% sodium perborate 1% emulsifier 0.5% hydrogen peroxide	1.90±0.37
R6	3% sodium perborate 1% emulsifier 0.5% oxalic acid	2.15±0.40
R7	3% sodium perborate 1% emulsifier 0.5% sodium thiosulfate	1.93±0.36

Oxalic acid combination provided the better bleaching than hydrogen peroxide and sodium thiosulfate with R6. On the other hand, sodium perborate by itself gave the more effect on the surfaces compared to the all combinations. R2 was selected the optimal parameter for the next processes to be carried out on the chromium defected surfaces of wet-blue leathers. Sodium perborate bleaching and its combinations were also applied on the chromium surfaces, and Table 4 shows all the bleaching process combinations on both dyed and wet-blue sheepskins.

Table 4. Color differences on the wet-blue and dyed surfaces

Runs	Wet-blue sheepskins Bleaching agents			ΔE
R8	3% perborate	-	-	18.61±1.97
R9	3% perborate	1% emulsifier	0.5% hydrogen peroxide	10.74±1.02
R10	3% perborate	1% emulsifier	0.5% oxalic acid	11.02±1.11
R11	3% perborate	1% emulsifier	0.5% sodium thiosulfate	10.91±1.07
Runs	**Dyed sheepskins Bleaching agents**			**ΔE**
R2	3% perborate	-	-	5.44±0.52
R5	3% perborate	1% emulsifier	0.5% hydrogen peroxide	1.90±0.37
R6	3% perborate	1% emulsifier	0.5% oxalic acid	2.15±0.40
R7	3% perborate	1% emulsifier	0.5% sodium thiosulfate	1.93±0.36

Table 4 gives a comparative examination for the bleaching process applied on the wet-blue and dyed surfaces. The most effective bleaching was obtained on the wet-blue leathers by only sodium perborate application. Color difference between the bleached surface and the control sample was 18.61±1.97. Oxalic acid combination was better than the other combinations on both chromium and dyed surfaces. Only sodium perborate application was also more advantage in terms of the economical costs compared to the combined processings.

It is possible for bleaching agents to cause the physical deformation of the materials that they were applied. That's why, mechanical durability of the leathers after bleaching was also examined. The best bleaching effect was obtained on the chromium defected surfaces, thus wet-blue bleached and then finished leathers were subjected to the tensile and tear strength tests. Table 5 gives the test results belong to the final products bleached and re-processed.

Table 5. Strength test results of bleached and re-processed leathers

Process	Tensile strength	Tear strength
Without bleaching	10.24 ± 2.16^a	36.02 ± 4.25^a
Sodium perborate bleaching	9.87 ± 2.24^a	34.98 ± 3.96^a
Combined 1	9.13 ± 0.98^b	28.54 ± 4.01^b
Combined 2	9.81 ± 1.99^a	31.15 ± 2.97^b
Combined 3	9.21 ± 2.02^b	28.65 ± 2.84^b

a, b; values in the same column with different superscript letters are significantly different ($p<0.05$).

Sodium perborate bleaching didn't affect the both tensile and tear strength of leathers as seen in Table 5. Oxalic acid combination did not also decrease the tensile strength values statistically as from 10.24±2.16 to 9.81±1.99. Hydrogen peroxide and sodium thiosulfate combinations statistically affected the tensile strength values. All the combined applications reduced the tear loads of bleached leathers with important differences. The highest decreases on the strength values were carried out by hydrogen peroxide bleach. Consequently, the best bleaching effect was obtained by sodium perborate without any physical deformation of leather.

CONCLUSION

Defected leathers having impurities on the surface are poor in terms of the aesthetics and resulted with low prices in sales for the companies. Therefore, such surface defects should be prevented before finishing with the effective ways. Chromium and dyeing spotted surfaces on the leathers were successfully eliminated by this work without any lose of strength properties as the quality indicator of leather. Sodium perborate application gained economical value for the leather products by sustaining the physical composition as safe and gentler bleach.

REFERENCES

CIE (Comission Interantionalle DeL'Eclairage) (1976), *Official recommendations on uniform colour spaces colour difference equations metric colour terms*.

Fujiwara, M., Tamura, T. and Akabane, Y. (1995), "Reaction of sodium perborate with a chlorinated cyclic hindered amine (TMP-CI): I. Quantitative evaluation of active oxygen species in fabric bleaching", *Journal of the American Oil Chemists' Society*, 72, 97-103.

John, G. (1996), *Possible defects in leather production: Definitions, Causes, Consequences, Remedies and Types of Leather*. Lampertheim, Germany.

Spicka, N. and Tavcer, P.F. (2015), "Low-temperature bleaching of knit fabric from regenerated bamboo fibers with different peracetic acid bleaching processes", *Textile Research Journal*, 85, 1497-1505.

TS EN ISO 2419 (2006), Physical and mechanical tests - Sample preparation and conditioning. Turkish Standards Institution, Ankara, 6p.

TS EN ISO 2418 (2006), Chemical, physical and mechanical and fastness tests - Sampling location. Turkish Standards Institution, Ankara, 11p.

TS 4117 EN ISO 2589 (2006), Physical and mechanical tests - Determination of thickness. Turkish Standards Institution, Ankara, 7p.

TS 4119 EN ISO 3376 (2006), Physical and mechanical tests - Determination of tensile strength and percentage extention, Turkish Standards Institution, Ankara, 9p.

TS 4118-2 EN ISO 3377-2 (2005), Physical and mechanical tests - Determination of tear load - Part 2: Double edge tear. Turkish Standards Institution, Ankara, 7p.

Valera, M.C., Camargo, C.H.R., Carvalho, C.A.T., Oliveira, L.D., Camargo, S.E.A. and Rodrigues, C.M. (2009), "Effectiveness of carbamide peroxide and sodium perborate in non-vital discolored teeth", *Journal of Applied Oral Science*, 17, 254-261.

Xu, C., Hinks, D., Sun, C. and Wei, Q. (2015), "Establishment of an activated peroxide system for low-temperaturecotton bleaching using *N*-[4(triethylammoniomethyl)benzoyl]butyrolactam chloride", *Carbohydrate Polymers*, 119, 71-77.

3D IMAGING CAPTURE OF THE FOOT AND DATA PROCESSING FOR A DATABASE OF ANTHROPOMETRIC PARAMETERS

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Manufacturing high quality footwear with an optimal degree of comfort, adapted to the needs of various pathologies of the consumer, with effects on the size and shape of the foot, is conditioned by mastering techniques of processing anthropometric data obtained by 3D foot scanning, transposition of the anatomical, morphological, and biomechanical information into parameters for the design and modeling of shoe lasts and models. Anthropometric measurements are also required because of the specificity of each population, either in terms of length, width, height and foot conformation and consequently, the correlation between them, or growth rate during certain periods of time, depending on socio-cultural conditions, climate, food, etc. The current technological progress makes it possible to obtain 3D anthropometric data and use them in many industrial and research applications. Setting up a database of anthropometric data for the citizens of a country becomes a necessity. Digitized anthropometric data management enables their application in various professional disciplines such as medical studies, anthropometry studies, CAD/CAM footwear design, design of medical devices. This study aims to demonstrate the necessity and importance of creating a database of anthropometric parameters for the Romanian population.

Keywords: anthropometric parameters, database, foot.

INTRODUCTION

The development of the practical application of anthropology in the world was determined by the war industry, that required anthropometric sizes to manufacture equipment (footwear, clothing, accessories), as well as to size weapons.

The first anthropometric measurements were performed in England, in the years prior to the Hundred Years' War (1337–1453).

In Romania, the first mass anthropometric measurements were performed in 1968. In 1981–1982 and 1994–1995, these measurements were performed again, using the same methodology, on a much lower number of subjects, however (0.02–0.03% of the population). The analysis of anthropometric parameters measured in the two periods shows a series of changes, as well as size increments for manufacturing footwear.

The anthropological structure of a population, its dimensional and conformational variability are genetically and mesologically determined. When analyzing the anthropological structure of a population, many factors that influence this structure and make the distinction between different populations or within these populations are taken into account (gender, ontogenetic evolution, geographical factor) (Kozma *et al.*, 2014).

Thus each country builds its own anthropometric database necessary to establish dimensional standards for shoes, clothing, adapting environmental objects in the workplace, in private life, medicine, etc.

MEASURING INSTRUMENTS AND METHODS

Anthropometric foot data determine the spatial shape of shoe lasts. Over time different ways of measuring the human foot were developed in order to get initial data for shoe last design process.

Direct Method (Direct Contact with the Subject)

In the classical method or the method using direct contact with the subject, measurements are taken manually and specific tools are used: graded tape, caliper, pen, tissue paper, photographic developer.

Indirect Method (No Direct Contact with the Subject)

Advanced and integrated technologies, such as optical measurement, electronic signal and digital data processing, software and hardware, have directed the traditional 2D anthropometric data measurement to a new trend, the use of the 3D scanning system. In this case, foot conformation and specific sizes are obtained by means of 3D scanning systems that enable the storage and processing of data using specialized software.

The main advantages of 3D scanning systems are the accuracy and correctness of measurements, high speed scanning and processing, the possibility of data storage and database creation, the possibility of saving the 3D shape of the foot for further use and processing using dedicated software for modeling shoe lasts (Mortazavi *et al.*, 2008), designing shoes (Zhao *et al.*, 2010) and prototyping (McPoil *et al.*, 2009).

In the ongoing anthropometric survey in Romania, in order to calculate anthropometric parameters, the 3D shape of the foot is taken using USB INFOOT system (Fig. 1) consisting of a 3D scanner and dedicated software MEASURE 2.8. The system scans the foot shape and can recognize and automatically place up to 20 anatomical measurement points. It is intended for research, designing shoe lasts, selecting proper footwear, etc.

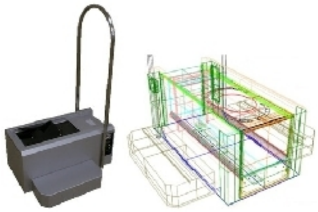


Figure 1. INFOOT USB 3D scanner

Configuration of INFOOT Application and Scanning Conditions

Prior to foot scanning and obtaining anthropometric parameters, equipment calibration is required, through the software's configuration interface (Scanning config), as well as verification of scanning conditions, setting recognition mode of anatomical points (manual or automatic), position of foot axes and toe angles, data processing module, printing template, saving format, etc.

Obtaining the 3D Shape of the Foot

Steps to obtain the 3D shape of the foot using the INFOOT scanner and dedicated software MEASURE 2.8 are: input of subject-specific data and of the scanning conditions (full name, year of birth, height, weight, gender, practiced sports, position of the subject during scanning, country, ethnic group), placing the foot in the working area of the scanner, scanning using the "Scan to" command and saving the data on the subject and the scanning results.

Positioning Anatomical Points and Measuring Anthropometric Parameters

After obtaining 3D foot shape in order to determine anthropometric parameters, the MEASURE 2.8 software displays the scanned foot, section by section (Fig. 2) and enables correction of any scanning errors, viewing the 3D foot shape (Fig. 3) in order to check and possibly correct the location of anatomical points, manual placement of anatomical points that have not been recognized by the software, viewing the 3D shape of the foot with anatomical points in various forms (cloud, polygon, surface) (Fig. 4). Anthropometric data sheet can be visualized and analyzed after positioning anatomical points (Sarghie *et al.*, 2013).

Scanning errors can occur due to penetration of light, lint, dust, sweat stains in the working area of the scanner. We recommend checking and cleaning the work area before each scan. In case of errors that cannot be corrected, or that require too much time for correction, the scanning operation is repeated.

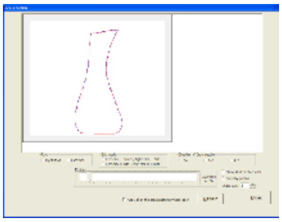


Figure 2. Sectional view of the foot

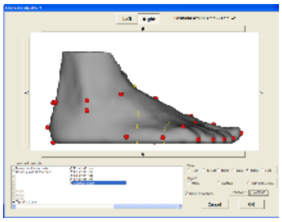


Figure 3. 3D foot shape and placement of anatomical points

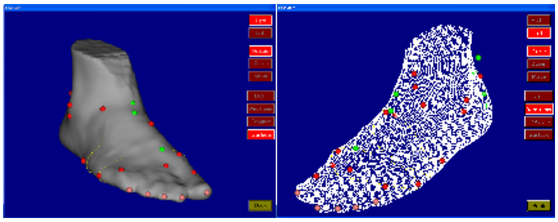


Figure 4. 3D foot shape and anatomical points in different forms (surface - a, cloud - b)

In order to calculate anthropometric sizes characterizing the foot of analyzed subjects, foot size parameters are thus processed: 16 significant anatomical points are manually set for each foot (left and right); These points are predefined and displayed in the scanning software, but are specific to the spatial conformation of each foot, and may be, therefore, changed. These anatomical points are presented in Figure 5.

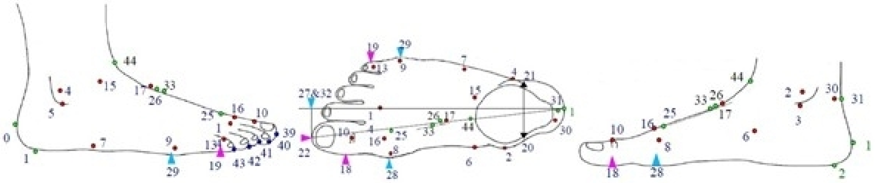
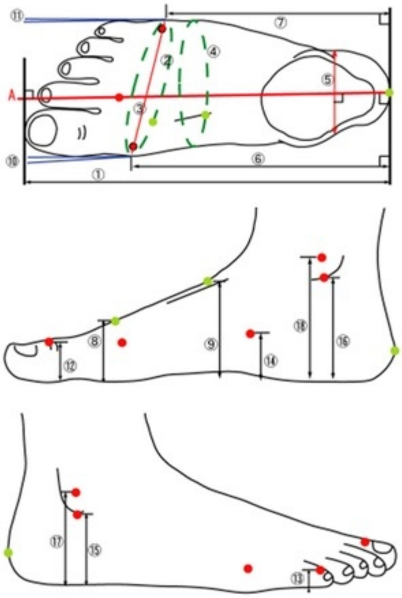


Figure 5. Significant anatomical points of the foot:

0 - Pternion
1 - Landing point
2 - Medial malleolus
3 - Sphyrion
4 - Lateral malleolus
5 - Sphyrion fibulare
6 - Navicular
7 - Tuberosity of 5th metatarsalis
8 - Metatarsale tibiale
9 - Metatarsale fibulare
10 - Toe 1 joint
13 - Toe 5 joint
14 - Tip of toe 2
15 - Tentative junction point
16 - Tip of toe 1
17 - Top of instep point

Following placement of anatomical point, foot-specific sizes will be automatically calculated (fig. 6).



1. Foot length
2. Ball girth circumference
3. Foot breadth
4. Instep circumference
5. Heel breadth
6. Instep length
7. Fibulare Instep length
8. Height of top of ball girth
9. Height of instep
10. Toe 1 angle
11. Toe 5 angle
12. Toe 1 height
13. Toe 5 height
14. Height of navicular
15. Height of sphyrion fibulare
16. Height of sphyrion
17. Height of the most lateral point of lateral malleolus
18. Height of the most medial point of medial malleolus

Figure 6. Foot-specific sizes (http://www.iwl.jp/main/mark_dimension.html)

Following foot scanning operations and placing anatomical points onto the surface of the scanned foot, values for a number of 21 anthropometric parameters, lengths, breadths, girths and angles are determined (Table 1).

Table 1. Analyzed anthropometric foot parameters

1.	Foot length	Lp	(mm)
2.	Ball girth circumference	Pd	(mm)
3.	Foot breadth	Ld	(mm)
4.	Instep circumference	Pr	(mm)
5.	Heel breadth	Lc	(mm)
6.	Instep length	Lm1	(mm)
7.	Fibulare Instep length	Lm5	(mm)
8.	Toe height	Hd	(mm)
9.	Height of instep	Hr	(mm)
10.	Toe 1 angle	Ud1	(^{o})
11.	Toe 5 angle	Ud2	(^{o})
12.	Toe 1 height	Hd1	(mm)
13.	Toe 5 height	Hd5	(mm)
14.	Height of navicular	Hn	(mm)
15.	Height of Sphyrion fibulare	Hsf	(mm)
16.	Height of Sphyrion	Hs	(mm)
17.	Height of the most lateral point of lateral malleolus	Hme	(mm)
18.	Height of the most medial point of medial malleolus	Hmi	(mm)
19.	Heel angle	Uc	(^{o})
20.	Heel girth	Pc	(mm)
21.	Ankle girth	Pg	(mm)

Transposition of anthropometric data into last design and manufacture is not carried out using a standardized method or international system – this is not possible due to the size specificity of each country and each population – but depends on the intuition, artistic sense and professional training of the designer, and is eventually confirmed by test wearing. One must know the correlations that must exist and be determined among the specific sizes of each foot, and among foot, last and footwear (Rigal, 1991; Deselnicu *et al*., 2014).

DATA ANALYSIS AND MANAGEMENT

Database analysis and management consists in: (i) primary 3D database; (ii) statistical processing and analysis of 3D data for the 3D anthropometric database; (iii) data processing and analysis to set footwear size increments; (iv) data processing and analysis to establish the morphological character of the foot; (v) data processing and analysis to establish size increments; (vi) data processing for medical research: diabetic foot, arthritic foot, etc.

APPLICATIONS

There are a number of current and potential applications for 3D scanners in commercial, clinical and research areas related to the human foot. The foot scanner's role in orthosis and customized shoe design and manufacture has been established.

The utility of scanning systems for clinical and research purposes has been successfully demonstrated, particularly for anthropometric measurement. 3D scanners allow large numbers of subjects to be scanned quickly and easily, with the data available for analysis at a convenient time for the researcher. There would appear to be scope for the expansion of scanner-based research into the investigation of a range of foot conditions, for example those that require monitoring the progression of a deformity over time. This approach could help to reduce x-ray exposure for the patient.

CONCLUSIONS

Making high quality footwear with an optimal degree of comfort, adapted to the needs of various pathologies of the consumer, affecting the size and shape of the foot, depends on mastering anthropometric data processing techniques obtained by 3D foot scanning, transposition of the anatomical, morphological and biomechanical information into shoe last and footwear model design and modeling parameters. Correlating internal dimensions of footwear with anthropometric measurements of the foot is particularly important in order to meet comfort conditions. Setting up and using an anthropometric database are compatible and competitive with the European research area. 3D scanning technology is new to Romania and is one of the sources for new research projects. Statistical data analysis will allow the development of anthropometric standards in compliance with the real sizes of the population. 3D scanning technology can and should be the source of innovation in various industrial fields.

Acknowledgements

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REFERENCES

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A., Militaru, G. (2014), "Sustainable Consumption and Production in the Footwear Sector", *Leather and Footwear Journal*, 14(3), 159-180, ISSN: 1583-4433.

Kozma, A., Glavce, C., Balaceanu-Stolnici, C. (2014), Anthropology and Environment, NICULESCU, ISBN PDF: 978-973-748-859-6.

McPoil, T.G., Vicenzino, B., Cornwall, M.W., Collins, N. (2009), "Can Foot Anthropometric Measurements Predict Dynamic Plantar Surface Contact Area?", *Journal of Foot and Ankle Research*, 2, 28

Mortazavi, S.B., Joze Kanani, M., Khavanin, A., Mirzaei, R., Rasoolzadeh, Y., Mansourizadeh, M. *et al.* (2008), "Foot Anthropometry by Digital Photography and the Importance of its Application in Boot Design", *Journal of Military Medicine*, 10(1), 69-80.

Rigal, R. (1991), *La Forme*, Editions C. T. C., Lyon.

Sarghie, B., Costea, M., Liute, D. (2013), "Anthropometric Study of the Foot Using 3D Scanning Method and Statistical Analysis", International Symposium in Knitting and Apparel, ISKA 2013, *Textiles of the Future*, organized by "Gheorghe Asachi" Technical University of Iasi, Faculty of Textiles – Leather and Industrial Management, 21-22 June, 2013, Iasi, Romania, 428-433, ISSN 2069 – 1564.

Zhao, J., Xiong, S., Bu, Y., Goonetilleke, R.S., Yuan, Z., Zhang, Y., Long, C. (2010), "An Automatic Method of Measuring Foot Girths for Custom Footwear using Local RBF Implicit Surfaces", *International Journal of Computer Integrated Manufacturing*, 23(6), 574-583.

*** http://www.iwl.jp/main/mark_dimension.html

INCREASING FOOT COMFORT INSIDE THE SHOES BY OPTIMIZING THE LAST SHAPE

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Throughout life, the human foot undergoes permanent transformations. Results of medical studies show that foot size stabilizes at the age of 18, when the ossification process ends. Therefore, special attention should be paid to quantifying constructive parameters of the shoe last so as not to adversely affect the natural development of the foot. To increase foot comfort inside the shoes, lasts will be designed based on anthropometric measurements by 3D imaging capture. For last and footwear prototyping, 17 children, eight girls and nine boys, aged between 3 and 7, were chosen for the study. The following steps were taken: (i) anthropometric data collection from female and male children and statistical processing of these data in order to establish constructive parameters of lasts; (ii) designing and manufacturing the last - average size - according to destination; (iii) designing and manufacturing the footwear model; (iv) creating a batch of shoes - test wearing; (v) analyzing the results of test wearing. As a result of designing and creating the new prototype lasts, and their testing through test wearing, it was concluded that: (a) the back of the last can be standardized in a proportion of 2/3 of the total length; (B) in terms of size, footwear corresponds to the shape of the foot. It follows that the new prototype lasts provide comfort inside the footwear.

Keywords: comfort, anthropometric parameters, last.

INTRODUCTION

The last is one of the most important factors in providing comfort (Zhao *et al*., 2008).

Currently, most footwear producers import last models from different countries (Italy, Turkey, etc.) that do not always comply with dimensional correlations between foot and last (Deselnicu *et al*., 2014). This leads to footwear being manufactured on lasts whose sizes are not consistent with the foot sizes of Romanian population, with repercussions on comfort in the wearing process.

To determine the correct shape and size of lasts, anthropometric measurements of the lower limb are required, so that there would be a close correspondence between these measurements and those that amount to the internal volume of the shoe (Mihai *et al.*, 2009; Nácher *et al.*, 2006).

The objective of this study is to increase foot comfort inside the shoe by optimizing the shape of the last.

EXPERIMENTAL

To construct the spatial shape of the last, the starting point was scanning the foot and measuring its various total and partial sizes.

Method

Scanning Method Using the INFOOT System – 3D Imaging

The INFOOT USB system, model IFU-S-01 & IFU-H-01, was used for three-dimensional foot scanning (Fig. 1).

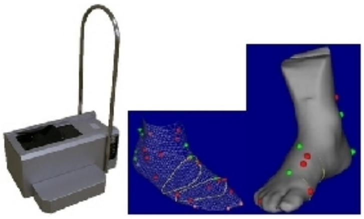


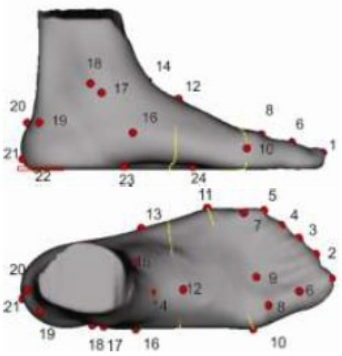
Figure 1. 3D Scanner (INFOOT) - Capturing foot sizes with the 3D Scanner

Data was processed by correctly identifying and positioning anatomical points that define anthropometric parameters of the foot. These points are predefined and are displayed by the scanning software, but they are specific to the spatial conformation of each foot, and therefore can be changed.

Positioning Anatomical Points and Measuring Anthropometric Parameters

Using INFOOT system, the right and then the left foot were scanned in a total of 17 subjects, of which eight girls and nine boys, aged 3-7.

In order to calculate foot anthropometric sizes for the analyzed subjects, 26 significant anatomical points were positioned manually for each foot (right and left). These anatomical points are shown in Figure 2:



Anatomical points of the foot: 1 - Tip of toe 1; 2 - Tip of toe 2; 3 - Tip of toe 3; 4 - Tip of toe 4; 5 - Tip of toe 5; 6 - Toe 1 joint; 7 - Toe 5 joint; 8 - Tip of metatarsal 1; 9 - Tip of metatarsal 2; 10 - Metatarsale tibiale; 11 - Metatarsale fibulare; 12 - Instep point; 13 - Tuberosity of 5th metatarsal; 14 - Tibiotalar joint; 15 - Tentative junction point; 16 - Cuboid; 17 - Sphyrion; 18 - Medial malleolus; 19 - Subtalar joint; 20 - Top of calcaneus point; 21 - Pternion; 22 - Landing point; 23 - Fore point of longitudinal arch; 24 - Rear point of longitudinal arch; 25 - Sphyrion fibulare; 26 - Lateral malleolus.

Figure 2. Positioning anatomical points (Pastina, 2012)

After positioning anatomical points, foot-specific sizes were automatically calculated. Anthropometric measurements were used to determine the shape and contour of the foot by outlining (plantar footprint). Metatarsal joints and toe directions were marked on the footprint and outline.

RESULTS AND DISCUSSIONS

To design the insole of a last, one needs to know: foot length (outline), toe and heel breadth (outline and footprint), heel amplitude length, distance between the pternion and the first and fifth metatarsal joints, the angle between the longitudinal axis (tip of the toes-heel) and the line that defines toe breadth, the angle between the longitudinal axis and the shank axis, foot angle (formed at the intersection of tangents drawn through the first and fifth metatarsal joints and heel contour), the ratio of longitudinal axis dividing toe breadth.

To set these parameters, children's foot scans were processed.

Anthropometric parameter values resulting from processing the imaging capture using 3D scanner (INFOOT) compared to those resulting from processing plantar footprints are given in Table 1.

Table 1. Comparison of average values of anthropometric parameters – INFOOT plantar footprint

Parametri antr. (INFOOT)	**Picior stang**	**Picior drept**	**Media INFOOT**	**Media Plantogr.**
L (mm)	193.3/190.5/194.7/190.1/162.5/170.6/197.6/165.8/190.7/188.5/185.1/163.9/184.8/177.6/188.6/184.7/153.7	192.0/192.6/198.0/191.7/162.8/171.8/197.2/164.7/190.1/187.6/183.6/162.5/187.3/182.2/188.0/184.4/152.3	181.52	181.16
LI (mm)	143.9/136.8/144.4/140.7/117.2/130.8/148.7/122.4/143.4/140.1/135.3/121.3/127.9/130.6/138.6/133.1/105.6	141.9/142.7/151.6/142.5/118.4/126.9/148.7/119.6/145.5/138.2/138.8/120.7/133.0/135.9/137.9/135.6/114.9	134.82	133
LV (mm)	120.4/121.2/122.8/116.8/104.6/107.5/128.4/102.1/120.4/115.2/116.1/99.0/112.4/112.9/118.2/112.8/96.8	123.0/121.1/129.1/126.8/104.2/110.2/114.6/101.1/118.9/114.0/115.0/100.8/114.9/114.8/117.2/114.9/90.5	113.8	110
ldc (mm)	80.8/71.9/74.1/79.6/66.3/69.7/82.6/75.4/80.8/73.1/73.4/70.1/78.6/71.2/80.0/76.7/62.9	78.1/72.3/72.7/75.4/66.0/66.7/86.9/71.6/81.2/71.5/74.4/69.3/78.4/72.4/78.8/73.4/66.7	74.21	73
Lcc (mm)	53.4/53.2/56.8/54.8/44.1/48.0/54.2/50.2/54.0/51.9/49.7/51.8/57.5/50.8/55.1/55.8/48.6	53.1/52.7/56.9/55.7/43.2/47.1/55.7/51.0/54.8/54.0/51.1/50.3/58.2/50.9/55.3/54.0/47.7	52.4	50
Pd (mm)	194.2/177.6/180.4/191.0/165.9/171.2/197.9/181.1/192.0/177.8/178.6/173.8/199.7/180.4/192.4/188.1/158.9	192.7/175.3/177.0/183.7/165.6/163.8/209.4/177.1/193.0/176.5/180.8/169.9/198.6/179.7/191.0/182.3/164.0	181.8	-
Pr (mm)	196.4/183.5/186.8/193.9/169.3/165.5/200.8/180.3/192.2/187.3/179.3/172.2/199.3/182.9/190.2/186.9/163.0	194.9/180.3/185.7/193.6/168.4/165.7/203.4/177.0/190.2/176.5/179.1/168.9/199.4/182.1/191.0/183.2/164.1	183.4	-

The analysis of anthropometric parameters shows that the average size is 18 (metric system) or 27 (French system), with the following parameters: α = 13°, β = 5-6°, γ = 74°, ldc = 73 mm, lda = 62 mm, Lcc = 50 mm, Lca = 36 mm, Ace = 6 mm, Aci = 7 mm, ldic = 32 mm, ldia = 41 mm, ldec = 25 mm, ldea = 37 mm, where: α = foot angle (formed at the intersection of tangents drawn through the first and fifth metatarsal joints and heel contour); β = the angle between the longitudinal axis and the shank axis; γ = the angle between the longitudinal axis and the line that defines toe breadth; L = foot length; LI = distance between the pternion and the intersection of the perpendicular from the first metatarsal joint on the longitudinal axis; LV = distance between the pternion and the intersection of the perpendicular from the fifth metatarsal joint on the longitudinal axis; Lc = distance between the pternion and the heel center; ldc = contour toe breadth; lda = footprint toe breadth; ldic = inner contour toe breadth; ldia = inner footprint toe breadth; ldec = outer contour toe breadth; ldea = outer footprint toe breadth; Lcc = heel contour breadth; Lca = heel footprint breadth; ac = heel amplitude; Ade = outer toe amplitude; Adi = inner toe amplitude; Ace = outer heel amplitude exterior; Aci = inner heel amplitude; Pd = ball girth circumference; Pr = instep circumference.

Another element to be considered, in designing both the insole and the last, is the tip of the toes-heel axis. In designing the insole, everything was calculated in relation to the axis that unites the pternion and the head of the second toe (Fig. 3).

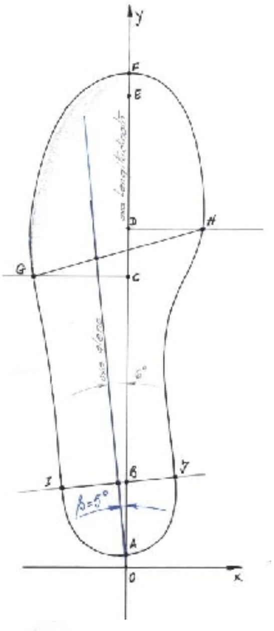


Figure 3. Insole design Nm 18 (Fr 27)

The insole of the last is designed as follows:

First, a system of axes is drawn, XOY. Starting from O, 0.025 Lp are measured (value of heel amplitude) and the rearmost point of the insole is defined, point A. Point B marks the center of the heel (0.18 Lp). Points C and D define on the axis of the first and fifth metatarsal joints, 0.62 Lp and 0.72 Lp, respectively. OE distance is the foot length. EF distance is the tolerance (2-3 mm movement tolerance and 5-15 mm toe tip shape tolerance). The shank axis is drawn through point A, forming an angle of 6° with the longitudinal axis. This angle corresponds to angle β = 5° resulting from plantar footprint processing. On the shank axis, through point B, a perpendicular is drawn, whose intersection is marked K. KI = KJ, where IJ is heel breadth, 42.5 mm. GH is toe breadth, 66.3 mm. This line segment intersects perpendiculars drawn through C and D, so that PH = 44 % ld and HG = 56 % ld. Insole contour is obtained uniting points O, J, H, F, G, I, O, and the appearance of the contour is similar to that of the average contour between the footprint and foot outline. The length of the insole is calculated using equation 1:

$$lb = Lp - ac + am + av \qquad (1)$$

where: lb = insole length;
Lp = foot length;
ac = heel amplitude (0,025 Lp);
am = movement tolerance (2-3 mm);
av = toe tolerance (5-20 mm, depending on fashion).

Starting from the average size insole template, all other sizes were obtained. To check the correctness of these insoles, footprint outlines and insole outlines were overlapped, with positive results.

Making the last requires knowledge regarding anatomical and functional parameters of the foot, elements of biomechanics, method of transposing anthropometric parameters in designing last execution and control templates. In determining the spatial shape of the last, an important part is played by values of ball girth circumference, instep circumference and heel circumference (entry circumference). Another element the last designer should consider is the parallelism of planes in the heel and metatarsals I and V.

Based on the above, two prototype lasts were created using the conventional method, for children's footwear, size 18, breadth 4 and 5, respectively (Fig. 4). Breadth 4 is indicated for girls' shoes, while 5, for boys' shoes.

Figure 4. Prototype lasts

LAST-MAKER software was used to check the position of the foot inside the shoes, i.e. the compatibility between foot and last in terms of size (Fig. 5).

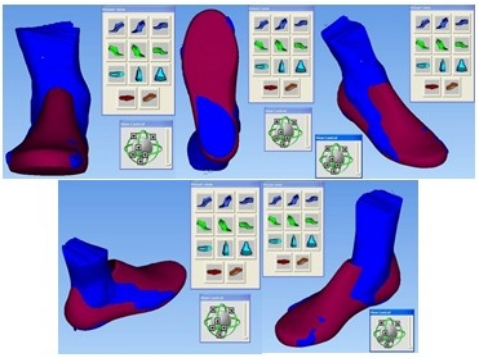


Figure 5. Compatibility between foot and last in terms of size

A correct correspondence is noted between the spatial shape of the foot and that of the last, the accuracy in terms of the method of transposing anthropometric parameters in designing lasts for children's shoes. This sets the premises for the possibility of standardizing (normalizing) the back part of the last. Figure 6 presents this suggestion.

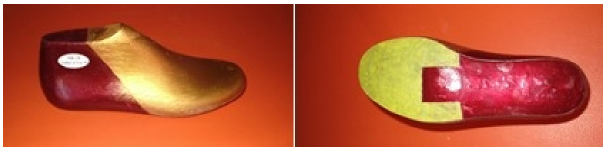


Figure 6. Standardization of the back part of the last

Compared to the 1972 standardization, the new concept is much easier, and does not hinder the act of creation (the fashion factor), but only standardizes certain parts that make up the footwear (metallic shank, counter, insole reinforcement). Prototype lasts were tested in manufacturing and in terms of size fit by test wearing, with very good results.

CONCLUSIONS

Children's footwear is recommended to be manufactured using breadth 4 for girls and 5 for boys, and for boots, breadth 5 and 6, respectively.

When designing the insole of the last, the angle between the tip of the toe-heel axis and the shank axis should be 6°.

Given the results of test wearing and checking the compatibility between foot, last and footwear, the back of the last, a portion of 2/3 of the total length, is considered standardisable.

The lack of abrasions, fatigue, redness of the foot surface in areas of maximum pressure, the walking mobility and safety demonstrates that in terms of size, the shoes conform to the spatial shape of the child's foot (aged 3-7). Therefore, the new prototype lasts provide foot comfort inside the shoes.

Acknowledgements

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REFERENCES

Deselnicu, V., Crudu, M., Zainescu, G., Albu, M.G., Deselnicu, D.C., Guta, S.A., Ioannidis, I., Gurau, D., Alexandrescu, L., Constantinescu, R.R., Chirila, C., Macovescu, G., Bostaca, G. (2014), "Innovative Materials and Technologies for Sustainable Production in Leather and Footwear Sector", *Leather and Footwear Journal*, 14(3), 147-158, ISSN: 1583-4433.

Mihai, A., Sahin, M., Paştină, M., and Harnagea, M.C. (2009), Footwear Design, Performantica, Iaşi.

Nácher, B., Alemany, S., González, J., and Alcántara, E. (2006), "A Footwear Fit Classification Model Based on Anthropometric Data", *SAE Technical Paper*, 2006-01-2356.

Pastina, M.. (2012), "Contributions to 3D Modelling and Fast Prototyping of Prophylactic Footwear for People with Special Needs", "Gheorghe Asachi" Technical University of Iaşi.

Zhao, J. *et al.* (2008), "Computerized girth determination for custom footwear manufacture", *Computers & Industrial Engineering*, 54(3), 359-373.

CAD-CAM PRESCRIPTION AND DESIGN ITEMS OF CUSTOM MADE MEDICAL FOOTWEAR SOLES

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Custom made medical footwear is a prescription-based medical device. In order for the end-product to achieve the expected therapeutic effects, several conditions have to be fulfilled, such as: diagnosis and assessment of pathology and establishing the treatment goals, knowledge of the prescription items and of those technical characteristics of the product that allow to achieve these goals, the existence of flexible technologies enabling the design and practical execution of the prescription in the shortest possible time in terms of economic efficiency. In the absence of these factors, achieving the goals of the conservative treatment is difficult and frequently leads to failure. The present article proposes a review of the prescription parameters of custom made medical footwear soles and presentation of CAD-CAM solutions for their design. For this purpose, some sole prescription parameters were transposed into a virtual model ready for execution on automatic milling machines. CAD-CAM technologies enable the flexibilization of the custom made footwear sole design and manufacture processes and reduces the waiting times of patients. The implementation of these technologies requires significant investments for their purchase and for training medical specialists and technicians involved in establishing and implementing the treatment.

Keywords: soles, medical footwear, CAD-CAM

INTRODUCTION

Custom-made medical footwear is a prescription based medical device. Custom-made medical devices refer to “any device specifically made in accordance with a duly qualified medical practitioner's written prescription which gives, under his responsibility, specific design characteristics and is intended for the sole use of a particular patient. The abovementioned prescription may also be made out by any other person authorized by virtue of his professional qualifications to do so.” (Directive 93/42/EEC). Seen from this perspective, the medical prescription must include the “constructive characteristics” of the used medical device having a therapeutic role in the conservative treatment of foot pathomechanics.

The medical literature lists a number of therapeutic effects and necessary prescription parameters of the medical devices used in the implementation of conservative treatment of foot pathomechanics. In this regard, as a pathology, therapeutic effects and specific design features for medical footwear soles include:

- **pathologies:**

- *hallux rigidus* or *hallux limitus*, metatarsalgia, healed plantar ulcerations, *pes planus* or foot with medially deviated subtalar joint, *pes cavus* or foot with laterally deviated subtalar joint, minor pains in ankle joint, blocked ankle joint, diabetic foot (Nawoczenski *et al.*, 1988; Praet and Louwerens, 2003), rheumatoid arthritis foot (Cho *et al.*, 2009), knee injuries, etc.

- **therapeutic effects:**

- modification of the range of motion in the angle joint (Petcu *et al.*, 2013), decrease of the pressures from the distal metatarsal heads ulcerations area (Nawoczenski *et al.*, 1988; Mueller, 1999), decrease of the supporting time on the affected joints, influencing the supinator or pronator moments through the modification of the ground reaction force moment arm relative to the anatomical joints of interest, modification of shock absorbing properties, etc. As main adverse effects we can note an increase of the postural instability as a result of sole height, hardness of the sole material and decrease of the contact surface with the supporting surface (Hijmans *et al.*, 2007) or decrease of the impact shock absorbing properties and increase of the energetic consumption as a result of pronounced sole wearing (Saito *et al.*, 2007).

- **constructive characteristics / prescription parameters:**

- the shape of the superior longitudinal sole profile and of superior surface will influence the way in which the foot will stay in contact with the sole. Together with the inferior longitudinal profile, foot stability inside the shoes, body posture and pressure distribution at the plantar foot surface could be influenced (Dananberg, 1998),

- the shape of the inferior longitudinal sole profile and of bottom surface. The effect of this type of profile is best exemplified in the case of the rocker sole (Hutchins, 2009). In this way, the main constructive characteristics which can be considered prescription parameters are: sole's rocker profile, proximal and distal contact lines with supporting surface, the orientation of these lines or of the inferior profile relative to the walking direction (Petcu *et al.*, 2013), sole's height in different areas, medial or lateral extension of the distal area of the sole's heel with the purpose of influencing the stability or the pronator and supinator moments around the anatomical joint axis of interest (known as medial or lateral "Thomas heel"),

- the sole's lateral flange. This could be applied on different areas as the heel area or entire lateral area. When this flange is applied on both medial and lateral sides it will generate a trapezoidal shape of the sole section,

- the physico-mechanical properties of the soles such as the hardness of the used material or the properties which are defining the slipping phenomenon relative to the supporting surface (friction coefficient).

It should be mentioned that these constructive characteristics and their link with the therapeutic effect should be known by the medical device prescription specialist. They should be included in the prescription file which is the communication base between diverse categories of specialists involved in the conservation treatment. Knowledge of these constructive characteristics by the medical specialist will allow him to select the optimal sole shape in the case of a prefabricated product or an efficient communication with the technical specialist involved in the design and their practical manufacture in the case of custom-made devices. It should be mentioned that, in general, in Romania, these design parameters are not known to the medical specialists, the medical prescription being limited to an indication such as "orthopedic footwear". Meanwhile, in the case of technical specialists there is no knowledge of the potential biomechanical effect which can be created by a specific technical characteristic of the sole at the level of the musculoskeletal system. Another important aspect is the sole's design and manufacture process flexibility. In this respect it is worth mentioning the practical difficulty of manufacturing a footwear sole with a rocker profile oriented relative to the walking direction and not to the longitudinal foot axis. The use of 3D modeling software solutions with automatic milling technologies or, more recently, 3D printing process allows to maximize the flexibility of the design and manufacture process of soles, facilitating practical implementation of the prescriptive features otherwise very difficult to achieve practically.

METHOD

The modern design of medical footwear soles could be done based on many 3D modeling software solutions. The purpose of this paper is to exemplify one working method which can be used in the virtual modeling of the custom-made medical footwear. In this respect it is necessary first to know:

- the prescription parameters / constructive characteristics of the medical device as indicated by the medical specialist,
- the design and 3D modeling methods of the prescription parameters. This requires knowledge of generic operations that allow shaping lines, surfaces or volumes (for example extrusion lines or surfaces, cutting volumes, etc.),
- how these design methods can be put into practice through specific functions of available 3D modeling software solutions.

The software used to exemplify the work method was PowerSHAPE 2016 from Delcam company.

RESULTS

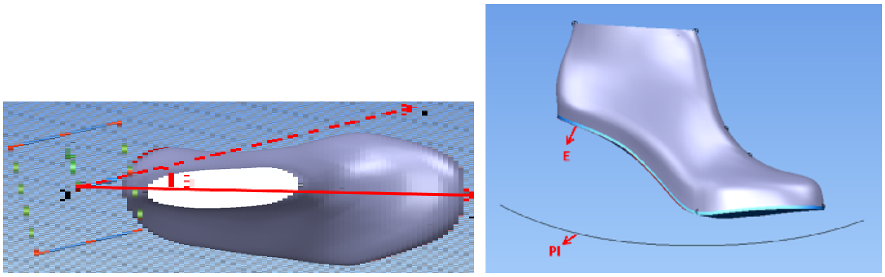
In this work a simulation was carried out for designing a rocker sole. In describing the design process only the generic names of the performed operations will be indicated and not the specific features or functions of the chosen software solution. This approach is justified by the fact that an operation can be performed using different functions or commands. How they are selected depends only on the ability and knowledge of the designer.

The selected prescription parameters for this simulation were as follows:

1. the angle of the longitudinal foot axis with walking direction, a=12° (Figure 1a),
2. footwear last's heel height of 65 mm (Figure 1b). In the absence of the virtual last, the longitudinal profile of the last bottom and its orientation relative to the prescribed heel height can be indicated,
3. the rocker profile as indicated in prescription, (Figure 1c). In this example, the longitudinal rocker profile corresponds to an antero-posterior rocker profile where the contact with supporting surface is represented by a line. In this situation the proximal and distal contact lines are confounded,
4. the orientation of the inferior longitudinal profile of the sole is parallel with walking direction. This option facilitates walking in the forward direction in the case of the feet having the longitudinal axis (the heel-toe axis) at a higher angle with walking direction than the normal one (approximately 7 degrees),
5. the lateral surface of the sole is vertical.

The main basic steps need to be taken are:

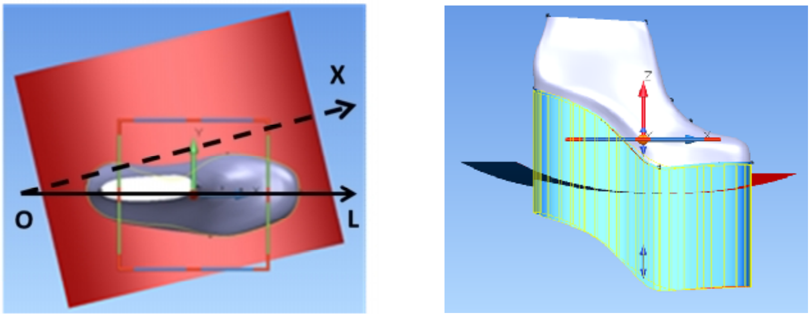
1. the virtual last is oriented in a such way as the longitudinal last's axis (the heel-toe axis) is making the prescribed angle, "a", with walking direction (Figure 1a),
2. an extrusion, E, of the last's plantar surface (bottom last) is made in order for the superior sole's surface to be obtained, the extrusion height being equal with the sum of the last insole thickness and upper thickness in the lasting area. ("E" - Figure 1b),
3. the rocker profile is designed in the last' sagittal plane (Figure 1b),



a) b)

Figure 1. Last orientation and design of the inferior profile: a) the last's longitudinal axis orientation, OL, relative to the walking direction, Ox, b) the design of the inferior profile, Pl, relative to the last's oriented position

4. the rocker profile's extrusion in the transversal (horizontal) plane is made. Its orientation relative to walking direction is presented in Figure 2a,
5. the vertical extrusion of the superior outline of the sole obtained in step no. 1 is made (Figure 2b),



a) b)

Figure 2. Sole design: a) inferior profile extrusion, b) superior surface extrusion

6. the lateral sole surface is obtained by cutting the vertically extruded surface with the horizontally extruded rocker profile (Figure 3a).

The extrusion options allow the design of soles having oblique or vertical walls. These options are useful in two situations:

- when the aim is to design a sole with a larger inferior surface area than the last's bottom area, in order for the footwear stability to be increased (the transversal section having a trapezoidal shape),
- when the aim is to modify the pronatory or supinatory moments through the modification of the reaction force moment arm relative to the anatomical joint axis of interest. In this case, a lateral (for the pronatory moment modification) or medial (for the supinatory moment modification) inclined transversal profile will be created.

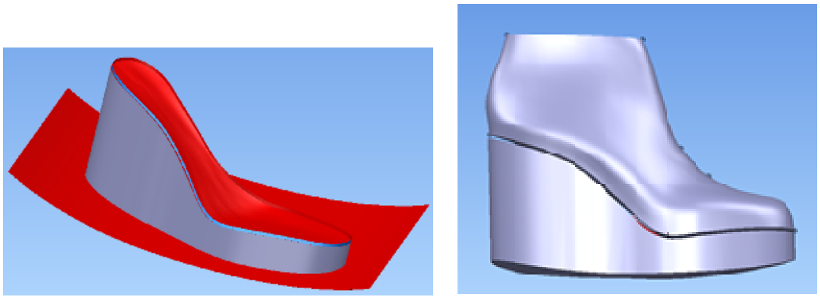

a) b)

Figure 3. Rocker sole: a) cutting of the extruded volume, b) the final sole shape and the last

7. the superior surface of the sole is obtained based on the outline of the surface resulted through the extrusion of the last's bottom (step no. 2),
8. the inferior surface of the sole corresponding to the wearing area is obtained using the outline resulted through the intersection of the lateral surface with the surface obtained through the inferior longitudinal profile's extrusion,
9. the surface defining the sole's volume is obtained through the union of all three anterior designed surfaces: superior plantar surface, lateral surface and inferior surface (wearing area),
10. for the automated milling or printing manufacture the designed virtual sole's volume is exported in a format which can be recognised by the CAM (Computer Aided Manufacture) software (.stl format for example).

The final shape of the sole and the last shape are presented in figure no.3b. The space created through the extrusion of the plantar surface ("E", step no. 2) can be seen, determined by the last insole and upper thickness.

CONCLUSIONS

Using CAD-CAM technologies allows the flexibilization of the designing and manufacturing process of the medical footwear soles and the reduction of the patient's waiting time. New prescription / constructive characteristics could be introduced and easily manufactured.

The use of these technologies requires significant investments for the acquisition and especially for the training of the medical and technical specialists involved in the treatment prescription and implementation processes.

In order for the final product to produce the estimated therapeutic effect, several conditions must be met, such as: the diagnosis and the evaluation of the pathology and establishing the treatment's objectives, knowledge of the prescription elements and of those technical characteristics of the product that will achieve the treatment's objectives, availability of flexible technologies which will allow the design and manufacture of the prescription in a shorter time and with economical efficiency. In the absence of these factors, achieving the conservative treatment's objectives is difficult, leading, not infrequently, to failure.

REFERENCES

Cho, N.S. *et al.*, (2009), ”Randomized controlled trial for clinical effects of varying types of insoles combined with specialized shoes in patients with rheumatoid arthritis of the foot”, *Clinical Rehabilitation*, 23, 512–521.

Dananberg, H. (1998), “Comfortable High Heel Shoe”, US Patent 5782015, Jul. 21.

Hijmans, J.M., Geertzen, J.H.B., Dijkstra, P.U., and Postema, K. (2007), “A Systematic Review of the Effects of Shoes and Other Ankle or Foot Appliances on Balance in Older People and People with Peripheral Nervous System Disorders” *Gait & Posture*, 25(2) 316–23, doi:10.1016/j.gaitpost.2006.03.010.

Hutchins, S., Bowker, P., Geary, N., and Richards, J. (2009), “The Biomechanics and Clinical Efficacy of Footwear Adapted with Rocker profiles—Evidence in the Literature”, *The Foot*, 19(3), 165–70, doi:10.1016/j.foot.2009.01.001.

Mueller, M.J. (1999), “Application of plantar pressure assessment in footwear and insert design”, The Journal of Orthopaedic and Sports Physical Therapy 29, 747–755.

Nawoczenski, D.A., Birke, J.A., Coleman, W.C. (1988), “Effect of rocker sole design on plantar forefoot pressures”, Journal of the American Podiatric Medical Association, 78, 455–460.

Petcu, D., Bucur, D., Karavana, H.A., Nistor, M., Pantazi, M., Georgescu, M. (2013), “The influence of the rocker sole profile’s orientation on the sagittal ankle range of motion”, The 4th IEEE International Conference on E-Health and Bioengineering - EHB 2013, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania, November 21-23, 2013.

Praet, F.E., and Louwerens, J.K. (2003), “The Influence of Shoe Design on Plantar Pressures in Neuropathic Feet”, *Diabetes Care*, 26(2), 441-445.

Saito, S., Muraki, S., and Tochihara, Y. (2007), “Effects of Worn-Out Soles on Lower Limb Stability, Shock Absorption and Energy Cost during Prolonged Walking”, Journal of Physiological Anthropology, 26(5), 521–526, DOI: 10.2114/jpa2.26.521.

*** Council Directive 93/42/EEC of 14 June 1993 concerning medical devices, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31993L0042.

*** Delcam PowerShape Documentation, http://www.delcam.com/software/powershape

BEHAVIOR IN FINISHING OF PCM FIBERS IN BLENDS WITH NATURAL, MAN-MADE OR SYNTHETIC FIBERS

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Phase Change Material (PCM) is a substance with a high heat of fusion which melts and solidifies at a certain temperature, being capable of storing and releasing large amounts of thermal energy. This behaviour of PCM is the basis for its temperature regulating effect, as a component of textile functionalized fibres and helps the body to stay within the comfortable temperature range. For the finishing of textiles containing PCM fibers, a great importance should be given to each technologic operation, so that the PCMs from the fiber's structure would not be eliminated by preliminary treatments, dyeing or by the final finishing applied procedures. In this study, laboratory pre-treatment and dyeing experiments were made, on knitted fabrics with content of PCM fibers in blends with natural, man-made or synthetic fibers, to enable the overall finishing processes influence on the main physical-mechanical characteristics, dyeability and color fastness properties. Various methods of preliminary treatment and dyeing were applied, specific to each fiber composition of yarns, by using the optimum results obtained in previous performed studies. The behavior during finishing of knitted fabrics varies, depending on the fiber components blended with PCM fibers, without affecting the physical-mechanical characteristics of knitted fabrics. As regards the dyeing process, all samples are uniformly dyed, with color differences depending on the applied finishing process. Dyeing fastness is very good in the case of blends with man-made cellulosic fibers and good in the case of blends with cotton or with synthetic fibers.

Keywords: PCM fibers in fiber blends, physical-mechanical characteristics, dyeing behavior

INTRODUCTION

The modern lifestyle creates today the situation in which the actual aim of textile materials is changing from traditional simple protection to the necessary functionality and added-value (e.g. comfort). Textile material is still considered as the interface between the wearer and the environment, but it needs more active role of adapting to the drastic changes of the physiological needs, in the accordance with the changes in the environment (Jocic, 2010). Development of functional fibers is based on the process of incorporating active chemicals into the fiber in its manufacturing stage, and this technology is rather increasing today, mainly being supported by fiber manufacturers. Integration of PCMs in clothes is a straightforward method to obtain thermo-regulating properties. When body temperatures increase, the PCM melts and absorbs heat from the body. Then, when the temperature drops, the PCM solidifies and the stored heat is released again and thus has the ability to stabilize body temperature. This behavior of PCM is the basis for its temperature regulating effect as a component in textile fibers (Mondal, 2008). In previous studies performed (Popescu *et al*., 2011), the influence of chemical auxiliaries used in the preliminary process, as well as of the applied technological parameters, on the heat of fusion of textile materials made of cellulosic man-made fibres containing PCM, were determined. In this paper, the influence of finishing operation on knitted fabric with Cell Solution™ Clima fibers content was studied. This functionalized man-made cellulosic fiber is the newest fiber with thermo-regulating features of Smartpolymer GmbH (Germany) and TITK group, developed by a new concept for incorporating PCMs in wet spun cellulose fibers based on direct addition of free PCM to a cellulose solution (***Cell Solution).

EXPERIMENTAL PART

Materials

The knitted fabrics used in this study are made of different construction of yarns, as mentioned in Table 1.

Table 1. Knitted fabrics yarns composition

Fabric code	Yarn count (Nm)	Yarn construction (%)	Yarn mixture
A	50/1	50/50	Tencel/ PCM fibers
B	50/1	70/30	Tencel/ PCM fibers
C	60/1	50/20/30	Cotton/ PA/ PCM fibers
D	65/1	70/30	PES/ PCM fibers

Finishing Procedures

Pre-treatment and dyeing procedures were applied on piece laboratory dyeing apparatus “Redkrome” (Ugolini-Italy), at a bath ratio of 1:20, according to Table 2.

Table 2. Finishing procedures applied on knitted fabrics with content of PCM fibers

Code	Finishing operations
Finishing procedures applied on 50% Tencel/ 50% PCM fibers knitted fabrics	
AV_1	scouring (40º, 30 min)→reactive dyeing
AV_2	scouring (85º, 30 min)→reactive dyeing→bio-polishing (acid cellulase)
AV_3	scouring (40º, 30 min)→causticizing→bio-polishing (acid cellulase)→reactive dyeing
Finishing procedures applied on 70% Tencel/ 30% PCM fibers knitted fabrics	
BV_1	scouring (40º, 30 min)→reactive dyeing
BV_2	scouring (85º, 30 min)→reactive dyeing→bio-polishing (acid cellulase)
BV_3	scouring (40º, 30 min)→causticizing→ bio-polishing (acid cellulase)→reactive dyeing
Finishing procedures applied on 50% cotton/ 20% PA/ 30% PCM fibers knitted fabrics	
CV_1	mild alkaline treatment→bleaching→ 2 steps dyeing (reactive/PA reactive dyestuffs)
CV_2	enzymatic→pre-treatment (alkaline pectinase)→bleaching→2 steps dyeing (reactive/acid dyestuffs)
CV_3	causticizing→bleaching→bio-polishing (acid cellulase →2 steps dyeing (disperse/ reactive dyestuffs)
CV_4	single step scouring and bleaching→2 steps dyeing (PA reactive/reactive dyestuffs)
Finishing procedures applied on 70% PES/ 30% PCM fibers knitted fabrics	
DV_1	scouring (60ºC)→drying→dry heat-setting→dyeing in 2 steps (disperse and reactive)
DV_2	scouring→drying→dry heat-setting→causticizing→bio-polishing→2 steps dyeing
DV_3	scouring→drying→dry heat-setting→causticizing→2 steps dyeing→bio-polishing V_1
DV_4	scouring→drying→dry heat-setting→causticizing→2 steps dyeing→ bio-polishing V_2

Physical-Mechanical and Color Measurements

Physical-mechanical characteristics and color assessment have been made on raw knit fabrics and on final product, as follows:

- *physical-mechanical characteristics:* mass (SR EN 12127-2003), density (SR 5903-1993), thickness (SR EN ISO 5084/2001), maximum force - GRAB method (SR EN ISO 13934/2-2002), elongation at maximum force (SR EN ISO 13934/2-2002), abrasion resistance - NU MARTINDALE method (SR EN ISO 12947-2/2002);

- *dyeing performance:* color differences (SR EN ISO J01:2003), washing fastness (SR EN ISO 105-C 06:1999), acid perspiration fastness (SR EN ISO 105-E 04:2009), alkaline perspiration fastness (SR EN ISO 105-E 04:2009), rubbing fastness (SR EN ISO 105-X12:2003).

RESULTS AND DISCUSSIONS

Finishing Operations Influence on Physical-Mechanical Characteristics of Knits

Table 3. Physical-mechanical characteristics of 50% Tencel/ 50% PCM fibers

Code	Mass, g/m^2	Density/10 cm course no./ horizontal	Density/10 cm row no./ vertical	Thickness mm	Maximum force, N horizontal	Elongation, % horizontal	Abrasion resistance, cycles no.
Raw	225	110	130	0.93	193.0	168.0	18810
AV$_1$	213	109	112	0.85	198.8	164.0	14243
AV$_2$	202	106	107	0.83	209.0	171.0	14652
AV$_3$	220	104	115	0.85	176.6	156.0	15079

As regards the influence of the applied finishing processes on the physical-mechanical characteristics of the knitted fabric made of 50% Tencel/ 50% PCM fibers the following can be concluded (Table 3):

- the finishing processes induce the decrease of mass, density and thickness characteristics, within normal limits, as compared to raw knitted fabric, indicating the knitted fabric relaxation during finishing: mass decreases by 2-10%, density decreases horizontally by 1-6 course/10 cm and vertically by 15-23 rows/10 cm and the thickness decreases to less than 0.1 mm;
- maximum force and elongation at maximum force are not negatively influenced by the finishing process, except AV$_3$ sample (which was subjected to causticizing), to which there is a decrease of approx. 8.5% of maximum force and by 7% of elongation at maximum force, but these decreases are not significant;
- abrasion resistance decreases as compared to raw knitted fabric by a percentage between 19% and 24%, this behavior being correlate with the decrease of density in horizontal and vertical direction.

Table 4. Physical-mechanical characteristics of 70% Tencel/ 30% PCM fibers

Code	Mass g/m^2	Density/10 cm course no./ horizontal	Density/10 cm row no./ vertical	Thickness mm	Maximum force N, horizontal	Elongation, % horizontal	Abrasion resistance, cycles no.
Raw	208	114	107	1.01	158.4	207.0	21441
BV$_1$	199	103	103	0.83	188.6	194.0	18861
BV$_2$	208	105	112	0.83	214.0	161.0	16601
BV$_3$	199	105	112	0.86	211.0	159.0	18130

The analysis of physical-mechanical characteristics of knitted fabric made of 70% Tencel/ 30% PCM fibers (Table 4) indicates the following:

- variation in mass, density and thickness of finished knitted fabric as compared to raw knitted fabric is insignificant; there is a tendency of knitted fabric relaxation in horizontal direction for all the finishing variants and contraction in the vertical direction for BV_2 sample (scoured at 80ºC) and BV_3 sample (causticized at 70ºC);

- maximum force recorded higher values as compared to raw knitted fabric, but the elongation at maximum force decreased by approx. 22% in BV_2 sample and by 23% in BV_3 sample;
- abrasion resistance decreases as compared to raw knitted fabric between 12% and 23%, this behavior being correlate with the density decrease in horizontal and vertical direction.

Table 5. Physical-mechanical characteristics of 50% cotton/ 20% PA/ 30% PCM fibers

Code	Mass g/m^2	Density/10 cm		Thickness mm	Maximum force, N horizontal	Elongation, % horizontal	Abrasion resistance, cycles no.
		course no./ horizontal	row no./ vertical				
Raw	174	110	109	0.92	128.4	181.0	28240
CV_1	197	115	139	0.88	215.0	167.0	60135
CV_2	222	113	144	0.95	242.0	177.0	55141
CV_3	180	115	116	0.87	219.0	189.0	56374
CV_4	200	115	139	0.87	210.0	145.0	52557

In terms of physical and mechanical properties of finished fabrics made of 50% cotton/ 20% PA/ 30% PCM fibers (Table 5), the following can be noticed:

- after finishing there is observed the shrinkage of knitted fabrics, leading to increased values of the mass (g/m^2) and density/10 cm, characteristics compared with untreated knit; at the same time, the increasing of maximum force takes place; this behavior is correlated with increased density on the horizontal and vertical direction;
- also, the abrasion resistance is almost doubled as compared to raw knit, this behavior being correlated with increased density on the horizontal and vertical direction.

Table 6. Physical-mechanical characteristics of 70% PES/ 30% PCM fibers

Code	Mass g/m^2	Density/10 cm		Thickness mm	Maximum force, N horizontal	Elongation, % horizontal	Abrasion resistance, cycles no.
		course no./ horizontal	row no./ vertical				
Raw	111	111	107	0.88	115.0	143.0	22689
DV_1	150	129	129	0.98	120.0	105.6	36058
DV_2	167	140	139	1.05	110.6	91.2	38299
DV_3	173	146	149	0.97	127.7	119.0	39303
DV_4	149	124	123	0.95	118.6	105.2	29780

The finishing process influences the physical-mechanical characteristics of knitted fabric made of fiber blends 70% PES/ 30% PCM fibers, as follows (Table 6):

- increased values of the mass, density and thickness, for all types of finishing, as compared with raw knitted fabric, due to the contraction of knitted fabric in wet environment;
- at the same time the increasing of maximum force and abrasion resistance take place;
- however, elongation at maximum force decreases for all types of finishing applied, with values ranging between 16.8% and 36.2%.

Dyeing Process Performance

Table 7. Dyeing process performance - Colour differences

Code	X	Y	Z	Colour difference					
				DL*	DC*	DH*	DE*	Mark	Observation
AV_1	54.23	58.91	11.55	REFERENCE					
AV_2	55.44	60.42	11.41	0.82	1.85	0.35	2.05	4-5	lighter, saturated, greener
AV_3	55.48	60.68	14.56	0.96	-5.88	1.36	6.11	3-4	lighter, unsaturated, greener
BV_1	55.34	60.10	11.10	REFERENCE					
BV_2	55.73	60.88	12.76	0.42	-3.66	1.08	3.84	4	lighter, unsaturated, greener
BV_3	56.01	60.96	12.20	0.47	-2.17	0.47	2.27	4-5	lighter, unsaturated, greener
DV_1	21.55	12.01	11.27	REFERENCE					
DV_2	20.19	11.26	10.95	-1.22	-1.38	-1.11	2.15	4	darker, unsaturated, bluer
DV_3	18.35	10.15	9.61	-3.12	-2.51	-0.32	4.02	3	darker, unsaturated, bluer
DV_4	20.04	11.23	11.05	-1.26	-1.94	-1.44	2.73	4	darker, unsaturated, bluer

Evenness of dyeing was obtained for all finishing variants. In the case of 50% Tencel/50% PCM fibers and 70% Tencel/ 30% PCM fibers, color differences between samples were of ½ - 1 ½ tone, which is normal when different types of pretreatment are applied. In finishing processes which include bio-polishing operations (AV_2, BV_2) and causticizing-bio-polishing (AV_3, BV_3), lighter colors are obtained, as compared to the reference sample (AV_1, BV_1). Knit sample made of 70% PES/ 30% PCM fibers which were subjected to causticizing (DV_2, DV_3, DV_4) are more intensely dyed than the reference sample (non-causticized DV_1), the color difference ranging between 1-2 tone.

Table 8. Dyeing process performance – Color fastness

Code	Washing				Acid perspiration				Alkaline perspiration				Rubbing	
	Color change	Color staining			Color change	Color staining			Color change	Color staining			Dry	Wet
		CO	PA	PES		CO	PA	PES		CO	PA	PES		
AV_1	4/5	5	5	5	5	4/5	5	5	5	4/5	5	5	5	45
AV_2	5	5	5	5	5	4-5	4/5	5	5	4/5	5	5	5	4
AV_3	5	5	5	5	5	5	5	5	5	5	5	5	5	4/5
BV_1	4-5	4	5	5	5	4/5	4/5	5	4/5	4	4/5	4/5	5	5
BV_2	5	5	5	5	5	5	5	5	5	5	5	5	5	5
BV_3	5	4-5	5	5	5	4/5	5	5	5	4/5	5	5	5	5
CV_1	4/5	4/5	4/5	4/5	4	4	4/5	4/5	4	3/4	4/5	4/5	4-5	3/4
CV_2	4/5	4	4	4/5	4/5	4/5	4	4/5	4/5	4	4	4/5	5	4/5
CV_3	5	5	5	5	4/5	5	5	5	4/5	4/5	4/5	5	5	4/5
CV_4	4/5	4	4-5	4/5	4	4	4/5	4	4	4/5	4/5	4/5	4-5	3
DV_1	4/5	4	3	3/4	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	5	4/5
DV_2	4/5	4/5	3/4	4	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	5	4/5
DV_3	4/5	4	2/3	3/4	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	5	4/5
DV_4	4	4	2	3/4	4/5	4	4	4/5	4/5	4	4	4	4	4

In terms of color fastness properties (Table 8) it has been noticed that the dyeing fastness to washing, acid/alkaline perspiration, wet/dry rubbing is very good in blends of PCM fibers with Tencel fibers dyed with a bi-functional reactive dyestuff, the marks obtained in this case for change of shade and staining of the multi-fiber standard ranging between 4-5/5 (predominantly 5). An improved fastness to washing and alkaline perspiration is noted, in case of samples which include bio-polish operation, marks obtained in the change of shade being higher by ½ tone. The dyeing fastness is generally good in blends of PCM fibres with cotton/PA or with PES, most of marks being 4-5/5 (predominantly 4-5). Lower fastness to alkaline perspiration and wet rubbing (marks 3/3-4) are obtained in the case of 2 steps dyeing with reactive/selected reactive dyes (for wool/PA type fibers) of cotton/PA/PCM fiber blends (CV_1, CV_4). Also, lower washing fastness is obtained in the case of PES/PCM fiber blends subjected to bio-polishing subsequent to the dyeing operation (DV_3, DV_4) where the staining of PA and PES standard fibers is high (marks 2/2-3/3-4). From this reason it is recommended to perform the bio-scouring operation before the dyeing operation.

CONCLUSIONS

All finishing processes applied on knitted fabrics made of PCM fibers in blends with natural, man-made or synthetic fibers are appropriate and do not negatively affect the physical-mechanical characteristics of knitted fabrics. The behavior during finishing of knitted fabrics varies, depending on the fiber components blended with PCM fibers. In the case of blends with Tencel fibers, the knitted fabric relaxes during finishing, inducing decrease under normal limits of mass, density and thickness characteristics, without negatively influencing maximum force and elongation at maximum force. In the case of blends with cotton/PA fibers or with PES fibers, the knitted fabric shrinks, leading to increased values of mass, density and thickness characteristics. At the same time maximum force and abrasion resistance increase. The decreasing of the elongation at maximum force after finishing of knitted fabric containing 70% PES is under normal limits. This is caused by the heat-setting operation. As regards the dyeing process, all samples are uniformly dyed with color differences depending on the applied finishing process. Dyeing fastness is very good in blends of PCM fibers with Tencel fibres and good in blends of PCM fibres with cotton/PA or with PES fibers.

Acknowledgement

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REFERENCES

Jocic, D. (2010), "Functional finishing of textiles with responsive polymeric systems", *Surface modification systems for creating stimuli responsiveness of textiles*, D. Jocic Ed., University of Twente, Enschede, The Netherlands, pp. 37-59.

Mondal, S. (2008), "Phase change materials for smart textiles – An overview", *Applied Thermal Engineering*, 28, pp. 1536-1550.

Popescu, A., Mocioiu, A.M. and Hulea, C. (2011), "Study procedures for finishing textile materials with PCM content", *Proceedings of The 4th TexTeh International Conference*, Bucharest, pp.67-75.

***http://www.cellsolution.eu/products/cell-solution-climafiber/

HARNESS/CONTAINER EQUIPMENT FOR PARACHUTES - SAFETY AND PERFORMANCE DESIGN

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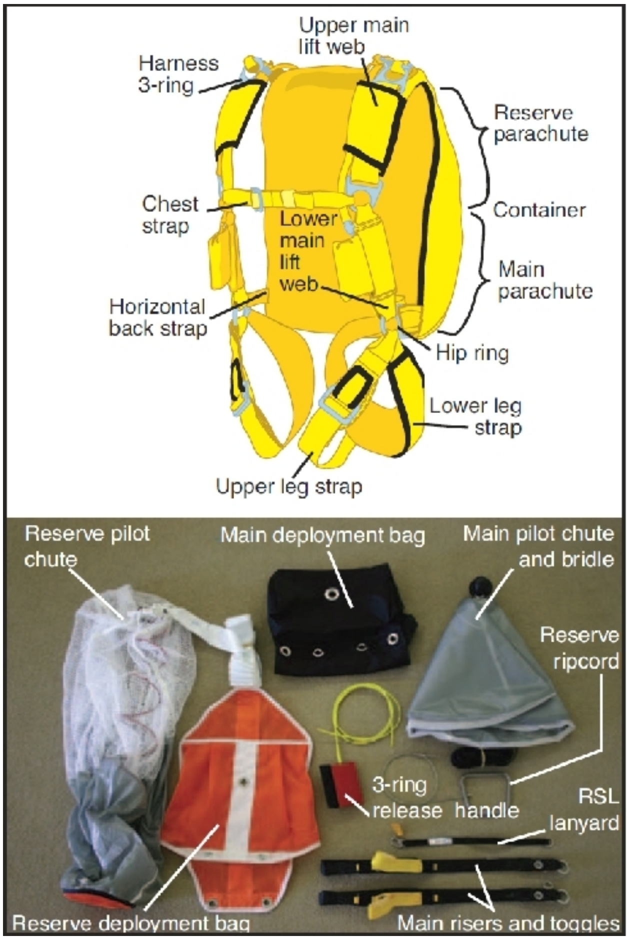
The overall objective of the research is to achieve a multifunctional harness / container assembly for parachutes, adaptable to a big range of canopy types and packing volume. The container is designed to take over and maintain main and reserve parachute canopies in a folded stage. Container / harness assembly is the safety system of the parachutist; it controls the deployment and opening of parachutes. The main components and subassemblies of container are specific with the type of parachutes connected to the container (Romanian Air Club, 2013). Containers for sport parachutes have two compartments, the bottom compartment for main parachute and its subassemblies and the upper compartment for reserve parachute and its subassemblies. Sports containers have distinct requirements for main parachute compartment and reserve parachute compartment. Currently the sizes of reserve canopy parachute and main parachute canopy compartments are manufactured in accordance with the volume of folded canopies. The project innovation is to develop a multifunctional container with adjustable variable compartments volume that allow the hosting of three different sizes of main and reserve parachutes. We propose for the variable volume compartments to be achieved by adjusting the container side panel in three positions adequate with the three packing volume of the parachutes.

Keywords: main container, reserve container, harness, main parachute, reserve parachute

INTRODUCTION

Harness / container assembly is the safety system of the parachutists; it controls the deployment and opening the parachutes. Any round or wing canopy can be connected to the container in specific compartments of the container so that they are open. The container controls the canopies opening and the harness ensures the link of the parachutist with canopy. The assembly harness / container include all parts needed to make a parachute airworthy. Basic assembly harness / container is what remains when all detachable assemblies (without being fixed by stitching) are removed.

Containers for sport parachutes have 2 compartments, one for main parachute with its subassemblies (pilot chute for main parachute, riser webbing; piloting controls; main risers and quick connectors; main deployment bag; cord connecting the main parachute with spring pilot chute; cord contact pin curved to pull main parachute; control handles; automatic opening system; RSL system.) and one for reserve parachute with its subassemblies (pilot chute; piloting controls; Reserve Static Line system; soft links on the riser webbing; reserve deployment bag; metal handle for manual opening of reserve parachute; AFF), Figure 1 (US Dept. of Transportation, 2005).



Source: Parachute Rigger Handbook; Chapter2-Design and Construction

Figure 1. Harness/container assembly and Subcomponents

Sports containers have distinct requirements for main parachute compartment and for reserve parachute compartment.

To achieve operational requirements it takes a well thought out design so, the reserve parachute compartment is generally small, tight, and especially wedge shaped when used for a wing type canopy.

Main parachute compartment design is less restrictive than the reserve parachute.

Currently the compartment sizes canopy parachute container reserve and main parachute canopies are made in accordance with the volume in folded state of the canopy. For example ICON container size (code) is designed to host only a certain range of parachute packing size for reserve and main parachute, Table 1 (Aerodyne Systems, 2005):

Table 1. Correspondence between the volume of the reserve parachute and main parachute and container size

Code container ICON	Reserve parachute volume / Max. volume accepted (m^3)	Main parachute volume / Max. volume accepted (m^3)
I2	0,0016 – 0,0018/0,0045	0,0014 – 0,0017/0,0053
I3	0,0018 – 0,0020/0,0048	0,0018 – 0,0022/0,0060
I4	0,0020 – 0,0024/0,0056	0,0021 - 0,0024/0,0064
I5	0,0024 – 0,0028/0,0063	0,0024 – 0,0028/0,0068
I6	0,0028 – 0,0036/0,0078	0,0028 – 0,0034/0,0076
S7	0,0031 – 0,0041/0,0080	0,0037 -0,0041/0,0083
S8	0,0036 – 0,0041/0,0080	0,0041- 0,0044/0,0086

Harness standard configuration is equipped so as to ensure the torso, head, arms and legs with straps, adding later survival kits or pillows.

In the past the parachute systems had detachable harness from the container, allowing the interchangeability of different models. With the development of sport parachuting the suspension system (harness) began to be integrated into the container, resulting assembly harness /container. This was accomplished by sewing the harness in the container. One of the most innovative models adopted in recent years is “articulated” harness that incorporates metal rings at the junction hip and a chest strap, Figure 2 (US Dept. of Transportation, 2005).

ICON harness is also produced in several sizes, coded according to Table 2 (Aerodyne Systems, 2005).

a) b)

Figure 2. a) Articulated harness configuration b) Icon harness

Table 2. ICON harness sizes coding

Harness code	Harness size
A	XXSpecial
B	XSmall
C	Small
D	Medium
E	Large
F	XLarge
G	XXLarge
H	XXXLSpecial

The materials used in the manufacture of container must be standardized as materials for parachutes. The well-known fabrics are Cordura Type III, 1000 denier coated one side with polyurethane film, with high durability and resistance to abrasion, tears and scuffing (MIL-C-7219, 1987).

Harness webbing must be woven with the shuttle because both sides look the same. This type of weaving creates a connection lock, which prevents it from unraveling if the edge is jagged or torn webbing, in conformity with MIL standards (MIL-STD-1480, 1988; MIL-W-27265, 1988). The tensile strength must be minimum 3000 daN.

Metallic accessories will be made of carbon steel, special steel and corrosion-resistant metals.

EXPERIMENTAL PART

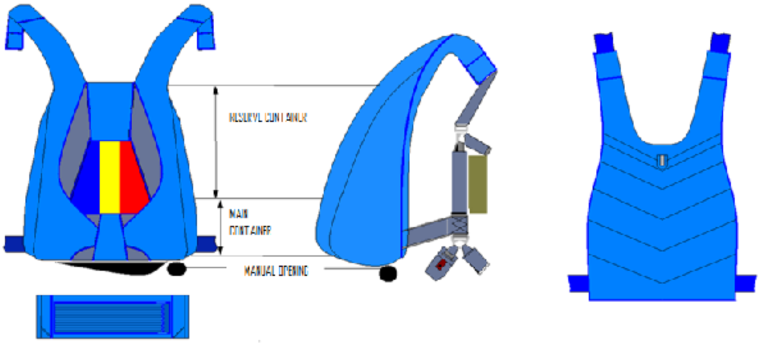
Multifunctional Container Design

The designed container model is presented in Figure 3. To achieve multifunctional use, a container that can be used with main and reserve canopies of different volumes, our innovation consists in an adjustable side panel of the container. We will obtain a container that will be able to accommodate three different volumes of main and reserve parachutes. This will be done in two ways:

Option 1: using string and rig rings, which will raise the container side panel in three positions adequate to the three volumes of parachutes, Figure 4 a). In order to preserve the shape of the container side panel edges will be strengthened, achieving volume variation on the middle panel. Also to avoid exposing the cord during flight this panel will be covered with a flap;

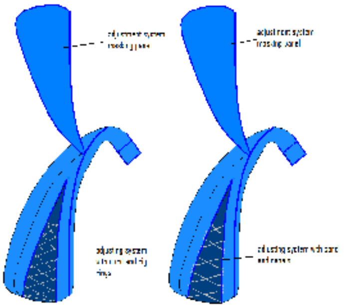
Option 2: with cord passing through the side channels. Tightening positions and dimensions are identical to the former. In this version the possibility of hanging in flight is eliminated Figure 4 b).

Sizing multifunctional container was made for a container with a larger volume, which can be reduced to a smaller volume of folded canopies. The adjustment will be done in three steps, marked on the tightening cords, and will be locked in these positions.



a) b)

Figure 3. Multifunctional container. a) Front and side view; b) Back view



a) b)

Figure 4. Adjustable side panel. a) O1: with cord and Rig Rings; b) O2: with cord and canals

Harness Design

The harness will be adjustable on the parachutist body and incorporated into the container. The parts of the harness are shown in Figure 5.

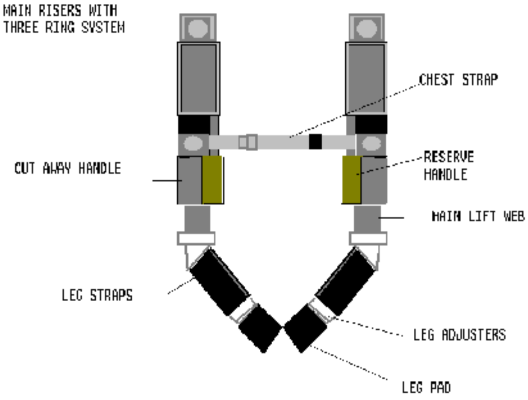


Figure 5. The parts of adjustable harness

RESULTS AND DISCUSSION

The suspension system (Harness) and container are components of parachutes.

To achieve the objective, namely designing a multifunctional container that can be used with main and reserve canopies with different volumes we have proposed an innovation that consists in adjustable side panel of the container. This will be done in two ways:

- Option 1, with cord and rig rings;
- Option 2, with cord and canals.

The adjustment of compartment volume will be done in 3 steps, marked on the tightening cords, and will be locked in these positions.

The container was custom designed for Romanian parachutists participating in international competitions.

CONCLUSION

As a general conclusion the performances of the multifunctional harness/container, experimental model, will be tested and verified on the ground.

Acknowledgements

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REFERENCES

Aerodyne Systems (2005), “ICON Harness Container Packing Manual”, South Africa.
Romanian Air Club (2013), “Know your parachute”.
U.S. Department of Transportation Federal Aviation Administration-Flight Standards Service (2005), “Parachute Rigger Handbook”.
*** MIL-C-7219 (1987), Military Specification: Cloth, Duck, Nylon.
*** MIL-STD-1480 (1988), Color Codes for Webbing, Textile Manufactures ID.
*** MIL-W-27265 (1988), Military Specification: Webbing, Textile, Woven Nylon.

IMPROVED FIT AND PERFORMANCE OF FEMALE BULLETPROOF VESTS

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The body armor for military personnel should provide a balance of protection and comfort. For both men and women, if body armor does not fit correctly and provide adequate coverage, it can adversely affect the safety and effectiveness. The design and manufacture of the body armor for female officers can present issues of particular concern. For now, Romania's female soldiers don't have any other choice than to wear men's body armor of smaller sizes, even though their body shape is different. For many women, that means wearing a standard issue vest that is tight across the torso and too loose around the middle can cause discomfort and offers less protection. The aim of the project was to develop a new protective bullet proof vest that is manufactured taking into account the female body shape, that provides comfort, mobility and adjustment possibilities, while superior ballistic resistance properties will be similar to the existing body armors. For morphological characterization of the target group, the women recruited in the national defense system, it was made an anthropometric survey using 3D scanning technology the human body in order to provide the necessary body size database to design the ballistic body armor. From primary anthropometric data we extracted the body dimensions required in designing the protective bullet proof vests. The novelty in the body armor design consist in taking into account the shape and cup size of Romanian female body. For the determination of the cup size we selected these critical dimensions: bust and under-bust circumferences.

Keywords: Body armor, anthropometric survey, female bulletproof vest, 3D simulation.

INTRODUCTION

The main function of the bulletproof vests is to provide protection, in order to reduce the impact and prevent the penetration of weapons of any kind. Women's participation in various activities that were previously allowed only to men grew over the years. Thus an increasing number of women are employed in jobs that require high physical stress, such as military or fireman. Since women are not the majority, the equipment they wear has not been specially designed for use by them. Historically, all uniforms and protective equipment for military and paramilitary personnel have been designed taking into account the sizes of the men personnel. Additionally, most manufacturers design bullet proof equipment for certain types of threats and not for certain users (Chen and Yang, 2010). Women working in the military field are ordered to wear bullet proof equipment that does not correspond with their body shape. As the responsible for US Programme for bullet proof equipment for female staff it must be taken into consideration that "women are not small man". Current studies, made by the US Department of Defence (DoD) shows that when the bulletproof vests are worn by women, there is a free space between the breasts and the protection panel, and this free space can be large enough to fit a grenade, creating an additional risk for women soldier. The smallest size of bulletproof vest is too wide or too long for 85% of female personnel. Moreover it is difficult to breathe due to pressure from the front ballistic panel on the chest area. These factors increase the risk of injury for women who wear these types of bulletproof vests. There are several properties of the bulletproof vests that define them. Of these impact resistance and comfort, defined as body adjustment and mobility are the most common and most necessary needs of wearers of such equipment. Nowadays we cannot talk about the design of new, comfortable and ergonomic bullet proof equipment without performing in-depth anthropometric studies.

The aim of the project was to develop a new protective bullet proof vest that is manufactured taking into account the female body shape, that provides comfort, mobility and adjustment possibilities, while superior ballistic resistance properties will be similar to the existing body armors.

METHOD

For the morphological characterization of the target group for women in the national defence system, in order to provide the necessary database for designing the bulletproof equipment, as part of the project, an anthropometric survey was performed by using 3D body scanning technology. The equipment used in performing the anthropometric survey is a mobile system 3D Body Scanner VITUS XXL Anthroscan Professional based on laser triangulation optical system technology. 3D scanning methodology complies with *EN ISO 20685:2005*-3-*D scanning methodologies for internationally compatible anthropometric databases.* The standard protocol is to use 3D surface scanning systems in the human body shape data acquisition. Measurements that can be extracted from 3D scans are according to: *ISO 7520-1:2008 Basic human body measurement for technological design-Part 1: Body measurement definitions and landmarks and ISO 8559:1989 Garment construction and anthropometric surveys-Body dimensions*.

Following the established protocol of body scanning, there were measured by 3D scanning a number of 105 subjects, women in the national defence system (Niculescu *et al.*, 2010). The 150 3D sizes automatically retrieved during a scan for each of the 105 subjects were stored in a database. 56 anthropometric sizes of the 150 sizes measured in 3D scanning were selected and described and will be subject to statistical analysis.

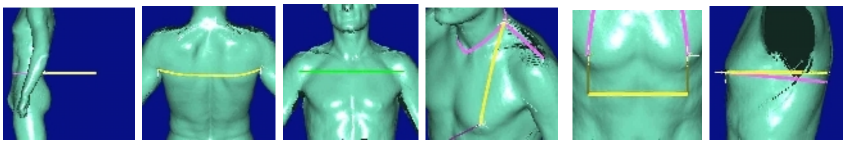


Figure 1. Examples of selected anthropometric sizes

After completing the anthropometric survey, each anthropometric size selected for morphological indicators specific to the target group is subject to unidimensional statistical processing by using EXCEL software package. The calculated statistical parameters allow the anthropomorphological assessment of women in national defence system and the establishment of a primary database required in the design of protective bullet proof equipment.

From primary anthropometric data resulted from the pilot sample scans, there were extracted the body sizes required in designing the protective bulletproof vest: bust and under-bust circumferences. The dispersion diagram and the correlation between the two body sizes are presented in Figure 2.

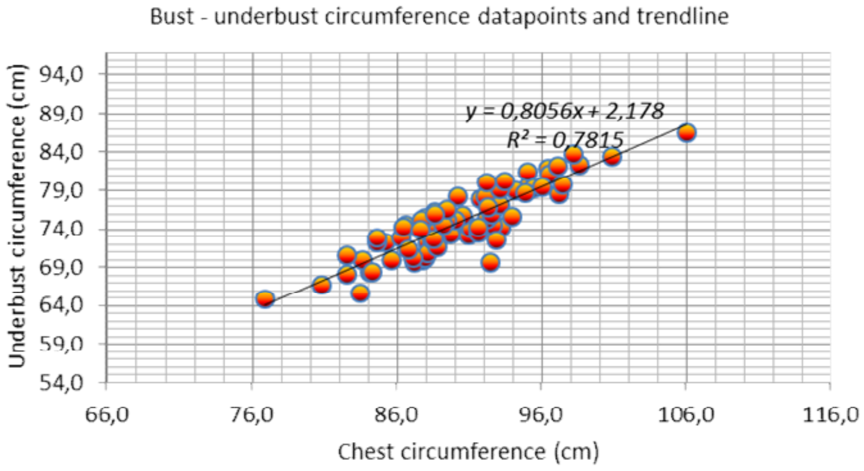


Figure 2. Dispersion diagram and regression line

The age group of the pilot sample under anthropometric pilot survey is 18-23 years. The cup size distribution in the pilot sample is shown in Figure 3.

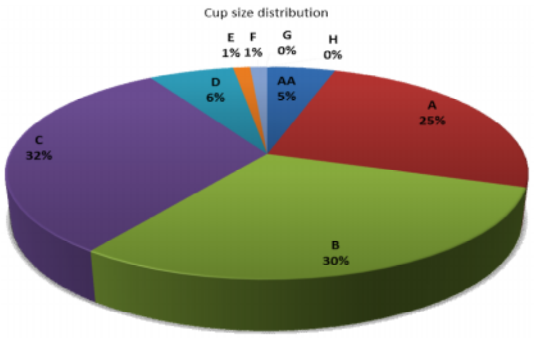
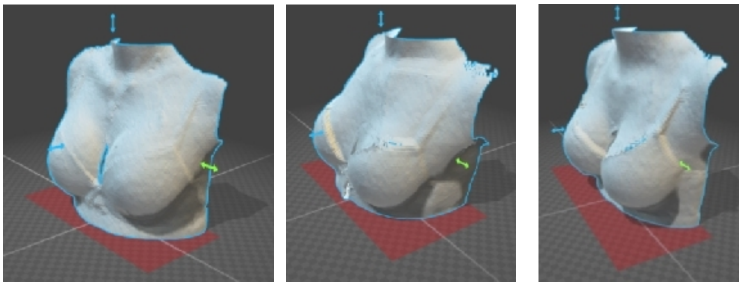


Figure 3. Cup size distribution

Cup size distribution analysis of the pilot sample presented in Figure 3 showed the highest percentage for cup size C (32%), followed by size B (30%) and size A (25%). Anthropometric data of the pilot sample, selected as needed to design the bullet proof vest for cup sizes with the highest frequency, was subjected to statistical analysis by calculating averages on both the total sample and the representative sample of each type of cup.



Cup A Cup B Cup C

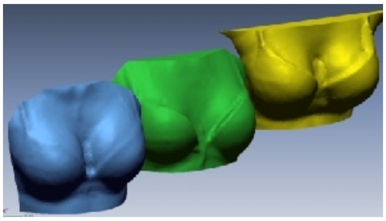


Figure 4. 3D shapes of avatar body anterior parts a) size S cup A, b) size S cup B, c) size M cup C

It was established the size of bulletproof vest presented in Table 1.

Table 1. Size of bulletproof vest

Body sizes		Size of bulletproof vest		
		Size	Cup	
Bust circumference, cm	Waist circumference, cm		Cup size	Note
87-89	68-72	S	A	For bra 75, cup A
89-91	68-72		B	For bra 75, cup B
91-93	73-78	M	C	For bra 75, cup C

The functional model of bulletproof vest was developed for size S cup A, in accordance with anthropometric measurements of this size. Matrices for preforming the ballistic package were made in accordance with 3D shapes of the anterior parts of the avatar bodies corresponding to size S cup A and B and size M cup C, Figure 4 a) b) and c).

The physical development of the functional model by STIMPEX SA was preceded by its virtual development using solutions for digital design of the vest patterns, modelling and 3D simulation of the vest on an avatar from the sample scanned corresponding to size S. It was used the software suite OptiTex. The virtual development of the functional model was made in two versions:

V1: *3D knitted inner layer* with a mass of 756 g/m^2 and a thickness of 6.31 mm followed by *ballistic protection package no. 1* with a thickness of 5.93 mm and 4,000 g/m^2 (formed of 20 layers of Twaron fabric CT 709);

V2: *3D knitted inner layer* with a mass of 756g/m^2 and a thickness of 6.31 mm followed by *ballistic protection package no. 2,* with a thickness of 2.7 mm and 1800 g/m^2 (formed of 9 layers of Twaron fabric CT 709).

The steps to develop the virtual functional model were: a) 2D pattern design from data in the dimensional table of the model and in correlation with the real sizes of protective bulletproof vest using Pattern Design Software (PDS) from OPTITEX; b) Simulation bulletproof vest functional model on the avatar using Optitex 3D Suite Software; c) Evaluating the fit of the product on the body, such as the distance between the textile material and the body surface, tensions developed in the textile material and their orientation by using 3D software functions such as tension scale, technical characteristics of the material.

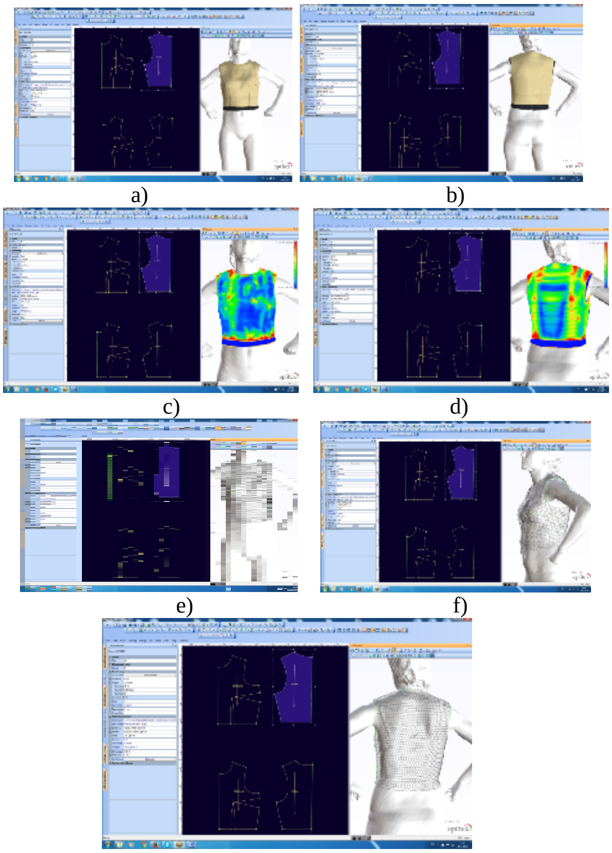

a) b)
c) d)
e) f)
g)

Figure 5. Simulation of the vest developed from the ballistic package 1 on the avatar a), b) checking the aspect of the vest front / back, c) and d) checking the matching body-product, e), f) and g) 3D visualization of the vest as a mesh of triangles

Checking compliance of the functional model with the anthropometric survey results revealed the following:

- patterns of the vest in ballistic package 1 and ballistic package 2 should be modified by increasing the length of the front side and the back of the vest;
- rechecking patterns of the vest in ballistic package 1 and increasing the addition of looseness;
- rechecking the simulation and a new evaluation of the correspondence body-product.

CONCLUSIONS

The body armor for military personnel should provide a balance of protection and comfort. For both men and women, if body armor does not fit correctly and provide adequate coverage, it can affect safety and effectiveness.

Designing, manufacturing and testing female specific body armors present a complex technical challenge. A material that works perfectly well as a flat armor may not perform well when subjected to folding, cutting, stitching or even changes in the stresses in the materials as it is shaped to provide protection. Additionally, there are comfort factors that impact the wearability of armor, and if an armor is uncomfortable, it is less likely to be worn, leaving the officer unprotected.

The female body armour technologies need to provide the right shape together with the right ergonomics for the benefit of female wearers in terms of protection, wearability and comfort.

The possibility to check the aspect of the product and how it is matching on the 3D model of the human body, without requiring physical development of a prototype is the main advantage of the virtual prototypes. If the product does not fit properly on 3D surface of 3D model (dummy/ avatar), the designer can easily modify the shapes of the parts and then check the new shape by repeating the steps of 3D simulation process in virtual space.

Acknowledgement

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REFERENCES

Chen, X. and Yang, D. (2010), “Use of 3D angle-interlock woven fabric for seamless female body armor: part 1: ballistic evaluation”, *Textile Research Journal*, 80(15), 1581-1588.

Niculescu, C., Filipescu, E. and Avadanei, M. (2010), “General aspects concerning the development of a female dimensional tipology using 3D body scanning measurements”, *Industria Textila*, 61(6), 271-275, Ed. CERTEX-Bucharest, ISSN 1222-5347.

Smith, B.L. and Ting, C.L. (2009), “Molded torso-conforming body armor including method of producing same“, United States, Patent No: US 2009/0255022 A1. 2009.

Ter Haar, F.B., Reulink, H.G.B. and Daanen, H.A.M. (2013), “3D Scanning of Dutch Military - Secular Trends in PCA for 18,000 Soldiers”, *4th International Conference on 3D Body Scanning Technologies*, pages 144-150, ISBN 978-3-033-04300-8, Long Beach CA, USA.

*** OptiTex 3D Suite. Available from: http://www.optitex.com.

INVESTIGATION OF BIOMECHANICAL PARAMETERS AND THEIR ASSOCIATION WITH AGE IN THE CASE OF ELDERLY WOMEN

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Gait and balance disorders are common in older adults and they are independent predictors for the functional decline resulting in various degrees of disability and loss of independence. Gait disorders have multiple causes, they have a progressive course and may negatively impact the functional performances. Many gait disorders appear in connection with underlying diseases. The incidence of gait deficiencies is greater in older women. Non-neurological causes are more often identified. Painful joint deformities are common causes of gait disturbances in elderly. The study proposes to identify the influence of age on the walking speed and the support phase during walking. The study included 100 female volunteers, aged between 55 and 87. The subjects participated in the measurement of biomechanical parameters using AMTI's AccuGait System, while speed was determined using a speed measurement system placed above the ground reaction force measuring platform. It was found that there are statistically significant differences among the three age groups in terms of walking speed and support phase.

Keywords: elderly, gait disorders, travel speed, stance time

INTRODUCTION

Walking and the postural balance are fundamental physiologic processes which enable the individual to properly respond to challenges and to integrate in the environment. Both are motor functions of great complexity relying on the anatomic and functional integrity of the musculoskeletal system as the peripheral organs of locomotion, the somatosensory pathways and of the motor cortex and the brain stem control as well.

Gait and balance disorders are common in older adults and they are independent predictors for the functional decline resulting in various degrees of disability and loss of independence. They often involve multiple contributing factors and a comprehensive assessment is essential to determine the targeted intervention. The functional limitations increase with age and the physiologic senescence processes are complicated by underlying medical condition not always attributable to aging itself. Gait disorders have multiple causes, they have a progressive course and may negatively impact the functional performances. Appropriate preventive and rehabilitation interventions are needed in order to avoid loss of independence and limited quality of life (Alexander and Goldberg, 2005).

Early identification of the most appropriate interventions relies on the diagnostic approach of the walking impairments emphasizing on the level of the deficit and the underlying medical condition. The clinical classifications categorize the deficits according to various criteria (Jahn *et al.*, 2010; Nutt *et al.*, 1993; Nutt, 2001; Thompson, 2007). Clinically oriented classifications are preferred in the current practice.

The classification proposed by Nutt and Marsden (1993), for example, is a three-level classification of the gait and postural balance disorders according to the level of the deficit (Nutt *et al.*, 1993).

1. Higher-level disturbances
    - deficit of the cortical control of gait
    - anxiety-related gait disorders
    - psychogenic gait disorders
2. Intermediate-level disturbances
    - efferent and afferent sensorimotor pathways of the CNS (paresis after stroke, Parkinson’s disease - extrapyramidal manifestations, cerebellar dysfunction - incoordination)
3. Lower-level disturbances
    - arthritis
    - myopathy
    - polyneuropathy

The classification above provides valuable information on the level of the deficit. Nevertheless, clinicians often prefer categorizing the impairments strictly based on the clinical observation (Snijders *et al.*, 2007). Clinical evaluation is a sufficient as an initial approach and offer a precise guideline for the next step of the diagnostic approach, in most cases.

Walking deficiencies and balance impairment are frequent in older adults as a consequence of aging itself or as a result of age-related diseases.

Population-based studies review show that gait and balance disorders occur in 14% of individuals aged over 65, about 20% of people aged 80 report difficulties in locomotion and postural balance and the corresponding figure is about 50% beyond 85 years old (Martin and O'Neill, 2004; Sanders *et al.*, 2010).

It was found in one study performed in a family medicine setting that gait and balance disorders had multiple causes in 75% of the older adults. In most of the older patients examined in family settings, the self-reporting walking difficulties appeared in connection with underlying diseases (Hough, cited by Salzman, 2010): painful joints (deformities, arthritis) 37%, orthostatic hypotension 9%, stroke 10%, visual impairment 1%, back pain 5%.

RESEARCH METHODOLOGY

The study used quantitative methodology, and an exploratory and descriptive approach. The research methods which best fit the objective of the study were the biomechanical gait measurement for ground reaction force, the questionnaire-based survey, and observation (Vasilescu *et al.*, 2015; Mihai *et al.*, 2016; Gherman *et al.*, 2016).

The data was collected in Bucharest over a period of three weeks. The ground reaction force was measured using AMTI´s AccuGait System (www.amti.biz). The respondents were also applied a short questionnaire consisting of questions referring to anthropometric and demographic data, mainly enquiring about the respondents' age, height, weight and medical conditions.

The main statistic indicators characterizing the sample are presented in Table 1.

Table 1. Statistic indicators for the main demographic and anthropometric parameters

		Height *(cm)*	Weight *(kg)*	Age *(years)*
N	Valid	100	100	100
	Missing	0	0	0
Mean		161.75	72.96	67.05
Median		163.00	71.00	64.00
Mode		165	80	59
Std. Deviation		6.663	11.414	8.916
Minimum		148	50	55
Maximum		184	112	87

Source: SPSS software

The age of the participants varied between 55 and 87 (Fig. 1), with the eldest female subjects (over 71 years) making up most of the sample (34%), followed by the subjects in the 60-64 (27%) and 55-59 (26%) age groups, respectively). The average height of the subjects of the studied sample was 161.75 cm, the average weight was 73 kg and the average age was 67 years (Deselnicu *et al.*, 2016).

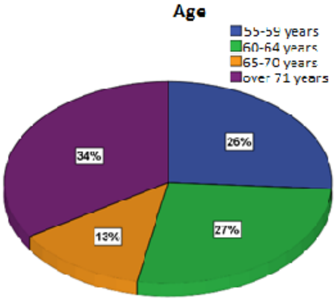

Figure 1. Sample structure by age (Source: SPSS software)

Data Analysis and Results Interpretation

Health Conditions Analysis

Important information correlated with the biomechanical parameters was provided by the answers to the health condition questionnaire. The elderly women participating in the study declared the illnesses that they suffer from.

Age is a very important parameter for the investigated sample. The participants have been divided into three age groups, to further investigate significant differences between the three groups in terms of important parameters. Figure 2 illustrates the differences regarding the declared health conditions across age groups:

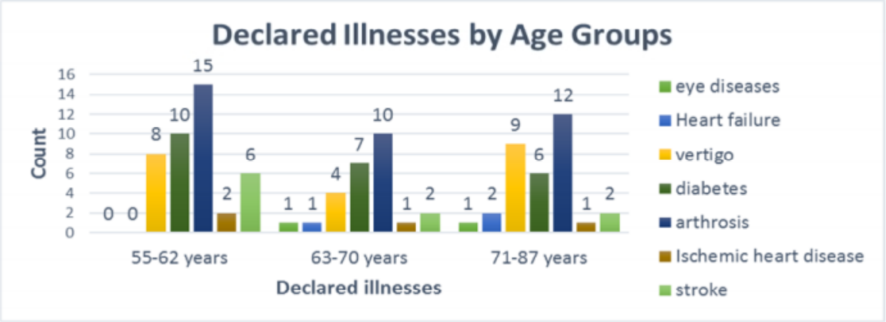

Figure 2. Distribution of declared illnesses by age groups

Data analysis shows that walking and balance disorders have multiple causes: arthrosis, diabetes, vertigo, eye diseases, heart failure, ischemic heart diseases, and stroke. In all three age groups, the most common diseases are: arthrosis, diabetes, and vertigo.

The distribution of the main biomechanical parameters that were investigated with the force platform are presented in Table 2.

Table 2. Biomechanical parameters statistic indicators

		Travel speed (m/s)	Stance time (s)
N	Valid	100	100
	Missing	0	0
Mean		.7004	.89870
Median		.7300	.82250
Mode		.49	.740
Std. Deviation		.18170	.277616

Source: SPSS software

The average support phase period when the foot is in contact with the ground is 0.89870 seconds, and the average walking speed is 0.73 m/s.

Furthermore, the authors investigated if the variance of the main biomechanical parameters which were tested was statistically significant across the three age groups. Two working hypotheses were formulated as follows:

H_1: There are statistically significant differences between the three age groups in terms of the travel speed.

H_0: There are no statistically significant differences between the three age groups in terms of the travel speed.

The analysis was performed using the SPSS software. The results are presented in Table 3.

Table 3. One-Way ANOVA test results for the variance of travel speed across age groups

		Sum of squares	df	Mean Square	F	Sig.
Travel speed	Between Groups	1.697	2	.848	52.362	.000
	Within Groups	1.572	97	.016		
	Total	3.269	99			

Source: SPSS software

The significance level for hypothesis H_1 is 0.000 (p=0.000), which is smaller than the significance level of 0.05 established for this analysis. Hypothesis H_1 was therefore accepted, confirming that there are statistically significant differences between the three age groups in terms of the travel speed. Consequently, the null hypothesis was rejected.

The second working hypothesis tested the variance of the support phase of elderly women across age groups:

H_2: There are statistically significant differences between the three age groups in terms of the support phase.

H_0: There are no statistically significant differences between the three age groups in terms of the support phase.

Table 4. One-Way ANOVA test results for the variance of the support phase across age groups

		Sum of Squares	df	Mean Square	F	Sig.
Support phase	Between Groups	.477	2	.239	3.237	.044
	Within Groups	7.153	97	.074		
	Total	7.630	99			

Source: SPSS software

The significance level of 0.044 (p=0.044) for hypothesis H_2 was calculated, which is smaller than the significance level of 0.05. Hypothesis H_2 was therefore accepted, confirming that there are statistically significant differences between the three age groups of elderly women in the investigated sample in terms of the support phase.

Age is, therefore, an important factor that influences important biomechanical parameters such as the travel speed and support phase in the case of elderly women.

CONCLUSIONS

Data analysis shows that age is an important factor influencing significant biomechanical parameters, such as the support phase and walking speed in the case of elderly women.

Acknowledgements

This work was supported by UEFISCDI Bucharest under the Partnership Programme project “Preventing gait deficiencies and improving biomechanical parameters for the elderly population by designing and developing customized footwear”, acronym MOBILITY, code PN-II-PT-PCCA 2013-4, Contract no. 122/2014.

REFERENCES

Alexander, N.B. and Goldberg, A. (2005), "Gait Disorders: Search for Multiple Cause", *Cleveland Clinic Journal of Medicine*, 72(7), 586-599.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A., and Militaru, G. (2016), "New Products Development through Customized Design Based on Customers' Needs. Part 1: Footwear Comfort Parameters", *Procedia Technology*, 22, 1043-1050.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A., and Militaru, G. (2016), "New Products Development through Customized Design Based on Customers' Needs. Part 2: Foot Pathology Manufacturing Parameters", *Procedia Technology*, 22, 1059-1065.

Henry, G.T. (1990), *Practical Sampling*, Sage Publications, Thousand Oaks.

Hough, J.C., McHenry, M.P., and Kammer, L.M. (1987), "Gait Disorders in the Elderly", *American Family Physician*, 35(6), 191–196.

Jahn, K., Zwergal, A., and Schniepp, R. (2010), "Gait Disturbances in Old Age, Classification, Diagnosis, and treatment From a Neurological Perspective", *Deutsches Ärzteblatt International*, 107(17), 306–316, published online Apr 30, 2010. doi: 10.3238/arztebl.2010.0306, PMCID: PMC2872829 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2872829/figure/F3/

Martin, M.P., and O'Neill, D. (2004), "Vascular Higher-level Gait Disorders--A Step in the Right Direction?" *The Lancet*, 363(9402), 8, http://www.ncbi.nlm.nih.gov/pubmed/14723986?dopt

Nutt, J.G. (2001), "Classification of Gait and Balance Disorders", PMID: 11347216 [PubMed - indexed for MEDLINE] http://www.ncbi.nlm.nih.gov/pubmed/11347216.

Nutt, J.G., Marsden, C.D., and Thompson, P.D. (1993), "Human Walking and Higher-level Gait Disorders, Particularly in the Elderly", *Neurology*, 43, 268–279.

Salzman, B. (2010), "Gait and Balance Disorders in Older Adults", *American Family Physician*, 82(1), 61-68.

Sanders, R.D. *et al.* (2010), "Gait and Its Assessment in Psychiatry", *Psychiatry* (Edgmont), 7(7), 38-43, published online 2010 Jul. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2922365/

Snijders, A.H, van de Warrenburg, B.P., Giladi, N., Bloem, B.R. (2007), "Neurological Gait Disorders in Elderly People: Clinical Approach and Classification", *The Lancet Neurology*, 6(1), 63–74.

Thompson, P.D. (2007), "Higher Level Gait Disorders", *Current Neurology and Neuroscience Reports*, 7(4), 290-4. Review. PMID:17618534 http://www.ncbi.nlm.nih.gov/pubmed /17618534.

ANALYSIS OF SELECTED ANTHROPOMETRIC PARAMETERS OF ELDERLY WOMEN

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This study was conducted to establish a set of major causal factors that influence foot pathologies and deformities for the elderly, analyzing variables such as weight and height of subjects. The study included 100 elderly female volunteers weighing between 50 and 112 kg and measuring between 148 and 184 cm in height. The sampling method used was the non-probabilistic one, based on a mixture of Henry's sampling types, using the questionnaire, and testing was conducted using measurement and observation instruments. Subjects participated in biomechanical measurements using AMTI's AccuGait System force platform, performed in Bucharest for a period of three weeks. The results show a strong correlation among the tested variables. Weight proves to be one of the most important causative factors, as there were statistically significant correlations between it and most biomechanical parameters measured. Thus, it can be concluded that as the elderly grow in weight, they undergo significant changes in biomechanical parameters, reflected in foot deformities and pathologies.

Keywords: elderly, anthropometric parameters, weight, height

INTRODUCTION

Walking is a complex motor activity, that is a physiological response to sensory information from the external environment, integrated at all levels of the nervous system.

Gait and postural balance disorders are common in geriatric clinical practice. As with any other aspect of elderly care, the boundary between the deterioration of locomotor functions caused by senescence processes and pathological processes, to which the elderly are most vulnerable by definition, is often difficult to establish. According to the literature, most gait and postural balance disorders are due to identifiable medical causes and are not only physiological consequences of aging (Jahn *et al.,* 2010; Salzman, 2010). Senescence is an independent risk factor for gait and balance postural dysfunctions. The assertion is reinforced by the high prevalence of these disorders in the geriatric population. The importance of the phenomenon results from its association with increased risk of falls, immobilization, increasing dependency and decreased quality of life (Jahn *et al.,* 2010; Stolze *et al.*, 2004).

Gait deficiencies and impaired postural balance are common in the elderly both in the physiological processes of senescence, and as a result of specific diseases. Clinical studies show that 14% of people over 65 have gait disabilities, in the age group up to 80 around 20% of individuals claim difficulties in walking and maintaining balance while walking, and after the age of 80, the percentage of people with postural and walk abnormalities reaches 50% (Martin and O'Neill, 2004; Sanders *et al.*, 2010).

RESEARCH METHODOLOGY

The study was conceived as a quantitative approach, mainly exploratory and descriptive. The research employed the following methods: biomechanical gait measurement for ground reaction force, the questionnaire-based survey, and observation (Vasilescu *et al*., 2015; Mihai *et al.*, 2016; Gherman *et al.*, 2016, Sarghie *et al.*, 2013, Costea *et al.*, 2014).

Data Collection

The data was collected in Bucharest over a period of three weeks. The ground reaction force was measured using a platform - AMTI´s AccuGait System (www.amti.biz). The respondents were also given a short questionnaire consisting of questions referring to anthropometric and demographic data, mainly enquiring about the respondents' age, height, weight and medical conditions.

Research Sample

The sample was composed of 100 elderly persons, all female. The method used for sampling was the non-probabilistic one, based on a mixture of Henry's types (1990) - convenience, typical cases, critical cases, “snowball”. The female volunteers weighed between 50 and 112 kg and measured between 148 and 184 cm in height.

The participants’ age was a very important criterion in the analysis. The sample structure by age distribution is described in Figure 1. The majority of tested elderly women were aged over 71 (34%), followed by those in the 60-64 age group (27%) and those aged 55-59 (26%). The 65-70 age group consisted in only 13% (Deselnicu *et al.*, 2016).

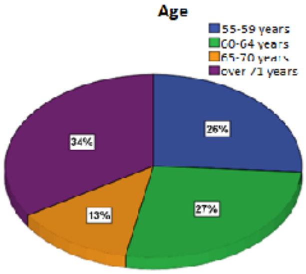

Figure 1. Sample structure by age (Source: SPSS software)

In order to have a more complete characterization of the sample, it was determined that the average height of the subjects was 161.75 cm, the average weight was 73 kg and the average age was 67.

Data Analysis and Interpretation

The sample was divided based on age groups of the elderly females, in order to emphasize the significant differences between them (Table 1).

Table 1. Statistic indicators of the variation of height and weight over age groups

Age groups (years) / Statistic indicators			Height (cm)	Weight (kg)
55-62 years	N	Valid	41	41
		Missing	0	0
	Mean		**161.54**	**71.71**
	Median		163.00	70.00
	Mode		165	62
	Std. Deviation		5.840	9.185
	Minimum		150	56
	Maximum		174	93
63-70 years	N	Valid	26	26
		Missing	0	0
	Mean		**162.19**	**75.92**
	Median		164.50	72.50
	Mode		165	70[a]
	Std. Deviation		6.165	12.709
	Minimum		150	60
	Maximum		173	112
71-87 years	N	Valid	33	33
		Missing	0	0
	Mean		**161.67**	**72.18**
	Median		163.00	69.00
	Mode		163	60[a]
	Std. Deviation		8.049	12.714
	Minimum		148	50
	Maximum		184	110

a. Multiple modes exist. The smallest value is shown

Source: SPSS software

It is seen that the sample is much more homogeneous in terms of height, registering minor variations in relation to age. Mean height for all age groups was placed around 161 cm. The sample is much more heterogeneous with respect to weight (large standard deviation), the largest weight recorded in the 63-70 age group (75.92 kg). The average weight registers a decrease in the oldest age group, so the weight does not increase linearly with age.

The weight distribution can be analyzed in Figure 2.

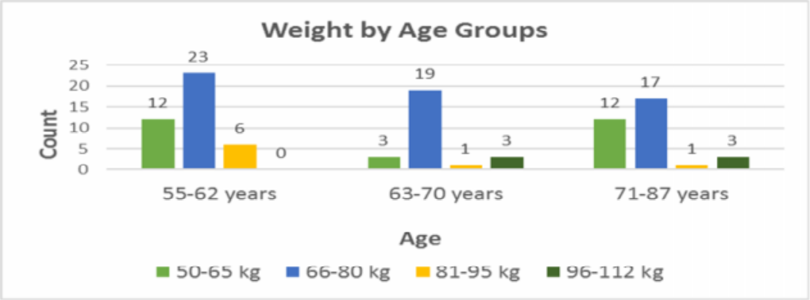

Figure 2. Weight distribution by age groups

Sample structure according to the variables of weight and age in Figure 2 is as follows: in the 55-62 age group, most subjects weigh between 66-80 kg, followed by those weighing between 50-65 kg and then those weighing between 81-95 kg; in the 63-70 age group, most subjects weigh between 66-80 kg, followed by those in the 50-65 kg range, then those weighing between 96-112 kg and then those weighing between 81-95 kg; in the 71-87 age group, most subjects weigh between 66-80 kg, followed by those in the 50-65 kg range, then those weighing between 96-112 kg and then those weighing between 81-95 kg.

Figure 3 illustrates the height distribution over the three age groups:

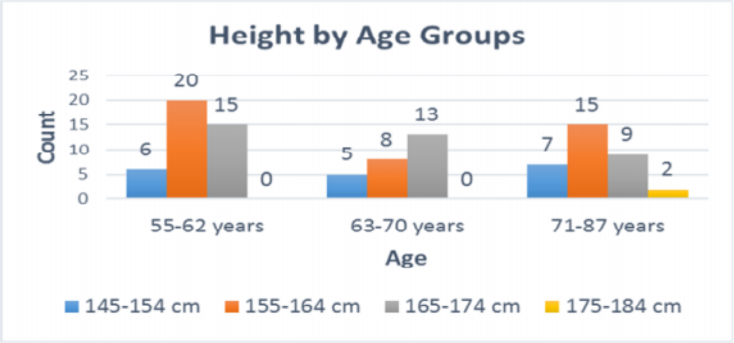

Figure 3. Height distribution by age groups

Height distribution in the three age groups shown in Figure 3 is as follows: in the 55-62 age group, the height of most subjects was between 155-164 cm, followed by those with 165-174 cm and 145-154 cm in height, respectively; in the 63-70 age group, the height of most subjects was between 165-174 cm, followed by those with 155-164 cm and 145-154 cm in height, respectively; in the 71-87 age group, the height of most subjects was between 155-164 cm, followed by those with height between 165-174 cm, then those with 145-154 cm and 175-184 cm in height.

Hypotheses Testing

In order to emphasize the variation of the demographic and anthropometric parameters across the investigated sample, One-Way ANOVA (Analysis of Variance) method was used. The method compares the means of three or more independent groups (in the present case, the three age groups of the investigated sample). One working hypothesis (H_1) was formulated:

H_1: There are statistically significant differences between the three age groups regarding weight.

H_0: There are no differences between the three age groups regarding weight.

In order to test the two hypotheses, the results of the One-Way ANOVA test are presented in Table 4.

Table 4. One-Way ANOVA test results for the variance of weight over age groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.761	2	.881	1.505	.227
Within Groups	56.749	97	.585		
Total	58.510	99			

Source: SPSS software

As p=0.227, greater than the confidence level of 0.05 established for this analysis, the alternative hypothesis has to be rejected, therefore the null hypothesis has to be accepted: there are no statistically significant differences between the three age groups of elderly women in terms of weight.

H_1: There are statistically significant differences between the three age groups regarding height.

H_0: There are no differences between the three age groups regarding height.

The results of the One-Way ANOVA to test these hypotheses are presented in Table 5.

Table 5. One-Way ANOVA test results for the variance of height over age group

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.238	2	.119	.201	.818
Within Groups	57.472	97	.592		
Total	57.710	99			

As p=0.818, greater than the significance level of 0.05 established for this analysis, this alternative hypothesis has also to be rejected, therefore the null hypothesis has to be accepted: there are no statistically significant differences between the three age groups of elderly women in terms of height.

CONCLUSIONS

To highlight anthropometric parameter variation in elderly women by age, One-Way ANOVA statistical test was used and no significant differences were found among the three age groups investigated in terms of weight and height.

Acknowledgements

This work was supported by UEFISCDI Bucharest under the Partnership Programme project “Preventing gait deficiencies and improving biomechanical parameters for the elderly population by designing and developing customized footwear”, acronym MOBILITY, code PN-II-PT-PCCA 2013-4, Contract no. 122/2014.

REFERENCES

Costea, M., Vasilescu, A.M., Hortal, G., and Mihai, A. (2014), “Plantar Footprints Analysis - Case Study (Part 2)”, *Leather and Footwear Journal*, 14(4), Certex Publishing House, ISSN 15834433, 243-250.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A., and Militaru, G. (2016), “New Products Development through Customized Design Based on Customers’ Needs. Part 1: Footwear Comfort Parameters”, *Procedia Technology*, 22, 1043-1050.

Deselnicu, D.C., Vasilescu, A.M., Mihai, A., Purcarea, A.A., and Militaru, G. (2016), “New Products Development through Customized Design Based on Customers’ Needs. Part 2: Foot Pathology Manufacturing Parameters”, *Procedia Technology*, 22, 1059-1065.

Gherman, D., Vasilescu, A.M., Tudorache, D.L., Oancea, C., Tudorache, D.L., Ciuvica, M.M., and Mirica, R. (2016), “Promoting Active Aging by Innovative Technologies in Developing and Manufacturing of Customized Assistive Products for Elderly”, *21st EUMASS Congress and the 4th International Congress of Medical Assessors in the Republic of Slovenia*, 09-11 June 2016.

Henry, G.T. (1990), *Practical Sampling*, Sage Publications, Thousand Oaks.

Jahn, K., Zwergal, A., and Schniepp, R. (2010), “Gait Disturbances in Old Age, Classification, Diagnosis, and treatment From a Neurological Perspective”, *Deutsches Ärzteblatt International*, 107(17), 306–316, published online Apr 30, 2010. doi: 10.3238/arztebl.2010.0306, PMCID: PMC2872829 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2872829/figure/F3/

Martin, M.P., and O'Neill, D. (2004), “Vascular Higher-level Gait Disorders--A Step in the Right Direction?” *The Lancet*, 363(9402), 8, http://www.ncbi.nlm.nih.gov/pubmed/14723986?dopt

Mihai, A., Vasilescu, A.M., Costea, M., Sarghie, B., Pantazi, M., and Deselnicu, D.C. (2016), “Customized Comfort in Footwear for the Elderly Female Population”, *The 19th International Technical Footwear Congress of the International Union of Shoe Industry Technicians (UITIC)*, 3-5 February 2016, Chennai, India.

Salzman, B. (2010), “Gait and Balance Disorders in Older Adults”, *American Family Physician*, 82(1), 61-68.

Sanders, R.D. *et al.* (2010), “Gait and Its Assessment in Psychiatry”, *Psychiatry* (Edgmont), 7(7), 38-43, published online 2010 Jul. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2922365/

Sarghie, B., Costea, M., and Mihai, A. (2013), “3D Modelling of Shoe Lasts Using Templates Based on Anthropometrical Measurements of the Foot – Case Study”, *Leather and Footwear Journal*, 13(3), 221-234.

Stolze, H., Klebe, S., Zechlin, C., Baecker, C., Friege, L., and Deuschl, G. (2004), “Falls in Frequent Neurological Diseases - Prevalence, Risk Factors and Aetiology”, *Journal of Neurology*, 251, 79–84.

Vasilescu, A.M., Pantazi, M., and Mihai, A. (2015), “Research on the Scientific Design and of Footwear for the Elderly”, *III International Leather Engineering Congress: Innovative Aspects for Leather Industry*, May 21-22, 2015, Izmir, Turkey.

EFFECT OF VARIOUS TANNING PROCESSES ON CHARACTERISTICS OF LINING LEATHERS

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Wear comfort, fit, and durability and more significantly linings are the main factors that affect the quality of shoe upper leathers. Lining leathers are generally produced from goat skins, cowhides as well as sheepskins mostly depending on the customer demands. Over the last few decades, soft, breathable and comfortable linings that provide a healthy environment for the feet skin have gained importance. As it is well known that tanning procedure has a direct influence on the above mentioned properties of the manufactured lining goods, this study aimed to determine the effect of different tanning processes on the wear comfort and lining characteristics of leathers for the foot health and wear comfort. For this purpose, the effect of four different tanning techniques was investigated on properties of lining leathers produced from goat skins in terms of dynamic and static water absorption, color fastness to perspiration, to and fro and crockmeter rubbing fastness tests. The tanning process of pickled goat skins was differentiated by applying wet-white, vegetable, mineral, and semi tanning procedures. Post tanning processes were carried out in accordance with the same conventional formulation throughout the production of the lining leathers. The findings reveal that different tanning techniques had a significant impact on the lining characteristics and the tanning method could be selected according to the use of the linings in variable shoe types and the customer demands.

Keywords: lining leathers, tanning, perspiration, water absorption

INTRODUCTION

Footwear is an important wear material used by human life. Various types of footwear produced by various types of materials have been developed. The footwear produced for industrial, agricultural, military, athletic and artistic purposes have certain differences and directly affect the wear comfort of the foot wears. Therefore, appropriate materials and manufacturing techniques should be taken into consideration for the footwear manufacturing (Bitlisli *et al*., 2005; Śmiechowski *et al*., 2014).

Lining leathers are one of the main factors that affect the quality of shoe upper leathers in addition to wear comfort and durability. The comfort of lining leather could be explained in terms of a comfort provided by the leather characteristics including various physical properties such as water absorption, rubbing and perspiration properties as well as water vapor permeability (Sari and Basaran, 1996; Sari and Bitlisli, 1996; Bitlisli *et al*., 2005). Nowadays soft, breathable and comfortable linings that provide a healthy environment for the feet skin have gained importance due to the customer demands. Although limited number of literatures describing the lining leather properties was found, they were published in earlier times (Diebschlag *et al*., 1976; Hernon, 1981; Shuttleworth, 1973) and no report was found addressing the effect of tanning technique on the lining leather properties and their effect on wear comfort in the current literatures.

In this study, the effect of four different tanning techniques such as wet-white, vegetable, mineral, and semi tannings was investigated on physical and comfort properties of lining leathers produced from goat skins in terms of dynamic and static water absorption, color fastness to perspiration, to and fro and crockmeter rubbing fastness tests.

MATERIAL AND METHOD

Material

In this study, domestic pickled goat leathers were used for the tanning process of the lining leathers. The conventional leather chemicals were used in the leather manufacturing processes.

Method

The Tanning Procedure

Chrome, vegetable, wet white and semi (chrome and vegetable tannin combination) tanning techniques were selected for the study. After conventional depickling, bating and degreasing processes, the tanning began with the pickling procedure. Chromium (8% chromium salt), vegetable (16% vegetable tanning, 5% synthetic tannin), wet-white (2.5 phosphonium and 3% aluminum tri formiate), and semi (3% chromium salt, 13% vegetable tannin) tanning procedures were performed conventionally. The tanning processes were followed by post tanning operation in terms of neutralization and fatliquoring using the combination of sulphited, phosphate and lubricating synthetic fatliquoring agents. After drying and mechanical operations, the goat skins were converted into crust lining leathers.

Sampling and conditioning of the lining leathers were performed according to TS EN ISO 2418 (2006) and TS EN ISO 2419 (2006) prior to physical tests. Also, the thickness of the leathers was determined with TS 4117 EN ISO 2589 (2006) standard.

Static Water Absorption

The static water absorption behavior of the lining leathers was tested according to TS 4123 EN ISO 2417. Kubelka apparatus was used for determination of the water absorption of the leathers under static condition and the measurements were carried out at 15 minutes, 30 minutes, 1h, 2h and 24h of the test.

Dynamic Water Absorption

The dynamic water absorption property of the lining leathers was examined with the standard of TS 8541 EN ISO 5403. For this study, the aim of this test was to determine the degree of the lining leathers wet-ability.

Color Fastness to Perspiration

TS EN ISO 11641:2012 standard was used as a method for determining the color fastness to perspiration. This method was for all kinds of leather at all stages of processing.

To and Fro Rubbing Fastness

The rubbing fastness properties of leathers were examined by Bally Finish Tester 9029 according to TS EN ISO 11640 standard (100 rubs in dry and 25 rubs in wet). Prior and subsequent to the rubbing tests, color measurements were performed and the changes in color were compared.

Crock-meter Rubbing Fastness

The crockmeter rubbing fastness of the leathers was performed in accordance with ASTM D5053 standard. The dry and wet rubbing fastness (10 rubs for each) was tested by Atlas CM5 (SDL ATLAS Company, USA) test device. The fabric used in the test was evaluated according to the standard ISO 105-A03 while the leather samples were evaluated as follows; 1 = Good, 2 = Medium, 3= Poor.

RESULTS AND DISCUSSION

The thickness values of the lining leathers were determined as 0.83±0.16; 0.83±0.05; 0.78±0.03; 0.99±0.07 for the chrome, vegetable, semi and wet-white tanning respectively.

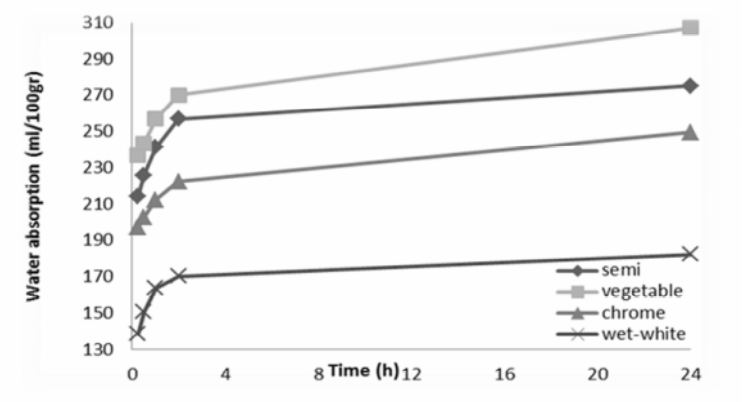

Figure 1. Kubelka (Static water absorption) values of lining leathers (ml/100gr)

Static water absorption (Kubelka) test results are shown in Figure 1. The highest water absorption was determined from the vegetable tanned lining leathers due to their hydrophilic structure. Semi tanned lining leather was in the second place while the wet-white tanned lining leather had the minimum water absorption value as 182.54 ml water for 100gr leather.

The recommended water absorption is a minimum of 100% for linings and insoles for 24 hours (TS EN 344-1, 1998; Bitlisli *et al*., 2005) that is a well indication of high water absorption quality requirements for linings and insoles. Our static water absorption results were found two fold higher than this requirement such as 274.55, 307.19, 249.57, 182.54 mL/100 gr for semi, vegetable, chrome and wet-white tanned lining leathers.

Table 1. Dynamic water absorption (%) and penetration time of lining leathers

Tanning techniques	Water absorption %	Time (seconds)
Chrome	52.06±8.8	9±4.6
Vegetable	99.61±5.9	3±0.4
Wet-white	21.62±6.0	52±6.7
Semi	102.72±0.8	3±0.4

Dynamic water absorption (%) results showed that less than minute water was penetrated into the leather in a dynamic condition. Wet-white tanned lining leathers had the longest penetration time (52 seconds) in comparison to other tanning techniques with minimum water absorption (%) value. Vegetable and semi tanned lining leathers had similar and the highest dynamic water absorption values due to their hydrophilic structure (Table 1). The high water penetration ability of the lining leathers lead to minimum complaints by users who had a feeling uncomfortable feeling in their shoes (Bitlisli *et al*., 2005).

The color fastness results of the lining leathers differentiated in tanning techniques were found satisfactory for dry rubbing fastness with the value of 5 in terms of felt and leather. The wet felt resulted in 4/5 for all the tanning techniques. However, wet dry rubbing fastness of tanned leathers was determined quite low especially for vegetable and wet-white tanned lining leathers. These results indicated that tanned leathers meet the standards of UNIDO recommended for lining leathers (UNIDO, 1996) except wet leather rubbing results of vegetable and wet-white lining leathers (Table 2).

Table 2. To and fro rubbing fastness results of lining leathers

Tanning techniques	Dry		Wet	
	Felt	Leather	Felt	Leather
Chrome	5	5	4/5	3
Vegetable	5	5	4/5	2
Wet-white	5	5	4/5	1
Semi	5	5	4/5	3

The crockmeter fastness results of the lining leathers tanned with different tanning materials are given in Table 3. For wet and dry crockmeter fastness results were found quite satisfactory and correlated with the 'to and dry' rubbing fastness results. On contrary to 'to and fro' wet rubbing fastness result of chrome tanned lining leathers, the crockmeter result of chrome tanned lining leather had higher fastness values.

Table 3. Crockmeter fastness results of lining leathers

Tanning techniques	Dry		Wet	
	Fabric	Leather	Fabric	Leather
Chrome	5	1	4/5	1
Vegetable	5	1	4	2
Wet-white	4/5	1	4/5	1
Semi	5	1	4	2

The evaluation of the leather for crockmeter; 1 good; 2 medium; 3 poor

Color fastness to perspiration tests results were presented in Table 4 in terms of wool, acrylic, polyester, nylon, cotton, and acetate. According to the UNIDO quality standards, the felt wouldn't be under 3 according to grey scale for the lining leathers with aniline finishing including the color fastness to perspiration (UNIDO, 1996) which indicates the tanned leathers were significantly fulfilled the requirements of UNIDO.

Color fastness to perspiration values of lining leathers were evaluated both for grain and sued side of the leathers (Table 4). The perspiration results indicated that all lining leathers had quite good values and not fewer than 3 according to grey scale. Only the vegetable lining leather had 3 grey scale values for cotton and acetate.

Table 4. Color fastness to perspiration values of lining leathers

	Chrome		Vegetable		Wet-white		Semi	
	Grain	Sued	Grain	Sued	Grain	Sued	Grain	Sued
Wool	4/5	4/5	3/4	3/4	4/5	5	3/4	4
Acrylic	4/5	5	4	4	4/5	4/5	4/5	4/5
Polyester	5	5	4	4	4/5	4/5	4/5	4/5
Nylon	5	5	4	3/4	4/5	4/5	4/5	4/5
Cotton	5	5	3	3	4/5	4/5	4	4
Sec. Cell. Acetate	5	5	3	3	4/5	4/5	4/5	4/5

Water absorption and perspiration properties of vegetable and semi tanned lining leathers were found higher and comparable respectively than that of chrome and wet-white tanned leathers although the rubbing fastness values were no better. These characteristics investigated in the study made the all leathers to be used in the producing of lining leathers.

CONCLUSION

In this study, the effect of four different tanning techniques was investigated on properties of lining leathers produced from goat skins in terms of dynamic and static water absorption, color fastness to perspiration, to and fro and crockmeter rubbing fastness tests. The results of the study showed that all tanning techniques provided high quality lining leathers. But in the ecological point of view, semi and vegetable tanned lining leathers could be selected for the production of lining leathers due to their high water absorption capacity.

Acknowledgement

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REFERENCES

ASTM D5053. (2003), "Standard test method for color fastness of crocking leather", Annual book of ASTM standards United States, 15(04, 2.

Bitlisli, B.O., Karavana, H.A., Basaran, B. and Aslan, A. (2005), "Importance of Using Genuine Leather in Shoe Production in Terms of Foot Comfort", *Journal of the Society of Leather Technologists and Chemists*, 89, 107-110.

Diebschlag, W., Muller-Limmroth, W. and Mauderer, V. (1976), "The Influence of Several Socks and Linings on the Microclimate in Shoes with Upper Material of Leather or Synthetic", *Journal of the American Leather Chemists Association*, 71(6), 293.

Hernon, J.T. (1981), "Alum-Formaldehyde Tannage V. with Zirconium. Domestic U.S. Sheepskins For All-White Tanned Reverse Sueded Shoe Linings - Technical Note", *Journal of the American Leather Chemists Association*, 76(6), 223.

ISO 105-A03 (1993). "Textiles-Tests for color fastness - Part A03: Grey scale for assessing staining", International Organization for Standardization (ISO), Geneva, Switzerland.

Śmiechowski, K., Żarłok, J., and Kowalska, M. (2014), "The Relationship between Water Vapour Permeability and Softness for Leathers Produced in Poland", *Journal of the Society of Leather Technologists and Chemists*, 98, 259-236.

Sari, Ö. and Basaran, B. (1996), “Various physical properties of uppers in shoes with lining and their effect on foot comfort”, II. National Shoe and Side Industry Symposium Papers, Konya Chamber of Commerce, Konya, Turkey, Publication No. 2, 49-66.

Sari, Ö. and Bitlisli, B.O. (1996), “Sole leather production in Turkey and evaluation of its quality levels”, II. National Shoe and Side Industry Symposium Papers, Konya Chamber of Commerce, Konya, Turkey, Publication No. 2, 94-106.

Shuttleworth, S.G. (1973), “Future Developments in Leather Manufacture”, *Journal of the Society of Leather Technologists and Chemists*, 57, 1-4.

TS EN 344-1 (1998), “Safety, protective and occupational footwear for Professional use-part 1: requirements and test methods”, Turkish Standardization Institute, Ankara, Turkey.

TS EN ISO 2418 (2006), “Leather - Chemical, physical and mechanical and fastness tests-Sampling location”, Turkish Standard Institute, Ankara, Turkey.

TS EN ISO 2419 (2006), “Leather - Physical and mechanical tests - Sample preparation and conditioning”, Turkish Standard Institute, Ankara, Turkey

TS 4117 EN ISO 2589 (2006), “Leather - Physical and mechanical tests - Determination of thickness”, Turkish Standard Institute, Ankara, Turkey.

TS 4123 EN ISO 2417 (2005), “Leather - Physical and mechanical tests - Determination of the static absorption of water”, Turkish Standard Institute, Ankara, Turkey.

TS 8541 EN ISO 5403 (2006), “Leather - Physical and mechanical tests - Determination of water resistance of flexible leathers (ISO 5403:2002)”, Turkish Standard Institute, Ankara, Turkey.

TS EN ISO 11641 (2012), “Leather - Tests for color fastness - Color fastness to perspiration”, Turkish Standard Institute, Ankara, Turkey.

TS EN ISO 11640 (2013), “Leather-tests for color fastness-Color fastness to cycles of to-and-fro rubbing”, Turkish Standard Institute, Ankara, Turkey.

UNIDO (1996), “Acceptable quality standards in the leather and footwear industry”, Vienna.

IV.

INDUSTRIAL ECOLOGY & CIRCULAR ECONOMY

CO_2 EMISSION REDUCTION: AN EUROPEAN APPROACH FOR ROMANIAN TANNERIES

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The paper presents the objectives and results of the project IEE/11/949/SI2 615946 IND-ECO developed under the Intelligent Energy Europe - Executive Agency for Competitiveness and Innovation EACI umbrella, by a consortium of 16 participants with reference to Romanian tanneries in order to obtain a CO_2 emission reduction.

Keywords: tannery, energy efficiency, investment direction.

CO_2 EMISSIONS

Industrial activity, transports and also daily existence generate CO_2 directly or indirectly, by using energy, fuels or by its emanation, as a result of the technological process. CO_2 is the main generator of greenhouse gas, producing climate changes at planetary scale. It is estimated that, since the beginning of the industrial revolution, mankind has produced and pumped in the atmosphere 30% more carbon dioxide than there was naturally on Earth 200 years ago.

The Kyoto Protocol of December 1997 provides reduction of polluting emissions at global scale. In October 2004, EU state members, together with Romania, Bulgaria and the Republic of Moldova ratified the Kyoto agreement.

IND-ECO PROJECT

According to EU action plan of 2011 regarding energy saving, under Intelligent Energy Agency for Competitiveness and Innovation - EACI, a number of 16 entities representing European and national owners' associations from the footwear and leather industries, producers, research institutes from Italy, UK, Spain, Portugal, Romania and Bulgaria formed a consortium which started the project Industry Alliance for reducing energy consumption and CO_2 emission IND-ECO (2012-2015) with the following main targets:

- to obtain initial primary energy savings by its end;
- to create favourable conditions for much more investments by 2020.

SPECIFIC OBJECTIVES OF THE PROJECT

The objectives are as follows:

- Inventory of energy consumption. Development of benchmarks at the levels of footwear and leather industries.
- Verification of energy consumption. Identifying vulnerable areas requiring improvements.
- Identifying providers of financial solutions - bank credits for energy investments.
- Developing a database with technical and technological solutions for reducing energy consumption.

- Drawing up investment plans.
- Dissemination of results and access to databases created in the project.

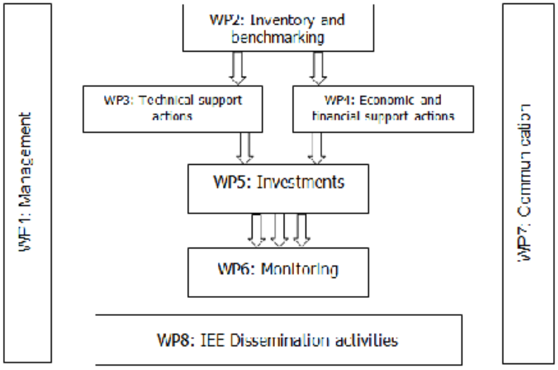

Figure 1. The structure of the IND-ECO work programme (WP)

CO$_2$ EMISSION REDUCTION APPROACH

The algorithm of CO$_2$ emission reduction is outlined in the following draft:

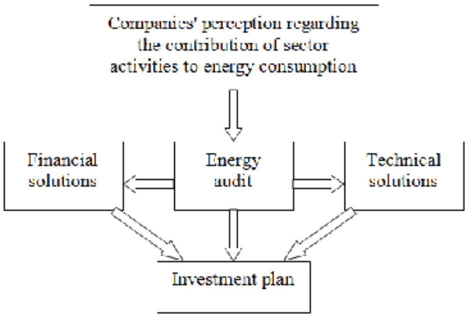

Figure 2. Algorithm of CO$_2$ emission reduction

EU TANNING SECTOR BENCHMARK

In the project a number of 85 inventories were collected from six EU countries regarding energy consumption. Tanneries surveyed used conventional, manual, semi-automatic, as well as in certain cases fully automated equipment and installations as demonstrated by the analysis of the relative data collected with the tool inventories. The output products regarding animal origin are mainly focused on: cattle, sheep, goat.

Main product groups are as follows:

Products sold by m^2:

Finished leathers	Semi-processed leathers

Products sold by kg:

Finished (full veg tanned leathers)	Semi-processed leathers

Table 1. Energy consumption and CO_2 equivalent resulted mainly from start population

Input processing Material subgroups	Energy consumption Kwh/m^2 average	CO_2 equivalent Kg/m^2 average
Finished leathers sold by m^2		
Raw to finished	7.3	2.9
Semi-processed to finished	7.0	2.7
Products sold by kg		
Full veg tanned starting from raw	1.86	0.79
Semi-processed pelts	0.65	0.46

TANNERIES. ACTION DIRECTIONS

For CO_2 reductions, based on project evaluation, the main directions for action seem to be the following:

- systems and lighting sources with economic consumption;
- checking electrical engines and replacement of underperforming ones;
- building insulation. Seals of access areas to reduce energy losses;
- extension of drying system using residual heat from working spaces;
- preparation of water process in centralized system and temperature steps;
- rehabilitation of distribution system for compressed air;
- checking and rehabilitation of steam boilers.

COMMUNICATION / DISSEMINATION

Communication / dissemination was mainly directed to: website set-up, flyers and other documents containing the basic information in a format that provides instructions at first sight.

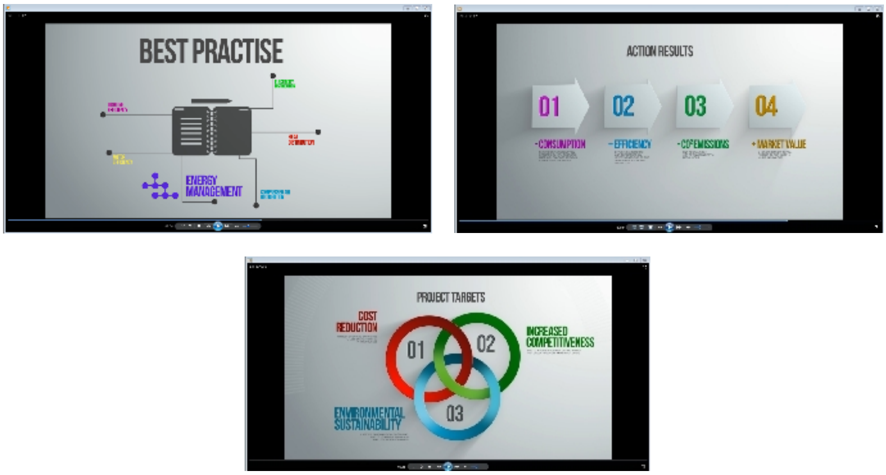

Figure 3. Communication components

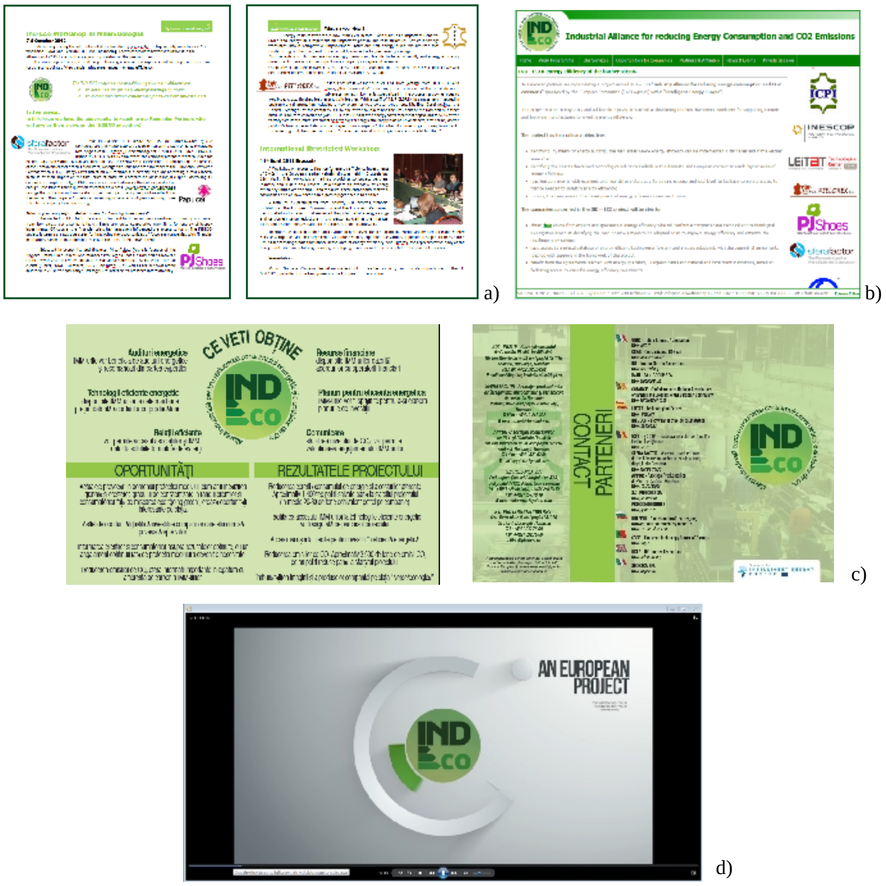

Figure 4. Dissemination material: a) newsletter, b) website (http://www.ind-ecoefficiency.eu), c) flyer, d) film

CONCLUSIONS

CO_2 emission reduction on technological processes, but not only, is a priority at planetary scale.

For this reason, the paper, after defining the notion of CO_2 emission, presents the European project IND-ECO and also its objectives that respond to announced desideratum in the leather production field.

The benchmark was conducted based on a number of 85 tanneries inventories at EU level. The directions of action to achieve goals as well as communication and dissemination activities of project results are inventoried.

REFERENCES

***, http://www.ind-ecoefficiency.eu/index.php

CONSIDERATIONS REGARDING THE ESD TYPE TEXTILE WASTE RECOVERY

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The Textile and Clothing Industry affects the environment through the use in the manufacturing process of very large quantities of water, energy and chemicals. This Industry generates a lot of waste, due to the use of a very large number of chemicals and technological processes. Waste generation implies a loss of materials and energy and imposes high environmental and economic costs to society for their collecting, treatment and processing. A permanent activity of finding technological solutions for the textile waste processing is also supported by the implementation in the economic and social life of multifunctional and adaptive textile systems for whose manufacturing smart elements are used. The work presents aspects on textile waste preliminary processing in the form of knitted bilayer textile structures containing conductive yarns. During the garments manufacturing have resulted textile waste. For these waste were conducted technological experiments of preliminary processing. This processing of the textile waste as yarns, trimmings from knitted fabrics, trimmings from woven fabrics includes the following operations: sorting depends on base colors, cutting, opening. The opening process can be characterized by: opening efficiency; unopened yarns percentage; unopened patches percentage; the average length of recovered fibers. In order to assess the fibers structures aspect, has been used the electron microscopy by analyzing their longitudinal and cross section images.

Keywords: conductive yarns, textile waste recycle, electron microscopy

INTRODUCTION

The causal system in implementing the *Europe 2020* objectives includes technological objectives as general support of economic, social and educational development. Technological objective, which is in direct relation to the economic one, is based on the principle of sustainable growth and generates emblematic initiatives referring to: “Resource Efficient Europe” and “An Industrial Policy for the Globalization Era”. In this context, the Textile & Clothing Sector has a special role that is associated with major environmental impacts (Clay, 2004).

Natural resources are consumed each year. Therefore, for the fibers production, about 132 million tons of coal and 6-9 trillion liters of water (Rupp, 2008). Cotton represents about 82.5% of consumption of natural fibers used in the textile industry (Shangnan and Plastina, 2010). Cotton crops require large quantities of water, for production of a T-shirt the consumption of water is about 2700 liters (Wallander, 2014). Also, natural resources are consumed for synthetic fibers production that represents about 60% of total fibers produced worldwide (Shangnan and Plastina, 2013). Globally, the Textile Industry consumes 1 trillion kWh yearly, 10% of the carbon footprint equivalent (Textile Exchange 2010 Global Market Report on Sustainable Textiles). Dyeing processes are also environmentally unfriendly activities, for dyeing of 1 kg textile fabric, 70-150 liters of water are used (Chakraborty *et al.*, 2005; Babu *et al.*, 2007). Like all waste, the textile waste are generated throughout the entire lifecycle of the textile products, from the fibers production process, textile and clothing industry, consumers, industries and commercial services (Caulfield, 2009; Dziedziczak, 2015).

Resource saving: 4.2 trillion gallons of water would be saved, that's enough to supply 27.8 million homes (Cuc, 2011; Chavan, 2014); 17 million tons of CO_2 would be saved, that's equivalent to taking 3.5 million cars off the roads; 7.5 million cubic yards of landfill space will be saved, that could fill the Empire State building 5.8 times.

With the implementation of textile fibers with advanced characteristics, the matter of textile waste gained new dimensions by the need of technological capacity of recovered fibers exploitation, through defining of new value-added products and not least by establishing the physical-mechanical characteristics of the products for new niches in the market.

MATERIALS AND METHODS

Conductive fibers and yarns are means for development of textile products meant for use in cases where uncontrolled static discharges may cause quality, health and safety issues. Type bilayer approach of the knitted structures for ESD garments allows delimitation of the accidental discharge track from the controlled discharge track of electrostatic charged fabric. The outer layer is mainly dissipative providing protection against short circuit and limiting the amount of static electricity that can be dissipated to the working environment, and the inner layer is mainly conductive, ensuring controlled draining of static electricity. An additional requirement for the inner layer is to ensure the comfort for the user.

The knitted structures for ESD garments, included the following variants:

- 100% cotton yarns;
- 100% wool yarns;
- Nega-Stat P210, 112dtex multifilament conductive yarns with trilobal carbon core and surface from polyester and Nega-Stat type P190, 156dtex;
- nylon yarns with surface saturated with carbon particles.

During the garments manufacturing have resulted textile waste. For these waste were conducted technological experiments of preliminary processing at MINET SA Ramnicu Valcea.

EXPERIMENTAL

The preliminary processing of the textile waste as yarns, trimmings from knitted fabrics, trimmings from woven fabrics includes the following operations: sorting depends on base colors, cutting, opening.

Sorting of the recyclable textiles is done manually, depending on the cleaning degree, fibers composition, color, type of materials and their destination.

Cutting of the textile waste is done to reduce their size, ensuring a good opening. If the recyclable textile materials contain chemical fibers, they are sprayed with substances to neutralize electrostatic charges and to eliminate the danger of ignition.

Opening is an operation carried out progressively, with increasingly intensities, which results in getting the opened material (M_D), which is composed of both recovered fibers (M_{D1}) as well as unopened yarn ends (M_{D2}) and small pieces of unopened fabrics (M_{D3}).

$M_D = M_{D1} + M_{D2} + M_{D3}$

Shares of the 3 components after opening are:

$P_1 = M_{D1} / M_D .100$

$P_2 = M_{D2} / M_D .100$ $\quad\quad P_1 + P_2 + P_3 = 100\%$

$P_3 = M_{D3} / M_D .100$

The number of opening drums is selected considering the following aspects: feed speeds of waste from one drum to another are equal; the density and gauge of the nails is increasing; drums speeds is increasing; the quantity of fed material, from one group to another, is decreasing.

The opening process can be characterized by:

- opening efficiency (70-80%);
- unopened yarns percentage (30-50%);
- unopened patches percentage (3-20%);
- the average length of recovered fibers (depending on their fibrous composition and the condition of waste/ 7-85 mm).

The percentage of unopened yarns is a parameter that contribute to increase the average length of recovered fibers. Regarding the unopened patches, the value obtained requires a careful analysis to establish the classic or unconventional technological processing flow, as well as the components of blending formula (recovered fibers/ bonding fibers).

The selection of cutting and opening technological parameters in correlation with the processing method and the characteristics of resulted material takes into account the following factors:

- type of processed raw material and processing method;
- cost - quality - efficiency balance;
- the accuracy of the sorting operation;
- technical evaluation of the equipment;
- establishing the number of opening modules;
- technological parameters processing optimization in correlation with structural and physical-mechanical characteristics of recovered fibers.

RESULTS AND DISCUSSIONS

The technological adjustments were taken into account fiber structure preservation, getting a reduced content of opened yarns and patches as well as a length of recovered fibers that allows further processing on classic and unconventional technologies. The physical-mechanical properties of opened material are shown in table 1.

Table 1. Basic characteristics of the opened material

No.	Characteristics	Value	Standard
1.	Average length	27,77 mm	STAS 12206/1983
	CV	19,23%	
2.	Unopened patches	17,47%	STAS 12206/1983
3.	Unopened yarns	47,96%	STAS 12206/1983

By analyzing the longitudinal and cross section aspect of the fibers structures, made by electron microscopy, stand out:

- a high opening degree of knitted waste (fig. 1);
- insignificant damages of the fibers on longitudinal surface (fig. 2);
- melts at the end of recovered fibers (fig. 3);
- broken cotton fibres (fig. 4).

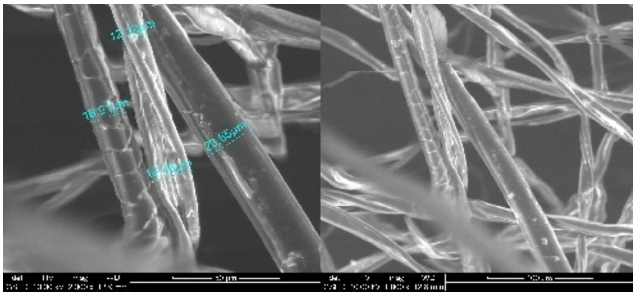

Figure 1. Opened fibres

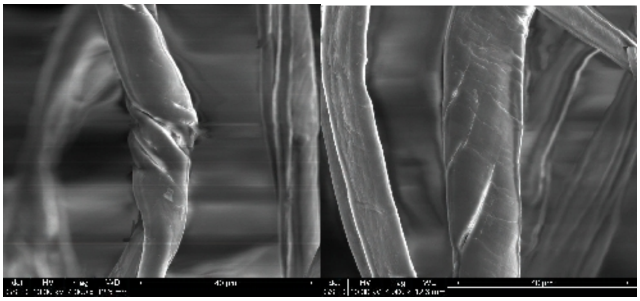

Figure 2. Damages of the fibers

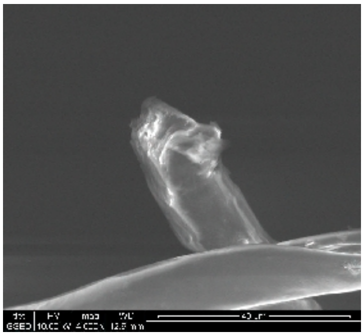

Figure 3. Melted fibre

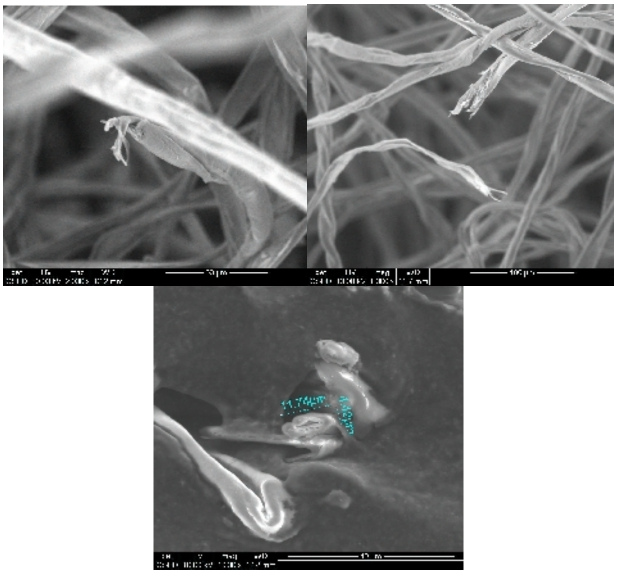

Figure 4. Broken cotton fibres

CONCLUSIONS

- The textile industry is facing a new emerging mind-set, with increasing awareness and appreciation of the resources already available today.

- Three key criteria have been identified in order to reduce the amounts of textiles in final waste disposal: i) reduced consumption of new textiles, ii) prolonged lifespan of existing textile products, and iii) reuse and recycling of textiles that no longer fit for its first intended use.

- The preliminary processing experimentations (cutting, opening) of the textile waste with conductive yarns content demonstrate technological potential of these recovered fibers which can be processed into unconventional textile elements.

REFERENCES

Babu, B.R., Parande, S.R. and Kumar, T.P. (2007), “Cotton Textile Processing: Waste Generation and Effluent Treatment”, *Journal of Cotton Science*, 11, 141-153.

Caulfield, K. (2009), *Sources of Textile Waste in Australia*, Apical International, Australia.

Chakraborty, S.S., De, S., Basu, J.K. and Das Gupta, S. (2005), “Treatment of a Textile Effluent: Application of a Combination Method Involving Adsorption and Nanofiltration”, *Desalination*, 174, 73-85.

Chavan, J. (2014), “Environmental Sustainability through Textile Recycling”, *Textile Science & Engineering*, S2, http://dx.doi.org/10.4172/2165-8064.S2-007.

Clay, J. (2004), *World Agriculture and the Environment: A Commodity by Commodity Guide to Impacts and Practices*, Island Press.

Cuc, S. (2011), “Environmental and socioeconomic sustainability through textile recycling”, *TEX TEH IV Conference, Textile Research - Active Factor For Increasing Performance And Competitiveness*, June 23-24.

Dziedziczak, K. and Bogusław, K. (2015), *Problemy Eksploatacji – Maintenance Problems*.

Pepper, L.R. (2010), “Textile Exchange 2010 Global Market Report on Sustainable Textiles”.

Rupp, J. (2008), “Ecology and Economy in Textile Finishing”, *Textile World*, Nov/Dec.

Shangnan, S. and Plastina, A. (2010), “EST/FAO and ICAC world apparel fiber consumption survey”.

Shangnan, S. and Plastina, A. (2013), *World Apparel Fibre Consumption Survey*, ISBN 9780979390395, Library of Congress Control Number 2013947672.

Wallander, M. (2014), “Increasing the diversion of textiles”, USAgain.

ECO-INNOVATIVE PRODUCTS AND TECHNOLOGIES BASED ON THE RECYCLING OF CERTAIN WASTES FROM TANNERIES AND NON-FERROUS METALS INDUSTRY FOR THE TRANSITION OF THE LEATHER INDUSTRY TO A CIRCULAR ECONOMY MODEL

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This paper presents experiments of recovery/recycling/reuse of as much tannery waste as possible to obtain new auxiliary materials with high added value and low eco-aggressiveness intended for leather processing. The paper also deals with conversion of waste difficult to manage (some of which is unrecyclable) from the non-ferrous metal industry into factors for increasing the eco-efficiency of the leather industry.

Keywords: Waste recovery-recycling-reuse, leather industry, non-ferrous metallurgy, circular economy

INTRODUCTION

Circular economy is one of the topical themes of the European environment policy, in the context of the increasing resource demand and the pressure it exerts on the environment (www, 2014).

The focus is on the transition from the linear economic model in which each product has a limited lifetime, to the circular economy, whose goal is to keep the added value in products for as long as possible and reduce waste to zero.

This concept of the modern world means shifting towards an economy based on reducing energy and raw materials, where the waste resulting from the multitude of activities has precise destinations from the design phase, through its integration in the cyclic spiral of reuse. In other words, waste occurring in a manufacturing process can constitute a raw material for other processes or even for the same technological process which produced it.

It is known that leather processing industry is currently facing serious environmental problems related to high consumption of water, use of chemicals with negative impact on people and the environment and, last but not least, due to the generation of large quantities of solid and liquid waste difficult to manage.

This paper presents experiments of using protein waste from the leather industry and metal waste from the titanium metallurgic industry to obtain new eco-friendly auxiliaries for the leather industry.

The processes of waste recycling and recovery do not follow the usual course for the other types of waste that can be reused as raw material (such as metal, glass, textiles, etc.) due to association of hide protein with a variety of other chemical species, some of which are eco-aggressive, which imposes additional costs of separation and co-recovery and also neutralization of aggressive effects.

This is why we need to establish technologies for extraction of collagen protein - the basic component of hides - in the form of short or solubilized fibers, aiming to achieve as high an yield and recovery (conversion) as possible (Paul *et al.*, 2013).

In this regard, we studied the recovery and superior exploitation of titanium waste resulting from the technological process of obtaining extra pure titanium (Crudu *et al.*, 2009a; Crudu, 2008; Crudu *et al.*, 2009b; Crudu *et al.*, 2008; Crudu *et al.*, 2013; Buzatu and Moldovan, 1994) and considered unrecyclable in the industry that produced it due to the high level of impurities.

MATERIALS AND EQUIPMENT

Raw materials and auxiliaries: solid titanium waste (filings-SC ZIROM SA Giurgiu Romania), limed and delimed bovine hide waste (trimmings-pilot-scale tannery INCDTP-ICPI Bucharest, Romania), technical auxiliaries used in leather processing, wet salted bovine hides, 20-25 kg (Constanta County, Romania), organic wet-white (Czech Republic), wet-blue (Romania, Netherlands).

Equipment: reaction vessel with stirring and temperature adjustment system (Caloris, Bucharest, Romania), experimental drum (DOSE MAT-Germany), device for shrinkage temperature determination (GIULIANI Italy), device for thermogravimetric analyses STA 449F1 Jupiter (Netzsch-Germany), DSC analyser 200F3 Maia (Netzsch-Germany), scanning electron microscope (SEM) equipped with Qanta 200 (FEI Netherlands) energy dispersive X-ray analysis system (EDAX) for qualitative, quantitative and elemental mapping.

EXPERIMENTS

Figure 1 shows the experimental model for obtaining the new innovative low eco-toxicity materials based on leather and titanium waste, intended for leather processing.

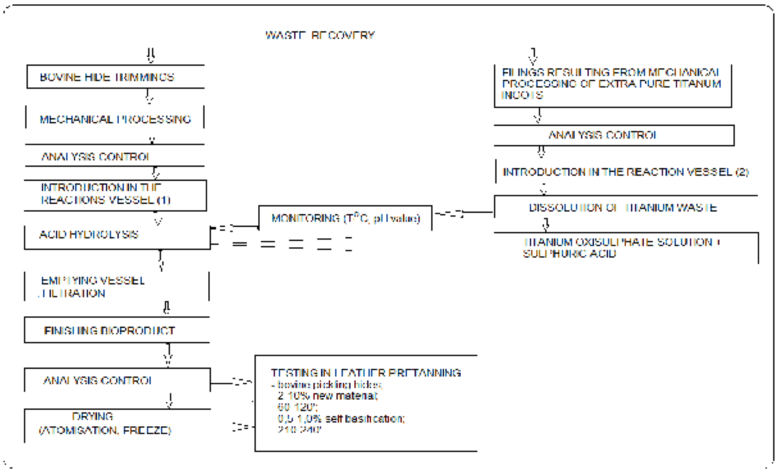

Figure 1. The experimental model for obtaining the new low eco-toxicity products

RESULTS AND DISCUSSIONS

The biomaterials obtained from leather waste and titanium waste were characterized and the results are presented below.

Table 1 presents the physical-chemical characteristics of new biomaterials.

Table 1. Physical-chemical characteristics of new biomaterials

No.	Characteristics	Determined values
1.	Dry substance, (%)	18-22
2.	Ash, (%)	3-5
3.	Total Nitrogen, (%)	7-8
4.	Protein substance, (%)	35-50
5.	Metal oxide, (%)	2.5-5.0
6.	pH, (unit. pH)	1.6-2.2

Table 2 presents values of shrinkage temperatures, reflecting hydrothermal resistance and therefore pretanning degree.

Table 2. Values of shrinkage temperatures, reflecting hydrothermal resistance and therefore pretanning degree

No.	% Material amount	Shrinkage temperature °C
1.	1-2	65-68
2.	3-5	70-75
3.	6-10	78-85

Table 3 presents the physical-chemical characteristics of wet-white pretanning using the new biomaterial in comparison with wet-blue and other wet-white (polyaldehydes).

Table 3. Physical-chemical characteristics of wet-white pretanning using the new biomaterial in comparison with wet-blue and other wet-white (polyaldehydes)

No.	Characteristics	Determined values		
		Our Wet-white	Wet-blue	Wet-white (polyaldehydes)
1.	Dry substance, %	60-80	60-80	60-80
2.	Ash, %	2-8	9-12	0-1
3.	Total nitrogen, %	12-17	12-14	14-18
4.	Protein substance, %	82-91	75-85	85-93
5.	Metal oxides, %	4-6	5-8	-
6.	pH	3.5-3.7	3.7-3.9	4.2-4.6
7.	Shrinkage temperature, ^{o}C	70	98	68

Table 4 presents elementary composition of the our wet-white, wet-blue and wet-white (polyaldehydes) by EDAX.

Table 4. EDAX elementary composition of the our wet-white, wet-blue and wet-white (polyaldehydes)

No.	Element	Determined values		
		Our Wet-white, %	Wet-blue, %	Wet-white (polyaldehydes), %
1.	**C**	45.85	51.08	64.90
2.	**N**	11.12	12.26	9.71
3.	**O**	26.24	26.12	20.29
4.	**Na**	2.98	1.40	2.04
5.	**Mg**	1.29	0.27	0.07
6.	**Al**	0.21	0.06	0.08
7.	**Si**	0.00	0.10	0.11
8.	**S**	2.71	2.64	1.65
9.	**Cl**	4.51	2.11	1.61
10.	**Ca**	0.20	0.33	0.25
11.	**Ti**	4.89	0.07	0.00
12.	**Cr**	0.00	3.57	0.00

CONCLUSIONS

Romania is known to have a waste recycling degree of 3%, while the European Union recycles on average 36%, but even so, there is the potential for approximately 600 million tons of waste recovery at Community level.

This paper attempted to propose new relevant solutions in the direction of recovery-reuse-recycling of industrial waste generated by the leather industry and other industries.

As a result of experiments, the following conclusions can be drawn:

- Using simple methods of processing untanned hide waste and unrecyclable titanium cuttings, new relatively low-cost but high added-value pre/tanning agents may be obtained;
- Their use in total or partial replacement of chromium in the leather tanning operation induces a series of technical, economic, social advantages, as well as major implications in environmental protection, due to recovery and reuse of waste difficult to manage and to the reduction of the negative impact on the environment of the leather processing industry, as tanning effluents and waste from mechanical operations no longer contain chromium compounds and large amounts of salts;
- The new semi-processed wet-white leather obtained by pretanning using the proposed materials are comparable to those obtained at international scale, allowing further mechanical and physical-chemical processing without any problems, even providing a superior technological versatility due to structural stability and light color (almost white);
- Compared to wet-blue, the new wet-white leathers, even with a lower hydrothermal stability, have the advantage of a lower content of metal oxide and ash, but a higher content of protein substance, while the color is also an advantage, not to mention the lack of chromium compounds;
- The use of the new tanning agents in leather pre/tanning does not involve changes in logistics or technology;

- The encouraging results will prompt further research in streamlining technologies for developing and using the new products from the waste used in this paper, as well as from other waste from leather processing and from other industries.

Acknowledgements

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REFERENCES

Buzatu, M., and Moldovan, P. (1994), Reacting and Light Alloys – Part I: Alloys Based on Titanium, UPB Lecture.

Crudu, M. (2008), Study regarding the use of inorganic compounds in natural leather processing in view of preventing environmental pollution (in Romanian), Ph.D.Thesis, “Gh.Asachi” University, Iasi.

Crudu, M., Boca, N., Sutiman, D., Ioanid, A., Caileanu, A., and Sibiescu, D. (2009a), Compounds with tanning capacity obtained from Ti-end unrecyclable waste of the Ti-metallurgic industry (in Romanian), CERTEX Press, ISBN 978-973-1716-54-1.

Crudu, M., Deselnicu, V., Albu, L., Niculescu M., Roşca, I., Sutiman, D., Sibiescu, D., and Cailean, A. (2008), “Eco-friendly tanning agents to be used in leather manufacture”, 10th International Chemical and Biological Engeneering Conference, Braga-Portugal, ISBN 978.972.97810.3.2. 498-499.

Crudu, M., Gaidau, C., Crudu, A., Popescu, M., and Ionescu, M. (2013), “Innovative material technology system with contribution to increasing eco-efficiency of industrial leather processing”, Annals of the University of Oradea, Fascicle of Textiles, Leatherwork, Vol: XIV, ISSN: 1843-813X, 95-100.

Crudu, M., Niculescu, M., Deselnicu, V., Sutiman, D., Sibiescu, D., Cailean, A., and Boca, N. (2009b), Romanian Patent App. A/00941/17.11.2009 « The process of obtaining and use of a range of chemicals with low toxicity to (pre) tanning natural leather »

Paul, H.L., Antunes, A.P.M., Covington, A.D., Evans, P., and Phillips, P.S. (2013), Towards zero solid waste: Utilising tannery waste as a protein source for poultry feed, Paper presented in: 28th International Conference on Solid Waste Technology and Management, Philadelphia, PA, USA, 10-13 March 2013. Philadelphia USA: *The Journal of Solid Waste Technology and Management*, ISSN 1091-8043.

*** http://greenly.ro/deseuri/green-week-2014-si-economia-circulara

APPLICATIONS OF LIFE CYCLE ASSESSMENT TO LEATHER INDUSTRY - AN OVERVIEW AND A CASE STUDY

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Global temperature increase is attributed to industrial pollution, rising number of automotive vehicles, deforestation, growing use of chemical fertilizers etc. In the future the potential increase of temperature even by 2°C could have a dramatic impact on life on Earth. Leather industry activities use hazardous chemical substances that generate an important quantity of CO_2 with a direct impact on global warming. The first part of the paper presents the LCA concept, methodologies and some relevant LCA studies for leather industry as a means to identify the main environmental impact “contributors”. In the second part the paper presents a gate-to-gate Life Cycle Assessment (LCA) case study for processing a leather assortment. The analyzed assortment is natural full grain, bovine leather, thickness 1.4-1.6 mm, domestic raw material, manufactured by a Romanian tannery in one year period. The study was conducted using GaBi ts - Life Cycle Assessment software, version 7.2.2.28, 2016.

Keywords: life-cycle assessment, leather processing, carbon footprint

INTRODUCTION

There is a direct correlation between the increase of anthropogenic green house gases [Methane (CH_4), Nitrogen Oxide (N_2O) etc.], especially of Carbon dioxide (CO_2), and the increase of global average temperature - global warming. These gases warm the Earth by absorbing energy and slowing the rate at which the energy escapes to space. The CO_2 concentration has constantly increased from 357 ppmv (parts per million by volume) in 1992 to 389 ppmv in 2011 (Brugnoli and Kráľ, 2012). At the end of 2015 The United Nations Climate Change Conference was held in Paris, France, which debated the issued of global warming. The participating 195 countries agreed, by consensus, on the reduction of climate change by reduction of greenhouse gases and keeping the global temperature increase below 2°C. But even that limit will have a dramatic effect on the world's climate (Wikipedia, 2016).

Leather industry which transforms raw skins and hides of animals, a by-product of meat industry, into a stable material called leather, by using, among other materials, hazardous chemical substances, is in the top of world pollutants. Hence leather processing activities have a direct impact on global warming by CO_2 emissions. The paper will present a study that aims to reveal the environmental impact of leather processing activities in the form of CO_2 emissions. Impact of different products, processes on the environment is determined by using Life Cycle Assessment (LCA) approach. LCA is a technique of evaluation of the actions exerted on the environment by all associated production phases, which transform raw skins/hides into finished leather. An LCA study proposes an *“environmental picture” of* manufacturing processes offering valuable information regarding inputs and outputs of the materials used, energy consumption, carbon footprint.

LIFE CYCLE ASSESSMENT IN LEATHER INDUSTRY

This section presents some relevant LCA studies conducted until 2016 for the leather sector. Special attention was given to the LCA studies for the Italian and Spanish leather sector as these are the most representative at European level.

Currently there is neither a unique methodology nor a general accepted agreement on calculating the environmental impact of leather processing (Brugnoli and Kráľ, 2012). Nonetheless most of the LCA studies are conducted partially or sometimes fully in accordance with the ISO standards namely ISO 14044 and ISO 14040 (ISO 14044, 2006; ISO 14040, 2006). In the case of an LCA study conducted for Italian and Spanish bovine leather production it is asserted that LCA methodology was not applied completely in accordance with the requirements of the ISO 14040 (Notarnicola *et al.*, 2007). The ISO 14044:2006 titled "Environmental management-Life cycle assessment-Requirements and guidelines", specifies requirements and provides guidelines for life cycle assessment (LCA), the ISO 14040:2006, titled "Environmental management-Life cycle assessment - Principles and framework" - describes the principles and framework for LCA.

The ILCD International Reference Life Cycle Data System handbook is a useful document that provides detailed guidance for planning, developing, and reporting life cycle emission and resource consumption inventory (LCI) data sets and Life Cycle Assessment studies (ILCD handbook, 2010). Likewise the document "Best Available Techniques (BAT) Reference Document for the Tanning of Hides and Skins" provides valuable insights into leather industry, thus very useful for conducting LCA in the mentioned industry (BAT, 2013).

Moreover conducting an LCA study is a complex task with numerous variables that hardly can be controlled and frequently the result does not have mathematical precision. Leather processing implies the use of a wide variety of chemical substances, some of them not presented in the current software for generating a LCA report. Hence the researchers have four options: 1) to choose from software database a substitute chemical substance that might have a similar environment impact, 2) if the consumption of the substance is lower than a certain percentage, e.g. 0.5 %, reported to the quantity of leather, it can be excluded, 3) when possible, to create a custom chemical flow in the LCA database software "mixing" the existing database chemical substances, components of the absent substance to generate the needed substance, 4) to exclude the chemical substance.

Impact categories are scientific definitions that link the specific substances to specific environmental issues:

- Abiotic Depletion (ADP) - unit: MJ, depletion of nonrenewable resources, i.e. fossil fuels, metals and minerals;
- Global Warming Potential (GWP) - unit: kg CO_2-Equiv., the impact of greenhouse gases on global warming;
- Acidification Potential (AP)- unit: kg SO2-Equiv., impact on environment of acid gases released into the air, acid rain
- Photochemical Ozone Creation Potential (POCP) - unit: kg Ethene-Equiv., change in the amount of the ozone formed due to various gas emissions
- Ozone Layer Depletion Potential (ODP) - unit: kg R11-Equiv., is the relative amount of degradation to the ozone layer ;
- Eutrophication Potential (EP) - unit: kg Phosphate-Equiv., Over-fertilization of soils and water.

One form of environmental impact assessment, LCA, in leather processing is the Global Warming Potential (GWP 100 years) [kg CO_2-Equiv.] for manufacturing 1 m^2 of finished leather. In the Table 1 is depicted the range of values of CO_2 indentified in literature:

Table 1. World Leather Industry CO_2, values of CO_2 emissions/m^2 of leather

Category	Unit	Total	Performance level	Source
GWP_{100}	$KgCO_2$	2.70	-	Naviglio, 2015
CO_2 Guide for Ambush, $KgCO_2$ of leather produced				
		0 - 6.58	Good	www.liteleather.com ISA Tan Tec
		6.58 - 8.04	Average	
		8.04 - 9.65	Fair	
		9.65 - 15	Low	

According to www3.epa.gov (2016), CO_2 has a GWP of 1 regardless of the time period used as reference and its concentrations in Earth's atmosphere will last thousands of years.

We mention here some LCA studies for leather industry. A LCA of Italian and Spanish Tanning Systems was conducted by Notarnicola *et al*. (2011). The scope: manufacturing of chrome tanned leather for women's shoes. The approach was cradle-to-gate, functional unit 185.8 m^2 of chrome tanned bovine leather equivalent to 200 kg of leather, thickness 1.3 mm. Methodology adopted: standards ISO 14040, ISO 14044. System boundaries include upstream, core and downstream processes of Spanish and Italian tanning systems. Location of the study: Santa Croce, Italy; Igualada, Spain. A comparative LCA cradle-to-gate study of Romanian tanning system for two different tanning technologies was conducted by researchers from Leather and Footwear Research Institute, Bucharest, Romania (Deselnicu *et al.*, 2014a, 2014b). The authors asserted that the new tanning technology developed within the institute generates 15 % less carbon footprint than classical chrome tanning system.

CASE STUDY

The case study presented in this section is a part of a product system life cycle assessment, namely a gate-to-gate LCA, evaluating the environmental impact of the core processes of leather processing. The LCA case study was conducted in Romania during 2015-2016, using data from 2014, briefly described in Table 2. Leather processing data were collected from a tannery from Ilfov, Romania. The recipe and all the processing operations for manufacturing finished leather for footwear industry were analysed.

Table 2. Quantity of leather processed, and consumptions recorded for 2014

	Quantity	Unit
Full grain leather for footwear industry, bovine leather tanned with chromium salts	58797	m^2
Electric energy	411272	kWh
Natural gas	170 776	m^3
Water (process water)	13179	m^3
Diesel oil for movement of vehicles	2421	l

The study was conducted using GaBi ts - Life Cycle Assessment software, Software - System and Database for Life Cycle Engineering, compilation 7.2.2.28, database version 6.115, (GaBi, 2016). Hence the results of the LCA presented in the current section were generated by the mentioned software solution.

The scope of the analysed leather production system is: production of finished leather for footwear industry. The functional unit of LCA is 1 square metre (m^2) of finished bovine leather, thickness 1.4-1.6 mm, assortment shoe upper leather. As a gate-to-gate approach, system boundaries include the following leather processing operations: Desalting, Soaking, Liming, Fleshing - Trimming - forming Beam House phase, Deliming - Degreasing, Bating, Pickling, Tanning, Wringing Pressing, Splitting after Tanning (e.g. Wet Blue Splitting) - forming Tanyard phase, Shaving - Trimming, Clearing, Neutralisation, Retanning, Dyeing - forming Dyeyard phase, Fatliquoring, Sammying - Setting out, Vaccum Drying, Hang Free Drying, Conditioning, Staking, Trimming, Roller Coating - Drying, Spray Coating, Drying, Embossing, Cutting - forming finishing phase. The plan from GaBi ts software of the conducted LCA which includes all the processing operations and corresponding phases for leather processing is presented in Figure 1.

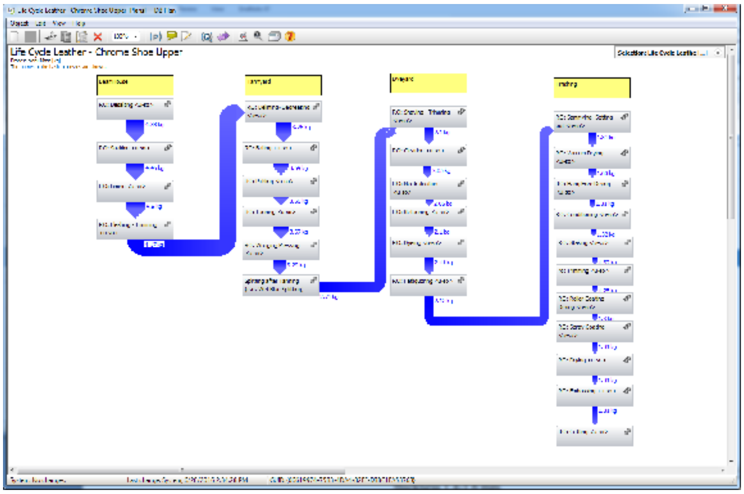

Figure 1. Plan of the LCA from GaBi ts software

Technological reference flows mentioned below are the ones used in LCA case study for manufacturing 1 square metre (m^2) of finished bovine leather: 1) bovine wet salted hides, bovine pelts, wet blue, crust etc. - equivalent weight in kg; 2) equivalent quantity of natural gas (kg) consumed for water and air heating; 3) electricity in kWh consumed by tannery machineries; 4) Process water - equivalent in kg; 5) chemical substances - equivalent quantity in kg; 6) waste for recovery in kg resulted from the technological process; 7) waste water in Kg resulted from leather processing.

Modeling of the LCA for beamhouse operations is depicted in the Figure 2, which shows the aggregated data plan from GaBi ts. Due to space constraints we present only the first main processing phase of leather processing.

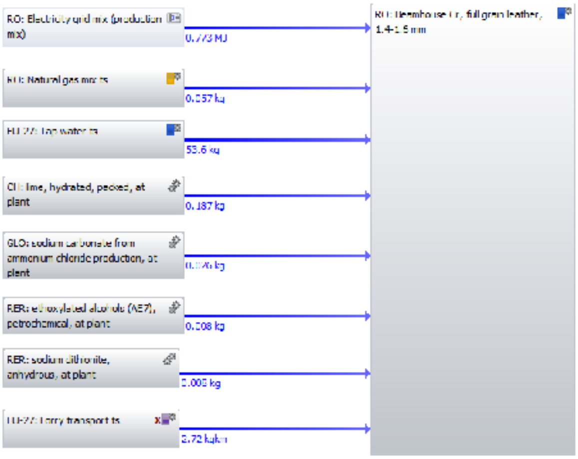

Figure 2. Beamhouse phase, modelled by LCA software GaBi ts

The results of the LCA study regarding the GWP impact category are presented in Table 2. From the data we had it seems that finishing phase generates the highest pollution in terms of CO_2, followed by Dyeyard and Beamhouse phase. What stands out is that tanyard phase generates the lowest pollution from all processing phases. This is unusual for LCA studies in general.

Table 2. LCA impact categories for manufacturing natural full grain, bovine leather, thickness 1.4-1.6 mm

	GWP
Beamhouse	0.426
Tanyard	0.332
Dyeyard	0.983
Finishing	1.623
Total	**3.289**
Unit	kg CO_2-Equiv.

CONCLUSIONS

Life Cycle Assessment tends to become a common instrument in leather industry. Increasingly more studies are published, values that define the impact on the environment are made public. In this respect the current paper presented a gate-to-gate LCA case study based on data collected from an important tannery from Romania, for production made during one year; full grain leather assortment, bovine leather, chrome tanned. The resulting values of the presented LCA study, more precisely of the GWP in terms of kg CO_2 equivalent fall into the ranges of the ones published worldwide in various studies and publications.

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REFERENCES

Brugnoli, F. and Kráľ, I. (2012), "Life Cycle Assessment, Carbon Footprint in Leather Processing (Review of methodologies and recommendations for harmonization)", Eighteenth Session of the Leather and Leather Products Industry Panel, Shanghai, China, 01-05 September.

CO_2 Guide, available from: http://liteleather.com.

Deselnicu, D.C., Crudu, M., Ioannidis, I., Brugnoli, F. (2014a), "Environmental aspects for leather from a Life- Cycle perspective - Part I – Methodology", *Proceedings of The 5th International Conference on Advanced Materials and Systems - ICAMS 2014*, 23-25 October 2014, Bucharest.

Deselnicu, D.C., Crudu, M., Ioannidis, I., Brugnoli, F. (2014b), "Environmental Aspects for Leather from a Life–Cycle Perspective. Part II: Impact Assessment", *Proceedings of The 5th International Conference on Advanced Materials and Systems - ICAMS 2014*, 23-25 October 2014, Bucharest.

ECO_2L (2014), Climate Protection and Leather, ILM, 16 January 2014.

European Commission (2013), Best Available Techniques (BAT) Reference Document for the Tanning of Hides and Skins, available from: http://eippcb.jrc.ec.europa.eu/reference/BREF/TAN_Adopted552013.pdf

GaBi - the leading Life Cycle Assessment software (2016), available from: http://www.gabi-software.com/international/solutions/life-cycle-assessment, accessed: July 2016.

ILCD handbook (2010), available from: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC48157/ilcd_handbook-general_guide_for_lca-detailed_guidance_12march2010_isbn_fin.pdf, accessed: July 2016.

ISO 14040:2006 (2006), Environmental management - Life cycle assessment - Principles and framework, available from: http://www.iso.org/iso/catalogue_detail?csnumber=37456.

ISO 14044:2006 (2006), Environmental management - Life cycle assessment - Requirements and guidelines, available from: http://www.iso.org/iso/catalogue_detail?csnumber=38498.

Naviglio, B., Calvanese, G. and Caracciolo, D. (2015), "La sostenibilita ambientale del cuoio", *Cuoio Pelli Materie Concianti*, 91(3/4), Maggio/Agosto, 2015, p92.

Notarnicola, B., Puig, R., Raggi, A., Fullana, P., Tassielli, G., Tarabella, A., Petti, L., De Camillis, C. and Mongelli, I. (2007), "LCA of Italian and Spanish Bovine Leather Production Systems in an Industrial Ecology Perspective", in: *Industrial Ecology in the Cattle-to-Leather Supply Chain*, Franco Angeli, Milano, Italy.

Notarnicola, B., Puig, R., Raggi, A., Fullana, P., Tassielli, G., Tarabella, A., Petti, L., De Camillis, C. and Rius, A. (2011), "Life cycle assessment of Italian and Spanish bovine leather production systems", *Afinidad*, LXVIII, 553, Mayo - Junio 2011.

Understanding Global Warming Potentials, available from: https://www3.epa.gov/climatechange/ghgemissions/gwps.html, accessed: 15 July 2016.

Wikipedia, 2015 United Nations Climate Change Conference, https://en.wikipedia.org/w/index.php?title=2015_United_Nations_Climate_Change_Conference&printable=yes, accessed: 19 July 2016.

ISOCYANATE-FUNCTIONALIZED COLLAGEN HYDROLYSATES AS PRETANNING AGENTS FOR ORGANIC WET-WHITE LEATHER

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The present paper aims to synthesize and test a pretanning agent based on isocyanate-functionalized collagen hydrolysates of low molecular mass (0.9 ÷ 3.6 kDa; polypeptides that include 8 to 32 amino acids), obtained starting from hides wet wastes, and used without further purification. Raw colloidal suspension resulted after hydrolysis was centrifuged to separate insoluble particles, and then was repeatedly filtered to retain coarse particulate and fatty maters. The clarified solution was concentrated by vaporization up to 35 % w/v dry matter, mixed with 10 % v/v dimethyl sulfoxide (DMSO), and maturated overnight under efficient mixing, in a hermetically closed reaction vessel, at ambient temperature. Using a solution of diisocyanate in DMSO, the molecular mass of the product was further increased by cross-linking, in parallel with the functionalization in the virtue of a small amount of free isocyanate groups that remain unreacted. The functionalized collagen hydrolysate was characterized by chemical (total nitrogen content, the amount of free carboxyl, amino, and isocyanate groups) and instrumental methods (infrared spectroscopy). The fraction of increased molecular mass after cross-linking was determined by comparative dialysis through membranes with 3.5 and 12 kDa. Pretanning ability of the functionalized hydrolysates was estimated by gelatin precipitation, and tested on sheep pelts. An increase of 6 ºC was measured for the shrinkage temperature. Pretanned pelts were gradually dehydrated (preventing the local drying) and drum-tumbled, and then was treated once again with the same functionalized product, in a short concentrated float. A supplemental increase of shrinkage temperature with 8 ºC was measured.

Key words: collagen hydrolysate, functionalization, diisocyanate, pretanning agent.

INTRODUCTION

Coordinative chromium salts still remain the preferred industrial tanning agents, and the classical vegetable tannins were only partially replaced in retanning recipes. The objective of replacing these "old fashioned" products currently faces to two challenges: (i) the fact that the definitions of the most important leather characteristics and properties were developed in relation with them, and were imposed by their use, and (ii) their technological efficiency and their tanning efficacy / price ratio are unrivalled in comparison with other tanning / retanning products. The race to develop replacing products is imposed nowadays by the ecological constraints, and by the increasing demands of the modern consumer, the "natural" or "organic" terms being enforced by marketers.

During the time, different products were tested as organic tanning / retanning agents. Due to their crosslinking potential, small-molecular, oligo- and poly-meric chemical compounds are sporadically used at industrial level, but, excepting some (di)aldehydes, none of them were largely accepted.

Functionalized compounds of natural origin represent a current investigated alternative. Protein hydrolysates could support a range of functionalizing reactions to become the "core" of poly-functional crosslinking (and possibly tanning) capable products.

The present paper discuss the way to produce, and then tries to validate, a "reconstructed" artificial polypeptide bearing isocyan chemical groups, in small but necessary amount to endow the "core" with tanning / retanning ability.

The high reactivity of isocyanate group and the rapid kinetics of its reaction with dehydrated dermis gave tanners hope for finding a practical solution to the issue of producing organic tanned leather. Seventy years ago, the crosslinking ability of small-molecular diisocyanates was first exploited to replace the classical tanning agents (Putnam, 1947). The method remained inapplicable at industrial scale because it imposes the work in organic solvents.

The use of oligo- and/or poly-isocyanate blocked derivatives as (pre)tanning agents is also well known and was proposed long time ago (Milligan *et al*., 1983; Träubel *et al*., 1998; Ebbinghaus *et al*., 2006), but their practical application is severely limited by three drawback factors: (i) the costs of producing them, (ii) their limited diffusivity into the pelt, when working in usual tanning conditions, and (iii) the need to work at relatively high temperatures to complete the de-blocking and effective crosslinking.

Collagen hydrolysates were also tested, individually or in association with other small molecular compounds, as polyfunctional physical crosslinkers, which could assist tanning / retanning processes (Cantera *et al*., 2000; Cantera *et al*., 2002; Aslan *et al*., 2006). The main drawbacks in using protein hydrolysates consist in: (i) their intrinsic putrescibility, (ii) their small molecular volume, and (iii) their low reactive chemical groups. A combined chemical transformation which concomitantly increases the molecular mass and the overall reactivity of protein hydrolysates could be beneficial in producing tanning / retanning compounds. The controlled reaction between some diisocyanates and collagen hydrolysates was used in our work in order to diminish the above mentioned deficiencies.

EXPERIMENTAL DATA AND DISCUSSIONS

The collagen hydrolysate we used was obtained according a classical method, starting from pelt / limed split wastes (Constantinescu *et al*., 2015). The physical-chemical characteristics of the final liquid form are resumed in Table 1.

Table 1. The characteristics of the collagen hydrolysate, in liquid form

The determined characteristics	Measuring unit	Determined value
Dry substance	% w/v	9.64
Minerals content in dry substance	% w/w	0.39
Total fatty matters in dry substance	% w/w	8.74
Total nitrogen in dry substance, TN	%	14.47
"Leather substance" in dry matter (TN x 6.28)	% w/w	90.85
Amount of carboxyl groups in dialyzed product	mEq / g	2.92
Amount of α-amino groups in dialyzed product	mEq / g	0.71
Amount of ε-amino groups in dialyzed product	mEq / g	1.74
Mass fraction of the molecules below 3.5 kDa	% w/v	57.41
Mass fraction of the molecules between 3.5 and 12 kDa	% w/v	39.12
Mass fraction of the molecules above 12 kDa	% w/v	3.47
Hydrolysis yield (= total solubilized / initial mass)	% w/v	84.21

The raw collagen hydrolysate in liquid form was further chilled at 17 °C, filtered through a coarse mesh, centrifuged at 3200 g, and ultrafiltered through a membrane having a MWCO value of 3.5 kDa (in tangential flow regime, discarding the retentate). A final dry matter of 5.84 % and a "leather substance" (equivalent with the total polypeptide content) of 5.45 % resulted, representing a purifying yield of about 56.53 %. The fatty maters were completely removed by the purifying method. Figure 1 depicts the acid/base bounding capacity and the buffering capacity of the purified product, based on which were calculated the amounts of reactive groups of polypeptides.

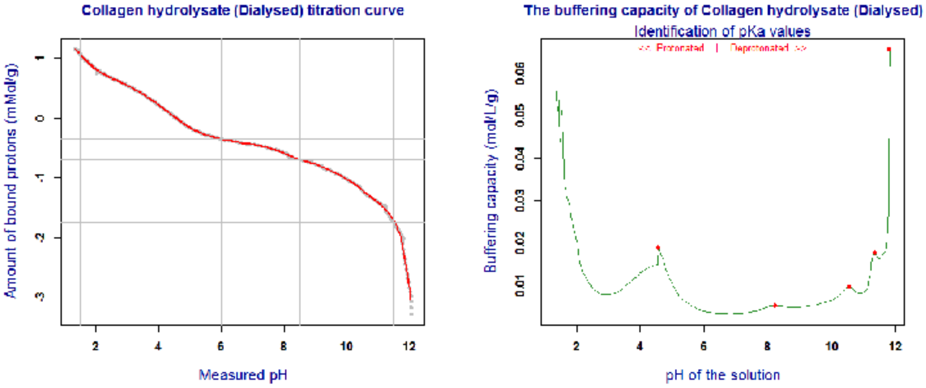

Figure 1. Proton bounding curve and buffering capacity of the purified collagen hydrolysate

The purified hydrolysate was first concentrated by evaporation up to a total polypeptide content of about 35 % w/v, and then 10 % v/v dimethyl-sulfoxide (DMSO) was slowly added, under efficient mixing. The resulted solution was maturated overnight under efficient mixing, in a hermetically closed reaction vessel, at ambient temperature (of about 18 °C). The traces of the resulted colloidal solid (about 0.16 % w/v) were removed by a second centrifugation.

In order to "reconstruct" an extended macromolecular edifice, the polypeptide mixture (initially having a molecular mass in the range of 0.9 ÷ 3.6 kDa, which represents the equivalent of 8 to 32 amino acids length polypeptides) was subjected to a controlled cross-linking with 0.3 % w/w hexamethylene diisocyanate (HMDI) dissolved in 5 % v/v anhydrous DMSO (relative to the volume of the hydrolysate solution). To prevent local precipitation, the HMDI solution was added dropwise, under efficient stirring. Concomitantly, the temperature of the reaction mixture was increased up to 60 °C. After a warm maturation of four hours (at 60 °C, under stirring), the resulted colloidal suspension was further maturated, overnight, at the ambient temperature. Second maturation concluded with the suspension coarsening and settling. The resulted flocks were then separated by centrifugation (3200 g, 30 min.). The slurry was re-suspended in water and centrifuged for three more times. The final sediment was suspended in 10 % v/w DMSO, and, after a warm maturation (4 hours, at 60 °C, under stirring in a hermetically closed autoclave; the dry matter content was finally determined), 3 % w/w isophorone diisocyanate (IPDI) (relative to the volume of the dry matter of the suspension), dissolved in 10 % v/v anhydrous DMSO, was dropwise added. At the end of four hours of efficient stirring, the reaction mixture was decanted and the resulted flocks were separated by centrifugation. The sediment was further slowly dried, at an increasing temperature from 60 to 95 °C, under permanent hot air flow. After each hour of drying, the final product was crushed and the grinded, to obtain a powder. Finally, the obtained solid powder was ball-milled in the presence of solid starch, in a ratio of 1 : 3 w/w, and of $NaHCO_3$, in a ratio of 1 : 0.5 w/w.

Figure 2 presents the ATR FTIR spectra of the initial purified collagen hydrolysate, the HMDI crosslinked product, and the final IPDI functionalized product.

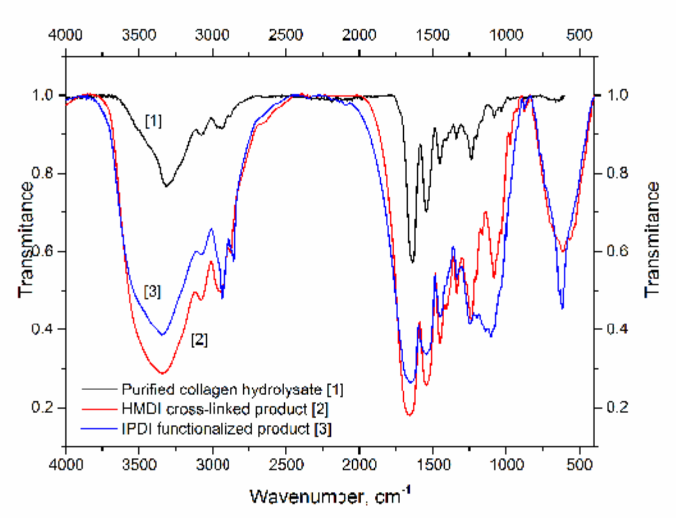

Figure 2. The FTIR spectra of the raw and functionalized products

The increase of molecular mass after the HMDI mediated crosslinking was determined by the amount of un-dialyzed soluble product, using membranes with 3.5 and 12 kDa. The obtained values were of 0.64 % w/v, and 81.30 % w/v, respectively.

The amount of free isocyanate groups was measured by gas chromatography, according the method of Xie and Chai (Xie and Chai, 2016). The values were 0.03 mEq/g in the case of the HMDI crosslinked product, and of 0.84 mEq/g in the case of the IPDI functionalized product.

The (pre)tanning ability of the final functionalized product was estimated by gelatin precipitation, and tested on sheep pelts. An increase of 6 ºC was measured for the shrinkage temperature after a treatment with 6 % w/w in a float of 150 % warm water (30 °C). Pretanned pelts were further gradually dehydrated (preventing the local drying) and drum-tumbled, and then was treated once again with the same amount of functionalized product, in a short float (80 % water, 45 °C). A supplemental increase of shrinkage temperature with 8 ºC was measured. The treated sheep leather were partially dried under vacuum, tumbled for 15 min., two time staken on a hydraulic machine, dried, re-humidified, staken again, wheeled in two steps, and finally tumbled. Soft, light brown sheep leather with a shrinkage temperature of 61 °C resulted.

CONCLUSIONS

We developed a procedure to „reconstruct” an extended polypeptide edifice starting from low molecular collagen hydrolysates, which, after the functionalization with a nonsymmetrical diisocyanate, gained (pre)tanning properties.

1. The molecular mass increasing was induced by the controlled crosslinking of collagen hydrolysates with hexamethylene diisocyanate.
2. The functionalization was performed using isophorone diisocyanate.
3. An increase of 14 °C was measured for the shrinkage temperature of the sheep leather in humid state; after drying and mechanical finishing the measured shrinkage temperature was of about 61 °C.

Acknowledgements

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REFERENCES

Aslan, A., Gülümser, G. and Ocak, B. (2006), “Increased chromium tanning efficiency with collagen hydrolysates”, *Journal of Society of Leather Technologists and Chemists*, 90(5), 201-204.

Cantera, C., Goya, L. and Mingo, R. (2000), “Collagen hydrolysate: ‘Soluble skin’ applied in post-tanning processes. Part 1: Characterisation”, *Journal of Society of Leather Technologists and Chemists*, 84(1), 29-37.

Cantera, C., Martegani, J., Esterelles, G. and Vergara, J. (2002), “Collagen hydrolysate: ‘Soluble skin’ applied in post-tanning processes. Part 2: Interaction with acrylic retanning agents”, *Journal of Society of Leather Technologists and Chemists*, 86(5), 195-202.

Constantinescu, R.R., Zainescu, G., Stefan, D.S., Sirbu, C. and Voicu, P. (2015), “Protein biofertilizer development and application on soybean cultivated degraded soil”, *Leather and Footwear Journal*, 5(3), 169-178.

Ebbinghaus, M., Heinzelmann, F., Kleban, M., Reiners, J. (2006), *US Pat. Appl. No 0080784/2006*, “Isocyanate-based tanning agents”.

Milligan, B., Buechler, P.R., Feairheller, S.H. (1983), *US Pat. No 4413997/1983*, “Dicarbamoylsulfonate tanning agent”.

Putnam, R.C. (1947), *US Pat. No 2523326/1947*, “Tanning with organic isocyanates innonaqueous medium”.

Träubel, H., Novotony, F. and Reiff, H. (1998), *US Pat. No. 5820634/1998*, “Process for tanning leather”.

Xie, W.-Q. and Chai, X.-S. (2016), “Determination of isocyanate groups in the organic intermediates by reaction-based headspace gas chromatography”, *Journal of Chromatography A*, 1468, 241-244.

SOLUTIONS AND EQUIPMENT FOR LEACHATE TREATMENT

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Without a proper waste management, leachate may contaminate the environmental; landfill gas can be toxic and lead to global warming and explosion and landfill wastewater can pollute the land and surrounding waters. The major factors, which affect the production of leachate and landfill gas are waste type, pH, moisture content, particle size and density, temperature, landfill type etc. Concentrations of various substances occurring in leachate are too high to be discharged to surface water or into a sewer system. These concentrations, therefore, have to be reduced by removal / treatment. Common toxic components in leachate are COD, BOD, ammonia, heavy metals etc. Process combinations for the treatment of landfill leachate have been thoroughly tried and tested over the past years by many researchers. A new solution and equipment were proposed by a team of researchers from DFR Systems SRL in order to treat the leachate. Several treatment stages were implemented in the wastewater treatment technological flux: physical treatment, biological treatment (with artificial mobile support), ultrafiltration, dissolved air flotation, UV disinfection etc. The new technology was tested on a lab-scale facility and the results were encouraging. In the present paper are presented the obtained results as well as the treatment technology and equipment.

Keywords: leachate, wastewater treatment, equipment for leachate treatment

INTRODUCTION

This paper aims to present the problem of collection and treatment of leachate from municipal landfills to reduce pollution levels by developing modern technologies and equipment/installations for leachate treatment. In order to treat the leachate is very important to know its composition that is influenced by the following factors: the composition of the waste being deposited and the degree of decomposition; the age of the landfill; mass of humidity existing in wastes; temperature; storage system and technological solutions for compacting, stratification, coating, period of execution etc.

For Romania, the general characteristics of the landfills in the country are the lack of treatment before storing and poor selective extraction by collecting a small percentage of recyclable components / recoverable and this in a narrow range (cardboard, paper, plastics and metals). Leachate generated in different phases has different quality characteristics. The acid phase is characterized by an acidic pH and a high content of volatile organic compounds like CO_2 and H_2S. The formation of volatile fatty acids is the main reason for the low pH and high organic content. Methane phase under aerobic or anaerobic conditions is characterized by a neutral pH and slow rate of fermentation. The main emissions are methane and CO_2. The organic matter content of the leachate is expressed through the necessary biochemical oxygen demand (BOD_5), total organic carbon (TOC) and chemical oxygen demand (COD).

It should be noted that metals do not significantly vary during different phases. Elements such as sodium, sodium chloride, AOX (halogenated) show the dependence of conditions prevailing circumstances for degradation. The same cannot be said of phosphorus whose concentrations decrease from the acid to the metanogenic stage.

The nitrogen concentrations show a downward trend explained by nitrification and denitrification processes in the warehouse and / or the incorporation of nitrogen in biomass. Variations are found throughout the browsing phase pH, BOD_5, COD, magnesium, calcium, iron, manganese and zinc. With the exception of pH most of the components have a higher level in the acid phase than in the metanogenic phase. Other indicators showing significant variations in the acid phase are: total organic carbon (TOC), conductivity, nitrogen, phosphorus, sodium, sulfur, potassium and arsenic. There are some exceptions, such as lead, copper, aluminum and barium which may have higher concentrations of methane during the stage.

The amount of leachate and its degree of contamination are dependent on: the type of waste deposited, deposit age, landfill height, meteorological characteristics of the location area, quality of deposit isolation.

a. The variation of leachate composition - high degree of waste composition diversification generates a similar high degree of leachate composition diversification.

b. Age deposit - over time, the leachate content (consisting in water, dissolved gases and biomass) vary. Leachate quantitative increase in the first 4 years, decrease until the 8th year and reaches a constant value that represents ≈1‰ of the maximum amount.

c. Temperature - the temperature affects biological processes and chemical reactions that occur in the deposit. Deposits with heights/depths greater than 15 m are not influenced by seasonal temperature variations.

d. The available oxygen content of the waste. Landfill and release of chemicals occurs are different in aerobic or anaerobic conditions. Deposits with thicker layers of waste are favored to anaerobic conditions.

e. Moisture waste. Location deposits in areas characterized by predominantly rainy weather generates a higher quantity of leachate. Climatic conditions lead also to leachate seasonal variations.

f. Homogeneity of mass storage. A municipal landfill is not homogeneous and several wastes adsorb water more easily (cardboard or paper) and at the other extreme waste such as plastics, glass or construction waste do not adsorb water.

A typical concentration of the average leachate pollutants is presented in Table 1.

Table 1. The main physico-chemical characteristics of an average leachate

	Values	Nature of pollution
pH	6.1	Acid
COD	1650 mg/l	Organic pollution
Organic compounds	4800 mg/l	Organic pollution
Na; K;	2900 mg/l	Mineral pollution
Ca; Mg;	2000 mg/l	Mineral pollution
Cl; SO_4;	4800 mg/l	Mineral pollution
NH_4	650 mg/l	Mineral pollution
Fosfor total	6 mg/l	Mineral pollution
Fe	900 mg/l	Metal pollution
Mn	25 mg/l	Metal pollution
Zn	10 mg/l	Metal pollution
Other metals like: Co, Ni, Cu, Cr, Pb, Mo, As, Hg	10 mg/l	Metal pollution

MATERIALS AND METHODS

Taking into account the large number of the pollutants present in leachates, a special technological flux must be conceived in order to remove all the pollutants mentioned in Tabel 1. In this way the new leachate treatment technology was based on several stages: removal, of coarse solids; removal of fine solids; leachate homogenization and pH correction; filter; biological stage; dissolved flotation unit; settler; membrane biofilter; UV disinfection. Additional chemical stage can be introduced in the mentioned technological treatment flux. A special attention was given to the final settler because this equipment must remove both the foam resulted from flotation and suspended solids. The main purpose of sedimentation is to remove suspended solids (organic matter, very fine sand, etc.), thus decreasing loading subsequent purification steps (Metcalf and Eddy, 2003). The rate of settling in clarifiers depends on the size and density of the particles and the degree of flocculation (Figure 1). For a correct designing mathematical modelling and numerical simulations were used.

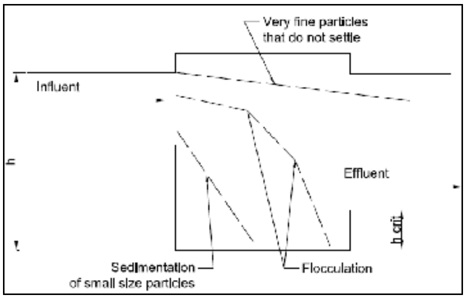

Figure 1. The rate of settling depending of particles size

The vertical section of the new vertical settler conceived, designed and patented is presented in Figure 2 (Moga, 2014). The vertical settler in used for the separation of phases from multiphase fluids. The principal characteristic of multiphase fluids (mixture) is the fact that, in static conditions, due to different specific weight, the phases will separate by gravity in two vertical directions: phases heavier than water will separate in a descending direction – in a process called sedimentation, and phases lighter than water will separate in the ascending direction, with floating materials rising to the surface – in a process called flotation.

The low flow velocity in a settler allows settleable particles to sink to the bottom, while constituents lighter than water float to the surface.

Sedimentation is also used for the removal of grit (pre-treatement technologies), for secondary clarification in activated sludge treatment, after chemical coagulation / precipitation, or for sludge thickening. Settlers can achieve a significant initial reduction in suspended solids (50-70% removal) and organic material (20-40% BOD removal) and ensure that these constituents do not impair subsequent treatment processes. The main purpose of a *settler* is to facilitate sedimentation by reducing the velocity and turbulence of the *wastewater* stream. Settlers are typically designed for a *hydraulic retention time* of 1.5-2.5 h. Less time is needed if the *BOD* level should not be too low for the following biological step. The tank should be designed to ensure satisfactory performance at peak flow.

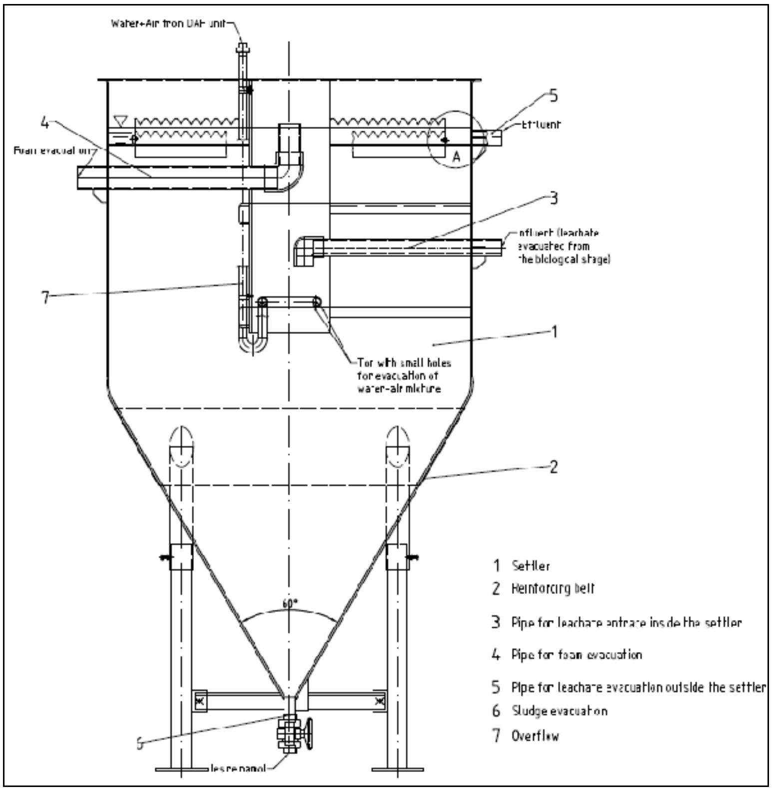

Figure 2. Vertical section of the new settler used for leachate treatment

EXPERIMENTAL

A laboratory installation was conceived, designed and realized. The technological flux of the developed facility is presented in figure 3. The biological treatment stage is also innovative and Mobile Bed Biofilm Reactor process is implemented. This technology utilizes free-moving biofilm carriers that represents a future evolution of the activated sludge process that allows a greater pollutant removal degree in smaller systems (Zafarzadeh *et al*., 2008). The biofilm grows protected within small plastic carriers, which are carefully designed with high internal surface area. These biofilm carriers are suspended and mixed throughout the water phase. The wastewater treatment with bio-media consists in adding biofilm carriers (small cylindrical shaped polyethylene carrier elements with specific density of 0.96 g/cm^3) in aerated or anoxic basins to support biofilm growth. When the microorganisms in the biological film die, the film breaks up and peels off from the solid support being carried away by the liquid current. The destroyed cellular material is directed to the vertical settler and detained as sludge. Hence, the need for the hydraulic scheme to include a secondary settling tank/filter (American Water Works Association, 2000). The new settler was included in the leachate treatment.

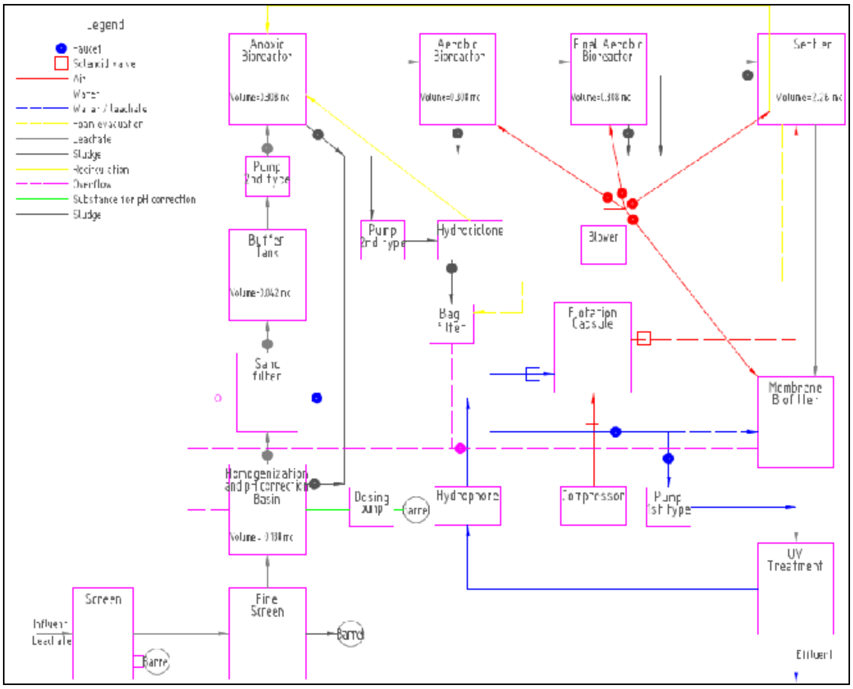

Figure 3. Technological flux of the laboratory installation for the leachate treatment

RESULTS AND DISCUSSIONS

The leachate flow-rate was established at 0.02 m^3/h. Experimental researched and the process efficiency of the laboratory leachate treatment plant was determined. Analysis for both influent and effluent were realized and the main quality indicators were measured. For BOD and COD removal an efficiency over 90% was obtained and all the quality indicators were significantly reduced.

CONCLUSIONS

Given the obtained results (the reduction of the leachate parameters values) from the experimental researches realised in laboratory conditions, the proposed technology may constitute solutions to modernize existing leachate treatment plants. Already some parts of the leachate treatment plant (the system of dissolved air flotation and vertical settler) have been tested with other types of wastewater (brewery) achieving a high efficiency of treatment and reduce the investment costs with (15-20)% compared to existing solutions for wastewater treatment.

Currently there are two patents for the components of the leachate treatment plant. The research team aims to develop technology in the future, analyzing the possibility of introducing advanced treatment steps to achieve a high treatment standards specified by the NTPA001 (effluent discharge into natural receivers).

Acknowledgments

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REFERENCES

American Water Works Association (2000), *Water Quality and Treatment, A Handbook of Community Water Supplies,* McGraw-Hill Handbooks, New York.

Metcalf and Eddy, Inc. (2003), *Wastewater Engineering: Treatment and Reuse, fourth edition*. McGraw-Hill Handbooks, New York.

Moga, I.C. (2014), *Technical reports for CESELEV project.*

Zafarzadeh, A., Bina, B., Nikaeen, M., Attar, H.M. and Hajiannejad M. (2010), “Performance of MBBR for Biological Nitrogen Compounds Removal from Wastewater by Partial Nitrification-Denitrification Process”, *Iranian Journal of Environmental Health Science and Engineering*, 7, 353-364.

STUDY ON OBTAINING KERATIN EXTRACTS FROM LEATHER INDUSTRY BY-PRODUCTS

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Keratin is a biopolymer with numerous functional properties for the production of biomaterials with applications such as: additives for cosmetics, biostimulator for growth and nutrition in agriculture, ecological treatments in reconstruction and protection of leather and furs, as alternative to pollutant chemical compounds. In most of the applications, in either large or niche industries, keratin is used as gels, films, nano- or micro-particles. This study presents the possibilities of using thermal and enzymatic processes of leather industry by-products to obtain keratin extracts. Keratin extracts were characterized by chemical and instrumental analyses: gravimetric, volumetric, potentiometry, Polyacrylamide Gel Electrophoresis, Infrared Spectroscopy, High-Performance Liquid Chromatography, Dynamic Light Scattering. Analytical investigation has shown that the keratin extracts obtained as polydispersions with 5% dry matter have 14% total nitrogen and contain 50% small and medium sized particles (10-500 nm range), such as free amino acids and oligopeptides and 50% larger sized particles (500-5560 nm ranges) such as polypeptides. The IR spectrum of keratin extract is similar to the IR spectrum of collagen from leather.

Keywords: keratin, molecular weight, amino acids

INTRODUCTION

Obtaining keratin hydrolysates from by-products/waste from the leather industry contributes to the recovery of these natural resources, to the reduction of the amount of stored waste and to environmental pollution prevention. The hydrolysates may be used to develop new biomaterials with multiple applications, as well as to design environmentally-friendly treatments for leather and fur with various functionalities.

Wool is a keratinous material with specific structure, mechanical behaviour and physical-chemical properties (Wang *et al.*, 2016). A clean wool fibre contains approximately 82% keratinous protein with high concentration of cysteine, approximately 17%, a protein material with a low cysteine content called “non-keratinous material” mainly localized in the complex of the cell membrane and approximately 1% of the non-protein material is made up of waxy lipids, plus a small amount of polysaccharide material (Lewis and Rippon, 2013).

Keratins are the most abundant structural proteins in epithelial cells and, together with collagen, it forms the most important biopolymer in the organic matter that constitutes animal tissue (Coulombe and Omary, 2002; McKittrick *et al.*, 2012). Keratin is among the most rigid biological materials, with high hardness and elastic modulus, although is is made up only of polymer compounds and rarely contains minerals (Wegst and Ashby, 2004; Szewciw *et al.*, 2010).

Keratinous materials are high in cysteine, which differentiates them from other biopolymers, are usually durable, rigid and non-reactive with the natural environment. They provide mechanical support and various protection functions in the adaptation of vertebrates to the external environment (Schweizer *et al.*, 2006).

Extraction of keratin from wool can be achieved in various ways, all of them involving the presence of reduction or distortion agents to break disulfide bonds (Aldemar *et al.*, 2005; Liu *et al.*, 2004; Aluigi *et al.*, 2007).

Keratin extracts are obtained using acid hydrolysis (Khosa and Ullah, 2014), hydrolysis in alkaline medium (Hill *et al.*, 2010; Staron *et al.*, 2014), enzymatic hydrolysis (Krejci *et al.*, 2011) and ionic liquid extraction (Ji *et al.*, 2014). The various methods of obtaining keratin extracts lead to variations in their composition and properties. These differences allow the use of keratin hydrolysates in the production of materials with multiple industrial applications, in agriculture and niche applications.

This study proposes to transform a low economic value material (sheep wool scraps) into a product with potential for exploitation in various fields: leather processing, cosmetics, agriculture, as wool is a source of organic nitrogen as macronutrient but also sulfur as essential mesonutrient for plant nutrition (for example, in leguminous plants sulfur deficiency leads to reduction of nodules on the roots, increase of soluble nitrogen and slow formation of protein substances).

EXPERIMENTAL

Materials

Raw materials: wool by-products from the leather industry with the following characteristics: dry substance, max. 87%; ash, max. 13%; total nitrogen, min. 13%.

Auxiliary materials: ammonia, 25% solution, CAS 1336-21-6; detergent; anhydrous sodium carbonate, CAS 497-19-8; distilled water; hydrated lime, p.a.; rotulis sodium hydroxide; potassium hydroxide, p.a.

Procedures

In order to obtain keratin extracts, raw wool (LB) was degreased in a FAVE vessel system using a solution of 1 g/L of 25% ammonia, detergent and sodium carbonate anhydrous, under stirring for 12 hours at 35°C.

After degreasing, the wool was dried in a ventilated open space and then fragmented.

Degreased wool (LD) was subjected to alkaline hydrolysis: (a) with 10% hydrated lime (b) with 5% sodium hydroxide, (c) with 5% potassium hydroxide and keratin hydrolysates were obtained according to the technological scheme of Fig. 1.

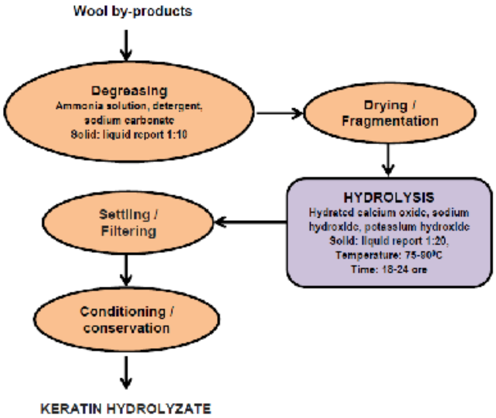


Figure 1. Technological scheme for obtaining keratin hydrolysates

Analytical Methods

The keratin extracts were analysed in terms of dry substance and total ash by gravimetric methods, total nitrogen and protein substance, aminic nitrogen by volumetric methods, pH by potentiometric method, molecular weight by SDS-PAGE electrophoresis. Particle size and distribution was determined by ZetaSizer device Nano ZS (Malvern, UK), IR spectral analysis by FT/IR-4200 (Jasco) with ATR device equipped. Static contact angle of keratine hydrolysate was evaluated with contact angle analysis equipment, VGA Optima XE system, AST Products SUA.

RESULTS AND DISCUSSIONS

Keratin hydrolysates with distinct colors were obtained depending on the alkaline hydrolysis medium: (a) clear orange (KHA1), (b) brown (KHA2), (c) tan (KHA3). The unsolubilized wool residue has a different appearance and consistency: a) RL1, from the hydrolysis with calcium oxide has a brittle consistency, b) RL2 from the hydrolysis with sodium hydroxide is compact and has a more rigid consistency, c) RL3 from hydrolysis with potassium hydroxide is voluminous and thick.

Keratin hydrolysates obtained by alkaline hydrolysis were analyzed in terms of chemical and physical-chemical properties and specific properties were identified, using different instrumental techniques: electrophoresis, DLS, IR spectroscopy.

Physico-chemical analyses showed significant and close values for total nitrogen, which reveals extraction of large amounts of proteins (Fig. 2). Proteinaceous material is between 65.29% (KHA3) and 79.02% (KHA1), and the amino nitrogen up to 3.21% (KHA1). The high values of ash range between 12.28% (KHA1) amd 25.62% (KHA3), showing that the keratin hydrolysates are rich in minerals used in hydrolysis (Ca, Na, K).

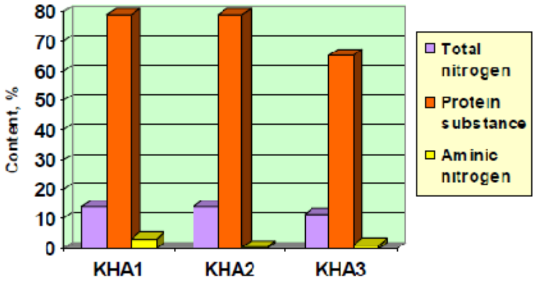


Figure 2. Protein composition of keratin hydrolysates

Determining Distribution of Molecular Weights in Keratin Hydrolysates by SDS - PAGE Electrophoresis

The electrophoretic pattern of keratin is associated with two main groups of protein specific to keratin, intermediate filamentous proteins and matrix proteins.

Figure 3 shows the distribution of molecular masses in keratin hydrolysates experimentally obtained by alkaline hydrolysis from wool by-products.

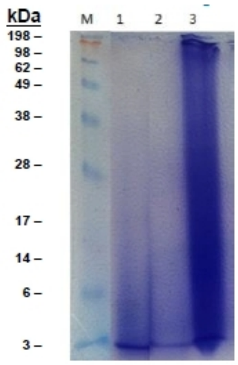
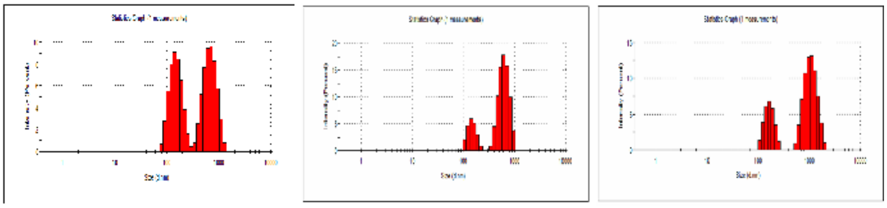


Figure 3. Distribution of molecural weights in keratin hydrolysates: M-marker;1-KHA1; 2-KHA2; 3-KHA3

Two high molecular weight bands are visible (45-60 kDa) attributed to filamentous proteins with a low sulfur content and more low molecular weight bands attributed to proteins with a high content of sulfur (20-10 kDa) and a high content of glycine/tyrosine (6-9 kDa) (Vasconcelos *et al.*, 2008). These considerations highlight the presence of proteins with a high content of sulfur (20-10 kDa) but rich in glycine/tyrosine (6-9 kDa) with low molecular weight, mainly in keratin hydrolysate (KHA1), but also in keratin hydrolysate (KHA3). Proteins with a low sulfur content and high molecular weight assigned to filamentous proteins are more evident in keratin hydrolysate (KHA3).

Determining Particle Sizes Using Dynamic Light Scattering (DLS)

Measurements of the intensity of reflected light indicate a narrow spectrum of particle sizes for the three types of keratin hydrolysate, Figure 4, including mostly medium-sized particles in the range of 100-1000 nm, specific to oligopeptides and larger peptides.



(a) (b) (c)

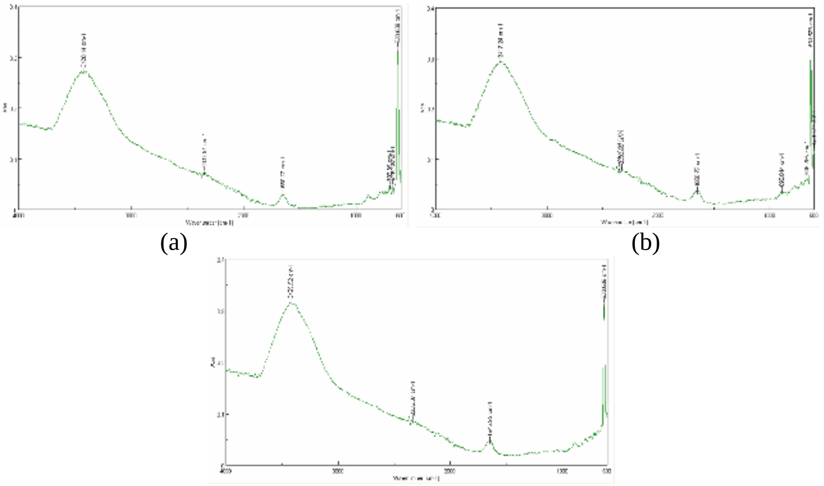
Figure 4. Particle size distribution in keratin hydrolysates: (a) KHA1, obtained with hydrated lime; (b) KHA2, obtained with sodium hydroxide; (c) KHA3, obtained with potassium hydroxide

However, the KHA1 hydrolysate also contains smaller particles in the higher area of the 10-100 nm range, specific to amino acids and small oligopeptides, but also a small percentage of particles greater than 1000 nm, namely polypeptides. Hydrolysates KHA2 and KHA3 do not have particles smaller than 100 nm, however, hydrolysate KHA3 shows a higher percentage of particles of over 1000 nm. These data are consistent with results of electrophoresis which reveal the presence of compounds with molecular weights lower than 20 KDa, and some compounds with molecular weight higher than 45KDa.

Structural Analysis of Keratin Hydrolysates Using FT-IR Spectroscopy

Infrared absorption spectra of keratin hydrolysates (KHA1, KHA2, KHA3) show characteristic bands attributed both to peptides (-CONH) of amide I, amide II, and amide III types, and to sulfur compounds.

Among these, there are bands specific to primary amides at 3400 cm^{-1} and secondary amides at 3440 cm^{-1} attributable to the stretching vibration $\nu_{N\text{-}H}$ and the band specific to tertiary amides at 1650 cm^{-1} attributable to the stretching vibration $\nu_{C=O}$, present in the three spectra of keratin hydrolysates (Fig. 5). The bands at 600-700 cm^{-1} attributed to stretching vibration $\nu_{C\text{-}S}$ and at 800-900 cm^{-1} attributed to rocking vibration $\delta_{S\text{-}H}$ are specific to sulfur compounds (Khosa and Ullah, 2014; Hill *et al.*, 2010; Staron *et al.*, 2014).



(a) (b)

(c)

Figure 5. FT-IR spectral analysis of keratin hydrolysates: (a) KHA1; (b) KHA2; (c) KHA3

Determination of Contact Angle and Surface Tension of Keratin Hydrolysates

Determining the contact angle value of keratin hydrolysates, in relation to glass as inert material indicates a strongly hydrophilic behavior, recording very low values: 9.25° for KHA1, 7.75° for KHA2, 6.32° for KHA3. These hydrophilic contact angles are the premises for good adhesion of keratin hydrolysates to non-hydrophobic porous surfaces, for example, unfinished tanned leather or plant seeds.

Surface tension values of keratin hydrolysates: 40.77 mJ/m^2 for KHA1, 46.49 mJ/m^2 for KHA2, 46.18 mJ/m^2 for KHA3, confirm the presence of low intermolecular forces that cause a high capacity for wetting surfaces.

CONCLUSIONS

Keratin extracts were obtained from wool by-products from the leather and fur industry, through alkaline hydrolysis.

Extracting keratin from by-products contributes to decreasing the amount of by-products stored and prevents pollution, while recovering and reusing residual protein as eco-friendly products for applications in industry and in bio-economy.

Using keratin extracts in agriculture has the advantage of providing organic nitrogen as macronutrient and sulfur as mesonutrient.

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REFERENCES

Alemdar, A., Iridag, Y., and Kazanci, M. (2005), “Flow behavior of regenerated wool-keratin proteins in different mediums”, *International Journal of Biological Macromolecules*, 35, 151–153.

Aluigi, A., Zoccola, M., Vineis, C., Tonin, C., Ferrero, F., and Canetti, M. (2007), “Study on the structure and properties of wool keratin regenerated from formic acid”, *International Journal of Biological Macromolecules*, 41(3), 266–273.

Coulombe, P.A., and Omary, M.B. (2002), “‘Hard’ and ‘soft’ principles defining the structure, function and regulation of keratin intermediate filaments”, *Current Opinion in Cell Biology*, 14(1), 110–22.

Hill, P., Brantley, H., and Van Dyke, M. (2010), “Some properties of keratin biomaterials: Kerateines”, *Biomaterials*, 31, 585–593.

Ji, Y., Chen, J., Lv, J., Li, Z., Xing, L., and Ding, S. (2014), “Extraction of keratin with ionic liquids from poultry feather”, *Separation and Purification Technology*, 132, 577–583.

Khosa, M.A., and Ullah, A. (2014), “In-situ modification, regeneration, and application of cheratin biopolymer for arsenic removal”, *Journal of Hazardous Materials*, 278, 360–371.

Krejci, O., Mokrejs, P., and Sukop, S. (2011), “Preparation and characterization of keratin hydrolysates”, in Mathematical Methods and Techniques in Engineering and Environmentyal Science: Proceedings of the 13th WSEAS International Conference on Mathematical and Computational Methods in Science and Engineering, 308-311.

Lewis, D.M., and Rippon, J.A., editors (2013), The coloration of wool and other keratin fibres, John Wiley.

Liu, Y., Shao, Z., Zhou, P. and Chen, X. (2004), “Thermal and crystalline behaviour of silk fibroin/nylon 66 blend films”, *Polymer*, 45, 7705-7710.

McKittrick, J., Chen, P.Y., Bodde, S.G., Yang, W., Novitskaya, E.E., and Meyers, M.A. (2012), “The structure, functions, and mechanical properties of keratin”, *The Journal of The Minerals, Metals & Materials Society*, 64(4), 449–68.

Schweizer, J., Bowden, P.E., Coulombe, P.A., Langbein, L., Lane, E.B., Magin, T.M. *et al.* (2006), “New consensus nomenclature for mammalian keratins”, *The Journal of Cell Biology*, 174(2), 169–74.

Staron, P., Banach, M., Kowalski, Z., and Staron, A. (2014), “Hydrolysis of keratin materials derived from poultry industry”, Proceedings of ECOpole.

Szewciw, L.J., de Kerckhove, D.G., Grime, G.W., and Fudge, D.S. (2010), “Calcification provides mechanical reinforcement to whale baleen alpha-keratin”, *Proceedings of the Royal Society B: Biological Sciences*, 277(1694), 2597–605.

Vasconcelos, A., Freddi, G., Cavaco-Paulo, A. (2008), “Biodegradable Materials Based on Silk Fibroin and Keratin”, *Biomacromolecules*, 9, 1299–1305.

Wang, B., Yang, W., McKittrick, J., and Meyers, M.A. (2016), “Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration”, *Progress in Materials Science*, 76, 229-318.

Wegst, U.G.K., and Ashby, M.F. (2004), “The mechanical efficiency of natural materials”, Philosophical Magazine, 84(21), 2167–86.

EXTRACTION OF COLLAGEN FROM BY-PRODUCTS, DESIGNED TO INCREASE THE QUANTITY AND QUALITY OF PRODUCTION IN AGRICULTURE

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Collagen polydispersions obtained by associated thermal and enzymatic processes for high-yield extraction of collagen from waste, under mild reaction conditions, are fit for application in agriculture. This study presents the possibility of using thermal and enzymatic processes of by-products from fishing industry to obtain collagen extracts, to increase the quantity and quality of oilseeds and horticultural production. Collagen polydispersions were characterized by chemical and instrumental analyses: gravimetric, volumetric, potentiometry, IR spectroscopy, tensiometer methods, Dynamic Light Scattering (DLS). Analytical investigation has shown that: the collagen polydispersions have bioactive properties due to the content of small sized particles (<100 nm range), such as free amino acids and oligopeptides, able to penetrate the cell membranes and seed coating; the larger sized particles (100-10000 nm ranges) such as polypeptides, ensure the bioactive deposit in the film matrix on the surface, with long term releasing ability; resistance of crosslinked film of fish gelatin for indehiscent treatment in rape crop. The synergy of collagen hydrophilicity, its known biodegradability, bio-active potential and film-forming properties recommend collagen polydispersions for applications in mixtures for treatment in agricultural crops.

Keywords: collagen by-products, surface properties, agriculture production.

INTRODUCTION

Since the main resource of collagen from the skin of terrestrial animals is susceptible to certain diseases (BSE, FMD), or is subject to religious restrictions when it comes to consumption, fish by-products as a source of collagen are an increasingly sought alternative, the more so as fish collagen has a lower phytotoxicity.

Fish processing generates a significant amount of waste, about 50-70% of the raw material (Pati *et al.*, 2010; Bae *et al.*, 2008), of which over 30% is made up of skin, scales and bones, extremely rich in collagen, which is why it has attracted the attention of many researchers (Lee *et al.*, 2001).

As expert studies show (Aberoumand, 2012), the methods used for isolating collagen from the skins of mammals are not effective enough for isolating collagen from marine resources, therefore, the processes of extracting collagen must be tailored to the resources and properties of collagen extracts, according to their applications (Gaidau *et al.*, 2013; Lee *et al.*, 2014).

However, thermal and enzymatic hydrolyses are the basic processes of extracting collagen from fish skin (Wang *et al.*, 2013; Zhang *et al.*, 2011).

To increase performance in obtaining collagen extracts from fish skin, processes can be improved by pre-treatment with ultrasound (Kim *et al.*, 2013; Zeng *et al.*, 2012), and by using a system of membrane ultrafiltration and nanofiltration (Kim, 2013; Picot *et al.*, 2010) to obtain bioactive peptides with a controlled molecular weight and functional properties.

Some properties required in various applications, for example elasticity of formed films, can be improved by the addition of crosslinking agents. Studies on improving the mechanical properties of fish skin collagen films (Liu *et al.*, 2014) have led to a reduction of film hygroscopicity from 295% to 54%, an increase in shrinkage temperature of the film (73.9°C) and film stability in distilled water at normal temperature.

This paper presents experiments for extracting collagen from fish skin waste, compared with extracting collagen from by-products of bovine leather, tanned or untanned, applying thermal-enzymatic hydrolysis processes, in terms of parameters specific to each type of leather processed.

EXPERIMENTAL PART

Materials

Fish skin was obtained from Danube Research-Consulting SRL, Romania. The leather by-products originated from wet blue shavings or pelt with the following characteristics: 50-70% volatile matter, 0.5-12.5% total ash, 14-17% total nitrogen, aqueous extract pH value of 3.5-8.0, were obtained in Leather and Footwear Research Institute, Bucharest, Romania. Sodium hydroxide, acetic acid were products of SC Chimopar SA Romania. Alcalase 2.4L was obtained from Novozymes Denmark.

Procedures

For collagen extraction from fish skin (sample 1), an alkaline pretreatment with Na OH 0.2 N, acid pretreatment with CH_3COOH 0.1 M, extensive washing, followed by thermal and enzymatic processing at 60±2°C during 6 hours at atmospheric pressure, for collagen hydrolysate obtaining (sample 2).

The method for collagen extraction from bovine pelt as gelatin (sample 3) and hydrolysate (sample 4) were conducted at 80°C. The method for collagen hydrolysate from wet blue shavings (sample 5) were conducted at 65-85°C with $Ca(OH)_2$ and Alcalase 2.4L for 6 hours (Niculescu and Gaidau, 2014).

To cross-link the fish skin collagen extracts, 2 mL/L glycerol and 2 mL/L glutaraldehyde solution was used in concentration of 15 g/L collagen (Liu *et al.*, 2014).

Analytical Methods

The collagen polydispersions were analysed in terms of dry substance and total ash by gravimetric methods, total nitrogen and protein substance, aminic nitrogen by volumetric methods, hydroxyproline content by spectrophotometry according to SR 13385/1997 and other amino acid composition by high-performance liquid chromatography (HPLC with Thermo Electron, Finningen Surveier with DAD detector), according to SR EN ISO 13903, pH by potentiometric method, molecular weight by SDS-PAGE electrophoresis. Particle size and distribution was determined by ZetaSizer device Nano ZS (Malvern, UK).

RESULTS AND DISCUSSIONS

The intermediary and final collagen extracts (gelatins and hydrolysates), described in Table 1, was assessed by chemical and instrumental analyses.

Table 1. Description of collagen extracts samples

Sample code	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Description	Gelatin from fish skin	Collagen hydrolysate from fish gelatin	Gelatin from bovine pelt	Collagen hydrolysate from bovine gelatin	Collagen hydrolysate from wet blue shaving

Results of analyses performed in order to establish the chemical composition of collagen extracts are presented in Table 2.

Table 2. Physical-chemical characteristics of collagen extracts

No.	Characteristics, MU	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	Dry substance, %	4.47	4.40	8.46	10.85	8.45
2	Total ash, %	0.67	4.09	0.47	5.53	7.57
3	Total nitrogen, %	14.76	14.77	15.60	15.48	15.03
4	Protein substance, %	82.95	83,01	87.67	87.00	84.47
5	Amino nitrogen, %	0.32	0.99	0.20	0.94	2.10
6	pH	6.35	7.94	3.70	7.56	8.31

Under laboratory conditions, the yields of extraction of nitrogen from skin were from 80.97% for the extraction of fish skin as gelatin, up to 90.90% for the extraction of the gelatin from bovine leather and 93.70% for hydrolysate extraction from tanned bovine leather.

As expected, the collagen hydrolysate derived from tanned skin (sample 5) has a higher value for ash, but also a higher amino nitrogen content compared with hydrolysates from gelatin (sample 4, sample 2). Amino nitrogen content of collagen hydrolysates is in strict correlation with average molecular weight, an important property of collagen extracts. In general, the average molecular weight of collagen hydrolysates is below 20 kDa. For experimental hydrolysates of collagen extracted by direct hydrolysis from tanned bovine leather (sample 5), according to Sörensen Method, amino nitrogen content of 2.10% indicates an average molecular weight of 2200 Daltons, while for the extracted collagen hydrolysates obtained by hydrolysis of gelatin, amino nitrogen content of 0.94% and 0.99% indicates much higher average molecular weights, of 13,800 Daltons and 13,000 Daltons, respectively. Large differences between the average molecular weights for untanned hide hydrolysates compared to tanned leather hydrolysates reveal that the destruction of links between the tanning agent and the collagen matrix during hydrolysis leads to fragmentation of polypeptides and getting a broader polydispersity. Previous research (Niculescu and Gaidau, 2014; Niculescu *et al.*, 2015) has shown, by means of HPLC analysis, that collagen hydrolysates with average molecular weight of up to 15,000 Daltons contain 4-8% free amino acids (histidine, alanine, glutamic acid, arginine, glycine, leucine, isoleucine, methionine, aspartic acid, valine, proline) and oligopeptides (phenylalanine/leucine, isoleucine/lysine) imparting bioactive properties to polydispersions.

The presence of very small components, such as free amino acids and oligopeptides, was emphasized by measurements with the ZetasizerNano ZS equipment, Malvern, for determining particle size and distribution, presented in histograms in Figure 1.

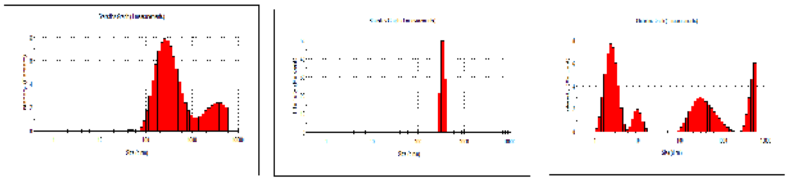

(a) (b) (c)

Figure 1. Particle size distribution in collagen hydrolysates obtained from: (a) fish skin gelatin; (b) bovine hide gelatin; (c) tanned bovine leather

For collagen hydrolysates obtained from tanned leather, histograms highlight the presence of several types of compounds, with various particle size distributions, while for hydrolysates obtained from untanned bovine hide and fish skin, the number of compound types is limited and sizes range mainly over 100 nm, which coincides with the presence of polypeptides, that give a higher average molecular weight, as indicated by amino nitrogen content. Table 3 presents the share of particle sizes in relation to size domains for analysed collagen hydrolysates.

Table 3. Share of particle sizes in collagen hydrolysates

Sample no.	Share of particle sizes, %			
	1-10 nm	10-100 nm	100-1000 nm	1000-5000 nm
Sample 2	-	1.5	76.4	22.1
Sample 4	-	-	100.0	-
Sample 5	47.9	4.9	30.2	17.0

The analytical results indicate that, under the technical conditions described in experimental models, hydrolysates with high polydispersity may be obtained, with a wide range of compounds in tanned bovine leather, or hydrolysates in the narrow domain with larger particles, from untanned bovine hides and fish skin.

FTIR-ATR spectral analysis revealed structural differences of the two types of collagen extracts, gelatin and hydrolysate: Figure 2 for collagen extracts from fish skin, Figure 3 for collagen extracts from untanned bovine hide gelatin.

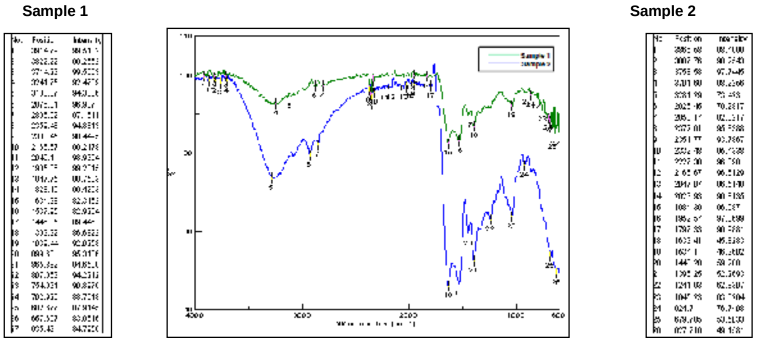


Figure 2. Comparative FT/IR spectra for gelatin and collagen hydrolysate derived from fish skin

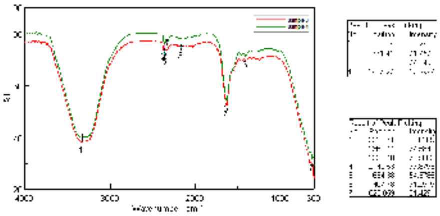
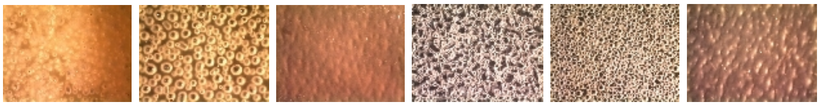


Figure 3. Comparative FT/IR spectra for gelatin and collagen hydrolysate derived from bovine hide

The experimental results confirm the spectral differences between the two types of collagen extracts, by the appearance of new peaks for hydrolysates compared to gelatin, at wavelengths of 2323.71 cm^{-1}, 2142.53 cm^{-1}, 1407.78 cm^{-1}, 623 859 cm^{-1}. However, in the range of 4000-3800 cm^{-1} and 1660-1600 cm^{-1}, no significant differences occur, which confirms the predominantly peptidic structure of collagen hydrolysate derived from gelatin.

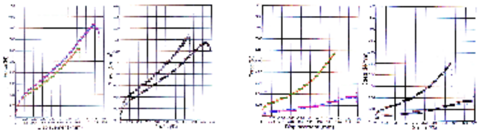
The presence of polypeptides is important for their known film-forming properties. Figure 4 presents the films of collagen extracted from fish skin, in the form of gelatin as such (a), gelatin with the addition of glycerol (b), and gelatin cross-linked with glutaraldehyde (c), and equivalent films (d), (e) (f), structured from fish collagen hydrolysate. The films obtained from raw collagen extracts are rigid and brittle, while the films made from extracts with the addition of glycerol, or crosslinked with glutaraldehyde are flexible and resilient.



(a) (b) (c) (d) (e) (f)

Figure 4. Optical microscopy (x20) of films: (a) gelatin; (b) additivated gelatin; (c) cross-linked gelatin; (d) collagen hydrolysate; (e) additivated hydrolysate; (f) cross-linked hydrolysate

Figure 5 presents the dynamics of tensile strength tests for fish gelatin additivated with glycerol (sample 1A) and for fish gelatin additivated with glycerol and cross-linked with glutaraldehyde (sample 1B).



(a) (b)

Figure 5. Tensile strength of collagen films: sample 1A; (b) sample 1B

The results confirm that collagen extracts are able to form films, whose flexibility, resilience and resistance can be changed depending on the degree of additivation using agents to improve flexibility and crosslinkers to increase resistance of films, to adapt them to the application requirements.

CONCLUSIONS

The experimental processes for extraction of collagen from by-products resulted in gelatin and collagen hydrolysates with broad polydispersity, smart environmental products for applications in agriculture.

The collagen hydrolysates obtained from untanned bovine hides and fish skin contain mostly large-sized particles, exceeding 100 nm, associated to polypeptides.

Collagen hydrolysates obtained from tanned bovine leather contain both large particles and small particles associated with free amino acids and dipeptides.

Gelatins and collagen hydrolysates are able to form films, and their flexibility, resilience and resistance can be improved by additivation and crosslinking.

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REFERENCES

Aberoumand, A. (2012), “Comparative Study Between Different Methods of Collagen Extraction from Fish and its Properties”, World Applied Sciences Journal, 16(3), 316-319.

Bae, I., Osatomi, K., Yoshida, A., Osako, K., Yamaguchi, A., Hara, K. (2008), “Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes”, Food Chemistry, 108, 49–54.

Gaidau, C., Niculescu, M., Stepan, E., Epure, D.-G., Gidea, M. (2013), “New Mixes Based on Collagen Extracts with Bioactive Properties, for Treatment of Seeds in Sustainable Agriculture”, Current Pharmaceutical Biotechnology, 14(9), 792-801.

Kim, H.K., Kim, Y.H., Park, H.J., Lee, N.H. (2013), “Application of ultrasonic treatment to extraction of collagen from the skins of sea bass Lateolabrax japonicas”, Fisheries Science, 79(5), 849-856.

Kim, S.-K. (2013), Marine Proteins and Peptides. Biological Activities and Applications, John Wiley & Sons, Ltd., New York, USA.

Lee, H.C., Singla, A., Lee, Y. (2001), “Biomedical applications of collagen”, International Journal of Pharmaceutics, 221, 1–22.

Lee, M.Y., Jo, Y.-J., Min, S.G., Hong, G.P. (2014), “Effects of High Pressure on the Porcine Placental Hydrolyzing Activity of Pepsin, Trypsin and Chymotrypsin”, Korean Journal for Food Science of Animal Resources, 34(1), 14-19.

Liu, H.Y., Zhao, L., Guo, S.D., Xia, Y., Zhou, P. (2014), “Modification of fish skin collagen film and absorption property of tannic acid”, Journal of Food Science and Technology-Mysore, 51(6), 1102-1109.

Niculescu, M.D., Becheritu, M., Gaidau, C., Epure, D.-G., Gidea, M., Stepan, E. (2015), “Extraction of collagen with bioactive properties for stimulation, nutrition and protection of plants”, Proceedings of III International Leather Engineering Congress, Izmir, Turkey, 259-264.

Niculescu, M.D., Gaidau, C. (2014), “New Collagen Extracts Conditioning for Applications in Crop Protection Against Pests”, Revista de Chimie, 65(12), 1457-1461.

Pati, F., Adhikari B., Dhara S. (2010), “Isolation and characterization of fish scale collagen of higher thermal stability”, Bioresource Technology, 101, 3737–3742.

Picot, L., Ravallec, R., Fouchereau-Péron, M. *et al.* (2010), “Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties”, Journal of the Science of Food and Agriculture, 90(11), 1819–1826.

Wang, W., Li, Z.L., Liu, J.Z., Wang, Y.J., Liu, S.H., Sun, M. (2013), “Comparison between Thermal Hydrolysis and Enzymatic Proteolysis Processes for the Preparation of Tilapia Skin Collagen Hydrolysate”, Czech Journal of Food Sciences, 31(1), 1-4.

Zeng, J.N., Jiang, B.Q., Xiao, Z.Q., Li, S.H. (2012), “Extraction of Collagen from Fish Scales with Papain under Ultrasonic Pretreatment, Advanced Materials Research”, 366, 421-424.

Zhang, F., Wang, A., Li, Z., He, S., Shao, L. (2011), “Preparation and Characterisation of Collagen from Freshwater Fish Scales”, Food and Nutrition Sciences, 2, 818-823.

INDUSTRIAL TRIALS FOR A MORE ECOLOGICAL CHROMIUM TANNING

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In leather production the chrome tanning method is the most widely used tanning system all over the world despite the storage and disposal of solid wastes and sludge containing high amounts of chromium poses a major challenge. For this reason many researches based on higher exhausting and lower chromium used technologies have emerged in the recent past. However, these technologies are not directly accepted by the industry due to risks and some possible changes in quality issues. Accordingly, in this project one of these approaches: chromium tanning without pickling process, using less chromium salts at higher initial pH is tried in pilot scale at a leading company in Turkish leather industry. The variables used in the research were; pre-tanning agents (aldehyde, sulphonyl chloride, and synthetic tanning agent), initial pH of tanning (5.0-5.5 and 6.0-6.5) and tanning agents (standard basic chromium sulphate and commercial tanning agent with lower chromium oxide content). The wet-end processes and finishing processes of the leathers were applied according to the company's production line. Chromium content of the leathers and the Cr_2O_3 remaining in effluents were determined for each tanning application. Also, the physical properties of the leathers were investigated. The Cr_2O_3 of the leathers were found to be varying between 3.12-4.83% while the remaining chromium in the effluents was between 16.9 - 1347 mg/L. Additionally, the properties of the final leathers were evaluated comparatively with company's regular products, considering the test results and organoleptical evaluations it was concluded that many of them were comparable to conventional chromium tanned regular products.

Keywords: Leather, Chromium Tannage, High Exhaustion

INTRODUCTION

Conventional chromium tanning process which is used for approx. 80% of produced leathers consists of three main steps namely pickling, tanning, and basification. Pickling; is being performed along with brine solutions and acids and tanning is being carried out by using 8-10% of basic chromium sulphate over pelt weight. Then, in basification step reactivity of chromium is increased and fixation is achieved by introduction of alkali salts. Since whole of the chromium used in process cannot be exhausted, approx. 1/3-1/4 of it (1500 – 5500 mg/L) remains in bath at the end of the process (R&D Dept Seta, Brazil, 2000; Sreeram *et al*., 2006; Tao *et al*., 2014). Treatment, storage and disposal of this this chromium containing effluents and sludge poses a major challenge. There are various approaches i.e. ameliorating the parameters of chrome tanning, modifying chrome tanning agents or the collagen and using auxiliary agents and/or combination tanning agents towards preventing these technical and environmental problems caused by conventional chromium tanning (Morera *et al*., 2006; Luan *et al*., 2007; Luo *et al*., 2009; Sundar *et al*., 2007; Thanikaivelan *et al*., 2002). Among these alternatives, higher exhausting chromium tanning technology applied at higher initial pH values without pickling by lower chromium offer is a promising one in recent years.

However this technology is not directly switched to application in the industry due to potential risks like incomplete penetration and precipitation of chromium on the leather surface due to high initial pH values and possible quality variations in the final products. In the present research adopting of higher exhausting ecological chromium technology instead of existing conventional chromium tanning is investigated in pilot scale at a leading company in Turkish leather industry by designing various experiments to optimize the process and to maintain similar properties and quality from the produced leathers.

MATERIAL AND METHODS

Lime split domestic pelts (to be approx. 50 kg per each trial) which were conventionally processed were used as material. They were delimed and bated according to company's production route. As blank, the first trial was performed according to company's conventional chromium tanning system with pickling. Other trials were performed without pickling and the necessary pH values depending on the pre-tanning agents were adjusted by using non-swelling acids. An aldehyde, a sulphonylchloride and a highly reactive syntan which are available in the market were selected to be used as pre-tanning agents. After pre-tanning stage, the pH of the pelts were adjusted to 5-5.5 and 6-6.5 respectively and chromium tanning was performed by using 5% standard basic chromium sulphate and 6% of a commercial chromium tanning agent having lower basicity and Cr_2O_3. The trial scheme is given in Fig.1.

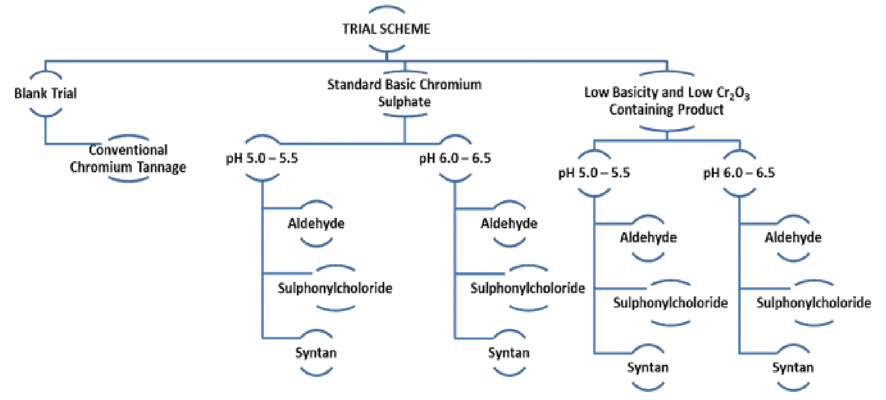

Figure 1. Trial scheme

In residual tanning baths total chromium and COD values were determined according to SM 3120 B and SM 5220 standard methods. After tanning, wet-end processes were carried out in one batch according to company's standard upper leather production route.

Cr_2O_3 contents (ISO 5398-1:2007), shrinkage temperatures (ISO 3380:2015), tensile strengths and percentage extensions (ISO 3376:2011), resistance to grain cracking and grain crack indexes (ISO 3378:2002) and tear loads (ISO 3377-2:2016) of the produced leathers were determined according to related standards.

RESULTS AND DISCUSSION

Cr_2O_3 contents of the leathers and COD values and amount of chromium remaining in residual baths are given in Table 1.

Table 1. Residual bath and leather parameters of tanning trials

		Leather Parameters			Residual Bath	
pH	**Trials**	**Homogeneity**	**Thickness (mm)**	**Cr_2O_3 (%)**	**Cr (mg/L)**	**COD (mg/L)**
-	Blank	Homogen	1.34	3.97	4142	7440
6.0-6.5	LowBasicity&Cr_2O_3 (**LBCr**)	Not Homogen	1.54	3.28	125	4480
6.0-6.5	Cr_ Aldehyde	Not Homogen	1.41	4.65	16.9	11400
6.0-6.5	Cr_ Sulphonylchloryde	Not Homogen	1.55	4.09	64.3	7840
6.0-6.5	Cr_ Syntan-1(F90)	Homogen	1.66	3.18	536	7800
6.0-6.5	**LBCr**_ Aldehyde	Not Homogen	1.46	4.17	88.9	8800
6.0-6.5	**LBCr**_Sulphonylchloryde	Homogen	1.41	4.02	219.2	51200
6.0-6.5	**LBCr**_Syntan-1(F90)	Homogen	1.51	3.38	81.3	4000
5.0-5.5	Cr_ Aldehyde	Not Homogen	1.56	4.37	217.8	760
5.0-5.5	Cr_ Sulphonylchloryde	Not Homogen	1.56	4.83	109.7	3200
5.0-5.5	Cr_ Syntan-1(F90)	Not Homogen	1.51	3.98	661.2	280
5.0-5.5	**LBCr**_Aldehyde	Homogen	1.51	3.96	629	6560
5.0-5.5	**LBCr**_Sulphonylchloryde	Not Homogen	1.43	3.16	340.5	760
5.0-5.5	**LBCr**_Syntan-1(F90)	Homogen	1.65	3.12	1347	8320

Considering the values given in the table it is seen that 4142 mg/L chromium remains in residual bath in conventional chromium tanning system which dramatically decreases to varying values between 16.9 - 1347 mg/L in chromium tanning trials at high initial pH values without pickling. In this new ecological tanning system amount of chromium remaining in residual baths can be reduced up to 67.5 % to 99.5% comparing to conventional tanning while 3.12-4.83% of Cr_2O_3 bound to the leathers depending on the type of system used in trials as presented in Fig.2.

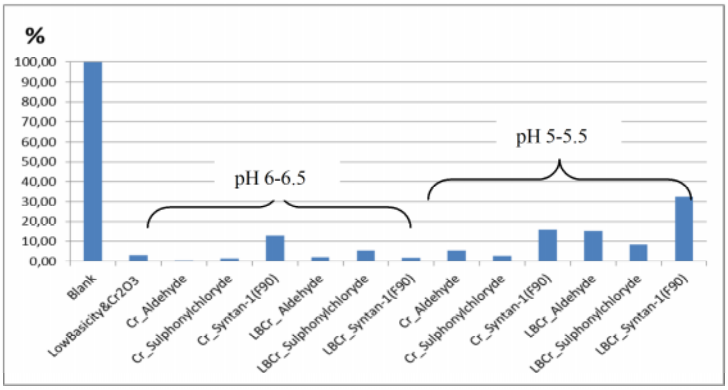

Figure 2. Decrease of chromium remaining in residual bath

From the evaluation and comparison of the physical test results of the leather samples produced higher exhausting technologies with conventional produced leather samples it was seen that most of the physical properties were found comparable with a few exceptions (Table 2).

Table 2. Physical properties of the final leather products

pH	Trials	Ts (^{o}C)	Leather Parameters				
			Tensile Strength (N/mm^2)	**Elongation (%)**	**Lastometer**		**Double Edge Tear (N/mm)**
					(kg)	**(mm)**	
-	Blank	119	18.4	46.1	18.6	7.37	74.18
6.0-6.5	LowBasicity&Cr_2O_3 (**LBCr**)	112	20.1	60.1	16.4	6.65	113.85
6.0-6.5	Cr_Aldehyde	118	12.9	40.8	19.7	7.42	65.21
6.0-6.5	Cr_Sulphonylchloryde	120	16.7	63.9	15.0	6.51	105.70
6.0-6.5	Cr_Syntan-1(F90)	115	14.4	46.0	21.9	7.61	91.43
6.0-6.5	**LBCr**_ Aldehyde	115	17.7	59.2	20.4	6.68	95.40
6.0-6.5	**LBCr**_Sulphonylchloryde	119	15.2	53.2	20.2	7.94	79.99
6.0-6.5	**LBCr**_Syntan-1(F90)	119	17.4	50.5	21.5	7.27	108.56
5.0-5.5	Cr_Aldehyde	119	10.1	46.6	15.8	7.10	70.45
5.0-5.5	Cr_Sulphonylchloryde	115	17.6	56.3	25.8	9.08	111.15
5.0-5.5	Cr_Syntan-1(F90)	121	16.2	56.2	20.8	7.10	113.55
5.0-5.5	**LBCr**_Aldehyde	118	15.4	57.9	21.4	7.54	80.72
5.0-5.5	**LBCr**_Sulphonylchloryde	119	12.7	68.7	25.8	8.37	64.92
5.0-5.5	**LBCr**_Syntan-1(F90)	119	13.5	61.1	23.1	7.52	84.77

Besides consideration and evaluation of chemical and physical data obtained from the analysis and tests, a committee comprising members from production supervisors, quality control and marketing departments of the company, made evaluations considering their existing product properties in terms of handle, touch and physical appearance and costumer demands. From the final evaluations considering both physical and chemical data and committee's remarks it was concluded that best results were obtained from Cr_Aldehyde, Cr_Sulphonylchloryde, Cr_Syntan-1(F90), LBCr_Syntan-1(F90) trials conducted at pH 5-5.5 and decided to make further studies to improve and verify these process designs in higher batches.

CONCLUSIONS

The approach handled in this research for ecological chromium tanning without pickling process and offering less chromium salts at higher initial pH values has been successfully applied in to the practice in pilot scale and chromium remaining in residual baths could be reduced up to 99.5% by alternative formulations comparing with conventional tanning.

Along with decreasing the amount of residual chromium, this approach has also the benefits of considerable decrease in load of treatment plant associated with noticeable decreases in chromium and salt in effluents, reducing treatment costs and potential utilization of sludge i.e. as compost.

Acknowledgement

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REFERENCES

Luan, S., Liu, Y. and Fan, H. (2007), "A novel pretanning agent for high exhaustion chromium tannage", *Journal of the Society of Leather Technologists and Chemists*, 91(4): 149-151.

Luo, Z., Zhang, X., Fan, H. *et al*. (2009), "Modification of collagen for high Cr(III) absorption", *Journal of the American Leather Chemists Association*, 104(4), 152-161.

Morera, J.M., Bacardit, A., Olle, L., Costa, J. and Germann, H.P. (2006), "Study of a Chrome Tanning Process without Float and With Low-Salt Content As Compared to A Traditional Process, Part II", *Journal of the American Leather Chemists Association*, 101(12), 454-460.

Research and Development Department, Seta S/A, Brazil (2000), "Rationalization of the tanning process: avoiding pickling and basification", *World Leather*, October, 67-71.

Sreeram, K.J., Raghava Rao, J., Chandrababu, N.K., Nair, B.U. and Ramasami, T. (2006), "High Exhaust Chrome-Aluminium Combination Tanning: Part 1. Optimization of Tanning", *Journal of the American Leather Chemists Association*, 101(3), 86-95.

Sundar, V.J., Rangasamy, T., Sivakumar, V. and Muralidharan, C. (2007), "A Novel Pickle-Free High Exhaust Chrome Tanning Method-An Approach for Total Dissolved Solids Management", *Journal of the Society of Leather Technologists and Chemists*, 88(5), 252-255.

Tao, E., Hong-Rui, M. and Hao, C. (2014), "The Quantitative Calculation Between Chrome Technological Parameters in Leather Making and Chrome Absorptivity" *Journal of the Society of Leather Technologists and Chemists*, 98, 63-68.

Thanikaivelan, P., Kanthimathi, M., Raghava Rao, J. and Unni Nair, B. (2002), "A Novel Formaldehyde-Free Synthetic Chrome Tanning Agent For Pickle-Less Chrome Tanning: Comparative Study On Syntan Versus Modified Basic Chromium Sulfate", *Journal of the American Leather Chemists Association*, 97(4), 127-136.

*** ISO 3376:2011 Determination of tensile strength and percentage extension

*** ISO 3377-2:2002 Determination of tear load -- Part 2: Double edge tear

*** ISO 3378:2002 Determination of resistance to grain cracking and grain crack index

*** ISO 3380:2015 Determination of shrinkage temperature

*** ISO 5398-1:2007 Chemical determination of chromic oxide content -- Part 1: Quantification by titration

*** SM 3120 B:2005, Standard Methods for Examinations of Water and Wastewater. Metals by plasma emission spectrometry, American Water Works Association, 2005.

*** SM 5220 B: Chemical Oxygen Demand (COD), Opened Reflux- Closed Reflux, Titrimetric Method.

USAGE OF STARCH IN LEATHER MAKING

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In recent years, various industries begin to head towards cleaner production due to increasingly restrictive regulations. Therefore, production from natural and renewable sources has become more important. Starch is a remarkable raw material because it is abundant, inexpensive and biodegradable natural polymer. The use of natural starch has remained limited, to be usually for food purposes, until recent decades. However, the usage of starch has increased significantly over the last few decades; via modification some of the properties of the starch and it has found wide range of applications in many industries such as plastics, agriculture, pharmaceutics, adhesive, textile, paper and food. In recent years, several studies have taken part in literature on possible use of modified starches also in leather industry – especially in tanning and retanning processes to ensure cleaner production. And it seems that starch products that modified with various methods in accordance their field of use will find more interest and use in leather making. For this reason in the present paper it is aimed to make an overview on the structure of starch as a renewable and natural resource and its potential applications in leather making.

Keywords: starch, structure, leather making

INTRODUCTION

In leather processing, raw skin/hide are transformed into leather by means of a series of chemical and mechanical operations. Tanning is the main process that converts the skin into leather material (Beena *et al*., 2011). Basic chromium sulphate salts are the most commonly used tanning agents in leather industry for many years. However, since chromium is a heavy metal and correspondingly its potentially harmful effects on soil, water and organisms, recent trends in leather tanning aimed towards avoiding chrome tannage due to environmental legislation and existing quantities of high grade chrome ore reserves. So, synthesizing green leather tannage has been paid more attention than ever (Lv *et al*., 2012). The usage of natural, biodegradable and environmental friendly products in retanning and finishing processes also will contribute to reduction of waste generated by leather industry. Therefore, the production of chemicals that will be used in leather making from natural and renewable sources is being paid importance.

Starch is a kind of rich, biodegradable, environmentally friendly and renewable resource, and is an important raw material made for green chemicals. It’s one of the main research directions that modified starch is used as green chemical material now and in the future (Liu *et al*., 2009).

In this paper, it is aimed to make an overview on structure of starch as a renewable and natural resource and its promising applications in leather making.

THE STRUCTURE OF STARCH

In plants, glucose is first produced via photosynthesis, and then stored in the form of starch granules (Song, 2011). It is widely distributed in the form of tiny granules as the major reserve carbohydrate in stems, roots, grains and fruits of all forms of green leafed plants (Neelam *et al*., 2012). The size and shape of starch granules vary for different biological sources (Song, 2011). Starch is made up of two fractions: amylose which is made up of essentially α-($1 \rightarrow 4$) D-glucopyranosyl units and amylopectin which is made up of a large number of short chains linked together at their reducing end side by a α-(1→6) linkage (Kaur *et al*., 2012). The structure of a starch macromolecule in most crops is a mixture of 20-30% amylose and 70-80% amylopectin. The degree of polymerization of starch is about 160 to 6000, and its relative molecular weight is about 25000 to 1000000 (Lu *et al*., 2005). It should be noted that the molecular structure (linear or branched) and molecular weight of starch molecules in a crop have a significant influence on its processability for various applications (Biresaw and Shogren, 2008).

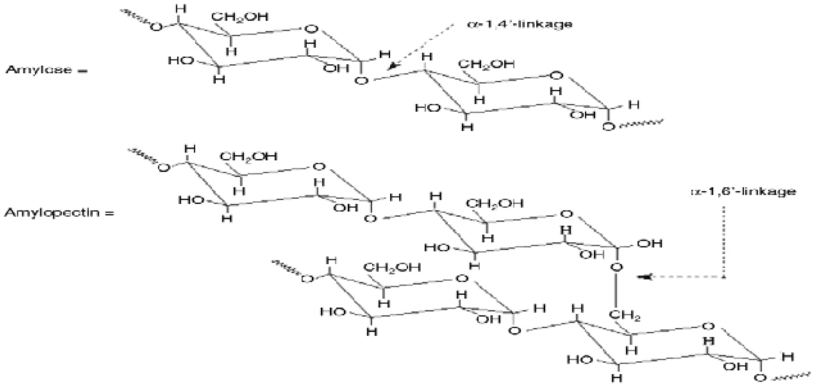

Figure 1. The structure of amylose and amylopectin

Starch itself is poor in processability, also poor in the dimensional stability and mechanical properties for its end products. Therefore, native starch is not used directly (Lu *et al*., 2009). Although some unsuitable characteristics of native starches limit their use in most applications, starch modifications can eliminate or reduce these undesirable traits (Chan *et al*., 2011). The available hydroxyl groups on the starch chains potentially exhibit reactivity specific for alcohols. In other words, they can be oxidized and reduced, and may participate in the formation of hydrogen bonds, ethers and esters (Lu *et al*., 2009).

There are three hydroxyls in 2, 3, 6 placement in each dehydrated glucose unit. Among these, the primary-hydroxyl in C6 has the strongest reactivity and then there are the secondary hydroxyls in C2 and C3 (Zhen and Ma, 2000). Hydroxyl groups have a particular affinity for other hydroxyl groups and can serve as a driving force in bringing starch chains together in an ordered manner through hydrogen bonding. Where such ordering occurs, crystalline regions are deposited in the granule. The remaining regions of unordered starch are referred to as amorphous. It is the crystalline regions that give a granule its structure and facilitate identification of raw starch (Murphy, 2000). The processing of native starch is often followed by disrupting its granular structure. When heated in the presence of water the starch granules swell, lose crystallinity and amorphous interior of granule diffuses into solution (Wilpiszewska and Spychaj, 2007). Therefore, the hydroxyl groups in starch structure can react with the different modifier reagents to enhance their processability for using different industrial areas.

USAGE OF STARCH IN LEATHER MAKING

Although the studies on possible use of starch in tanning process are rare, most of them is related to tanning of pelts with dialdehyde starch which can be produced by periodate oxidation of native starch. From the literature search, it was also noticed that the studies on possible use of starch as retanning and finishing agents have been increased.

Some of the studies in these fields are summarized below:

Celades *et al*. (1990) introduced a new method for a chrome-free tanning process. The authors gave the tanning steps as pre-tanning with oxidized starch, tanning with titanium salt, neutralization, retanning, dyeing and finishing. The authors thought that pre-tanning with selectively oxidized starch enhanced the interaction of titanium with collagen and the leather had excellent texture and handle.

Lv et al. (2012) studied on modifying of corn starch by glutaraldehyde and used it in tanning process. From the results they concluded that the modification of starch by glutaraldehyde was an efficient method to significantly improve its tanning property and the shrinkage temperature of the tanned leather was 85.2 °C. Accordingly, the authors thought that the modified starch could be used as a tanning agent and the pollution produced by chrome tanning could be significantly decreased.

Kanth *et al*. (2006) studied on stabilization of collagen by dialdehyde starch (DAS). DAS samples were autoclaved before the tanning experiments. The authors specified that autoclaving of DAS was resulting hydrolysis to lower molecular weight oligomeric species. These lower molecular weight species showed better tanning properties compared to un-autoclaved DAS and similar effect was also observed on use of DAS in alkaline conditions. It was stated that the leather samples tanned with autoclaved dialdehyde starch at high pH values had shrinkage temperatures higher than 84 °C and the leathers exhibited high stability against collagenase.

Tanning ability of graft copolymer of phenols and starch with horseradish peroxidase (HRP) catalysis was investigated by Lv *et al*. (2011). From the application results, the authors concluded that the phenols and starch graft copolymer had a good selective filling property and the retanned leather had an excellent thickening rate with higher shrinkage temperature.

Liu *et al*. (2009) synthesized a starch-polyacrylamide graft copolymer by means of HRP and graft copolymer was used in retanning process. From the evaluation of the results they have concluded that the increase of shrinkage temperature of the retanned leather proved that the graft copolymer played a certain role for wet blue, the thickness of retanned leather increased; also graft copolymer had a good selective filling property. It was also pointed out that the retanned leather had many advantages, including an excellent increased rate of thickness, good dyeing effect and eligible mechanical properties.

Lu *et al*. (2005) studied on graft copolymerization of oxidizing degradation of starch with acrylic acid, acrylonitrile and acrylamide as a leather retanning agent. They emphasized that the graft copolymer retanning had exceptional selective filling characteristics and could improve the uniformity of retanned leathers. It was also denoted that retanned leather had excellent softness, tightness and fullness, and its mechanical properties such as tensile, tear strength and elongation at break were improved. The authors also specified that the graft copolymer retanning will also be, biodegradable to some extent, and may reduce the environmental pollution caused by acrylic retans.

In another research, Xiaosheng *et al*. (2012) obtained two kinds of oligosaccharides by single (hypochloric acid) and double (hypochloric acid and hydrogen peroxide) oxidation. They used these products to fill wet-blue leather and determined their filling properties. As conclusion of their research the authors have remarked that the wet-blue leather samples filled with both oxidized starches had low moisture contents, good vapor permeability, and good separation characteristics of fibre bundles. Additionally it was stated that the application of double oxidized starch filling had favorable prospects for improving the sensorial characteristics of chromium tanned leather.

Beena *et al*. (2011) studied on possible use of graft starch in finishing and graft-starch products were used in finishing of leather as alternate for other leather finishing binders and it was reported that these products will be useful to minimize the pollution by other acrylic binders, etc. Regarding possible use of modified starch in finishing in A German patent (DE2, 756, 488) it was also reported that sodium carboxy methyl starch could improve the air-permeability of leather when it was used in finishing processes (Zhen and Ma, 2000).

CONCLUSION

In spite of taking many precautions and making attempts to develop more ecological way of production leather industry is one of the industries being debated widely due to its effluents and wastes having potential threats environmentally and day after day many stringent stipulations are being put into effect regarding this issue. In order to accord with these increasing restrictive regulations and improve environmental friendly products by more ecological ways of production, many researches are being started and conducted endlessly. In this point of view; to us, considering the unique properties of starch i.e. being abundant, inexpensive and biodegradable, it seems that it has a huge potential to be utilized in leather processing for many purposes. Despite native starch itself is poor in processability and it is not convenient to be used directly in leather processing, from the literature survey it is known that some unsuitable characteristics of native starches can be eliminated and can be modified in accordance with proposed field of use and many of the issues being debated can be overcome by including it in to leather processing.

REFERENCES

Beena, Z., Uzma, N. and Barkat, A.S. (2011), “Utilization of Starch in Leather Processing: An Environment-Friendly Polymer”, *Science, Technology & Development*, 30/2, 12-14.

Biresaw, G. and Shogren, R. (2008), “Friction Properties of Chemically Modified Starch”, *Journal of Synthetic Lubrication*, 25, 17-30.

Celades *et al*. (1990), *AQEIC Bol. Tech.*, 41(4), 190.

Chan, H.T., Leh, C.P., Bhat, R., Senan, C., Williams, P.A. and Karim, A.A. (2011), “Molecular structure, rheological and thermal characteristics of ozono-oxidized starch”. *Food Chemistry,* 126, 1019-1024.

German patent, DE2, 756, 488.

Kanth, S.V., Madhan, B., Rao, J.R., Nair, B.U., Sadulla, S. and Ramasami, T. (2006), “Studies on the Stabilization of Collagen Using Dialdehyde Starch: Part I: Effect of Autoclaving on Dialdehyde Starch”, *Journal of the American Leather Chemists Association*, 101/12, 444-453.

Kaur, B., Ariffin, F., Bhat, R. and Karim, A.A. (2012), “Progress in starch modification in the last decade”, *Food Hydrocolloids*, 26, 398-404.

Liu, G, Lv, S. and Ma, Y. (2009), “Synthesis and Leather-tanning Properties of a Starch-polyacrylamide Graft Copolymer by Means of HRP”, http://www.aaqtic.org.ar/congresos/china2009/download/2-4/2-122.pdf

Lu, S., Liang, G., Ren, H., Wang, J. and Yang, Q. (2005), "Synthesis and application of graft copolymer retannage of degraded starch and vinyl monomers", *Journal of the Society of Leather Technologies and Chemists*, 89/2, 63-66.

Lu, D.R., Xiao, C.M. and Xu, S.J. (2009), "Starch-based completely biodegradable polymer materials", *Express Polymer Letters*, 3/6, 366-375.

Lv, S., Gong, R., Duan, J., Ma, Y. and Gao, R. (2011), "Preparation and Properties of Graft Copolymer of Phenols and Starch with HRP Catalysis", *Advanced Materials Research*, 201/203, 2825-2828.

Lv, S., Gong, R. and Hu, J. (2012), "Study on Modified Starch by Glutaraldehyde and Its Properties and Application", *Advanced Materials Research*, 455/456, 575-581.

Murphy, P. (2000), "Starch", in Handbook of Hydrocolloids, CRC Press.

Neelam, K., Vigay, S. and Lalit, S. (2012), "Various techniques for the modification of starch and the applications of its derivatives", *International Research Journal of Pharmacy*, 3/5, 25-31.

Song, D. (2011), *Starch Crosslinking for Cellulose Fiber Modification and Starch Nanoparticle Formation,* In Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the School of Chemical and Biomolecular Engineering, Georgia Institute of Technology.

Wilpiszewska, K. and Spychaj, T. (2007), "Chemical modification of starch with hexamethylene diisocyanate derivatives", Carbohydrate Polymers, 70, 334-340.

Xiaosheng, D., Hui, C., Rui, D. and Zhihua, S. (2012), "Preparation and Application of Denatured Starches", *Journal of the Society of Leather Technologies and Chemists*, 96/5, 189-194.

Zhen, J. and Ma, J. (2000), "Modification of starch and its application in leather making", *Journal of the Society of Leather Technologies and Chemists*, 86, 93-95.

STUDIES ON THE ACID HYDROLYSIS OF CHAMOIS LEATHER WASTES

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The present paper aims to investigate a hydrolytic method, feasible to be applied at industrial level, to turn into useful products the Chamois leather wastes, mainly the buffing dust. The efficacy of acid treatments with 0.3, 0.5 and 1.0 % HCl solutions, by autoclaving at 105 °C, for reaction times of 6, 10 and 14 hours, was determined in an attempt to optimize the hydrolysis parameters. Based on the mass balance of performed experiments, the yields of hydrolysis processes were calculated for two of the products that were separated from the final mixture: collagen hydrolysates and fats. Optimal working parameters were: a concentration of 0.5 % HCl, and a duration of 10 hours, by simply boiling in autoclave, at 105 °C. The resulted polypeptides mixture was characterized by chemical (total nitrogen content) and physical-chemical methods (FTIR spectra, EDAX). The recovered greasy matters were purified by water-solvent partition, and the specific physical-chemical indicators were determined. The main advantage of the polypeptide mixtures obtained by Chamois wastes hydrolysis consists in the fact that they are chromium-free, and can be used for a large range of industrial (foaming agents, waste water treatment, chemical auxiliaries, fillers, adhesives, blends with synthetic polymers, concrete mixtures etc.) and agricultural applications (nitrogen and sulfur slow release fertilizers, animal feeding). The grease fraction can be used in leather wet finishing, or can be further chemically modified to produce surfactants. The small amounts of solid residues which resist to the acidic hydrolysis can be used, after neutralization, as additives of concrete mixtures, in producing decorative bricks.

Keywords: Chamois buffing dust, acid hydrolysis, polypeptide and greasy matters recovery.

INTRODUCTION

The stringent enforcement of the environmental protection has prompted technologists and scientists to re-look at the waste management and transform tannery solid and liquid waste materials into usable, value-added products. Thus, the advanced processing of the leather manufacturing solid wastes leads to the obtaining of protein-based materials that can be used for new building materials (concrete, thermal insulation panels and noise absorption panels (Sathish Kumar *et al*., 2015, UNIDO, 2000; Mafia *et al*., 2002), as biofertilizers to improve the soil quality (Zainescu *et al*., 2012), as additions for pigment pastes to obtain finishing films (Popa *et al*., 2016), composts (Sundar *et al*., 2011), biodegradable bioplastic composite materials (Deselnicu *et al*., 2014; Alexy *et al*., 2003), dyes adsorbents (Carpenter *et al*., 2013) or the production of biodiesel biofuel (Kolomaznik *et al*., 2009).

Only sheepskin is used in the production of genuine Chamois leather, due to its naturally open fibre structure. The manufacturing company has adhered to the framework agreement of the environmental reporting standard in the European leather and tanning industry, by using non toxic chemicals in any tanning treatments. This fact represents a major advantage for using the Chamois leather waste in hydrolysis processes, thus allowing for useful waste components to be recovered and then turned into eco-efficient bioproducts.

Accordingly, in the present paper we analyzed the yield and the mass balance of the hydrolysis process performed in acid conditions, at different concentrations of the HCl solution (0.3%; 0.5% and 1.0%) and durations of the hydrolysis (6, 10, and 14 hours).

The paper also deals with the extraction of useful components from the mixture resulted from the acid hydrolysis of the Chamois leather wastes, with the purpose of their further exploitation.

EXPERIMENTAL DATA AND DISCUSSIONS

The experimental work was carried out on Chamois leather buffing dust. According to the current standards, the following analyses were performed to characterize the buffing dust: the total nitrogen content (TKN=10.82%), the hide matter (HM=63.64% according to SR EN ISO 5397-1996); the mineral substances content (MS=13.17% according to SR EN ISO 4047-2002), the extractable fatty matters content (EFM=11.70%), and by deduction, other organic substances content was calculated (OS=11.49%), which includes the combined fatty matters. All the above mentioned values were expressed on the leather dry matter basis. Thus, the mass balance for the leather substance subjected to hydrolysis can be stated by the following equation (1):

$$\%\ \textit{Hide dry matter} = \%HM + \%MS + \%EFM + \%OS \tag{1}$$

In order to obtain a protein hydrolyzate in the laboratory, starting from the Chamois leather buffing dust, an experimental protocol was applied, with the purpose of analyzing the hydrolysis yield and the mass balance, dependent on the acid concentration (0.3%, 0.5%, 1%) and on the treatment time (6, 10, 14 hours), according to a procedure applied for all hydrolysis experiments: 50 grams of Chamois buffing dust (BDDM = 43.9901 g dry matter, of which BDPM = 27.9952 g protein matter) and 1 liter of HCl solution with a given concentration were introduced in an autoclave; the reaction mass was heated to 105 °C, for the time duration established by preliminary hydrolysis tests. The resulted product, in the form of a finely segregated gel, was centrifuged at 3000 RCF, for 30 minutes; the solid residue was collected and the supernatant was subjected to a liquid-liquid phase separation by using a mixture of 5:3 water : non-polar solvent (trichloroethylene), followed by vigorous stirring for 1 hour in a Vortex mixer, heating up to 60 - 70 °C and cooling down for 12 hours at room temperature for phase separation. Each separated layer was dried to constant weight in the oven at 100 ± 5 °C and then the dry substance mass was determined for each separated layer (Figure 1).

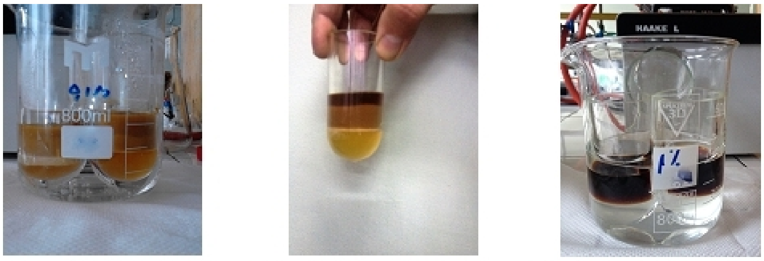
The mass balance for all hydrolysis variants was calculated according to equation (2), and the hydrolysis yield from equation (3). The dried product resulted from the aqueous layer, which contains hydrolyzed collagen fragments (polypeptides, oligopeptides, amino-acids) was analysed by infrared spectroscopy and X-ray dispersive energy (EDAX) spectral analysis. The total nitrogen content was determined by Kjeldahl method. In order to screen the collagen fragments by their molecular weight, the samples were dialysed using SpectraPor 3 (3.5 kDa) and SpectraPor 6 (1.6 – 2 kDa) membranes (Spectrum Laboratories Inc., Canada). Table 1 resumes the hydrolysis yield and the mass balance for the experimental variants.

$$\textit{Mass Balance}\ \% = \frac{TS(g) + EFM(g) + CFM(g) + TI(g)}{BDDM(g)} \cdot 100 \quad [\%], \tag{2}$$

where: DM is the total dry mass of the cumulated layers, TS is the total mass of soluble substances, EFM is the extractable fatty maters, CFM is the fatty matters combined with the collagen, TI is the total insoluble content, and BDDM is the dry matter content of the analyzed buffing dust (= 43.9901 g).

$$\textit{Hydrolysis yield}\ \% = \frac{PDM\,(g)}{BDPM\,(g)} \cdot 100 \qquad [\%]\,, \tag{3}$$

where PDM is the polypeptides dry mater content of the samples, and BDPM is the buffing dust protein matter content (= 27.9952 g).



3 phase separation with a concentration of 0,3 % HCl

4 phase separation with a concentration of 0,5 % HCl

2 phase separation with a concentration of 1 % HCl

Figure 1. Liquid – liquid phase separation

Table 1. Hydrolysis yield and the mass balance for the experimental variants

Components mass (g)	HCl concentration (%) / Hydrolysis duration (hours)								
	0.3			0.5			1.0		
	6	10	14	6	10	14	6	10	14
TS	21.7901	18.9226	18.5824	11.1203	13.8514	13.2096	28.2237	30.4022	31.3363
PDM	2.5082	2.2822	2.4138	2.1045	3.1054	3.1029	3.2848	4.1954	4.9391
CFM	10.8342	12.0456	14.1696	23.2162	26.3522	19.2131	2.9821	-	-
EFM	3.2313	5.5484	5.8216	0.8932	1.3507	5.2323	0.8236	5.9421	6.2816
TI	5.8726	5.4151	2.9605	6.2641	2.3974	4.3462	11.7946	7.2099	5.7627
BDDM	41.7282	41.9317	41.5341	41.4938	43.9517	42.0012	43.8240	43.5542	43.3806
Hydrolysis yield, (%)	8.96	8.15	8.62	7.52	11.09	11.08	11.73	14.99	17.64
Mass balance, (%)	94.86	95.32	94.42	94.33	99.91	95.48	99.62	99.01	98.61

Figure 2 illustrates the dependence of the total nitrogen content and of the hydrolysate proteins content on the HCl concentration and on the hydrolysis time. With a concentration of 0.3% HCl, a protein hydrolysate content of 12.99% is obtained after 14 hours, with a concentration of 0.5% HCl, a protein hydrolysate content of maximum 23.49% is obtained after 14 hours. The hydrolysis with 1% HCl provides the highest content of hydrolyzed proteins, i.e. 50.30% after a 14 hours treatment.

In order to assess the molecular weight of the resulting protein forms, the dialysis with SpectraPor 3 (3.5 kDa) and SpectraPor 6 (1.6 – 2 kDa) membranes was performed. For the hydrolysis variants with 1% HCl, no matter the duration, highly fragmented collagen forms are obtained, with a molecular weight of less than 1.6 kDa. The hydrolysis variants with 0.5% HCl provide molecular weights in the range 1.6 - 3.5 kDa, which, correlated with the hydrolysis yield, offer results in accordance with the aimed purpose. The 0.3% HCl variants offer less satisfying results correlated with the hydrolysis yield, even if the molecular weights are included in the expected range.

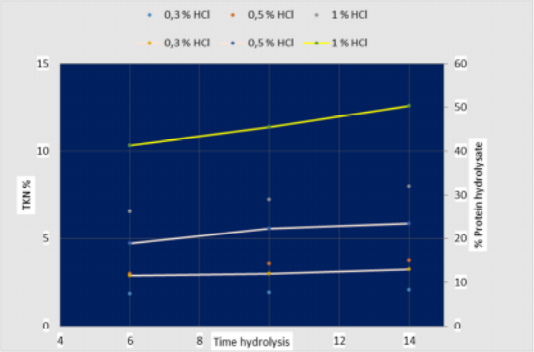
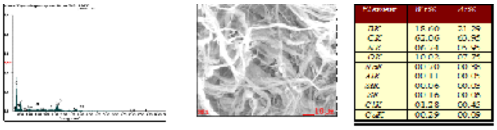


Figure 2. The TKN and the proteinshydrolysate content in the aqueous phase dependence on the HCl concentration and on the hydrolysis duration

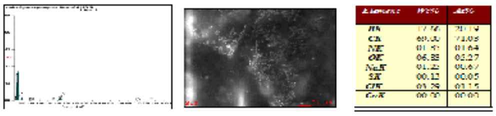
The EDAX charts are registered both for the Chamois leather powder and for the aqueous phases separated as described. The presence of the constituent C, N, O chemical elements can be noticed (Figure 3a) and also that of Na, B, Ca, Al, Si cations, which confirm the high content of mineral substances in the Chamois leather. They are present as sodium chloride, calcium oxide and traces of alumina and silicon oxide. Of all the mineral elements, the highest one is Boron, 18.6%, which can be explained by the fact that at the end of the oil tanning operation, in order to fix/mix the fatty substances with the functional groups of the collagen matrix in the cow-hide dermis, and also in order to shorten the oxidation stage, the modern technologies entail a rapid oxidation stage, using different oxidizing agents, such as perborate compounds (Zhou *et al*., 2003). Figures 3 (b, c and d) shows the EDAX charts for the collected aqueous phases. Regardless the hydrolysis version, the compounds of the Na, B, Al, Si, Ca cations are found in the aqueous phase, but the highest content is found for the concentration of 0.5% HCl. On the other hand, the acid concentration and the hydrolysis time have a major influence upon the degree of hydrolysis. The registered data show the advance of the hydrolysis at the same time with the increase of the acid concentration and of the hydrolysis duration, the aqueous phase is enriched in hydrolysate protein forms. Thus, for the 0,3% HCl version, in 14 hours, the nitrogen content is of 1.68%; With a concentration of 0,5% HCl, it reaches the value of 3.97%, and for 1% HCl, the nitrogen content is 6.64%. The registered values can be compared with those analytically determined by the Kjeldahl method.

Compared with the initial untreated sample, the IR spectra of the the aqueous protein phase (layer 1) are characterized by the distorsion of the specific peaks for amides I, II and III. The spectra in the figure 4 show a shift of the characteristic peaks in the range 3450-3300 cm^{-1} (of NH + OH groups) and of 3200 cm^{-1} spectral band (of free OH groups) of the initial sample to 3100 cm^{-1}, and 3000 cm^{-1} bands respectively, which could indicate possible new intermolecular interactions through hydrogen bonds.



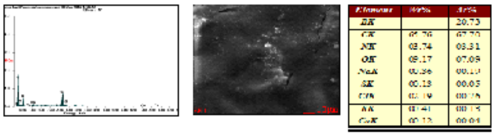
Element	Wt%	At%
BK	15.60	21.29
CK	62.06	63.95
NK	[illegible]	[illegible]
OK	10.02	07.75
NaK	[illegible]	[illegible]
AlK	00.11	00.05
SiK	00.06	00.03
SK	00.16	00.05
ClK	01.28	00.43
CaK	00.29	00.09

Figura 3a. EDAX chart for the Chamois leather powder waste



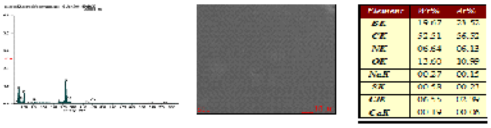
Element	Wt%	At%
BK	17.76	[illegible]
CK	69.00	71.05
NK	[illegible]	01.64
OK	06.38	05.27
NaK	01.25	00.67
SK	00.12	00.05
ClK	03.29	01.15
CaK	00.00	00.00

Figura 3b. EDAX chart for total soluble with a concentration of 0,3 % HCl



Element	Wt%	At%
BK		20.73
CK	65.76	67.70
NK	03.74	03.31
OK	09.17	07.09
NaK	00.36	[illegible]
SK	00.13	00.05
ClK	02.19	[illegible]
KK	00.41	[illegible]
CaK	00.12	00.04

Figure 3c: EDAX chart for total soluble with a concentration of 0,5 % HCl



Element	Wt%	At%
BK	19.67	[illegible]
CK	52.51	56.32
NK	06.64	06.13
OK	12.60	10.99
NaK	00.27	00.15
SK	[illegible]	[illegible]
ClK	[illegible]	[illegible]
CaK	[illegible]	[illegible]

Figure 3d: EDAX chart for total soluble with a concentration of 1,0 % HCl

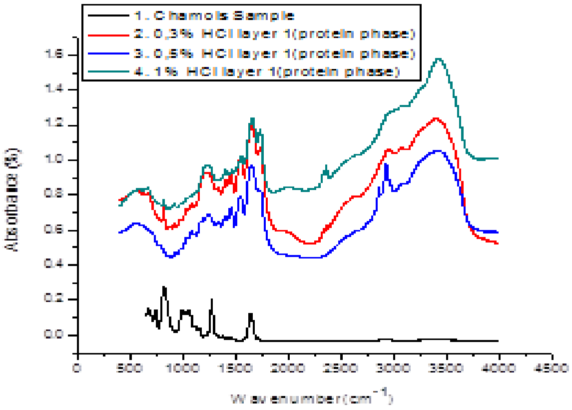


Figure 4. IR Spectra of layer 1 hydrolysates

It can be also seen a decrease in signal intensity of spectral bands characteristic to CO groups from amide I located between 1650-1650 cm^{-1}, of NH groups from amide II located between 1555 - 1545 cm^{-1}, and of NH +CH groups from amide III located between 1240-1230 cm^{-1}, for the hydrolisate samples (2, 3, 4) compared to the control sample (1).

CONCLUSIONS

As a result of the acid hydrolysis of the Chamois leather waste samples, it can be concluded that:

1. The processing of sheepskins in order to obtain Chamois leather implies the use of auxiliaries with a low toxic potential, which represents an advantage for the recovery of useful components with a positive impact on the environment.
2. For the 1% HCl hydrolysis, regardless the treatment time, highly fragmented collagen forms are obtained, with a molecular weight of less than 1.6 kDa. The variants with 0.5% HCl result in molecular weights of more than 1.6 kDa, which correlated with the hydrolysis yield, offer results in accordance with the aimed purpose. The 0.3% HCl concentration, even if it indicates molecular weights included in the expected range, correlated with the hydrolysis yield, offers less satisfying results.
3. After the hydrolysis treatment, the solvent extraction of the phases which contain useful components (peptides, oils, fats) makes it possible to be subsequently separated and used as biocomponents for different applications.

Acknowledgements

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REFERENCES

Alexy, P., Bakos, D., Crkonova, G. *et al*. (2003), “Poly(vinyl alcohol)–collagen hydrolysate thermoplastic blends: II. Water penetration and biodegradability of melt extruded films”, *Polymer Testing*, 22, 811–818.

Carpenter, J., Sharma, S., Sharma, A.K. *et al*. (2013), “Adsorption of Dye by Using the Solid Waste from Leather Industry as an Adsorbent”, *International Journal of Engineering Science Invention*, 2(1), 64-69.

Deselnicu, D.C., Militaru, G. and Deselnicu, V. (2014), “Obtaining of Biodegradable Plastic Materials”, *Revista de Materiale Plastice*, 51(1), 72-74.

Kolomaznik, K., Barinova, M. and Furst, T. (2009), “Possibility of using tannery waste for biodiesel production”. *Journal of American Leather Chemists Association*, 104, 177–182.

Mafia, G.J., Slomiana, M. and Davis, J.F. (2002), “Recent advances in collagen based technologies”, *Journal of American Leather Chemists Association*, 97, 74–82.

Popa, E., Bălău Mîndru, I., Pruneanu, M. *et al*. (2016), “Potential use of collagen hydrolysates from chamois leather waste as ingredient in leather finishing formulations”, *Annals of the University of Oradea fascicle of textiles, Leatherwork*, 17(2), 203-209.

Sathish Kumar, V. and Vijayaravind, S. (2015), “Utilization of Tannery Shredded Waste as Fine Aggregate in Concrete”, *International Journal of Engineering Research & Technology (IJERT)*, 4(04).

Sundar, V.J., Gnanamani, A., Muralidharan, C. *et al*. (2011), “Recovery and utilization of proteinous wastes of leather making: a review”, *Reviews in Environmental Science and Bio/*Technology, 10, 151–163.

UNIDO (2000), “Fourteenth Session of the Leather and Leather Products Industry Panel”, Zlin, Czech Republic, 1-58.

Zainescu, G., Deselnicu, D.C., Ioannidis, I. *et al*. (2012), “New Versatile Conversion Technology for Wet White Waste Transformation to Biofertilisers”, *Proceedings of the 4th International Conference on Advanced Materials and Systems - ICAMS 2012*, 71-76.

Zhou, H., Zhang, X., Chen, J., Jiang, X. *et al*. (2003), “Study of oxidating mechanism and development of technology about the unsaturated lipoids (II) —Characteristic properties of reaction and development of technology about lipoidic free radical [J]”; *China Leather*, 13, The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University, Chengdu 610065.

ECO-FRIENDLY SOLUTIONS FOR POLLUTION PREVENTION AND TEXTILE WASTEWATER TREATMENT

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The effluents resulted from the textile finishing generate the following pollution problems for the environment: concentration of pollutants in the discharged wastewater that leads to an increase of the main wastewater quality indicators: pH, matters in suspension, COD, BOD, chloride, sulphide, detergents, nitrogen; pollution of groundwater by a concentration of polluting minerals; pollution of soil in the area where textile and leather companies operate; pollution of natural receptors and the compelling of riverside industries to search for other clean water sources; persistence of dyes that are difficult to degrade in the natural receptors; impact on aquatic flora and fauna; decrease in photosynthesis due to water coloration; pollution of ground waters with dissolved solid substances; increase of alkalinity and of the content of mineral, organic substances and of soluble substances. All these problems can be minimized by adopting preventive measures and by pollution monitoring. Both pollution prevention solutions and new environmental technologies applied in the finishing textile processes are presented in this paper. There have been realized comparative studies between the conventional technologies, ecological technologies of textile finishing and advanced treatment technologies of wastewater. The impact of these technologies on the quality of wastewater has been studied with the demonstration of efficiency, through: decreasing of the amount of indicators, cost cutting of the treatment for each m3 of water, decreasing of the sludge quantity.

Keywords: pollution prevention, textile finishing, wastewater treatment.

INTRODUCTION

For the last 20 years, due to globalization and trade liberalization, the European textile sector has undergone changes such as the modernization of production processes refocused towards an increase of productivity, of product quality in line with the market and environmental protection requirements. This tendency has significant importance for Romania if we take into account that an important part of the exported products comes from the textile industry. Thus there is a need to diversify textile activities, to win new markets with ECO labeled competitive products, highly specialized products adapted to the demand, obtained through ecological technologies that do not influence the quality of the environment (Clay, 2004; Rupp, 2008).

In companies on the North Giurgiu Technological and Industrial Park - NGTIP studied the correlations between the main pollutants generated by the technological stages, with influences on the wastewater quality indicators and the methods for treating them in the treatment plants, in order to maintain them within the limits established by national and European norms (Pricop *et al*., 2013; Pricop, 2016). Pollution prevention can be achieved by various methods, as seen in Figure 1.

The textile materials processing uses a broad variety of chemical substances (detergents, alkali, acids, dyes, surfactants, surfactants etc) that contribute to the significant pollution of the environment. Wastewater from textile finishing raise serious problems related to the quantity of sediments, pH, temperature, color (group of dyes), content of organic substances (fiber particles, fiber materials, surfactants, phosphates, auxiliary chemical products, albumin, carbohydrates etc.), content in inorganic substances (salts, acids, alkali, chlorine, metals etc.). Due to the diversity of the production structure, the quality of wastewater varies not only from one company to another, but also within the same company.

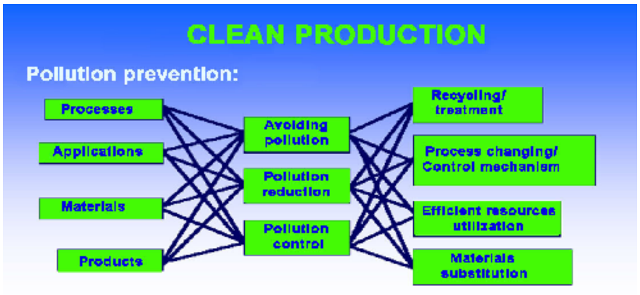


Figure 1. Pollution prevention through the application of clean production concept

MATERIALS AND METHODS

Sources of Pollutants in Textile Finishing

Textile finishing can be defined as the multitude of operations (mechanical, chemical and biochemical) that ensure the improvement of textile properties that is their aspect, comfort, durability and functionality. Most of the textile finishing operations can be effected over the entire technological flow on: fiber, sliver, yarn, fabric, knit and garment; however, finishing applied to fabrics or knits is predominant. In a simplified form, a textile finishing technological flow is represented in Figure 2.

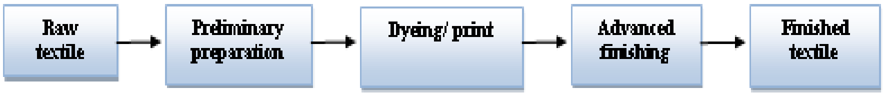


Figure 2. Textile finishing technological flow

In companies on the North Giurgiu Technological and Industrial Park - NGTIP the correlation between the main pollutants generated by technological phases was studied with influences over quality indicators of wastewater and the treatment methods in wastewater treatment plants in conformity with National and European Norms.

A correlation between technology stages of textile processing, pollutants and their influence on wastewater quality indicators and treatment methods is presented in Table 1 (Pricop *et al*., 2013; Pricop, 2016)**.**

Table 1. Correlation between technology stages of textile processing, pollutants and their influence on wastewater quality indicators

Technology stages generating polluting factors	Polluting factors and their effect on wastewater	Influences on wastewater quality indicators	Methods to remove pollutants
WARPING	Dust, fly	Suspended matter	-
SIZING	Sizing products,Starch, Polysaccharides, CMC, APV, Polyacrilates	COD, BOD, TSS	physical-chemical treatment, ozonation, biological treatment, filters, membranes
PRELIMINARY PREPARATION (Desizing, alkaline boiling)	surfactants, complexing agents, oils, sizing products, fibres, various waxes, mineral or vegetal impurities, enzyme products	COD, BOD, TSS, pH	physical-chemical treatment, ozonation, biological treatment, filters, membranes
BLEACHING	Chlorine or oxygen-based oxidizing agents (chlorite, hydrosulphite, thiosulphite,	COD, BOD, TSS, pH, sulphites, sulphates, chlorine	physical-chemical treatment, ozonation, biological treatment, filters, -membranes
DYEING	Wastes of sulfur dyes Wastes of indigosol dyes,Chemical auxiliaries, surfactants, complexing agents, heavy metals, dispersing agents, mordants	pH, color, TSS, metals, salts, temperature, COD, BOD, metals sulphates, sulphites, fixed residue	physical-chemical treatment, ozonation biological treatment, filters, membranes photocatalysis, advanced treatment
ROSS DYEING	Wastes of sulphur dyes Wastes of indigosol dyes, Chemical auxiliaries, surfactants, complexing agents, heavy metals	pH, TSS, metals, salts, temperature, water volume COD, BOD, fixed residue	physical-chemical treatment, ozonation biological treatment, filters, membranes photocatalysis, advanced treatment
WASHING/ RINSING	Wastes of sulphur dyes Wastes of indigosol dyes, Chemical auxiliaries	Influence in lower %, COD, BOD, water volume, fixed residue	physical-chemical treatment, ozonation, biological treatment, filters, membranes, photocatalysis, advanced treatment
STARCHING	Starching products (natural and synthetic polymers)	BOD (biochemical oxygen consumption), COD, TSS	physical-chemical treatment, ozonation, biological treatment, filters, membranes, photocatalysis, advanced treatment

EXPERIMENTAL

The Best Available Techniques (BAT), as well as the emissions associated to these, and/or the consumption level have been evaluated, taking into consideration the following elements: the identification of the elements that are important for the environment; the analysis of the most relevant general and activity specific techniques; the identification of the best environment performances based on the data that are available at the European and worldwide level; the examining of the conditions in which these performance levels could be obtained; the selection of the BAT techniques at the sector level, depending on the emissions and consumptions associated to these.

Further on, there are briefly presented some technologic possibilities of reducing the impact of the textile finishing over the environment (ecologic technological solutions of preliminarily preparation for cotton textiles, ecological technological solutions of dyeing the cotton textile materials, ecological solutions for final finishing), which can be applied or which have been implemented into the textile enterprises within the companies on the North Giurgiu Technological and Industrial Park – NGTIP.

Desizing the woven fabrics sized with starch with amylolytic enzymes:

- *Ecologic technology of removing the natural and accidental impurities from cotton:* The selection of the auxiliary chemical products depending on the impact over the environment; The enzymatic cleaning of the no cellulose impurities that are present in the cotton fiber.

- *Ecological technological bleaching solutions:* Ecological solutions of bleaching by replacing the sodium hypochlorite and the chlorine compounds; Avoiding the use of certain auxiliary products that are dangerous to the environment; The use of catalases for removing the H_2O_2 residue from bleaching.

- *Ecological technological solutions of preliminary preparing by cumulating certain stages of the technological process*: Technological processes combined by using enzymes; The usage of certain multifunctional auxiliaries; Employing the continuous processes of preliminary one stage preparing processes by the padding – steaming method;

- *Ecological procedures of dyeing with reagents*: The use of poly-functional reagents having a high fixation degree; The exhaust dyeing with reduced salt quantities; The substitution of salt in the reagent dyeing with biodegradable alternative products; The pad – batch procedure; The procedure of continuous dyeing; The elimination of detergents form the post dyeing washing operations; Enzymatic treatments of removing the hydrolyzed dyestuff after dyeing;

- *Ecological procedures of dyeing with sulphur dyestuff:* The use of sulfur dyestuffs and chemical auxiliaries having a minimum impact over the environment; Ecological procedure of dyeing with PAD-OX sulphur dyestuffs; The optimization of the equipment for continuous dyeing. In order to determine the main quality indicators of the wastewater per classical/ ecologic finishing processes of denim fabrics samples were collected resulting from both processes and environmental efficiency was determined.The comparative analysis of the parameters values (pH, suspension matter, BOD, COD, NH_4^+, sulfur and hydrogen sulfide, sulfates, free residual chlorine, detergents, fix residue) and ecological efficiency is found in Table 2.

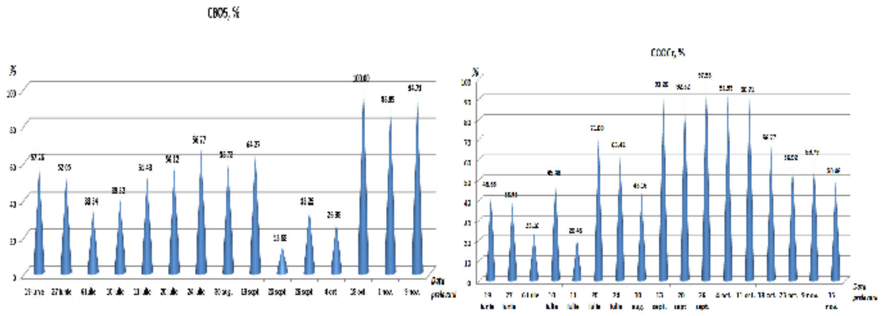
Table 2. Comparative characterization of the quality indicators of the wastewater resulted from the classic/ecological finishing processes of denim fabrics dyed with sulfur dyes

Parameter	pH	Suspension mater	BOD	COD	NH_4^+	Detergents
Admissible values according to NTPA002/2005	6.5-8.5	350 mg/L	300 mgO_2/L	500mgO_2/L	30 mg/L	25 mg/L
P.1 classical dyeing process	12.14	962	4424.6	7742.8	1.94	120
P.2 ecological process	8.1	330	410	610	1.14	35
Ecological efficiency (%)	33	68	91	92	41	71

RESULTS

The technical, economical and ecological advantages consist in: process flexibility and simplicity, good reproducibility, 90÷100% dye fixation, ecological process, less time/ increased productivity, small change of dye shade from batch to batch, great fastening resistance, reduced consumption of reducing agent – decrease of consumption with 40, reduced coloration of the residual water - economies made with water treatment; very low water consumption – water economy of about 50÷70%, reduction of water pollutants - with 15÷25%, reduction of sludge mass - with 10÷20%.

The assessment of the treatment installations from the textiles companies has lead to the conclusion that, in order to increase the pre-treatment efficiency the following upgrades are necessary at the wastewater treatment plant (WWTP): the existing used grills and sieves must be replaced with stainless steel grills and sieves with a greater capacity of retaining the impurities (holes smaller than 10 mm); mechanized scraping devices for impurities; automated systems for reading and adjusting the pH and turbidity; new basins for performing coagulation-flocculation; introduction of aeration systems in the 2nd treatment stage; settling basins and additional pumping plants for sludge will be installed. The efficiency of upgraded WWTP plant is higher than those of the old WWTP by 20÷50%. In the WWTP upgraded the water from the aeration basin is oxygenated by a pneumatic aeration system. Treated water, in a percentage of 94-97%, is separated from the sludge in the secondary settler. For a period of several months was studied the impact of using traditional technologies for textile finishing compared with the ecological technologies. The quality indicators (BOD and COD) for the generated wastewaters were compared for both technologies. A significant reduction in water pollution is found when using green technologies finishing, as seen in Figure 3.



a. b.

Figure 3. Graphical comparison regarding the treatment degree for: a. BOD, b. COD

CONCLUSION

The results of the project have benefited companies in the textile industry, which by applying the suggested solutions, manage to comply with the national rules relating to treated water discharge into sewer systems or natural receptors. In the future, in order to comply with the European regulations, which will become more stringent in the coming years, further investment will be need it, to upgrade wastewater treatment plants analyzed, in accordance with the solutions from this paper.

Acknowledgement

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REFERENCES

Clay, J. (2004), *World Agriculture and the Environment: A Commodity by Commodity Guide to Impacts and Practices*, Island Press.

Pricop, F., Popescu, A., Ghituleasa, C. and Moga, I.C. (2013), *Specific pollutants generated by the textile companies from the cross-border area and technological solutions for minimizing and monitoring of the wastewater pollution, Guide*, Certex Publishing House, Bucharest.

Pricop, F. (2016), *Technical reports for Biotehwater project*.

Rupp, J. (2008), *Ecology and Economy in Textile Finishing*, Textile World.

INNOVATIVE ENVIRONMENTAL TECHNOLOGIES INCLUDING WATER RECOVERY FOR REUSE FROM TANNERY AND INDUSTRIAL WASTEWATER – INDIAN AND ASIAN SCENARIO

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Wastewater discharge from world tannery sector is about 600 million m3/annum. The tanneries in Asia discharge more than 350 million m3 of wastewater per annum from the process of 8 to 10 million tons of hides and skins. The ground and surface water resources in many locations in and around tannery cluster contain high Total Dissolved Solids (TDS) and not fit for domestic and industrial use. The conventional treatment systems implemented all over the world reduce Biochemical Oxygen Demand, Chemical Oxygen Demand, Suspended Solids, Heavy Metals etc. and not TDS and salinity which are mainly contributed by chlorides, hardness and sulphates. The treatment plants are unable to meet the standards in terms of TDS, chlorides and salinity which are being enforced in India and many other countries. The pollution control authorities also insist on water recovery integrated with Zero Liquid Discharge (ZLD) system. Naval treatment systems such as special Micro Filter, Ultra Filtration, Membrane Bio-Reactor, Nano Filtration, Reverse Osmosis, etc. have been developed for recovery of water from domestic and tannery wastewater. The achievement of ZLD concept has got many technical challenges. Management of the concentrated saline stream treatment by adopting energy intensive evaporation system is one of the major sustainable issues. The innovative treatment technologies developed and adopted for water recovery, saline stream management, etc. are dealt in this paper.

Keywords: Tannery Wastewater, Treatment, Reverse Osmosis

INTRODUCTION

Annual leather process in Asian Countries is estimated at 8 to 10 million tons of hides and skins which is more than 50% of the estimated World leather production of about 16 million tons per year. Wastewater discharged from world tannery sector is about 600million m^3/annum. The tanneries in Asian countries including India, China, Vietnam, etc. discharge more than 350 million m^3 of wastewater per annum.

The conventional physiochemical and biological treatment systems are designed and implemented only to reduce Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), Heavy metals etc. and not TDS and salinity which are mainly contributed by chlorides, hardness and sulphates. Due to inherent quality of wastewater from tanning industry, the treatment plants are unable to meet the prescribed standards in terms of TDS, chlorides in salinity in the treated effluent.

There is not much scope in mixing the treated tannery effluent with domestic sewage to achieve the TDS level in many locations in Asia in the absence of organized sewage treatment plants of required capacity. Many polluting industries including tanneries are located in the land locked areas and there are constraints to discharge the treated effluent with high TDS in the Sea.

The TDS limit is being enforced in India and other parts of the World depending upon the final mode of disposal. In addition to the removal of TDS in the treated effluent, it is necessary to recover water for reuse to meet the challenge of water shortage. In many states in India, the pollution control authorities insist on water recovery integrated with Zero Liquid Discharge (ZLD) system. However, the achievement of Zero Liquid Discharge concept has got many technical challenges in addition to the application of various types of membrane systems. Management of the concentrated saline stream treatment by adopting energy intensive evaporation system seems to be one of the major issues in land locked areas.

This technical article deals with the recent developments on the environmental protection techniques in including water recovery from water discharged from tanneries and other industrial waste water treatment with focus on sludge reduction water recovery for reuse and salt recovery, marine disposal of saline reject with proper treatment and guard, etc. Case studies of major projects implemented in India, Spain, China, etc. and saline reject disposal coastal zones are covered in the novel technical paper.

NEED FOR IMPROVED TREATMENT SYSTEM FOR SLUDGE REDUCTION & WATER RECOVERY

Due to inherent quality of industrial wastewater such as textile dyeing units, tanneries etc., the conventional treatment plants are unable to meet the prescribed TDS level of 2100 mg/l in the treated effluent. In addition to TDS management the control of volatile solids in hazardous category sludge is also becoming a necessary.

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The TDS limit is being enforced in many parts of the world depending upon the final mode of disposal. In addition to the removal of TDS in the treated effluent, it is necessary to recover water for reuse to meet the challenge of water shortage. In many states in India the pollution control authorities insist on water recovery integrated with Zero Liquid Discharge (ZLD) system.

For control of sludge and recovery of quality water from wastewater, the required treatment steps are (i) Chrome recovery and other in process control including cleaner production (ii) Conventional physiochemical and biological effluent treatment systems to reduce BOD, COD, SS etc. and (iii) Tertiary treatment systems including, micro-filter, low pressure membrane units such as ultra-filtration etc., before the application of single or multiple stage Reverse Osmosis (RO) system. A special treatment process for recovery of water from waste water is given in Figure 1.

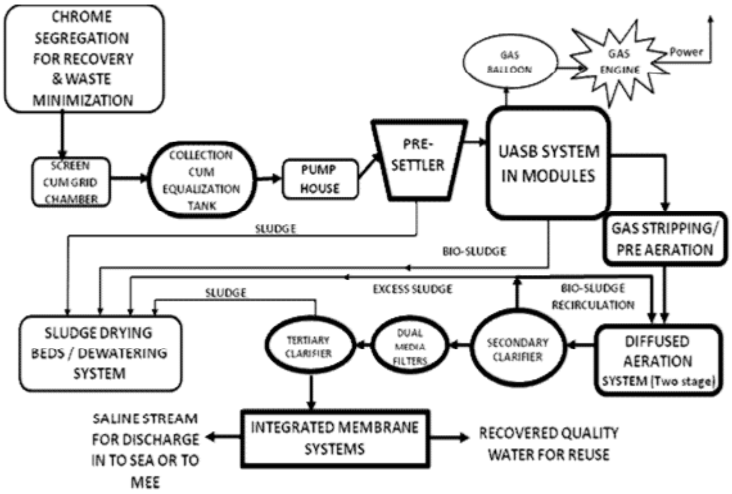

Figure 1. Process Flow Diagram for Tannery Waste Treatment & Integrated Saline Management – New and First of its kind

After primary and secondary treatment units, Reactive Clarifier, Dual Media Filter, Micro Filter, Ultra-Filter (UF) are installed prior to RO system for recovery of water.

The number of stages and types of RO system are based upon the TDS concentration in the feed water, estimated percentage of quality water recovery and reduction in volume of saline reject. High pressure Sea water membrane is adopted for handling treated effluent with TDS concentration more than 10,000 mg/l. The quality water recovery rate could be achieved to the level of 70 to 90% depending upon the feed water TDS level, type and stages of membrane system etc. In addition to recovery and reuse of quality water by the industry, the additional benefits are savings in chemical usage in the tanning process and reduction in pollution load in the effluent. The reject saline stream from RO system needs to be managed by adopting the options of forced / thermal evaporation system or disposal into Sea wherever feasible with suitable control.

Figure 2. Amiad Filter & Ultra-filter (UF)

Many full scale membrane systems have been installed for recovery of water from domestic and tannery wastewater with capacities ranging from 100 to 20000m^3/day.

MEMBRANE BIO-REACTOR (MBR) INTEGRATED WITH RO SYSTEM

Membrane Bio Reactor (MBR) system is commonly adopted in many countries to remove the residual BOD, suspended solids / coliform, etc. from the effluent. After treatment with MBR, the water is applied through RO system for removal of TDS and salinity to get drinkable quality water with TDS less than 500mg/l. A Common Effluent Treatment Plant (CETP) in Spain with MBR and RO system for water recovery was established in 2005. Recent times many CETPs in India have adopted MBR and other membrane system for water recovery and reuse from the tannery effluent. After MBR / UF treatment, the suspended solids and BOD values in the effluent are below detectable level and taken for treatment with RO system for recovery of water after the removal of TDS and salinity.

In China also water is becoming a scarce commodity in many locations. Expansion of high water consuming industries is allowed only if they are provided with water recovery system in the effluent treatment plants. To recover water from the tannery wastewater, submerged MBR linked with activated biological treatment is provided in the first stage. Following MBR system an RO plant in “Christmas Tree” configuration has been installed and operated at 12–16 bars. The RO plant produces about 70% permeate and 30% concentrate. The quality of the recovered water meets the drinking water standards. The saline water concentrate stream is further treated with Fenton process before disposal.

A view of the submersible MBR in one of the tannery effluent treatment plants in China is shown in Figure 3.

Figure 3. Submerged Membrane Bioreactor

The Nano Filtration (NF) is adopted for removal of colour and salts such as sulphates from the treated effluent after ultra filtration or MBR stage. Nano-filtration membranes are operated under low pressure with high yield of about 90%. Adopting NF will improve the efficiency of RO in water recovery and to decrease the volume of saline reject.

Multiple stage evaporators using thermal and electrical power have been installed for evaporation of the reject saline stream from RO system. However, there are many technical issues such as constrains in continuous operation of the system, meeting the required quality of the condensate water from the evaporator for reuse, management / utilization of the recovered salt with impurities etc., The capital and operational costs are also high. Further techno economical review and modified options are required on the sustainability of the system particularly in land locked areas.

NAVAL MARINE DISPOSAL OF TREATED SALINE STREAMS

A novel technological development has been made for the drawl of Seawater of 30,000 m^3/day from nearby Sea for the desalination plant integrated with a major leather complex in South India. Out of the total water quantity, freshwater of about 10,000m^3/day will be generated and the remaining 20,000m^3/day will be discharged into Bay of Bengal with special bio-control and dispersion system to safe guard the aquatic life. The leather complex will be using the freshwater generated by desalination plant for its process requirements and 9,000m^3/day wastewater will be treated, mixed with saline reject of the desalination plant, stored in a water tight pond for a capacity of about 10 days and discharged into the Sea by laying 5 km pipeline using high pressure HDPE pipe and special sprinkling system. The combined treated saline stream with a quantity of about 29,000m^3/day will be discharged once in a week under the overall control of environmental protection authorities.

With the support of many National Institutes and other organizations, model studies were carried out in finalizing the novel marine outfall. The spreading of an effluent cloud released in a marine environment is governed by advection caused by large scale water movements and diffusion caused by comparatively small scale random and irregular movements without causing any net transport of water. Hence, the important physical properties governing the rate of dilution of an effluent cloud in coastal waters are bathymetry, tides, currents, circulation and stratification.

A five port diffuser systems with 0.18 m diameter is planned with a jet velocity of 2.5 m/sec, for the release of treated effluents and reject water from the proposed desalination plant.

The Environmental Clearance (EC) and approval has been accorded by Government of India to this unique integrated project with water recovery using desalination process, tannery wastewater treatment, novel and safe saline reject disposal into Sea without affecting the marine life which is first of its kind in India.

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REFERENCES

Asian International Union of Environment (AIUE) (2014), *Commission documents on Environmental protection*.

Milá i Canals, L., Doménec, X. *et al*. (2002), “Use of LCA in the procedure for the establishment of environmental criteria in the catalan ecolabel of leather”, *The International Journal of Life Cycle Assessment*, 7, 39.

Rajamani, S. (2012), “Environmental update in Leather producing countries, Taiwan”, *Leather News India*.

Rajamani, S. (2013), “Concept and guidelines for Environmental Footprint for World Leather Sector”, *Leather News India*.

Rajamani, S. (2014), “Sustainable Environmental Protection System for tanning industry with viable sludge and saline stream management”, *Leather News India*.

Rajamani, S. and Casey, P. (2010), “Environmental update in Leather Producing Countries – Argentina”, *Leather News India*.

Rajamani, S., Chen, Z., Zhang, S., Su, C. (2010), “Environmental Update in Leather Producing Countries, China”, *Leather News India*.

RESEARCH ON THE HYDROLYSIS OF HIDE WASTE IN THE PRESENCE OF NATURAL POLYMERS

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Like other industries, the leather processing industry is forced to face high costs for waste treatment and disposal. Consequently, researchers are seeking solutions for effective recovery of organic waste from tanneries through various biochemical treatments in order to obtain protein biofertilizers. The novelty is based primarily on the fact that the starting point of the promoted technologies is the development of new complex products obtained by processing organic waste, called hydrogels with collagen structure, with applications in agriculture. This paper presents an innovative process for the biochemical degradation of pelt waste in order to obtain encapsulated protein biocomposites to be used as fertilizers for poor soils and for plant growth. The proposed method consists in the treatment of raw hide waste by direct hydrolysis of protein waste in an acidic environment, in combination with other polymers (polyacrylamide, starch, urea, acrylic, maleic, cellulose, etc.), thus obtaining hydrogels with collagen structure. For this purpose natural sources of protein are enriched by the addition of macro- and/or micronutrients, resulting in compounded complex systems, namely protein hydrogels. Following experiments it can be concluded that direct hydrolysis of a protein source in the presence of natural or synthetic polymers results in hydrogels with collagen structure to improve soil quality and horticulture.

Keywords: protein waste, hydrogel, tannery

INTRODUCTION

Organic biopolymers are a source of raw materials for agriculture, as protein waste composition provides sufficient elements to improve the composition of degraded soils and plants can harness some micro- or macroelements: nitrogen, phosphorus, calcium, boron, iron, magnesium, sodium, potassium, etc.

Obtaining hydrogels with collagen structure by pelt waste hydrolysis with applications in agriculture is a novelty, given that collagen is used only in medicine.

Compared to hydrogels based on synthetic polymers, hydrogels based on biopolymers have the advantage of biodegradability, biocompatibility and low toxicity (González *et al*., 1992).

Multicomponent absorbent hydrogel-type networks are next generation materials, with three-dimensional structure and high swelling capacity. The applications of these materials are diversifying, in recent years entering the fields of agriculture, food, pharmaceuticals, electrical devices and electronics, environmental protection and biomaterials (Kopeček, 2007). Hydrogels have a distinct three-dimensional structure and although they have a high water content, hydrogels are water-insoluble due to the crosslinked (physical or chemical) structure of the steric or crystalline linkages. The hydrogel swells when it comes into contact with the aqueous solution (Pooley *et al*., 2010).

Hydrogels can be obtained by two major mechanisms: hydrogels with covalent or irreversible links and hydrogels with reversible or physical links. The second category includes various subclasses such as ionic interactions (ionic hydrogels or cross-linked polyelectrolyte complexes) and secondary interactions ("entangled" hydrogels, grafted or complexed hydrogels, etc.) (Hennink *et al*., 2002).

The area of hydrogel synthesis was broadened by introducing natural polymer or composites of natural and synthetic polymers and continues to boom due to multiple and important applications of hydrogels particularly in medicine and pharmacy, as well as in other areas, such as agriculture.

The applications of hydrogels in agriculture aim at water retention in the soil or controlled release of pesticides or fertilizers (Azeem *et al*., 2014). In the first case, the application is based on hydrogels' ability to quickly absorb large amounts of water and then release it gradually, supplying plants with water for longer periods after watering the field (rain or irrigation) has ceased.

Hydrogels as controlled fertilizer release systems in agriculture have the major advantage of combining water absorption and its slow release in the interval between rainfalls or irrigations. Therefore they have the ability to retain water in the soil, with the property of slowly releasing fertilizer at the root, and the indisputable advantages presented above. For this reason, encapsulation of fertilizers in hydrogels with high water absorption ability has been researched more intensively in the past years, although there are not many papers published, particularly in the area of biodegradable polymers.

Literature on hydrogels with applications in controlled release of fertilizers (HECF) reveals some observations on the synthesis, structures, fertilizer release method, etc. In terms of the synthesis process HECF have been obtained in solution, in inverse suspension, by dispersing the aqueous solution of monomers into a water-immiscible organic solvent. The method has obvious advantages, as HECF are directly obtained as particles.

Depending on the structure of the resulting fertilizer particle, hydrogel may form the matrix where the fertilizer is embedded or the coating of the solid fertilizer (mono- or multi-layered particles) (Puccini *et al*., 2015).

Examples of synthesis of hydrogels for agricultural uses include: a hydrogel based on starch, carboxymethyl cellulose (CMC), and cross-linked polyacrylamide, obtained by radical polymerization of acrylamide in aqueous solution in the presence of N,N'-methylenebisacrylamide (MBA), crosslinking agent (Abd El-Rehim *et al*., 2006).

Hydrogels with applications in agriculture were also obtained by analogous polymer reactions. For example, in 2004 (Katima *et al*., 2004) the reaction between polyvinyl alcohol and phosphoric acid in an aqueous solution was used, followed by neutralization with sodium carbonate. The result was a hydrogel crosslinked by phosphate bridging and with sodium phosphate side groups. The hydrogel had the ability to slowly release phosphate groups in aqueous/wet medium.

Other researchers have obtained starch-sodium alginate hydrogels by mixing aqueous solutions of these polymers, which are composite hydrogels with applications in agriculture. Composite hydrogels have been used both as a matrix, and as a fertilizer particle coating (Gao *et al*., 2015).

EXPERIMENTAL DATA

Biotechnology for the preparation of protein biocomposites mainly consists of hydrolysis of pelt waste with the enrichment and/or compounding with polymers (corn starch, maleic copolymers, etc.) for use as conditioners and fertilizers (Zăinescu *et al*., 2014).

The research paper presents exploratory research that starts from obtaining new complex products - polymer multicomponents - called hydrogels, by processing pelt waste for applications in agriculture.

The paper presents the preparation of biodegradable polymer mixtures obtained from hydrolysis and enrichment of resulting hydrolysate with phosphorus and potassium.

In this study, we used limed hide waste from fleshing and trimmimg cattle hides (weight category 35 kg) from SC Pielorex Jilava tannery, Ilfov County, Romania. Raw hide contains (based on dry weight) 50-68% protein, 0.6-9% fat and 15-50% ash.

An innovative process has been proposed, namely a direct hydrolysis of pelt waste in an acid medium, combined with other polymers (polyacrylamide, starch, acrylic, maleic, urea, cellulose, etc.) to obtain hydrogels with collagen structure.

Figure 1. Pilot plant for developing hydrogel with collagen structure

The technological process for obtaining protein hydrogels is presented below:

- an amount of 4.6 kg of pelt waste is washed with water at 20-25°C in a drum for 30-80 minutes and delimed with 2-3.5% ammonium sulfate for 2-3.5 hours; waste is then ground using a TC 32 grinder (double knives) from SAP company, Italy;
- the mixture is hydrolysed at a temperature of 85-97°C for 2.5-3.5 hours, by adding about 4-6 liters of water in which were dissolved 1.3-2.8% concentrated sulfuric acid, 8-10% corn starch and 3-4.5% dipotassium phosphate $K_2HPO_4.3H_2O$ (to improve the nutritional properties by the addition of phosphorus and potassium required for plant growth and development), all quantities are relative to the mass amount of ground pelt waste;
- a natural polymer based on vegetable oil, labeled “EZ” (3-5 ml per kg of ground pelt) is then added and 2.5-4.5% urea can be added as plasticizer, continuing to stir in an autoclave at a temperature of 85-95°C for 1-1.5 hours, and then the mixture is removed from the autoclave in plastic drums.

Collagen-starch-urea hydrogels are thus prepared, which can be extruded into biodegradable films for use in agriculture (Zăinescu *et al.*, 2016).

RESULTS AND DISCUSSIONS

A hydrogel is defined as a polymer network which has the property of absorbing large amounts of solvent causing macroscopic changes in the polymer. The most important property of hydrogels is their degree of swelling and dissolution and water release of nutrients needed for plant growth.

An attenuated total reflectance spectrophotometer, FT/IR-ATR, Perkin Elmer USA was used to highlight the structural changes in the process of hydrolysis and interaction with various synthetic polymers. Knowing the main spectral characteristics of protein biopolymers in the IR and UV/Vis range, some significant bands for the amide structure were selected.

The IR spectrum shows amide I, II and III bands at about 1660, 1550 and 1240 cm^{-1}, respectively.

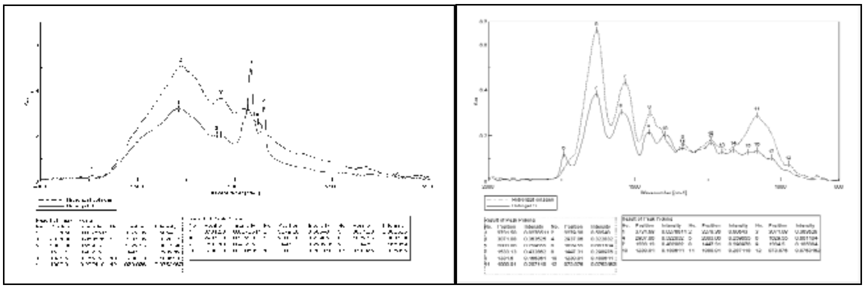

Figure 2. IR spectrum of collagen-starch-urea hydrogel

Hydroxyl groups and the hydrogen bonds are recorded between 3600 and 3100 cm^{-1}. According to the spectral assignments, in the case of collagen hydrolysate bands corresponding to amide groups were observed ($\gamma_{C=0}$ at 1645 cm^{-1}, δ_{NH} and γ_{C-N} at 1556 cm^{-1}). If the band at 1550 cm^{-1} shifts to 1530 cm^{-1}, the movement is considered a marker of distortion. In the case of collagen hydrolysates, some of the OH groups are replaced with methoxy groups (CH_3), attenuating hydrogen bonds, decreasing crystallinity of the collagen and increasing water solubility. Thus, the presence of collagen, urea and starch was confirmed in the molecular structure of the hydrogel.

The protein was encapsulated using a natural product based on vegetable oil labeled “EZ” (Figure 3).

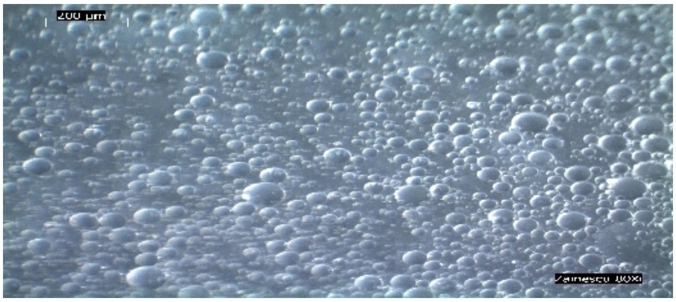

Figure 3. Microscopic image of encapsulated hydrogel with collagen structure (80x)

Physical-chemical analyses of hydrogels obtained in laboratory and pilot stage were carried out in the Quality Control and Testing Laboratory of ICPI (Table 1). The chemical analysis of hydrogel with collagen structure for field experiments indicated the $N_{13}P_9K_{12}$ composition (total nitrogen 13.71%; phosphorus P_2O_5 3.64%; potassium K_2O_5 6.18%).

Table 1. Physical-chemical properties of experimental samples

No.	Characteristics	UM	Sample code / Determined values		Standard method
			4L	5P	
1	Dry substance	%	24,60	10,72	SR EN ISO 4684: 2006
2	Ash	%	7,89	6,06	SR EN ISO 4047 : 2002
3	Total nitrogen	%	8,17	13,71	SR ISO 5397 : 1996
4	Protein substance	%	45,92	77,05	SR ISO 5397 : 1996
5.	pH	pH units	4,53	5.53	STAS 8619/3 : 1990

All these hydrogels with collagen structure may be used as smart biofertilizers, particularly as foliar fertilizers.

CONCLUSIONS

Obtaining hydrogels with collagen structure by hydrolyzing pelt waste for applications in agriculture is a novelty, given that the collagen structure is mainly used in the composition of hydrogels used in medicine. In support of this are the most recent scientific reports in this area related to the interest of researchers in interdisciplinary fields concerning the preparation of hydrogels with collagen structure. This scientific paper is based on a new concept, that of performing direct hydrolysis, using a polymer and nutritional multicomponent system required to obtain hydrogel for agriculture.

Thus experiments in the pilot phase have established the technological process for preparation of encapsulated starch-urea hydrogels with collagen structure starting from pelt waste. Optical microscopy and IR analysis confirmed that the protein source can be transformed by direct hydrolysis of the protein multicomponent system into collagen hydrogels.

Optimal parameters were set for preparation of encapsulated hydrogel with collagen structure with controlled release of nutrients (nitrogen, phosphorus, potassium, boron, iron, etc.) necessary for plant growth.

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REFERENCES

Abd El-Rehim, H.A., Hegazy, E.-S.A. and Abd El-Mohdy, H.L. (2006), “Effect of various environmental conditions on the swelling property of PAAm/PAAcK superabsorbent hydrogel prepared by ionizing radiation”, *Journal of Applied Polymer Science*, 101, 3955-3962.

Azeem, B., KuShaari, K.Z., Man, Z.B., Basit, A. and Thanh, T.H. (2014), “Review on materials and methods to produce controlled release coated urea fertilizer”, *Journal of Controlled Release*, 181, 11–21.

Gao, X., Li, C., Zhang, M., Wang, R. and Vhen, B. (2015), “Controlled release urea improved the nitrogen use efficiency, yield and quality of potato (*Solanum tuberosum* L.) on silt loamy soil”, *Field Crops Research*, 81, 60–68.

González, N., Vadillo, I., Trigo, R. and Teijon, J. (1992), “Hydrogeles: Síntesis, propiedades y aplicaciones”, *Rev. Ibe. Pol.*, 1(1), 79-83.

Hennink, W.E. and van Nostrum, C.F. (2002), “Novel crosslinking methods to design hydrogels”, *Advanced Drug Delivery Reviews*, 54, 13–36.

Katime, I., Katime, O. and Katime, D. (2004), *Los materiales inteligentes de este milenio: Los hidrogeles macromoleculares. Síntesis, propiedades y aplicaciones*, Servicio Editorial de la Universidad del País Vasco, Bilbao, España.

Kopeček, J. (2007), “Hydrogel biomaterials: A smart future”, *Biomaterials*, 28, 5185–5192.

Pérez-Limiñana, M.A., Escoto-Palacios, M.J., Martínez-Sánchez, M.A., Ferrer-Palacios, J., Arán-Aís, F. and Orgilés-Barceló, C. (2014), “Recovery of Tannery Wastes for Functional Microencapsulated Products”, *Proceedings of The 5th International Conference on Advanced Materials and Systems - ICAMS 2014*, Bucharest, 479.

Pooley, S.A., Rivas, B.L., Lillo, F.E., Pizarro, G. and Del, C. (2010), “Hydrogels from acrylic acid with N,N-dimethylacrylamide: synthesis, characterization, and water absorption properties”, *Journal of the Chilean Chemical Society*, 55, 19–24.

Puccini, M., Seggiani, M., Vitolo, S. and Lucio, L. (2015), “Utilization of starch films plasticized with urea as fertilizer for improvement of plant growth”, *Chemical Engineering Transactions*, 43, 7005-7010.

Zăinescu, G., Albu, L., Deselnicu, D., Constantinescu, R.R., Vasilescu, A.M., Nichita, P. and Sîrbu, C.A. (2014), New Concept of Complex Valorization of Leather Wastes, *Materiale plastice*, 51(1), 90-93.

Zăinescu, G., Coară, Gh., Florescu, S.M., Constantinescu, R.R. (2016), “Obtaining polymer hydrogels with collagenic structure from pelt waste”, *The SGEM Albena-Bulgaria Conference Proceedings, Book 4 - Energy and Clean Tehnologies*, Vol II, 73- 81.

CHARACTERIZATION OF WASTE SKINS USED IN TULUM CHEESE PRODUCTION

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Tulum cheese is a traditional cheese type of Turkey, which the ripening process of the cheese is carried out in sheep and goat skins. Although there are some industrial casings, sheep and goat skins are still the commonly preferred casing materials in traditional Tulum cheese production due to the natural favor and taste that give to the cheese characteristics. The difference of these natural casing materials, such as type, age, gender and sex, gives different tulum cheese quality. But not only a high quality cheese production but also the utilization of the skins used in tulum cheese production is carrying a great importance in environmental point of view. In this study, the utilization possibilities of raw skins that become the waste of the food industry after the tulum cheese production were investigated in terms of physical and chemical properties. For this purpose, forty eight (48) raw skins in different type (goat and sheep skins), age (6 months, 1 and 2 years old) and gender (male and female) were examined. The physical tests such as tensile strength and percentage of extension, double edge tear strength, shrinkage temperature, water vapor and air permeability determination; the chemical analysis such as pH, matters soluble in dichloromethane and Kjeldahl nitrogen analysis were performed to reveal the usage possibilities of these raw materials for the leather or relevant industries.

Keywords: Tulum cheese, goat skin, sheep skin, casing, utilization.

INTRODUCTION

Tulum cheese production is commonly performed nowadays with alternative casing materials such as plastic, jam, bowel, cloth and polyethylene instead of goat and sheep skins due to the factors such as decrease in the number of ovine, legal restrictions occurred by the law of forest, regulations of the European Union and ease of use (Tarakçı *et al*. 2005; Hayaloğlu *et al*. 2007; Duman-Aydın and Gülmez, 2008; Arslaner and Bakırcı, 2009). However, the quality characteristics of Tulum cheese such as natural flavor and taste as well as the standardization of the applications and the shortening of ripening period have not been achieved yet by the alternative casing materials (Güven and Konar, 1994). The most important properties of the raw skins are the water vapor, air and gas permeability in addition to their unique skin characteristics. Not only a high quality cheese production, but also the utilization of the skins used in tulum cheese production is carrying a great importance.

From the environmental point of view, to utilize the waste generated by cheese production of the food industry that consists primarily of the leather protein has been the focus of interest. Although the skin characteristics of the goat and sheep skins by different age and gender was described prior to Tulum cheese production (Gun *et al*., 2014), no report was found about the chemical and physical characteristics of raw skins obtained after the Tulum cheese production.

In this study, the utilization possibilities of raw skins that become the waste of the food industry after tulum cheese production were investigated in terms of physical and chemical properties. The physical tests such as tensile strength, percentage of extension, double edge tear strength, shrinkage temperature, water vapor and air permeability determination; the chemical analysis such as pH, matters soluble in dichloromethane and Kjeldahl nitrogen analysis were performed to reveal the usage possibilities of these raw materials for the leather or relevant industries.

MATERIALS AND METHODS

Materials

Forty eight (48) waste raw skins in different type (goat and sheep skins), age (6 months, 1 and 2 years old) and gender (male and female) were examined after Tulum cheese production. Analytical grade chemicals were used for the chemical analysis.

The codes given to raw skins after Tulum cheese production are presented in Table 1.

Table 1. The codes for the waste raw skins of sheep and goat in different age and gender

6MSF: 6 Months Sheep Female	6MGF: 6 Months Goat Female
6MSM: 6 Months Sheep Male	6MGM: 6 Months Goat Male
1YSF: 1 Year Sheep Female	1YGF: 1 Year Goat Female
1YSM: 1 Year Sheep Male	1YGM: 1 Year Goat Male
2YSF: 2 Year Sheep Female	2YGF: 2 Year Goat Female
2YSM: 2 Year Sheep Male	2YGM: 2 Year Goat Male

Methods

The raw skins obtained after Tulum cheese production as a waste of food industry were conditioned according to TS EN ISO 2419, at 23±2°C and 50%±5 relative humidity prior to physical and chemical characterization.

The Determination of Physical Characteristics

The tensile strength and percentage extension, double edge tear load, shrinkage temperature and water vapor permeability were tested in accordance with the standards TS EN ISO 3376, TS 4118-2 EN ISO 3377-2, TS 4120 EN ISO 3380 and TS EN ISO 14268 respectively.

The air permeability was tested by the brand of Devotrans, DVT-HG model device (Kanli *et al*., 2010). The measurements were performed at 200 Pa for 5 minutes.

The Determination of Chemical Characteristics

The matter soluble in dichloromethane (%), pH and total Kjeldahl nitrogen content and hide substance were investigated according to TS EN ISO 4048, TS EN ISO 4045 and TS 4134 respectively.

RESULTS AND DISCUSSIONS

Physical test results of waste sheep and goat skins obtained after Tulum cheese production are given in Table 1. Two different ripening periods were selected for the production of Tulum cheese as 60 and 120 days.

Table 1. Tensile strength (N/mm^2), percentage of extension (%) and double edge tear load (N/mm) test results of sheep and goat skins after Tulum cheese production

	0. day			60. day			120. day		
	TS	%E	DET	TS	%E	DET	TS	%E	DET
6MSF	8.25	57.60	29.36	11.98	63.66	69.33	10.02	47.48	43.77
6MSM	7.58	62.00	27.64	11.03	53.58	55.31	10.32	45.64	48.22
1YSF	9.16	65.74	31.18	8.07	45.11	43.56	11.65	51.94	55.65
1YSM	7.72	75.50	32.04	11.61	49.11	54.80	11.87	56.16	67.26
2YSF	7.75	83.50	29.77	14.82	65.18	78.03	10.22	45.94	43.33
2YSM	12.18	39.36	40.15	17.69	59.05	78.83	16.23	46.60	62.94
6MGF	12.13	38.16	34.75	21.05	48.22	91.92	15.20	46.38	112.82
6MGM	14.81	63.76	51.05	17.64	64.00	78.54	16.61	34.31	60.23
1YGF	12.38	55.64	41.67	19.14	72.78	93.41	16.16	47.38	63.90
1YGM	12.71	40.06	40.12	17.88	44.09	84.03	15.78	53.05	80.89
2YGF	15.34	65.92	56.29	17.97	41.53	80.88	21.38	42.13	75.70
2YGM	18.00	60.96	55.57	28.04	47.72	112.89	19.19	35.51	58.02

* TS.: Tensile Strength; %E.: Percentage Extension; DET.; Double Edge Tear Load; the mean values were presented

The tensile strength, percentage elongation and double edge tear values of sheep skins were found 8.77 N/mm^2, 63.97% and 31.69 N/mm respectively. The same tests were applied to sheep skins after 60 and 120 days of cheese ripening and the results were found 12.53 N/mm^2, 55.95%, .63.31 N/mm and 11.72 N/mm^2, 48.96%, 48.96 N/mm respectively.

And for the goat skins, the tensile strength, percentage elongation and double edge tear values were found 14.23 N/mm^2, 54.08% and 46.58 N/mm respectively. After 60 and 120 days of cheese ripening, the results were found 20.29 N/mm^2, 53.06%, .90.28 N/mm and 17.39 N/mm^2, 43.13%, 75.26 N/mm respectively.

The goat skins used as a casing material had better tensile, tear load and elongation at break values than the sheep skins. A difference in the physical properties of raw skins was determined depending on the ripening period. Generally, tensile strength and tear load values were increased, although percentage of extension values was decreased after the ripening process.

The shrinkage temperatures TS (°C) of sheep and goat skins were found 68°C prior to ripening process. After the ripening period, shrinkage values were found 65°C in common. This decrease was attributed to the long storage time during the ripening of Tulum cheese (Table 2).

Table 2. Shrinkage temperatures of sheep and goat skins after Tulum cheese production (°C)

	TS(°C) 0. day	TS(°C) 60. day	TS(°C) 120. day
6MSF	67	64	66
6MSM	67	64	66
1YSF	70	66	63
1YSM	69	65	65
2YSF	67	66	69
2YSM	68	63	63
6MGF	66	65	66
6MGM	70	64	5
1YGF	66	65	64
1YGM	65	65	65
2YGF	69	67	67
2YGM	70	65	65

*The mean values were presented in the table

The water vapor permeability of sheep and goat skins were found 0.48 mg/cm^2.h and 0.70 mg/cm^2.h respectively before the ripening process. After 60 and 120 days of cheese ripening, the results were found 0.53 and 0.35 mg/cm^2.h for sheep skins and 0.41 and 0.50 mg/cm^2.h for goat skins respectively. The water vapor permeability of goat skins was found higher than the sheep skins; however a decrease was determined after the ripening period for both skin types (Table 3).

Table 3. Water vapor (mg/cm^2.h) and air permeability (cm3/cm^2.sec) results of sheep and goat skins after Tulum cheese production

		0. day			60.day			120.day	
	WVP	AP wool side	AP flesh side	WVP	AP wool side	AP flesh side	WVP	AP wool side	AP flesh side
6MSF	0.56	43.97	6.07	0.42	3.78	8.55	0.27	5.97	23.67
6MSM	0.54	162.90	56.27	0.67	0.88	11.40	0.79	4.20	15.33
1YSF	0.31	52.93	5.70	0.57	2.22	15.18	0.17	19.57	20.80
1YSM	0.65	372.27	29.97	0.71	3.92	7.70	0.27	6.53	7.63
2YSF	0.41	134.60	4.93	0.35	7.57	20.72	0.20	5.85	7.48
2YSM	0.41	93.40	12.73	0.43	1.53	7.77	0.41	2.57	7.95
6MGF	0.82	277.90	33.53	0.49	10.82	15.08	0.17	9.68	17.35
6MGM	0.63	196.17	14.30	0.41	6.37	10.93	1.30	15.93	33.90
1YGF	0.80	155.87	10.17	0.34	12.08	16.70	0.36	11.13	31.40
1YGM	0.46	138.00	38.90	0.47	25.28	27.22	0.48	16.78	27.33
2YGF	0.69	63.60	9.27	0.35	14.50	27.77	0.49	20.62	32.62
2YGM	0.77	169.07	13.93	0.41	4.85	12.62	0.23	11.77	25.50

*WVP; Water vapor permeability; AP; Air permeability, the mean values were presented in the table

The air permeability values of waste sheep and goat skins are shown in Table 3 and the test were performed from wool and flesh side of the skins. A decrease from 2 to 10 times at 60 and 120 days of ripening was determined for the air permeability values compared prior to ripening process. This might be because of the protein materials filling the porous structure of the raw skins (Table 3).

Table 4. pH and matter soluble in dichloromethane (%) results of sheep and goat skins after Tulum cheese production

	0. day		60. day		120. day	
	pH	Matter soluble in dichloromethane (%)	pH	Matter soluble in dichloromethane (%)	pH	Matter soluble in dichloromethane (%)
6MSF	6.9	15.11	5.8	21.60	6.4	13.94
6MSM	6.8	7.00	6.1	13.50	6.5	12.23
1YSF	6.7	8.58	6.2	14.02	5.9	18.44
1YSM	6.6	8.99	5.9	14.10	6.2	12.25
2YSF	6.6	13.88	6.4	18.62	6.9	13.83
2YSM	7.0	8.29	5.7	16.77	6.1	15.88
6MGF	6.3	4.78	6.1	10.56	6.2	6.21
6MGM	6.6	5.98	6.1	8.81	6.4	10.16
1YGF	6.3	5.40	5.9	10.91	6.2	16.13
1YGM	6.6	2.77	6.1	5.71	6.3	9.46
2YGF	6.3	8.08	6.2	5.73	6.3	6.04
2YGM	6.3	4.31	5.8	7.64	6.1	6.23

*The mean values were presented in the table

The pH values were found 6.8 and 6.4 for sheep and goat skins before the cheese production. Later, these values were shifted to a little acidic side and then raised again during the ripening process. This was attributed to the fermentation during the cheese production. An alteration was found for the fatty substance (matter soluble in dichloromethane %) for the skins depending on the ripening time (Table 4).

Table 5. Nitrogen Content (%) and Hide Substance (%) results of sheep and goat skins after Tulum cheese production

	0. day		60. day		120. day	
	Nitrogen Content (%)	Hide Substance (%)	Nitrogen Content (%)	Hide Substance (%)	Nitrogen Content (%)	Hide Substance (%)
6MSF	7.33	42.89	9.14	51.34	10.70	60.15
6MSM	8.93	52.27	10.06	56.54	10.31	57.97
1YSF	8.75	51.21	10.07	56.58	10.59	59.52
1YSM	8.94	52.28	10.19	57.24	9.89	55.56
2YSF	8.16	47.71	9.72	54.65	9.77	54.88
2YSM	10.09	59.04	10.12	56.87	10.33	58.04
6MGF	10.94	62.89	11.02	61.94	10.69	60.05
6MGM	8.90	51.81	11.20	62.96	12.20	68.58
1YGF	10.66	61.33	11.02	61.94	10.20	57.32
1YGM	10.82	62.20	12.27	68.94	12.16	68.36
2YGF	9.64	55.44	11.38	63.98	11.52	64.75
2YGM	10.74	61.77	11.27	63.36	11.11	62.44

The hide substance % of sheep skins were found lower than the goat skins. Gun *et al*. (2014) was found the total nitrogen% and hide substance values as 10.77-16.85 and 86.32%. Dikmelik (1978) was determined the hide substance for raw goat skins as 81.72%.

CONCLUSIONS

In this research, physical and chemical characterization of the raw sheep and goat skins generated as a waste of food industry was investigated to determine the utilization possibilities in leather or relevant industries. And the following conclusions have been drawn: a) The physical strengths, matters soluble in dichloromethane, nitrogen content (%) and hide substance (%) values were increased in parallel with the ripening process time. On the contrary, percentage of extension, shrinkage temperature, water vapor and air permeability values were decreased. b) The physical strengths, water vapor and air permeability values of the goat skins were found higher than the raw sheep skins. This could be the reason of the goat skin preference for traditional Tulum cheese production. c) The matters soluble in dichloromethane of goat and sheep skins after the Tulum cheese production were increased in the proportion of 50% and 100%, respectively that was a well indication of the cheese production effect on the amount of raw skin fat.

REFERENCES

Arslaner, A. and Bakırcı, (2009), “Geleneksel peynir çeşitlerimizden Erzincan Tulum peynirinin farklı ambalaj materyallerinde olgunlaştırılması üzerine bir araştırma” *II. Geleneksel Gıdalar Sempozyumu*, 27-29 Mayıs 2009, Van, 147-152.

Dikmelik, Y. (1978), “İşlenmek üzere İzmir tabakhanelerine gelen kıl keçisi derileri üzerinde araştırmalar”, Ege Üni. Fen Bilimleri Ens. Deri Tek. ABD., Doktora tezi, İzmir, 104.

Duman-Aydın, B. and Gülmez, M. (2008), “Investigation of Alternative Methods in Making Erzincan Tulum Cheese”, *KafkasÜniv Vet FakDergisi*, 14(1), 67-73.

Gun, I., Mutlu, M.M., Adıgüzel Zengin, A.C., Sumer, G., Seydim, Z.B. and Zengin, G. (2014), “Determination of Skin Properties in Different Type, Gender and Age used for Ripening of Cheese”, *Proceedings of the 5th International Conference on Advanced Materials and Systems - ICAMS 2014*, 23-25 October, 61-66.

Güven, M. and Konar, A. (1994), “İnek sütünden üretilen ve farklı materyallerde olgunlaştırılan Tulum peynirlerinin fiziksel, kimyasal ve duyusal özellikleri”, *Gıda*, 19(5), 287-293.

Hayaloğu, A.A., Çakmakçı, S., Brechany, E.Y., Deegan, K.C. and McSweeney, P.L.H. (2007), “Microbiology, biochemistry and volatile composition of Tulum cheese ripened in goat’s skin or plastic bags”, *Journal of Dairy Science*, 90, 1102-1121.

Kanlı, N., Adiguzel Zengin, A.C. and Bitlisli, B.O. (2010), “The effects of different finishing types on water vapour and air permeability properties of shoe upper leathers”, *Proceedings of the 3rd International Conference on Advanced Materials and Systems*, 16-18 September 2010, Bucharest, Romania, 63-66.

Tarakçı, Z., Durmaz, H., Sağun, E. and Aygün, O. (2004), “Evaluation of Chemical Characteristics and Proteolysis and Lipolysis Levels in Hatay Sıkma Cheese”, *Vet .Bil.Derg*, 20(1), 53-59.

TS 4134. (1985), Leather - Determination of Nitrogen Content and Hide Substance. Titrimetric method, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 14268, (2004), Leather - Physical and mechanical tests - Determination of water vapor permeability, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 2419. (2012), Leather - Physical and mechanical tests - Sample preparation and conditioning (ISO 2419:2012), Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 3376. (2012), Leather - Physical and mechanical tests - Determination of tensile strength and percentage extension, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 4045. (2012), Leather - Chemical tests - Determination of pH, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 4048. (2013), Leather - Chemical tests - Determination of matter soluble in dichloromethane and free fatty acid content, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 3377-2. (2016), Leather - Physical and mechanical tests - Determination of tear load - Part 2: Double edge tear, Turkish Standard (TSE), Ankara, Turkey.

TS EN ISO 3380. (2016), Leather - Physical and mechanical tests - Determination of shrinkage temperature up to 100 °C, Turkish Standard (TSE), Ankara, Turkey.

V.
CULTURAL HERITAGE

SURFACE CHARACTERISATION OF PARCHMENTS BY THERMAL MICROSCOPY AND UNILATERAL NMR

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The protection of parchment collections in public and private libraries, archives, museums face nowadays several challenges such as environmental pollution, climate change, limited energy consumption, lack of testing/diagnosis protocols in conservation science as well as lack of standardisation of best practices in conservation. In this paper, the applications of thermal microscopy (imageMHT) and unilateral NMR to surface characterisation of parchments are presented. As parchment is a biomaterial with hierarchical structure characterised by the intimate relationship and connectivity between individual molecules and their organisation in micro-fibrils, fibrils and fibres, a micro to nano-scale investigation protocol is required for a reliable assessment.

Keywords: parchment, imageMHT, unilateral NMR.

INTRODUCTION

Parchment is a biomaterial endowed with great stability, strength and resilience, together with exceptional longevity. Studies carried out in the last decades showed that historical parchments are, however, especially sensitive to temperature and relative humidity variations in the storage environment, or elicited by the conservation and/or restoration methods (Hansen *et al*., 1992; Bowden and Brimblecombe, 2002). Deterioration is often more advanced on the parchment surface which may prevalently contain destabilised collagen or even gelatin. A stiff glassy surface with a flexible fibre layer below is a very risky condition for the preservation of writings and illuminations since the surface rigid layer is subjected to mechanical stress induced by even small variations of relative humidity and temperature due to its different capability of contraction and swelling compared with the underlying fibres. Characterisation of damage related to temperature and relative humidity was mainly achieved through the examination of parchments exposed to accelerated ageing procedures (Badea *et al*., 2012a; Della Gatta *et al*., 2007). Appropriate advanced physical-chemical and structural investigation techniques were developed in the compass of several EC, international and national projects, i.e. IDAP (EVK4-CT-2001-00061), OPERA (CIPE-D39) and COLLAGE (PNCDI II 224/2012) where over 300 samples were exposed to various hydrothermal ageing. In this paper, the use of thermal microscopy (imageMHT method and unilateral nuclear magnetic resonance (NMR)) to reveal whether there is any evidence for change to collagen on parchment samples'surface after their exposure to ageing in various controlled *T* and RH conditions is illustrated.

EXPERIMENTAL METHODS

Micro Hot Table Method (imageMHT)

The MHT method and equipment was already described (Badea *et al*., 2012b). Shrinkage motion of collagen fibres was digitally recorded and evaluated by the imageMHT software developed within the research project COLLAGE (www.collage.com.ro) (Miu *et al*., 2014).

Unilateral Nuclear Magnetic Resonance (NMR)

NMR measurements were performed at room temperature using a bar magnet NMR-MOUSE sensor with a double-D radio-frequency coil controlled by a Bruker Minispec spectrometer working at 20.05 MHz ^{1}H resonance frequency as described earlier (Badea *et al*., 2016). The NMR signal stems from a depth of about (0.5–1) mm from the surface of the sensor. The proton spin-lattice relaxation times T_1 were measured with a saturation-recovery pulse sequence using a Hahn-echo with an echo-time of about 25 μs for detection.

PARCHMENT DETERIORATION RELATED TO TEMPERATURE AND RELATIVE HUMIDITY

Standards and guidelines in force impose narrow *T* and RH intervals for parchments storage and display implying a high consumption of energy no longer sustainable. Loosening these tight environmental standards has thus become a much debated concept. In 1994, the Smithsonian Institution’s Conservation Analytical Laboratory issued revised guidelines allowing for as much as 15 % fluctuation in RH (35 % to 65 %) and 10 °C in temperature (11 °C to 31 °C), regardless of the materials from which objects were made. Progress in materials research pushed forward the conclusion the most museum objects can tolerate, without mechanical damage, larger fluctuations than previously thought (Erhardt and Mecklenburg, 1994). In the case of parchment and leather, reaction to RH changes is more complex as they are heterogeneous materials composed of distinct collagen populations and gelatin (Badea *et al*., 2012a; Della Gatta *et al*., 2007; Badea *et al*., 2012b; Badea *et al*., 2011). The differential behaviour of these components in terms of response to RH changes is a key factor to be considered for setting up appropriate storage and exhibition RH ranges. The intrinsic heterogeneity of historical parchment and leather has therefore great implications for setting environmental standards since not all artefacts react in a similar way to the same environmental RH. Consequently, some targeted research is required in this direction. The use of imageMHT and unilateral NMR as valuable techniques for characterizing parchment is illustrated in what follows.

Thermal Stability by Shrinkage Activity

The shrinkage temperature T_s of collagen fibres has been extensively as a gross metric of deterioration of collagen-based artefacts without considering their intrinsic structural heterogeneity. It has been reported that T_s very well relates with denaturation temperature T_d of fibrillar collagen measured by differential scanning calorimetry

(DSC) (Badea *et al.*, 2012a; Badea *et al.*, 2012b; Budrugeac *et al.*, 2010) as illustrated in Figure 1.

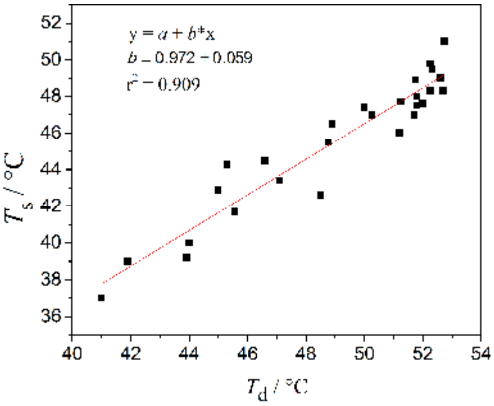

Figure 1. Plot showing the correlation between T_d and T_s for 32 parchments exposed to various hydrothermal ageing treatments

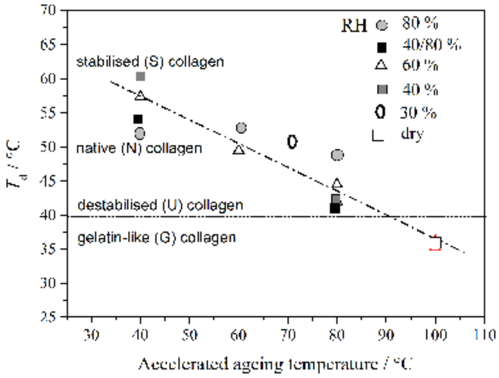

Figure 2. Variation of T_d depending on (T, RH) ageing conditions. T_d values correspond to parchment samples exposed to 32-day ageing.

Moreover, depending on T_d value parchments were previously classified in four groups: stable/native (N) for 48 °C < $T_d \leq$ 56 °C; stabilized (S) for T_d > 56 °C,: unstable (U) for collagen with 30 °C < $T_d \leq$ 48 °C and gelatin-like (G) for $T_d \leq$ 40 °C (Figure 2). Evaluation of both main shrinkage C and total shrinkage ΔT intervals provides further information about the level of structural heterogeneity and degree of damage. Based on the study of parchment samples exposed to thermal ageing at various T (e.g. 40, 60, 70, 80 and 100 °C) and RH (dry, 30%, 40%, 60% and 80%) we have found that ΔT interval increases as the structural heterogeneity increases, whereas it tends to decrease as a consequence of cross links formation (Badea *et al.*, 2012a; Della Gatta *et al.*, 2007; Badea *et al.*, 2016; Budrugeac *et al.*, 2010; Carşote *et al.*, 2016; Della Gatta *et al.*, 2005; Şendrea *et al.*, 2016b). On the other hand C interval generally tends to increase as a result of collagen thermal destabilization. However, in case of extended scission of primary peptide bonds, C interval dramatically decreases or even disappears. Accordingly, by measuring the shrinkage activity *in situ* with portable equipment, we can obtain important information about the thermal stability, structural heterogeneity and degree of deterioration of historical parchments (Figure 3).

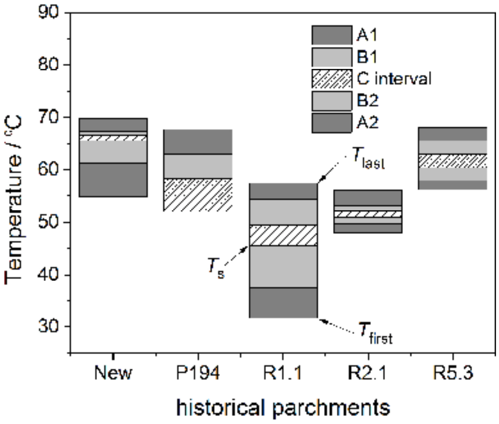

Figure 3. Shrinkage intervals for three parchment rolls (State Archives of Turin, XIV century), one parchment document issued by the chancery of the Stephen the Great (Library of the Romanian Academy, XV century) and one newly manufactured parchment. $\Delta T = T_{last} - T_{first}$, where T_{last} and T_{first} are the temperatures at which the very first and very last fibre motions are observed, respectively.

Unilateral Nuclear Magnetic Resonance (NMR)

It has been shown that longitudinal (or spin-lattice) relaxation time, T_1, can be used to qualitatively assess the changes of collagen structural water environment within historic parchments (Badea *et al*., 2008; Masic *et al*., 2012). This noninvasive and nondestructive approach proved to be very sensitive in studying the effects of deterioration factors on the organization of bound water within the collagen structure (Badea *et al*., 2016; Şendrea *et al*., 2016a; Badea *et al*., 2008; Masic *et al*., 2012; Şendrea *et al*., 2015). Specific trends were observed for T_1 during hydrothermal accelerated ageing of parchments. For example, parchments subjected to 80 °C and 80% RH showed a slight shortening of T_1 values, whereas in the case of parchments aged by a combined action of SO_2, light irradiation, and heating, an increase of T_1 relaxation values was found. A complex behaviour was displayed by the parchments exposed to ageing at fix temperature (i.e. 40 and 80 °C) and daily alternate RH (i.e. 40% and 80%) (Figure 4). This behavior was related to the competition of two deterioration processes, i.e. thermal stabilization through cross-links formation (responsible for T_1 value decrease as a result of the reduced chain mobility) and peptide bonds cleavage (responsible of T_1 value due to the opening of triple helix structure, which allows random exposure of hydrophobic residues to free water). In order to better discriminate the deterioration effects on the organization and properties of water and collagen within ancient manuscripts, the decays should be analysed by fitting the experimental data to a double or triple exponential function as reported for archival Italian documents (Badea *et al*., 2008) and for Dead Sea Scrolls (Masic *et al*., 2012).

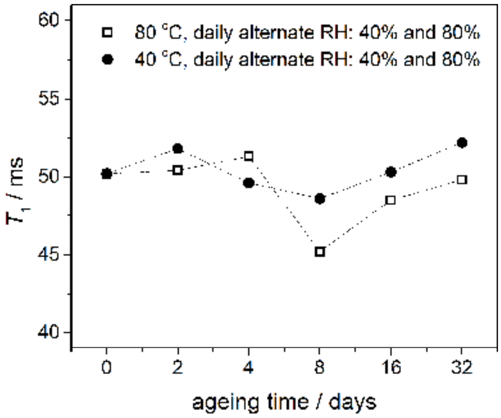

Figure 4. Variation of the proton relaxation time T_1 for parchments exposed to hydrothermal ageing at 40 °C/80 °C in controlled RH conditions as a function as ageing time

IMPLICATIONS OF THIS STUDY FOR CONSERVATION PROFESSIONALS

Analysis of the changes in collagen at the surface of parchment subjected to hydrothermal heating has shown that both thermal stability and structure of parchment are affected. Both imageMHT and unilateral NMR techniques provide valuable markers to assess such changes. Moreover, the availability of portable equipments (e.g. the imageMHT equipment developed within the project COLLAGE and NMR MOUSE from Magritek Gmbh) allows for *in situ* testing and evaluation of the 'health state" of historical documents and objects. For conservation professionals, the assessment protocols based on imageMHT and unilateral NMR represents a step forward in the routine conservation activity as valuable tools for diagnosis and monitoring the historical, archaeological and artistic objects made of collagen.

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REFERENCES

Hansen, E.F., Lee, S.N. and Sobel, H. (1992), "The effects of relative humidity on some physical properties of modern vellum: Implications for the optimum relative humidity for the display and storage of parchment", *Journal of the American Institute of Conservation*, 31(3), 325-342.

Bowden, D. and Brimblecombe, P. (2002), "The Thermal Response of Parchment and Leather to Relative Humidity Changes", in R. Larsen (Ed.), *Microanalysis of Parchment*, Archetype Publications Ltd., London, 63-72.

Badea, E., Della Gatta, G. and Usacheva, T. (2012a), "Effects of temperature and relative humidity on fibrillar collagen within parchment: a micro Differential Scanning Calorimetry (micro DSC) study", *Polymer Degradation and Stability*, 97, 346-353.

Della Gatta, G., Badea, E., Mašic, A. and Ceccarelli, R. (2007), "Improved Damage Assessment of Parchment (IDAP) Collection and Sharing of Knowledge", EC Research Report No. 18, R. Larsen (Ed.), Luxembourg, ISBN 987-92-79-05378-8, 89-98.

Badea, E., Sommer Dorte, V.P., Mühlen Axelsson, K., Della Gatta, G. and Larsen, R. (2012b), "Standardised methods for damage ranking in parchment: from microscopic evaluation to collagen denaturation assessment", *e-Preservation Science*, 9, 97-109.

Miu, O.A., Badea, E., Grigore, O. and Miu, L. (2014), "Automatic method for damage assessment of historical and archeological objects and artefacts and objects in leather and parchment", Patent application A/001253-05.07.2014.

Badea, E., Şendrea, C., Carşote, C., Adams, A., Blümich, B. and Iovu, H. (2016), "Unilateral NMR and thermal microscopy studies of vegetable tanned leather exposed to dehydrothermal treatment and light irradiation", *Microchemical Journal*, 129, 158-165.

Erhardt, D. and Mecklenburg, M. (1994), "Relative Humidity Re-examined", in Ashok Roy, Perry Smith (Eds.), *Preventive Conservation: Practice, Theory and Research*, Archetype Publications, London, 32–38.

Badea, E., Della Gatta, G. and Budrugeac, P. (2011), "Characterisation and evaluation of the environmental impact on historical parchments by DSC", *Journal of Thermal Analysis and Calorimetry*, 104/2, 495-506.

Budrugeac, P., Badea, E., Della Gatta, G., Miu, L. and Comănescu A. (2010), "A DSC study of deterioration caused by environmental chemical pollutants to parchment, a collagen-based material", *Thermochimica Acta*, 500, 51-62.

Carşote, C., Badea, E., Miu, L. and Della Gatta, G. (2016), "Study of the effect of tannins and animal species on the thermal stability of vegetable leather by differential scanning calorimetry", *Journal of Thermal Analysis and Calorimetry*, 124(3), 1255-1266.

Della Gatta, G., Badea, E., Ceccarelli, R., Usacheva, T. and Mašić, A. (2005), "Assessment of damage in old parchment by DSC and SEM", *Journal of Thermal Analysis and Calorimetry*, 82, 637-649.

Şendrea, C., Badea, E., Carsote, C., Stanculescu, I. and Miu, L. (2016b), "Thermal microscopy and ATR-FTIR study of gamma irradiation on vegetable tanned leather", submitted to *Revista de Chimie* (accepted).

Şendrea, C., Badea, E. and Adams, A. (2016a), "Unilateral NMR and micro DSC study of artificially aged parchments", submitted to *Revista de Chimie* (accepted).

Badea, E., Miu, L., Budrugeac, P., Giurginca, M., Mašić, A., Badea, N. and Della Gatta, G. (2008), "Study of deterioration of historical parchments by various thermal analysis techniques, complemented by SEM, FTIR, UV-VIS-NIR and unilateral NMR investigations", *Journal of Thermal Analysis and Calorimetry*, 91, 17-27.

Masic, A., Chierotti, M. R., Gobetto, R., Martra, G., Rabin, I. and Coluccia, S. (2012), "Solid-state and unilateral NMR study of deterioration of a Dead Sea Scroll fragment", *Analytical and Bioanalytical Chemistry*, 402, 1551–1557.

Şendrea, C., Badea, E., Stanculescu, I., Miu, L. and Iovu, H. (2015), "Dose-dependent effects of gamma irradiation on collagen in vegetable tanned leather by mobile NMR spectroscopy", *Leather and Footwear Journal*, 15(3), 139-150.

ADVANCED MATERIAL TECHNOLOGY FOR THE CONSERVATION OF HISTORICAL BUILDINGS

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Four different compositions were designed for restoration mortars reflecting the dominant ancient and historical types. Three of them (two lime and one hydraulic lime mortar) contained a pozzolanic member while the two of them (lime and hydraulic lime mortar) contained polypropylene fibers. Micro-morphological and elemental (SEM/EDAX), mineralogical (XRD) and thermal analysis (TGA) were performed in order to identify the microstructure, the chemical consistency, and durability of each composition. Although hydraulic lime presented a slightly better response on mechanical strength, lime mortar with pozzolan and polypropylene fibers selected, in terms of cost factor, as a sufficient composition for further research. Meeting the requirements for materials under the triptych of "compatibility-coherence-reversibility" with the authentic ancient matrix as well as resistant to climate changes, metakaolin was used as an improvement agent in the binder part at 25% replacement. The experimental mortar (EM) updated its characteristics in both mechanical and hydraulic aspects. Crystallization test, elevated humidity conditions, acid rain, freeze-thaw tests were performed under simulated conditions with respect to international standards. EM mortar responded adequately to extreme climatic conditions in contrast to the standard lime mortar. That attributed to the ability of metakaolin to resist in contraction-expansion processes by maintaining its matrix.

Keywords: mortars, culture-heritage, conservation

INTRODUCTION

Conservation of Historical Monuments and Buildings

The term of conservation includes an integrated management program under which environmental variations are taken into account in projects regarding the protection of historical monuments and building. As "damage" could be defined a deterioration mechanism on mortars which becomes apparent at a certain moment affecting the physical, chemical and/or mechanical properties. The most important of damage factors are humidity, air pollution, the presence of soluble salts and organic activity (Van Hees *et al*., 2004; Charola, 2004). All of them are interwoven to the climate factor, therefore, the conservation materials should be considered under this aspect as well. For example, precipitation which acts in the vertical line at the buildings and enhance the capture of humidity is dependent on climate change. Moreover, precipitation factor could be attributable for the import of dissolved acidic components in the material surface (Amoroso & Fassina, 1983). However, the triptych of "compatibility-coherence-reversibility" with the authentic matrix is of major importance in the design of restoration products. With respect to above lines, an attempt was made to design conservation mortars addressing improvements in binder materials.

Preparation of Standard Restoration Mortar

Initially, four different compositions were designed for restoration mortars reflecting the dominant ancient and historical types. (Table 1). The lime/pozzolan ratio was 1:1 for D1 while for D2, D3, D4 was 1:2 considering a better reaction between lime and pozzolan in order to achieve more efficient hydraulic characteristics in mortars (Massazza, 1998). For the aggregates, a silica sand was used according to standard BS EN 196-1:2005 (Methods of testing cement, sand for strength measurements) as well as crushed ceramics of properly gradation for a sufficient porosity (Figure 1). The weight ratio of binder to aggregate was 1:3 as it is accomplished the development of a porous system capable of delivering elevated mechanical strength reflecting ancient mortars. Mortars were prepared in standard 40 mm x 40 mm x 160 mm specimens. Mortar mixers were used and attention was paid to the intensity in order not to break the aggregates. Polypropylene fibers were added in the dry blend for D3 and D4 compositions. This admixture was chosen to protect mortars against “cracking” from capillary cracks during drying and temperature variations. The required amount of water estimated based on BS EN 1015-11: 1999. By the end of mortar preparation, the triplet metal matrixes were mechanically filled as described in BS EN 196-1:2005. After 24h where specimens allowed curing they placed in a temperature chamber at 20±1°C and 95% humidity for 28 days. Then placed at ambient conditions with relative humidity 65% and temperature 20±1°C for 9 months to develop their characteristics and to remove excess moisture.

Table 1. Composition of standard conservation mortars

		Standard Mortars			
	Raw material	D1	D2	D3	D4
Binder	Lime	+	+		+
	Hydraulic lime			+	
	Pozzolan		+	+	+
	Ceramic powder	+	+	+	+
Aggregates	Quartz sand	+	+	+	+
	crushed ceramics	+	+	+	+
Admixtures	polypropylene fibers			+	+

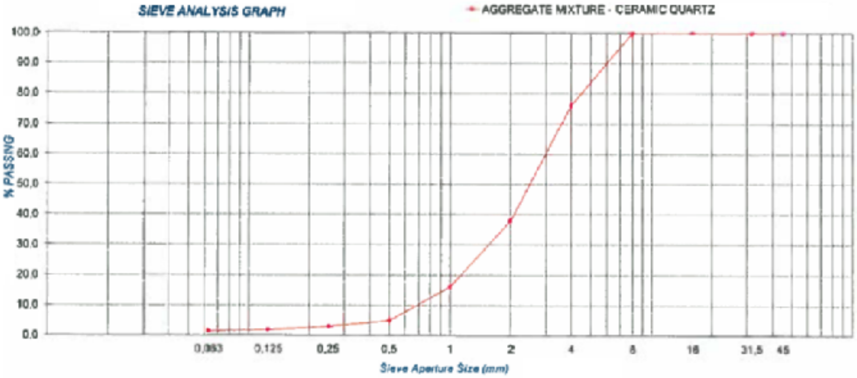

Figure 1. Grading curve of the aggregate in standard mortars

MATERIALS AND METHODS

SEM/EDAX methods were used to determine the morphological and elemental characteristics of mortars. Photomicrographs were selected with secondary and backscattered electron detector. Elemental analysis was performed by sequential scanning of an electron beam on the sample surface while mineralogical analysis conducted with X-ray diffraction (XRD). Mechanical properties related to flexural strength were measured according to BS EN 1015-11: 1999.

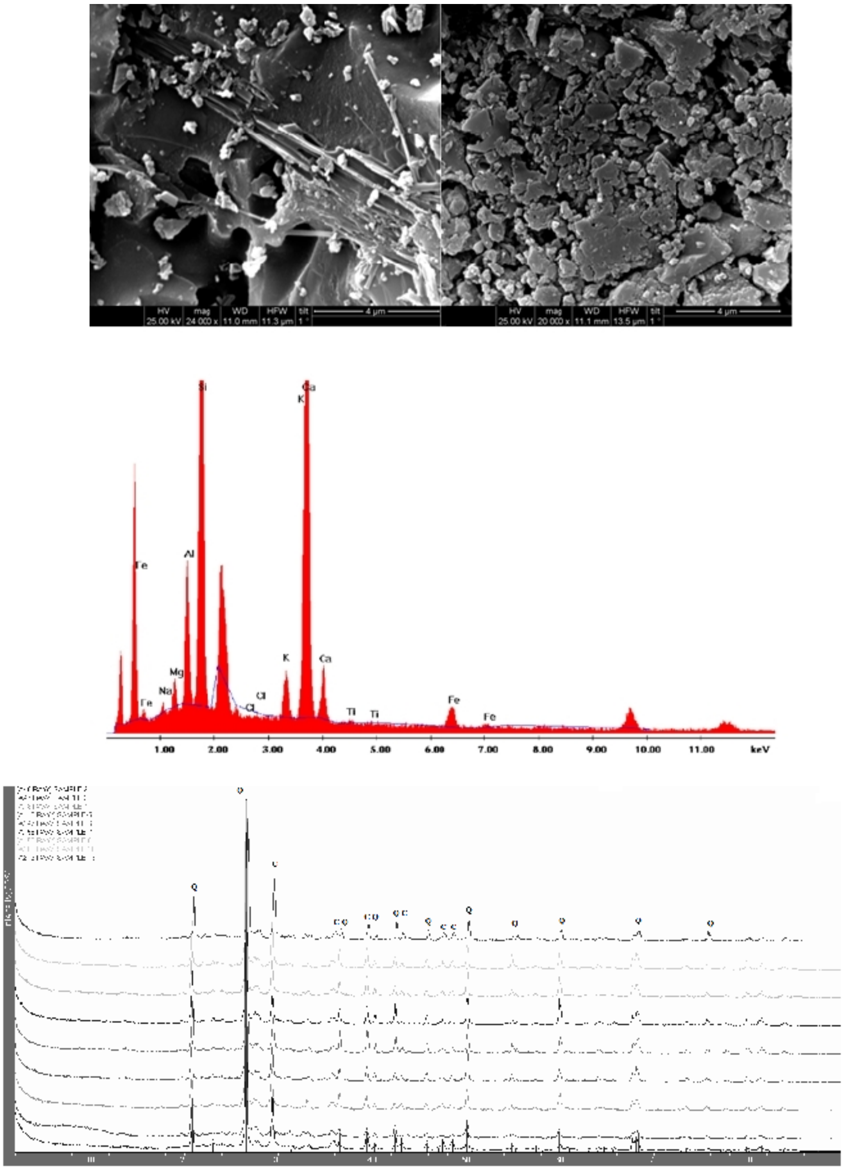

Figure 2. Morphological, chemical and mineralogical [Q-quartz; C-calcite] analysis in standard mortars

RESULTS AND DISCUSSION

Chemical analyses concluded on a high concentration of pozzolanic oxides (SiO_2: 48%-60% and Al_2O_3: 10%-18%) which were homogenized distributed. Calcium-silicate and calcium-aluminate oxides presented a linear relationship inversely related highlighting the formation of C-S-H and C-A-H active hydrated phases. This observation comes in agreement with the XRD qualitative characterization where quartz (Q) and calcite (C) minerals (Figure 2) mainly detected. Generally, the carbonation reaction in lime mortars with pozzolanic additives (D2, D3, D4) presented sufficient mechanical strength values with a median range of development. D4 sample presented the smaller flexural strength (0.9 MPa) and the higher total porosity (39%) in 94 days of maturation however was greatly improved in 289 days (1.8MPa fl.st. and 33% t.p.). The best respond was for D3 sample from the start (1.4MPa fl.st. and 31% t.p. in 94 days) as well as at the conclusion (1.9MPa fl.st. and 27% t.p. in 289 days) of the experiment. D1 and D2 samples exhibited lower values characterized as sufficient. D1 sample, despite the absence of pozzolanic member, exhibits excellent strength values 1.0MPa in 94 days of maturation and 1.7 MPa in 289 days (better that D2) indicating the ceramic powder as a valuable technical pozzolan in mortars. Finally, samples D3 and D4 became more resistible to "cracking episodes" as developed the higher strengths due to polypropylene fibers. Although D3 hydraulic mortar presented better characteristic than D4 lime mortar considering the higher cost as deterrent factor, D4 sample was chosen for further improvements.

Improvements on Standard Mortars

The rule under which the decision for the improvement agent was made based on the impact of climatic effects as deterioration factors. One of the main damages in historical monuments and building is the formation of plaster due to sulfates, especially in urban or industrial centers where air pollution is evident (Hossack and Thomas, 2015; Santhanam *et al*., 2002). Moreover, taking into account the climate change where the temperature rises and drying cycles become more intense (Lelieveld *et al*., 2012; IPCC, 2007) the design imposed the enhancement of resistance in cracking mechanisms during the maturation and strengthen period for the mortars, further than that imparted by polypropylene fibers. Attending to address the above parameters metakaolin (MK) was used in the binder part at 25% replacement. Moreover, it has been reported the increased resistance to sulfate attack with MK replacement (Wu *et al*., 2016; Nabil, 2006). The evaluation of the experimental mortar EM was based on hydraulicity, as expressed by CO_2/H_2O ratio, and mechanical strength. The hydraulicity was estimated with Thermal Gravimetric Analysis (TGA). The weight loss measured between 200-600°C and over 600°C. The range of 200-600°C represents the structural bound water which released with the dissolution of aluminosilicate bonds while the weight loss over 600°C represents the dissolution of CO_2 bonds (Frankeová *et al*., 2012). "Recarbonated" material was not detected resulting in reliable hydraulicity ratios. Flexural strength versus CO_2/H_2O ratio diagram (Figure 3) was constructed to standard and experimental mortars. Moreover, literature data were added (Frankeová *et al*., 2012).

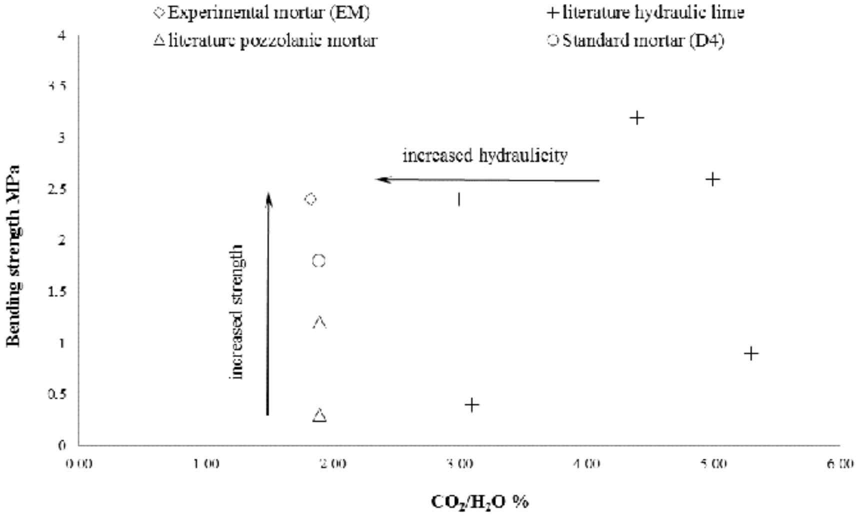

Figure 3. Flexural strength versus CO_2/H_2O ratio [literature data Frankeová *et al*., 2012]

The development of flexural strength increased in experimental mortar with respect to the standard mortar D4. Metakaolin, as presents large specific surface area and high pozzolanic factor, enhanced the initial mortar with a positive effect on microstructure (total porosity for EM 29% and for D4 33%) as well. Furthermore, it is observed that structurally bound water exhibited a higher ratio in EM sample (4.1%) than D4 sample (3.6%) suggesting the improvement of the pozzolanic activity in experimental mortal. In figure 3 it is evident that both EM and D4 samples grouped in the vertical axis with the pozzolanic samples, a binder of high hydraulicity, while in horizontal axis EM grouped with the hydraulic mortars presenting higher flexural strength than D4. This observation leads to the conclusion that the choice of MK as improvement agent gained the benefits regarding both mechanical and hydraulic aspects.

Finally, EM and D4 samples were tested in experimental environmental conditions. Many tests were run in order to detect the response of mortars under extreme condition. Crystallization test, elevated humidity conditions, acid rain, freeze-thaw tests were performed under simulated conditions with respect to international standards e.g. EN 12370 (1999), in order to detect the adequate response of EM sample. In more detail, variations in the weight of the samples were relatively small regarding crystallization and elevated humidity tests. However, in elevated humidity conditions, EM presented a better response with increased flexural strength until the second cycle. This is consistence with hydro-thermal TGA analysis where the enhanced pozzolanic properties were detected. The simulation of acid rain evaluated with three damage indicators DMC, DCC and DEC, concerning mass loss ratio, compressive strength variation, and variation of modulus of elasticity, respectively. Samples showed a constant rate of mass loss when exposed to acid solutions which increased gradually with increasing soak time. At the same time the strength gradually decreases with a total failure behavior at the pH 1.5 solution. Modulus of elasticity presented higher variations over 35 days in pH 1.5 solution and over 45 days in pH 2.5-3.5 solutions. In freeze-thaw tests, EM responded well enough without any failures, unlike D4 where the reduction of their strength was more pronounced when the porosity increased.

CONCLUSIONS

Four different compositions were designed for restoration mortars reflecting the dominant ancient and historical types. Micro-morphological and elemental (SEM/EDAX), mineralogical (XRD) and thermal analysis (TGA) were performed in order to identify the microstructure, the chemical consistency and durability of each composition. Lime mortar with pozzolan and polypropylene fibers selected in terms of cost factor as a sufficient composition for further investigation. Attending to address enhanced cracking mechanisms due to climate variations, metakaolin (MK) was used in the binder part at 25% replacement. Experimental mortar (EM) updated its characteristics in both mechanical and hydraulic aspects. Moreover, EM responded well enough to crystallization test, elevated humidity conditions, acid rain, freeze-thaw tests. The success of EM lies in the ability of metakaolin to resist in contraction-expansion processes by maintaining its matrix as produce strong bonds preventing the generation of large porous.

REFERENCES

Amoroso, G. and Fassina, V. (1983), “Stone Decay and Conservation”, *Materials Science Monographs*, 11, Elsevier Science, Amsterdam.

BS EN 196-1:2005. Methods of Testing Cement. Determination of Strength. British Standards Institute: London, UK, 2005.

BS EN 1015-11: 1999. Methods of test for mortar for masonry. Determination of flexural and compressive strength of hardened mortar. British Standards Institution: London 1999.

Charola, A. (2004), “Stone Deterioration in Historic Building and Monuments”, *10th International Congress on Deterioration and Conservation of Stone*, Stockholm, Sweden, 3-14.

EN 12370:1999. Natural stone test methods—determination of resistance to salt crystallization, 1999–2003.

Frankeová, D., Slížková, Z., Drdácký, M. (2012), “Characteristics of mortars from ancient bridges”, in Válek, J., Hughes, J.J., Groot, C.J.W.P. (eds.), *Historic Mortars: Characterisation, Assessment and Repair*, vol. 7, 165–74.

Hossack, A.M. and Thomas, M.D.A. (2015), “The effect of temperature on the rate of sulfate attack of Portland cement blended mortars in Na_2SO_4 solution”, Cement and Concrete Research, 73, 136–142.

IPCC (2007), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Solomon, S. *et al*. (eds.), Cambridge University Press, Cambridge, UK.

Lelieveld, J., Hadjinicolaou, P., Kostopoulou, E. *et al*. (2012), *Climatic Change*, 114: 667, doi:10.1007/s10584-012-0418-4.

Nabil, M.A. (2006), “Durability of metakaolin concrete to sulfate attack,” *Cement and Concrete Research*, 36, 1727-1734.

Santhanam, M., Cohen, M.D. and Olek, J. (2002), “Mechanism of sulfate attack: a fresh look. Part 1: summary of experimental results”, *Cement and Concrete Research*, 32(6), 915–921.

Van Hees, J., Binda, L., Papayianni, I. and Toumbakari, E. (2004), “Characterisation and Damage Analysis of Old Mortars”, *Materials and Structure*, 37, 644-648.

Wu, Z., Deng, Y., Liu, S., Liu, Q., Chen, Y., Zha, F. (2016), “Strength and micro-structure evolution of compacted soils modified by admixtures of cement and metakaolin”, *Applied Clay Science*, Volumes 127–128, Pages 44-51.

UNCONVENTIONAL COSTUME COLLECTION INSPIRED BY THE BRANCUSI ART. FROM THE ENDLESS COLUMN TO THE ENDLESS DRESS

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Opinions about the complexity of the Brancusi inspiration sources revealed that the sculptor statuary is a transcript, in a modern manner, of the influence at a stylistic and thematic level that Romanian folklore and folk art have exercised over his artistic genius. Starting from various native elements found in the sculptor's work, a shift was made from the shape of the popular objects, from the functionality and the decorative intimacy of the peasant interiors, directly connected with the spirituality and the ancient background, to the artistic essentialization and conceptualization characteristic to Brancusi. A transposition of Brancusi's work characteristic elements in an unconventional costume was achieved with the personal collection, through various experiments, modifying the materiality, the texture, the volumetry, the color and the transparency. Using the derridean example, the Endless Column became the Endless Dress. The garment - objects collection "Endless Dress", in its various versions, worked equally for fashion shows, costume exhibitions and for a play staged on "Masca" Theatre in Bucharest. The present research is an example of transposition of the high Romanian cultural spirituality in fashion and costume, which certainly may go further through stylization and simplification in a more functional, commercial direction, explored by the local creative industries.

Keywords: Brancusi, fashion, theatre

INTRODUCTION

Constantin Brancusi, the sculptor, an artist known worldwide, gave mankind creations materialized in an universal sculpture language, which were and still are loved and understood by a wide audience, upon whom produces a unique fascination. His creative genius has given a new brilliance to the ancient world of ideas and universal truths, has updated myths to the contemporary acceptance and thinking level in new spiritual forms. Brancusi appealed to essences in order to communicate philosophical ideas from the past thinking, bringing them to the modern and optimist spirit of the civilized man, hoping his works will express confidence in life and open the men souls to beautiful.

In the cultural context that followed Brancusi's creation, where the work of the great artist was base of inspiration for several art fields, appeared the interest for a personal interpretation of Brancusi's sculptures.

The "Endless dress" project had from the start as purpose the reinterpretation of Brancusi's sculptures through a garment-objects collection, preserving the balance between shape, proportions, volumes and fabrics.

THEORETICAL HIGHLIGHTS

A starting point for this research was the personal fascination for the Romanian sculpture, Constantin Brancusi, and especially for the "Endless Column", opera situated in the center of his artistic creation.

Resorting to many Brancusi's exegetes writings on symbolism and significance of the artist's sculptures, a rigorous documentation was made, which served as the foundation for a modern unconventional interpretation.

Brancusi appealed to essences in order to communicate ideas of the past philosophical thinking, bringing them towards the modern and optimistic civilized man spirit and by keeping the proportions, it has been attempted, through stylization and essentialization, a transposing of the “Endless Column” polyvalent symbolism in garment-objects.

“Endless Column” amazes with its litheness and produces admiration to anyone is given to see it: “When you look at this column in a photographic reproduction, it's hard to penetrate the strong sense of energy, the closed vigor into it,” said after a trip to Targu Jiu, Mario Michelli, Italian writer and art critic. He continues: “Only when I saw it so high, strong and right springing up suddenly, without pedestal, from the empty plain, it seemed that I snatched, that I understood at once all the Brancusi’s secret art”.

Watching the “Endless Column”, there is this impression of infinite succession, the idea of repetition, for the rhomboid overlapping is not isolated, nor accidental. The same phenomenon can be seen in “Portrait of James Joyce”, “Socrates”, “King of kings”, the artist oscillating between spiral and overlapping on an axis having a clear idea of becoming and perpetual return in a cycle that is endlessly repeated. Each column’s octahedron represents the end and beginning of each other, so that the work suggests a scale of generations, ages, years or moments; a relentless ascent.

The “Endless Column” rises directly from the ground to the blue sky, where is lost in the infinite, conquering space, becoming a new interpretation of the Universe with its sublime. This artwork is imprinted a continuous movement, life and glow.

“At Brancusi, form and light merge. It's hard to say what is most important in sculptor’s thinking. Both, however, shape and light are the most direct ways of communication.

The extraordinary mastery, reality contingent, discovering new monumental meanings, spiritual integrating modern sculpture in architecture, transformation of the sculpture into a hotbed light as the source of movement and life, have opened unexpected perspectives within this art”, wrote Mircea Deac, in “Brancusi, archetypal sources”.

Mircea Deac also reminded several discussions and interpretations of “Endless Column”, essential symbol of Brancusi’s creation: “... celestial and aerial trees of admirable proportions”, says Professor Sigfried Giedion; “a giant cactus in the deserts of California," wrote Malvina Hoffman; " a chain of perpetual kinks and rebirth,” added Andre Frenaud.

Petru Comarnescu considered “Endless Column” as inspired by the Gorj porches rhomboid pillars and “... designed to suggest in the wide space an infinity of human forms, shapes of birds and jugs, protrusions and eaves of rocks, towering in the horizon heaven”.

Brancusi himself considered that of all columns he made only one made it to rise up to heaven and confessed that through it finally reached perfection. He resembled it with “a song that leads to eternal infinity beyond all pain and factual joy”.

THE RESEARCH METHODOLOGY

An important first step towards the concept of sculptural expression of the costume made Brancusi himself in 1922, when for the ballet “Gymnopedies” staged by the nonconformist dancer, Lizica Codreanu, music by Erik Satie, he imagined a head accessory referring to “Witch”, a wood sculpture. Some have found symbolism in romanian witches tales and others in the book “La Sorcierese” - Jules Michelet, for they knew clearly that this writing was on the Brancusi’s library shelves.

Taking a leap into the future, 140 years since the birth of the great artist who brought a new vision in the universal plastic language, the essence of things is being celebrated, whether in Targu Jiu, Bucharest, Paris, London or New York.

In fashion, the sculptor’s works, but also the artist status and personality have inspired great designers to create clothes, shoes and accessories.

The Haute Couture collection signed by Valentino once again confirmed the strong symbolism of Brancusi’s works, in this case being about accessories. Creations with retro shapes and most varied materials, from leather to cashmere and fur, were inspired by Romanian sculptor’s Prometheus or The New Born.

Figure 1. Valentino, New York, 2014

Rick Owens creations had as inspiration source The Kiss symbol, the two halves of the circle found on the faces of each column gate.

Figure 2. Rick Owens, ss 2013

Lilly Heine relied on structure, volumetric and architectural lines. The aim behind her collection was to give life to the sculptures with the body motion of the models.

Figure 3. Lilly Heine, ss 2013

Also inspired by Brancusi's sculptures, Musette shoes and handbags for summer 2015 had one thing in common: bright golden skin, which remind the bronze works of the artist.

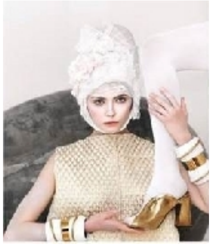

Figure 4. Musette, ss 2015

EXPERIMENTS, RESULTS AND DISSCUSIONS

“Endless dress” is a timeless garment-objects collection, including ten outfits with a rigorous image. The cut is precise, with straight lines and complex volumes resulting from unfolding of simple rhomboid forms, attention being directed to the clean and cared execution. However this strictness from the cutting is sometimes attenuated by the introduction of embroidery details obtained through various techniques of felting.

Figure 5. “Endless Dress”, Alina Gurguţă

For the technical realization, the patterns obtaining was made step by step, meaning that once getting the first piece, through numerous molds and corrections, the next one derived from it like the pieces of a telescope. Thus, parts of the first pattern were preserved, and through various additions and other modifications gave birth to other new parts.

Figure 6. “Endless Dress”, Alina Gurguţă

For the good achievement of all the outfits of “Endless dress” collection, were used both conventional materials (Merino wool, Eugenia lining, etc.) and unconventional (felt, which normally is used to make hats and some accessories like bags or purses). Felt was a choice because of its properties, being a stiff material and helping to produce the pieces according with the patterns and the sketches.

Figure 7. “Endless Dress”, Alina Gurguţă

Endless Dress is Endless Column transposed into garment; is a connection between body and spirit, interior - exterior, clothe – feeling, but also the connection between earth and sky. Borrowing the blue from the sky, chromatic comes to strengthen this meaning.

CONCLUSIONS

Brancusi certainly remains an actual artist, for many contemporary artists remember him and his work as inspiration. The artist reinvented sculpture returning to the creative artistic means used in the most remote and primitive arts, creating an universal art, unanimously appreciated, that cannot be erased with the twentieth century.

Figure 8. “Endless Dress” play for “Masca” Theatre, Bucharest

Figure 9. Living Statues “Endless Dress” for “Masca” Theatre, Bucharest

REFERENCES

Berger, R. (1978), *La mutation des signes*, Meridiane, Bucharest.
Deac, M. (1982), *Brâncuşi. Archetypal Sources*, Junimea, Iaşi.
Giedion-Welcker, C. (1981), *Constantin Brancusi*, Meridiane, Bucharest.
Lemny, D. (2009), *Lizica Codreano. Une danseuse roumaine dans l’avant-garde parisiene*, Vellant, Bucharest.
Noica, C. (1973), *Creation and beauty in Romanian utterance*, Eminescu, Bucharest.
Teja Bach, F. (1991), *Brancusi. Photo Reflection*, Didier Imbert Fine Art, Paris.
Velescu, R.C. (1999), *Concepts of Constantin Brancusi's poetics*, Univers Enciclopedic, Bucharest.

NANOMATERIALS FOR CONSERVATION / PRESERVATION OF CULTURAL HERITAGE

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In the last years the issue of conservation and preservation of cultural heritage has gained high interest both in scientific research and in the restoration of various monuments from universal heritage. The aim of this paper is the presentation of hydroxyapatite as a non-toxic and environmentally friendly nanomaterial that could be involved in the conservation of heritage monuments (chalk, marble or travertine), and the results obtained after applying it on these surfaces. Some scientific analytical investigations are used to detect the changes in the structure and colour of the surfaces where it had been applied. These nanostructured products for cultural heritage preservation highlight increased performances without changing the substrate color.

Keywords: hydroxyapatite, analytical investigations, nanomaterials.

INTRODUCTION

The study of architectural surfaces and materials used in restoration joined an increased interest in the last decades, in good connection with reviving some buildings with environmental and aesthetic decorative elements, and obeying art history and architecture elements, too. In such context, it is important to allow the interaction between the substrate and decoration, evaluation of the degradation processes, mostly physical, short or long term, physical and chemical changes of materials while restored and products for restoration, architectural surfaces and forms of biodegradation process (Clarke and Ashurst, 1972; De Witte *et al.*, 1985).

The sculptures and constructions made of marble, travertine and limestone have a special position among the unique architecture ensemble from many locations.

Limestone is a sedimentary rock composed mostly of calcite and aragonite, as different crystal forms of calcium carbonate. Travertine belongs to the limestone family also known as calcium carbonate. It is formed by minerals dissolving in ground water and then deposited on the earth’s surface by rivers, natural springs, or geysers. The major disadvantage of travertine is that it is brittle and susceptible to cracking, and sensitive to the freeze and thaw cycle. Marble is also a type of limestone that has had additional heat and pressure applied to it by the earth’s crust. Chalk is a soft, white, powdery limestone consisting mainly of fossil shells of foraminifers. External factors such as rain, snow, temperature, wind and atmospheric pollutants, could induce the weathering of all these stones. Rainwater and temperature can lead to deterioration, as well as the salt migration within the stone.

Nanomaterials exhibit excellent chemical-physical features for application in the conservation science of art works, being able to slow down the degradation processes of artifacts, by comparison with traditional methods, widely described in literature (Ion *et al.*, 2015a). The aim of this paper is to test some nano-consolidants based on hydroxyapatite, for consolidating the travertine, marble and limestone chalk stone, used as models for architectural monuments. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, has the advantage of similar crystal structure and close lattice match to calcite (Ion *et al.*, 2015b). The composition, the changes in the structure in time of the surfaces and color changes will be reported by using some analytical techniques: optical electron microscopy, Fourier transformed infrared spectroscopy (FTIR), colorimetry and Raman spectrophotometry tests.

MATERIALS

Specimens Samples Preparation and Consolidants

Different samples were prepared, such as travertine, marble and limestone chalk (40 mm × 40 mm × 40 mm) in order to achieve to distinguish the difference between non-treated samples and to observe the influence of consolidant used during the treatment. After the drying process the substrates were left to cool for two hours in a desiccator and their appearance was examined. Hydroxyapatite was used as consolidant, obtained in the lab by chemical precipitation method from calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$ and dibasic ammonium phosphate $(NH_4)_2HPO_4$, at room temperature. The powder was dry mortar in a mortar and pestle and then calcined in alumina crucible at 1200°C for 1h. The application of the consolidant was carried out by brushing. Dispersions have acquired a white opalescent appearance with uniform size of 30 nm.

Characterization Techniques

The following techniques were used: Fourier transformed infrared spectroscopy (FT-IR, Perkin–Elmer Spectrum One FT-IR Spectrometer), using the KBr pellets method, Dynamic Light Scattering (DLS) technique, for particles size and their size distribution, Stereozoom microscopy achieved with a binocular Optika microscope (100x – 4000x), for morphology evaluation of the analyzed stones.

Color measurements, achieved with a spectrophotometer (Carl Zeiss Jena M40) under a D65 light source and an observer angle of 10O. The CIELAB color parameters clarity (L*), red/green colour component (a*) and yellow/blue colour component (b*) and their derived magnitudes: chroma (C*) and tone (H*). The total color differences ΔE* were calculated using specific formulas (JIS, 2009).

RESULTS AND DISCUSSION

Stone materials of mineral-inorganic nature, due to their hydrophilic properties, need special treatments for their conservation. Stone can be deteriorated by acid attack (caused by rains and humidity condense in polluted urban atmospheres which induces corrosion to carbonatic materials such as calcium and/or magnesium carbonate based stones while silicatic stones are only poorly affected) and by soluble salts cyclic crystallization (Ferreira Pinto and Rodrigues, 2008). Some studies on environmental influences on degradation stone monuments, considering factors such as temperature (average, minimum, maximum), number of days of frost and sunny days, humidity annual average rainfall amount and chemical composition, air currents, will be evaluated, too, knowing that carbonates have been detected as the predominant in the body of the church with sulphates impurities (Price, 1996).

Stone consolidating materials as inorganic materials should produce insoluble phase within the voids and pores of a stone. The results show that some properties of conservation materials can be improved with the presence of nanomaterials (Mosquera, 2008). This should favor nucleation of HAp on calcite, and might permit formation of a coherent layer of HAp on the surface of marble.

Travertine is a form of limestone with a fibrous or concentric appearance formed by a process of rapid precipitation of calcium carbonate (Pentecost, 2005). Travertine is a porous limestone rock (composed of calcite, aragonite and calcite weak magnesium), which has a yellowish to brown. Travertine exists in two varieties: compact travertine, which is dense and thick stratified banks (20-40 m) and layered thin laminae, white or brown. In general, travertine is porous. If the pores are closed with cement dust by grinding and polishing travertine end a surface as smooth as marble.

Marble is a metamorphic rock that may be foliated or non-foliated, composed of recrystallized carbonate minerals, most commonly calcite or dolomite (Kearey, 2001). HAp has a much lower solubility and dissolution rate having the ability to confer protection in acidic environments, with a dense coating of only about 10 µm. The performance of HAp has already been proven in restoring the strength of weathered limestone (Sassoni *et al.*, 2011). Other workers have also investigated the use of HAp as a consolidant for marble (Yang *et al.*, 2011; Liu *et al.*, 2011; Matteini *et al.*, 2011). In this paper, we discuss its use as a surface-protective layer for chalk in order to obtain a noticeable retardation of the weathering. Chalk is a soft, white, porous sedimentary carbonate rock, a form of limestone composed of the mineral calcite. The infrared spectra, Figure 1, put into evidence different inorganic phases, as follows ($CaCO_3$, peaked at 1962, 1409, 715 and 603 cm−1; gypsum ($CaSO_4 \cdot 2H_2O$), centered at 667 cm^{-1}, clay minerals recognized, through the peaks from 975 cm^{-1} (Si–O–Si bond) from clay, and of calcite (1018 cm^{-1}) (Derrick, 1999).

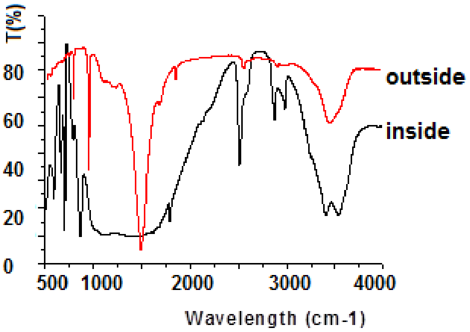

Figure 1. FTIR spectra of limestone from inside and outside building

Also, by analysing the interior and exterior limestone, the influence of the atmospheric pollutants on the limestone is visible by Raman spectra of HAp on limestone surface: it is visible the interaction between $CaCO_3$, peaked at 715 and 603 cm^{-1}; gypsum ($CaSO_4 \cdot 2H_2O$), centered at 690 cm^{-1}, clay minerals from 975 cm^{-1} (Si–O–Si bond) from clay, and of calcite (1100 cm^{-1}), Figure 2. Optical electron microscopy research was performed to investigate microstructure characteristic of the specimens.

Special attention was paid to the effectiveness of consolidation treatment observable namely in the pores and cracks. It is mostly the size of pore openings and the chemical/ mineralogical nature of the pore walls which are of relevance to the treatment by consolidant, Figure 3. Travertine treated with HAp, could be considered as effective in preserving stone material, Figure 4.

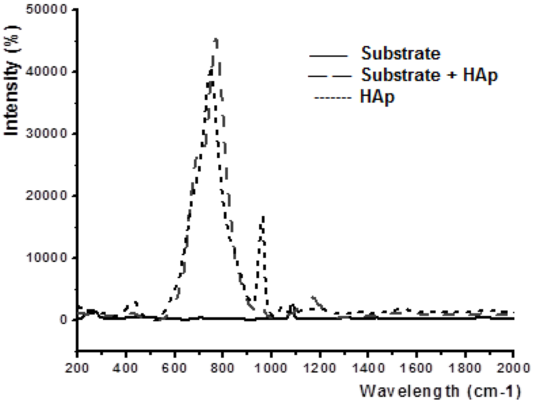

Figure 2. Raman spectra of limestone alone and covered with HAp

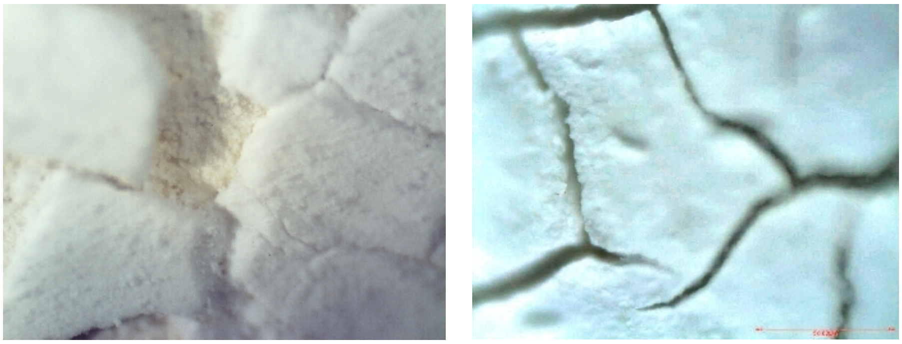

Figure 3. Optical microscopy of limestone surface after a long time after treatment

After treatment with HAp no significant coloristic changes will be observed, this being a proof of beneficial use of HAp (ΔE=0.91 for limestone, 1.65 for travertine and 2.2 for marble).

Figure 4. Travertine treated with HAp

However, as a weathering sign, the chromatic alteration is visible from the chromatic parameters: ΔE=3.91 for limestone, 4.65 for travertine and 3.2 for marble). These indicated that during the degradation, many islands are formed, containing white efflorescence, fragments loss, all together contributing to the coloring of the surface examined sample (Figure 5).

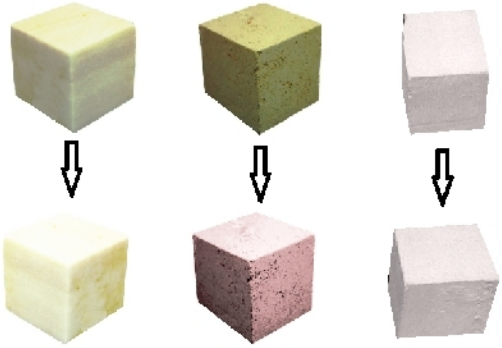

Figure 5. The chromatic aspects of marble (left), travertine (middle) and chalk (right). Above are the non-treated samples, and down the treated ones

The samples treated with HAp present a more uniform distribution of the consolidation product and homogeneous infilling of the matrix voids, and induced a slow white colour of the treated surface. Undoubtedly, this is caused by the network of hydroxyapatite, which can bind weathered stone blocks together providing a substantial reinforcement.

CONCLUSIONS

In this paper the structural, morphological and compositional aspects of some stone samples treated with HAp nanoparticles have been tested. A complex collection of analytical techniques has been used in order to identify the major aspects of restoration with HAp. Some weak bonds are forming between stones and HAp, and a strong homogeneous consolidation effect was visible, more pronounced for chalk limestone where local white area could be observed, assigned to a stronger interaction, followed by travertine and marble.

Acknowledgements

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REFERENCES

Clarke, B.L. and Ashurst, J. (1972), "Stone preservation experiments", in: Building Research Establishment Great Britain, Directorate of Ancient Monuments and Special Services, London.

De Witte, A., Charola, A.E. and Sherryl, R.P. (1985), "Preliminary tests on commercial stone consolidants", 5th International Congress on deterioration and Conservation of stone, Lausanne, 709-718.

Derrick, M., Stulik, D. and Landry, J. (1999), "Infrared Spectroscopy in Conservation Science", The Getty Conservation Institute, Los Angeles.

Ferreira Pinto, A.P. and Delgado Rodrigues, J. (2008), "Stone consolidation: The role of treatment procedures", Journal of Cultural Heritage, 9(1), 38–53.

Ion, R.M., Turcanu-Caruţiu, D., Fierăscu, R.C., Fierăscu, I., Bunghez, I.R., Ion, M.L., Teodorescu, S., Vasilievici, G. and Rădiţoiu, V. (2015a), "Caoxite-Hydroxyapatite Composition As Consolidating Material For The Chalk Stone From Basarabi-Murfatlar Churches Ensemble", Applied Surface Science, 358, 612-618.

Ion, R.M., Fierascu, R.C., Fierascu, I., Senin, R.M., Ion, M.L., Leahu, M. and Turcanu-Carutiu, D. (2015b), "Influence of Fântânița Lake (Chalk Lake) Water on the Degradation of Basarabi–Murfatlar Churches", Eng.Geol.Soc. Territ., Springer Ed., 8, 543-546.

JIS (2009) Z 8730, "Colour specification - Colour differences of object colours".

Kearey, P. (2001), "Dictionary of Geology", London and New York, 163.

Liu, Q., Zhang, B. (2011) "Synthesis and characterization of a novel biomaterial for the conservation of historic stone buildings and sculptures", Materials Science Forum, 317-320.

Matteini, M., Moles, A. and Giovannoni, S. (1994), "Calcium oxalate as a protective mineral system for wall paintings: methodology and analyses", Proceedings of the 3rd International Symposium on Conservation of Monuments in the Mediterranean Basin, Venice, 155–161.

Mosqueraa, M.J., Benıteza, D. and Perry, S.H. (2002), "Pore structure in mortars applied on restoration. Effect on properties relevant to decay of granite buildings", Cement and Concrete Research, 32, 1883–1888.

Price, C.A. (1978), "The use of the sodium sulphate crystallization test for determining the weathering resistance of untreated stone", Proceedings of the International Symposium On the Deterioration and Protection of Stone Monuments, Paris, 23p.

Sassoni, E., Naidu, S. and Scherer, G.W. (2011a), "The use of hydroxyapatite as a new inorganic consolidant for damaged carbonate stones", Journal of Cultural Heritage, 12, 346–355.

Yang, F., Zhang, B., Liu, Y. *et al.* (2011), "Biomimic conservation of weathered calcareous stones by apatite", New Journal of Chemistry, 25, 887–892.

PRELIMINARY CHARACTERISATION OF VEGETABLE LEATHER USED IN HERITAGE BINDERY

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Leather heritage and art bindery require special performances regarding physical, chemical, mechanical, organoleptic and esthetics characteristics but also a good stability over a long period of time. unlike chrome tanned leather, vegetable tanned leather is ecological, and provides better firmness, fullness, malleability and dimensional stability as well as heat and moisture plasticity, resistance to light radiations and hydrothermal stability. The leather quality requirements for niche domains like heritage and art bindery cannot be satisfied by the standardized industrial processes. In order to obtain the most appropriate mix of qualities, the selection of both fresh leather (e.g. animal species and age) and technology is required from batch to batch. Since vegetable leather is intrinsically an inhomogeneous material, still it is necessary an improved knowledge on the relationship between fabrication technology and leather properties. This paper therefore focuses on the mechanical characterisation of vegetable leathers in relation to their thickness and anatomic topography.

Keywords: vegetable leather, mechanical properties, anatomic topography

INTRODUCTION

The study of leather processing throughout history has importance for technology and science but also for history and art (Higham, 1999). Raw hide processing methods were numerous: smoke tanning, fat tanning, fermented milk, eggs, volcanic earth, vegetable tanning using plants extracts from barks, fruits, leaves, etc. Various ingredients such as fermented milk, eggs, volcanic earth, urine, excrements or combinations thereof were used in various periods and geographic areas (Bravo, 1964; Deselnicu *et al.*, 1984). Tanning with chrome salts came into use only by the end of the 19th century. Leather bookbinding originates in the East as a necessity, to protect the fragile paper. The most important types of leather used for bookbinding were made of goat and calf hides using sumac extracts. Leather processing for bookbinding up to the seventeenth century is described in the encyclopedias of Delalande and Diderot (Thomson, 2000). Bookbinding leather had to be fine, resistant to bending and compression, thin, vegetable tanned, with the characteristic book cover finishing. In the early 20th century many books from English and German heritage museums were found to be seriously degraded. The first investigation on leather degradation was conducted in 1842 by Atheneum Club in England in order to find the cause of damage to the bookbindings in their library (Thomson and Attenborrow, 2001; Haines, 1977; Cambras, 2004; Larsen, 1997). In 1911, the German authorities elaborated a series of regulations on the processing of leather for bookbinding: mineral acids, vegetable tanning agents such as spruce, birch, willow, chestnut, quebracho, and mirobolam, rapid tanning, bleaching agents, excessive leather stretching or thinning were banned.

Another issue, emerging in the early 20th century, was the unsuitableness of chrome leather for as new covers for valuable collections of books dating back several hundred years from the Hague library. Replacing of old vegetable leather with new chrome leather, later cataloged as totally wrong, led to the straining and contraction of the covers, including deformation of internal bindery and book within 10 years only (Thomson, 1983). This phenomenon was due to the ignorance of the chrome leather behavior over time. It is now well-known that chromium crosslink action continues long over the fabrication process and may cause significant surface shrinkage. To avoid further serious errors in conservation and restoration practice, an improved knowledge on the relationship between the fabrication technology and leather properties is still necessary. This study concerns with the relation between leather's mechanical (i.e. tensile stress, tensile strain, deformation) properties and anatomic topography (i.e. sampling position and direction).

MATERIALS AND METHOD

The mechanical tests were conducted on calf leather (fig. 1) manufactured at the INCDTP-ICPI through traditional methods. Commercial extracts from quebracho wood were used for tanning. The mechanical tests were performed with a Tinius Olsen Testing Machine with a 5 kN cell force accredited by the RENAR Romanian Authority. Determination of tensile strength and measurement of the percentage extension performed following the EN ISO 3376: 2011 – Leather – "Physical and mechanical tests". The constant load speed was 100 mm/min. The samples were cut in a bone shape specimen (ISO 527-2 type 5B - ISO 37 type 4, DIN 53504 type S3) of 35 mm in length, 6 mm shoulder width, 12 mm gage length, 2 mm specimen width. Using such small samples enabled us to obtain a high number of samples for a better characterisation of each leather, an especially of the thickness variation. A much bigger cutting device, specimen number 5 of ISO 527-3 (6 mm in width and 80 mm the testing length), was used in a previous study reported by some of us (Lungu, 2014) that resulted in only 11 samples per leather and not very clear results.

One of the major problems when working with natural materials is their intrinsic inhomogeneity. This is partly due to the animal anatomy and partly to the manufacturing process. In Figure 1a, it is shown the position of the sampling area which represents the spine area of the calf leather. The neck/head and tail directions are indicated. To avoid the scattering of results due to the mentioned topographical inhomogeneity, all samples were cut from the spine area, close to the back bone of the animal. The direction of sampling was perpendicular on the backbone (batch 1 being the closest and batch 4 the furthest) (Fig. 1b). The sampling area was: 120 mm wide and 370 mm long. Four batches were used for measurements (10 samples were collected per each batch).

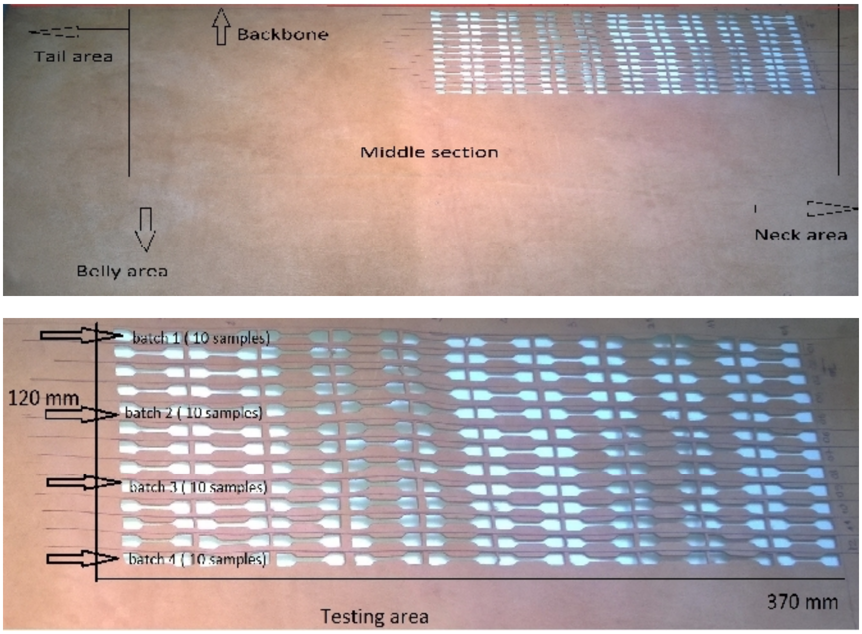

Figure 1. (a) Sampling area of the calf leather; (b) Detail of the sampling area

RESULTS AND DISCUSSIONS

The results obtained for each batch are shown in Tables 1-5. Due to the samples small dimensions, the leather's thickness non-uniformity impact was diminished, thus affecting in a small proportion the measurements. This is confirmed by the small values of the standard error of the mean and also by the small differences between the mean values for all 4 batches. Also, the standard deviation values are very low indicating that thickness variation is very close to the mean. The total variation of thickness along the tested area was 0.01 mm. The thickness and its coefficient of variation for all batches are listed in Table 1.

Table 1. Variation of thickness

Batch	Thickness (mm)	Coefficient of variation
1	1.003	0.027
2	0.999	0.041
3	0.012	0.038
4	1.003	0.039

These coefficients indicate the magnitude of variation for different means obtained from the 4 batches. It can be observed that the batch no 1 has the lowest variation, 0.027, while the other 3 batches have higher but close values, around 0.039. This indicates that thickness is more uniform closer it is to the backbone. Consequently, as the intrinsic variation of thickness is excluded, the mechanical tests results will indicate the behaviour of leather when exposed to stress, furthering from the backbone to the belly region.

In Table 2 are reported the results of the mechanical tests for the batch closest to the backbone. There are low values of standard deviation and standard error implying that the values are close to the mean and the mean is relatively accurate. This aspect is confirmed by the results obtained for the other 3 batches.

Table 2. Mechanical test and thickness results for batch no. 1

	Thickness (mm)	F_{break} (N)	F_{max} (N/mm^2)	Strain (%)
Mean	1.021	26.52	12.98	46.91
Standard deviation	0.028	3.038	1.433	9.660
Std error to the mean	0.009	0.961	0.453	3.055

Higher values for standard deviation and standard error are obtained for the values of deformation as illustrated in Fig. 2. They indicate a greater spread of the results from the mean. The deformation values indicate that the material is inhomogenous, which in our case represents an intrinsic characteristic of vegetable leather. Nevertheless, for batch no. 1 the values are lower than for the other 3 batches, indicating that the inhomogeneity increases as the distance from the backbone increases. In summary, for batch no.1 we found small variation in thickness and higher variation in deformation.

Comparing the variation of thickness and deformation it can be observed that the inhomogeneity of leather does not impact its thickness but rather its mechanical behaviour.

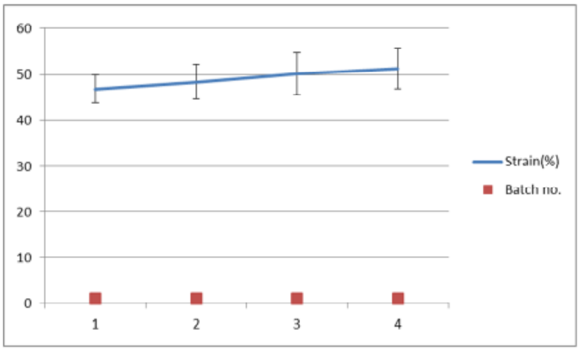

Figure 2. Variation of mean strain values

From the values reported in Tables 3-5 it can be observed that both the breaking force and tensile stress have a clear downtrend slope. This indicates a clear decrease of leather's mechanical resistance. In the same time, the deformation has an uptrend slope while distancing from the backbone, showing an increase in inhomogeneity. We can therefore state that leather's mechanical resistance is affected by the anatomical inhomogeneity which increases when moving from backbone to belly area.

Table 3. Mechanical test and thickness results for batch no. 2

	Thickness (mm)	F_{break} (N)	F_{max} (N/mm^2)	Strain (%)
Mean	0.999	23.72	11.87	48.45
Standard deviation	0.041	1.892	0.973	11.680
Std error to the mean	0.013	0.598	0.308	3.693

Table 4. Mechanical test and thickness results for batch no. 3

	Thickness (mm)	F_{break} (N)	F_{max} (N/mm^2)	Strain (%)
Mean	1.002	23.32	11.63	50.2
Standard deviation	0.038	2.891	1.390	14.335
Std error to the mean	0.012	0.914	0.439	4.533

Table 5. Mechanical test and thickness results for batch no. 4

	Thickness (mm)	F_{break} (N)	F_{max} (N/mm^2)	Strain (%)
Mean	1.003	21.45	10.673	51.4
Standard deviation	0.039	2.797	1.249	14.107
Std error to the mean	0.012	0.884	0.395	4.461

Figure 3 reports the values of the tensile stress (N/mm^2) in function of thickness for each batch. It can be observed that the tensile stress decreases as the distance from backbone increases (e.g. from batch no. 1 to batch no. 4) while the thickness remains almoust constant. This means that the material is becoming less hard as the distance from the backbone increases.

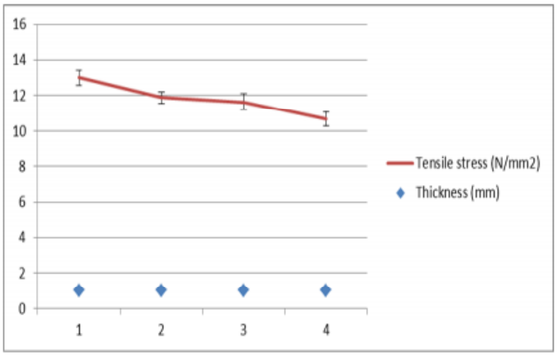

Figure 3. Mean tensile stress values related to thickness

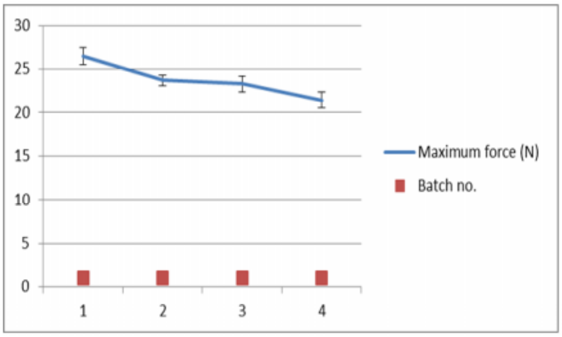

Figure 4. Mean maximum force values for 4 batches

The value of maximum force (N) (Fig. 4) is also decreasing as the distance from the backbone increases.

The accuracy of our results is supported by the low standard error and standard deviation values reported in Tables 1-4.

CONCLUSIONS

One of the major problems when working with natural materials concerns with their topographic inhomogeneity. The use of a big number of very small samples enabled us to eliminate the effect of thickness on leather mechanical properties and evaluate the influence of topography only. We found high values for standard deviation and standard error for the deformation values confirming the topographic inhomogeneity of leather and its increase as the distance from backbone increases. Moreover, the breaking force and tensile stress showed a clear downtrend slope indicating a decrease of leather's resistance supported by the increase of its inhomogeneity and distance from the backbone area.

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REFERENCES

Bravo, A.G. (1964), *Storia del cuoio e dell'arte conciaria*, Assoc. Ital. del Cuoio, Torino.

Cambras, J. (2004), *The Complete Book of Bookbinding*, New York: Lark Books.

Deselnicu, M., Olteanu, Ş. and Teodorescu, V. (1984), History of leather processing in Romania, Technical Press, Bucharest.

Haines, B.M. (1977), "Deterioration in leather bookbindings – our present state of knowledge", *British Library Journal*, http://www.bl.uk/eblj/1977articles/article9.html.

Higham, R.D. (1999), "The Tanner's Privilege – A Historical Review of the Tanner's Status in Different Societies", *Journal of the Society of Leather Technologists and Chemists*, 83, 25.

Larsen, R. (1997), *Environment Leather Project Deterioration and Conservation of Vegetable Tanned Leathers*, Final Report, Copenhagen.

Lungu, B.I., Moise, V.I., Cutrubinis, M. and Stanculescu I. (2014), "Study on mechanical proprieties of gamma irradiated leather and parchment", *Proceedings of the 5th International Conference on Advanced Materials and Systems - ICAMS 2014*, Bucharest.

Thomson, R. (1983), *The Nineteenth Century Revolution in the Leather Industries in Leather Manufacture Through the Ages*, Northampton, East Midlands Industrial Archaeology Group.

Thomson, R. (2000), "Bookbinding leather; yesterday, today and perhaps tomorrow", *Journal of the Society of Leather Technologists and Chemists*, 85, 66.

Thomson, R. and Attenborrow, G. (2001), "A Survey of Current Bookbinding Leathers", *Journal of the Society of Leather Technologists and Chemists*, 86, 163.

VI.

ECONOMICS, MANAGEMENT & MARKETING

EXPORT STRATEGY FOR A ROMANIAN FOOD PRODUCT

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As internationalization increases in nowadays competitive business environment, companies must consider it as a viable option to their growth and development. The paper analyses a wide range of factors to elaborate and finally decide upon the most appropriate export strategy of ROM chocolate from Romania to the Spanish market. It is concluded that indirect export is the most suitable entry mode, the marking and packaging regime is detailed, the quality assurance requirements are listed. The recommended delivery terms are FCA (Free Carrier) and CPT (Carriage Paid To), and the preferred method of payment is the letter of credit. The export strategy which is presented is viable and offers an efficient alternative to domestic operations for the producing company.

Keywords: export strategy, marketing, food, market entry

PRESENTATION OF THE PRODUCT

ROM is the first authentic chocolate bar on the Romanian market, launched in 1964. It has become a national symbol ever since. The brand is part of the KANDIA Dulce SA portfolio, the third player in the Romanian chocolate market (Official Kandia website). With a strong taste and the Romanian National three colored flag on the packaging, Genuine ROM chocolate bar was always preferred by Romanian consumers, resisting the invasion of imported products. The product has remained unchanged over the years: it kept the Romanian flag and symbols. The chocolate bar managed to retain its relevance, both for the audience that was raised tasting it during the communist period, and for the generations following 1990. The only changes were brought to its dimensions (ROM “The Great” or “The Double”), in response to the increased demand from consumers.

At present, Genuine ROM is available in four sizes, all made with the same recipe: ROM 32g, ROM the Great 45g (launched in 2006), the Double ROM 64g (launched in 2006) and ROM tablet 100 g (launched in 2010). The Best sold is the ROM 32 g format range, due to favorable price, but also to a very good distribution. This is also the classic format of the brand, which is present on the market since 1964. Therefore, packaging is the first representative element that makes the brand recognizable and which reproduces the national flag. Demonstrating consistency, ROM packaging has remained essentially unchanged since it emerged on the market in 1964. The only modification made concerned the logo to increase shelf visibility and confer the look of a modern brand. The long history and continuation which resisted social change and Western influences made ROM a real national symbol. Romanians take it with them to offer as a gift when they visit other countries, as an authentic Romanian product.

In terms of advertising, Genuine ROM is characterized by irony, but also boldness, having the courage and daring to evoke the delicate Romanian social phenomena (communism etc.). An important advertising element of the ROM brand identification is represented by its slogan (“Romanian sensations”) which supports both the product specifics (the strong dark chocolate taste combined with rum cream) and the brand's values: self-irony, courage, authenticity.

TARGET MARKET DESCRIPTION

The case study analyses the export strategy of ROM chocolate bar to a European market, namely Spain. At present, Spain holds the most numerous Romanian community in the world. Each country and culture affects in its own way the seller as well as the market. It is foreseen that as long as ROM Chocolate is sending a powerful message regarding the national culture of a country, the product will have a high absorption rate. Demographically speaking, Spain and Romania are similar in terms of culture (speaking languages, Latin values) and customer behavior.

To understand the profile of the buyer's habits, when exporting a product, it is important to study usage habits regarding similar products that are already being sold on that market. Buying habits are also extremely important.

One of the reasons why a company engages in export operations is when the local market is saturated with the company's product. As ROM chocolate is a phenomenon in Romania and is number 1 selling chocolate, the company decided to turn towards a new market with a major Romanian community.

There are several resemblances between the two countries. Another important aspect which is usually taken into consideration for the export strategy is the climate. Hot and wet climate tends to produce sociable and expansive people, while introspective and independent people populate a chilly landscape. In export operations, sellers are usually following the same geographic models when trying to convince a foreign population to adopt their product.

For the present export strategy, it can be used the Inshore Marketing strategy. It consists in introducing the product to one part of the population only, knowing that in time it will spread in the whole country to large segments of buyers. Following this strategy, ROM chocolate export strategy will target the Romanian community from Spain at first, then spreading to the rest of the territory by gaining market share. There could be linguistic problems, cultural challenges, regulation difficulties etc.

ENTRY MODE ON THE SPANISH MARKET

The Spanish market is a long-run economy which encourages manufacturers mostly through Government subventions. It creates good development opportunities for both domestic and foreign companies and it holds a good rank in the economic development of EU countries.

While there are several modes through which a company can enter international markets, they all have both advantages and disadvantages. Root (1994) and Kaffash (2012) conducted a study on "foreign market entry decisions", analyzing both internal and external factors for different entry modes. According to the resources, market conditions and product characteristics of ROM chocolate, the authors narrowed the number of foreign entry mode options to three: direct export, indirect export and licensing.

For ROM chocolate export strategy, direct export represents an important market entry mode, since the company can take control over the entire exporting activities: it does not involve intermediaries, it provides direct contact with the customers and it generates a better comprehension of the Spanish target market. In the same time, from a financial point of view, it is very expensive and it requires good local relations and knowledge of the domestic target market. Since the company produces and sells only in Romania, it has the disadvantages of low connections and knowledge of the Spanish market, higher costs for internationalization, and also the high risk borne only by the exporting company.

The second global entry mode analyzed involves licensing - transferring the production license for ROM chocolate from Kandia company to a specialized Spanish company, which will be responsible for all production and distribution of the product. Due to the strategic option of Kandia to keep its production in Romania and not externalize it elsewhere, this option is not acceptable.

Indirect export implies distributing the ROM chocolate in retailers' channels (supermarkets) operating on the domestic Spanish market which hold a good public image, have substantial sales, and possess a proven good understanding of local market particularities. This particular option ensures a quick penetration of the Spanish market, it does not require a large investment, and has a low risk attached. Even if the profitability is not as high as it would be if one of the previous two cases was chosen, indirect export entry mode suites best ROM chocolate, considering the elementary stage of its international activity. After the careful analysis of the retailers operating on the Spanish market, the envisaged partners and therefore distributors of ROM chocolate will be Alcampo and Carrefour.

MARKING AND PACKAGING REGIME

According to the European legislation every product which is classified as food must contain the following compulsory information on their label or package, such as name, list of ingredients, quantity of ingredients (percentage), net quantity (expressed in units of mass), date of minimum warranty, special storage conditions or conditions of use, name / business name and address of the manufacturer or packager, or of a seller established within the EU Community, the place of origin or provenance (in this case Romania). The individual package of the product must also contain the nutritional information of the product (Coles *et al*., 2003).

The packaging regime for exporting the product must therefore comply with the requirements mentioned above. More specifically, ROM chocolate bars are packed by the producer into cardboard boxes containing 36 pieces. These boxes are then packed in corrugated fiberboard boxes commonly named regular shipping container (RSC), which are the most widely used type of packaging export products. They hold several main advantages: they are cheap, easy to purchase and (an increasingly important feature), they are recyclable. They are also easy to print with labels containing important information. As a drawback, they lose their strength if they are wet (European Product Labeling Guide, http://eur-lex.europa.eu).

The fiberboard boxes will be stacked up in no more than five layers, because the weight might affect the formation, and put on euro-pallets. In order to protect them for water and also keep them together and protect the stack from falling, they will be shrink-wrapped. This will lead to a stable bundle pack, which will insure the

The fiberboard boxes (each one of them) must have labels imprinted containing the following information: country of origin and manufacture (Made in Romania), the final day of recommended consumption (Expiry date - dd/mm/yyyy), total weight of the contents, identification code of lot or batch (L - followed by the identification code), and special handling instructions, if applicable. For easy identification of the products in the logistic computerized systems, each box and each pallet must have a SJ quick identification code, such as a barcode, QR code or matrix code.

QUALITY CONTROL REGIME

The Spanish and European regulations concerning cocoa products and chocolate are focused on quality issues, to protect the names and prevent fraud in order to protect the prestige of chocolate. Spanish legislation is mostly based on the European Union rules created for food safety. The European Community is the largest importer - exporter of food products, so a solid legislation is needed to secure the safety of the consumers. The standard of the European legislation regarding chocolate is the Council Directive 1973/241/EEC in which chocolate products are categorized according to their ingredients. The fact that Romania is part of the European Union is a solid statement that the exported products fulfill the standards of Spain, and no further legislation barriers will be encountered.

According to the aforementioned standard, the ROM chocolate product can be included in the Milk Chocolate category, because its ingredients satisfy the regulations of this type of chocolate. European legislation specifies regulations for labeling, hygiene and weight of this category. The labeling Directive also specifies the composition, nature, durability, place of origin or method of production and the ingredients, sorted by the percent of the total composition.

Controls and standards are set for all the raw materials, for all the stages of the manufacturing process, the finish product inspection and the control over the storage and transportation (Importing – Exporting Goods, http://europa.eu). The ingredients required for the production of chocolate are mostly of natural origin: cocoa beans and sugar. The usual procedure used to evaluate cocoa beans is to study the appearance of the beans that are cut in half and the flavor of the chocolate made from the beans. Regarding sugar, the norms refer to storage and control of moisture and granulation. The EU law also specifies the additives which are allowed to be added to the cocoa powder. In this particular case, ROM chocolate contains E 322 - lecithin, which is mentioned as accepted additive.

The control of the manufacturing process is subject to clear conditions. From roasting to molding, all the production stages are inspected according to principles of quality and hygiene. The properties of the ingredients must be always checked before entering the process. During the process, physical and chemical characteristics are measured and compared with the accepted standards. These characteristics are checked also in the final product. At this stage, the EU legislation specifies that a microbiological test must be performed. The main health threat of chocolate is *Salmonella* bacteria which may be present in cocoa beans, although roasting the beans often kill it.

Although the Spanish legislation must be aligned with the European Directive, there are some peculiarities of the Spanish market: the main advantage of it is that it does not have a strong tradition regarding chocolate products, as France, Italy, Belgium and Switzerland have. Romania, as the exporting country, also shares most of the rules and regulations regarding chocolate with Spain, fulfilling therefore the Spanish market specifications.

TRANSPORT AND DELIVERY TERMS FOR THE EXPORT

In international trade, to ship a product overseas, exporters have at least four options: international freight forwarder, Shipper's Associations' services, express delivery or mail service and arranging its own shipping.

To establish the rules which define the responsibilities of seller and buyer for the delivery of goods under sales contract, the present case study uses the INCOTERMS (2010 version). Moreover, Incoterms reduce misunderstandings among traders, thus minimizing trade disputes and litigation (http://www.iccwbo.org).

In the case of ROM chocolate, where the export consists in food products with limited warranty, it is best to negotiate the terms of delivery as soon as possible. In addition, the conditions of transport are very important. The interest is for the goods to arrive at the destination in good conditions and without visible damage.

According to the arguments presented, the chosen method to penetrate the Spanish market is indirect export. As the product's size is small compared to other goods that can be sold or exported to the Spanish market, there are three INCOTERMS options that can be suitable for the delivery of ROM chocolate from Romania to Spain. The first appropriate INCOTERMS option is the FCA (Free Carrier) delivery term, which states that the seller delivers the goods to the carrier (transporter) or another person nominated by the buyer, at the seller's premises or another named place, as instructed by the buyer. The seller must deliver the goods already cleared for export at customs (all custom formalities, including export licenses). FCA has many advantages for the exporter. The risk and the cost borne by the Romanian company are low. However, in present conditions, the sea transport will not speed up the delivery process.

Another INCOTERMS option that can be used is CPT (Carriage Paid To). CPT states that the seller delivers the goods to the (first) carrier or another person nominated by the seller at an agreed place. The seller must contract for and pay the costs of carriage necessary to bring the goods to the named place of destination. A third INCOTERMS option that can be used in establishing the terms of delivery is EXW (Ex Works). In the case of EXW, the advantages of the exporter are even higher, as its cost and risks are very low compared to the ones carried by the buyer. As the chosen entry mode is indirect export, the Romanian company will work with retailers. One of them, namely Carrefour, is also present in Romania; therefore, it can be negotiated with it to include the transportation of ROM chocolate to Spain among other products Carrefour Spain is already purchasing and selling.

PAYMENT TERMS FOR THE EXPORT OPERATION

From the various international payment possibilities, for the present case study, the most appropriate method of payment was chosen the letter of credit, as it brings most advantages for both parties (www.escrow.com). The workflow of the letter of credit for the export of ROM chocolate to Spain market can be drafted as follows:

- The agreement - Kandia and the buyer reach an agreement on the transaction, which specifies the quantity and type of good(s) to be purchased, the purchase price, and the date/ place for delivery.
- The buyer initiates the letter of credit - it works with a commercial bank to have a letter of credit issued, having Kandia specified as beneficiary, which must be fully funded by the buyer before issuance. The issuing bank requires an insurance.
- The transmittal of the letter of credit - the buyer's bank issues the letter of credit.
- The receipt of the letter of credit – Kandia receives the letter of credit. The company then presents the letter of credit to the commercial bank. With the guarantees from the issuing bank, the company has a line of credit which it can potentially use for its operations. The final payment will be made once the delivery conditions of the contract are satisfied.
- The shipment is made - with the letter of credit in its possession, Kandia then provides the consignment of goods to the shipping company (regardless of the nature of transport - water, trucking, rail, air).
- The Bill of lading is issued - in exchange for the consignment the seller receives a bill of lading.

- The receipt of goods - after the shipment is made, the buyer actually takes ownership of the goods and confirms that the seller's consignment to them meets the specifications in the contract (quantity, quality conditions etc).
- The payment is made - once all necessary steps are completed and it is assured that the consignment meets the terms of the contract, Kandia can claim from the bank the final payment from the funds that were previously deposited for it.

CONCLUSIONS

The thorough analysis of different aspects concerning the export of ROM chocolate to Spain market, a complex export strategy has been documented and described in this paper. After analyzing possible suitable ways for the Romanian company Kandia to penetrate an international market, the most advantageous internalization method for the ROM chocolate was considered to be indirect export. This entry mode requires a lower investment by eliminating costs of promotion and brand reputation, which will be transferred to the local chosen partner. Terms and delivery of the ROM chocolate are highly dependent on the negotiation with the distributor and the importer. The most favorable INCOTERMS for ROM chocolate are FCA and CPT, and choosing one of them depends on the agreement with the importer. The chosen method of payment is the letter of credit. The analyzed export strategy is a viable option to complement the domestic operations of Kandia.

Acknowledgement

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REFERENCES

Coles, R., McDowell, D. and Kirwan, M.J. (2003), *Food Packaging Technology,* CRC Press, Blackwell Publishing.

Deselnicu, D.C. (2005), “Practical tools for change: A Software support for self-assessment of the business excellence implementation process in Romanian enterprises”, *Proceedings of The 4th International Conference on Management of Technological Changes*, 19-20 August, Chania, 2, 17-23.

Deselnicu, D.C., Albu, L., Swiger, J.A. and Cioponea, I. (2008), “Competitiveness and Innovation in Romanian Enterprises: A Case Study”, *Proceedings of The 2nd International Conference on Advanced Materials and Systems,* 23-24 October, Bucharest, 1, 545-550.

European Product Labeling Guide, Available from http://eur-lex.europa.eu, Accessed: 2015-07-9.

Importing – Exporting Goods, Available from http://europa.eu/voureurope/business/sell-abroad/import-export/index en.htm, Accessed: 2015-07-10.

International Chamber of Commerce: INCOTERMS rules, Available from http://www.iccwbo.org/products-and-services/trade-facilitation/incoterms-2010/the-incoterms-rules/, Accessed: 2015-07-10.

Kaffash, M.H., Haghighikhah M. and Hamidreza K. (2012), “Identifying Factors Influencing Entry Mode Selection in Food Industry of Small and Medium-sized Enterprises (SMEs) in Iran”, *International Journal of Marketing Studies,* 4(5), 47 – 55.

Kandia Dulce – Official website, Available from http://www.kandia-dulce.ro/, Accessed: 2015-07-08.

Root, F.R. (1994), *Entry Strategies for International Markets,* Jossey-Bass, San Francisco.

Securing the Transaction: The Advantages of Using Online Escrow Services versus Letters of Credit in International Tradel, Available from: https://www.escrow.com/news/Articles/the_advantages, Accessed: 2015-07-10.

SUSTAINABLE CONSUMPTION AND ETHICAL BEHAVIOR OF CONSUMERS IN THE FOOTWEAR INDUSTRY

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Traditionally shoes are made from leather and most of the worldwide population wear it, but there is a particular group of consumers, with an ethical behavior, who prefer shoes made from alternative materials. In our days, the main objective of the fashion industry (T&C, footwear) is to reduce the intensive use of fossil energy, non-renewable raw material and decreasing of landfill waste. In past years, sustainable and ethical consumption has become a popular topic in industries, has gained increasing importance in wealthy countries all over the world. This paper presents some point of view about ethical footwear and the consumer behavior, it also provides a brief overview of global consumption of footwear, as starting point for a new approach of the supply chain of footwear industry. The objective of this paper is to present consumers perception and interests regarding ethical footwear and ethical brands. To reach the main objective of the article, the methodology of the research consists in a survey applied to a target group of Romanian people, men and women, with average to high incomes, interested in buying less but sustainable products.

Keywords: sustainable footwear, ethical consumer, sustainable consumption.

INTRODUCTION

In recent years, the fashion industry environmental impact is growing considering the use of cotton, natural leather, wool and synthetics. It is known that leather production requires high water consumption and chemicals (used in leather tanning like chromium) with a negative impact on the environment. The production process, especially tanning, drying, dyeing and finishing uses chemicals and generate a high environmental impact (Caniato *et al*., 2011). A lot of fashion companies have requested assistance from foreign partners to produce their products, in order to subcontract some activities like cutting, sewing, dyeing to different firms across the world; leading to an improved supply chain (Kleindorfer *et al*., 2005; Elkington, 1994; Zhu *et al*., 2008). Therefor EU footwear industry is trying to cut costs by working together with vendors in low cost countries.

Global Footwear Market

In 2014, the worldwide production of footwear, according to APICCAPS, has increased from 2013 by 8% and reached 24.3 billion pairs. Asia accounts for 88% of global production, followed by South America 5%, Africa 3% and North America 2%. China remains a leader among footwear producing countries. The European footwear production shows a slightly decrease in the production, currently 3%.

In 2014 Asia was the biggest consumer of footwear, especially China is the largest market for footwear with 3.65 billion pairs followed by European Union with 2.77 billion pairs and United States with 2.29 billion pairs. US consumers bought per capita in 2013, 7 ½ pairs of shoes, 98% of shoes sold are imported from China, according to a report by the American Apparel & Footwear Association (AAFA). The highest consumption of footwear in Europe is United Kingdom with 523 billion pairs, and figure 1 presents the leading 10 global footwear consumers in 2014, by country.

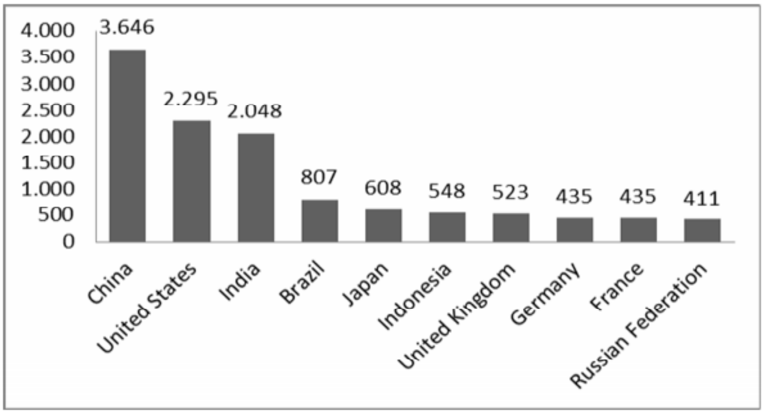

Figure 1. Top 10 of 2014 global footwear consumers (in number of pairs)
Source: World Footwear Yearbook 2015

European Footwear Market

Production

In 2014 the most important producer is Italy (Figure 2) accounted for 50% of total European production, followed by Spain (13%), Portugal (12%) and Romania (8%). The total value of EU production of footwear has increased from € 14.1 billion in 2010 up to € 17.4 billion in 2014.

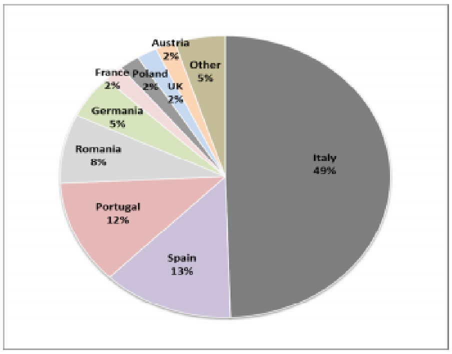

Figure 2. European production of footwear in 2014
Source: Eurostat, Prodcom, 2015

The EU footwear production industry focuses on adding value to products improving the design, comfort, quality, materials in order to respect the environmental rules. The EU has always been a significant provider of high quality footwear to the world market. But, the mass production was outsourced in Asia; currently 6 out of 10 pairs of shoes worldwide are produced in China.

Romania is an important player in this market and even feared by competitors since Romanian footwear reached 14% of total trade value.

Consumption

Between 2010 and 2014 the European consumption of footwear had a compound annual growth rate of 2.9%, reaching € 23.5 billion in 2014.

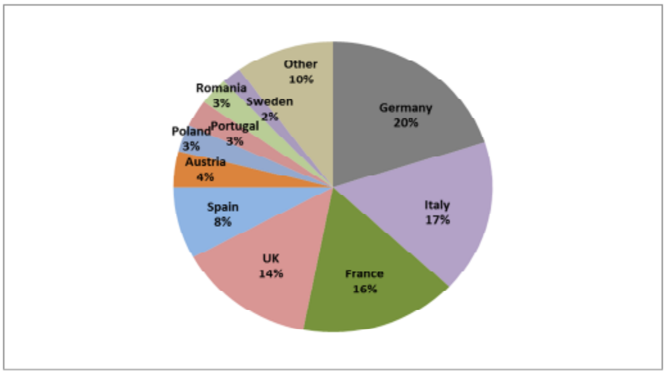

Figure 3. EU footwear consumption in 2014
Source: Eurostat / ProdCom, 2015

The consumption of footwear increased between 2010 and 2014 in developed countries (Denmark +7.6%, Austria 6.9%) and East European countries (Poland +6.6%, Estonia +19%). This growth in consumption of footwear is explained by population growth; market demand is growing; fashion changes very often. Another fact is that consumers are more informed about the international brands which means a developed and efficient supply chain.

In the same period of time southern European markets shows a decrease in consumption of footwear, for example Greece (-6%), Spain (-2.1%), Cyprus (-6.3%).

In 2014, over 52% of consumers bought footwear made of leather (Figure 4) and the EU demand for textile footwear increased between 2010 and 2014 with 7.7% per year; meaning that consumers are interested in other materials than leather.

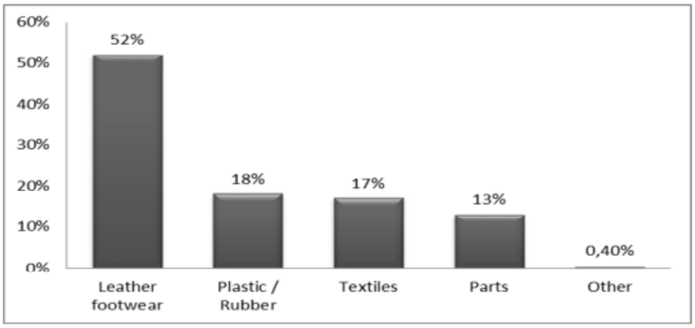

Figure 4. European footwear consumption by consumers choices

Sustainable Consumption & Ethical Consumer

The concept of sustainable consumption was defined for the first time in 1994 by the Oslo Symposium as "*the use of services and related products, which respond to basic needs and bring a better quality of life while minimizing the use of natural resources and toxic materials as well as the emissions of waste and pollutants over the life cycle of the service or product so as not to jeopardize the needs of further generations*".

Adopting the sustainable consumption patterns is the responsibility of all the people, but for this, population should be educated, motivated to change their habits and adopt a sustainable lifestyle.

An ethical consumer is a person who buy products which are eco - friendly from ethical companies, it is also aware about the negative impact on the environment of the production process, consumption and disposal. Generally consumers are influenced by many factors (Hoyer, 2012) divided in: demographic aspects such as gender, age, income, occupation, lifestyle, family size, religion, nationality; and social, personal, cultural aspects.

This paper investigates the awareness of the Romanian consumers towards ethical footwear. In Romania, consumers awareness is increasing in the last years, they are having various requirements and are influenced by several aspects but the footwear industry is responding with a wide range of products.

RESEARCH METHODOLOGY

The method used was primary market research, a survey was created. The questionnaire contain 15 items exploring the quantity of shoe purchases, the kind of raw materials they are made from, brand, price, interest of consumers about sustainable footwear, buying behavior of consumers of footwear, followed by four questions regarding demographic aspects such as gender, age, education level and income level of the respondent. Using two modes of administration: paper – and – pencil and computerized administration.

In the questionnaire closed-ended questions were used, as well a Likert scale for most of the questions, which is commonly used in marketing and can easily measure the satisfaction level of consumers.

The sample consists of consumers who participate at a footwear fair in Iasi. The clients were asked to be part of the study and complete the survey. The sample consists of 40 individuals, as shown in Figures 5-8.

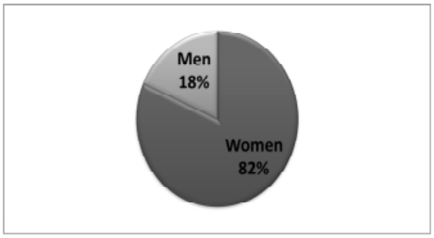

Figure 5. Sample structure by gender

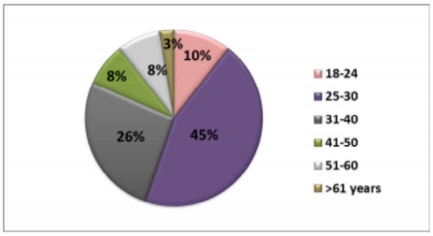

Figure 6. Sample structure by age

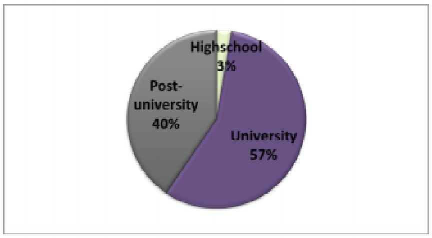

Figure 7. Sample structure by education level

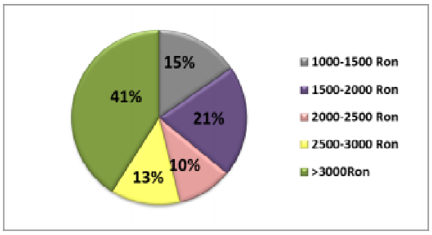

Figure 8. Sample structure by income level

RESULTS AND DISCUSSIONS

The data was collected in real time and analyzed using Google Forms.

The first questions in the questionnaire were designed to investigate the buying behavior of consumers and which aspects they follow in this purchase.

The first part of the survey aimed to investigate the amount of shoes bought in the last 3-6 months, the kind of raw materials they were made from, the brand, price and the place where they bought, and what are the reasons for this purchase.

In terms of quantity of shoes bought in the last 3-6 months 51.3% of respondents bought 2 pairs, followed by 33.3% with 3-4 pairs and 15.4% purchased over 4 pairs of shoes. As we can see more than half of respondents bought 2 pairs of shoes and this is a positive fact if we refer to environmental aspects, however for the industry and economy is not;

More than half of respondents (56.4%) prefer leather shoes because of their quality and lastingness; leather shoes are the most common in stores. 35.9% of interviewed are wearing shoes made from leather and textiles, such as snickers, and this is happening because 45% of the respondents have between 25 – 30 years old. Even so consumers (7.7% of respondents) turned their attention to friendly leather shoes such as fake leather made from a polyurethane synthetic microfiber or vegetable-tanned leather.

For this purchase, 43.6% of respondents bought form the mall, followed by 23.1% bought online, 20.5% from outlet and second hand stores and 12.5% from local producers. Buying footwear from the mall is the simplest option, but if consumers were more informed about the local producers perhaps they could have more options.

The second part of the survey is to find out the interest of consumers regarding sustainable footwear. The results reveal that 71.8% of respondents heard about sustainable footwear but the knowledge and information they have are limited.

Most of respondents do not trust in these shoes and their characteristics because they do not have enough information (65%), the prices are higher than regular shoes (80%) and the design / style is different and more daring (52%).

When they were asked about the reasons of buying sustainable footwear most respondents said that the most important factor is that shoes are eco – friendly (85%), followed by the treatment of employees (70%) and the manufacturing process affects less the environment (67.5%).

Only 43.6% of respondents believe that can help to protect the environment using / buying shoes from second hand stores for several reasons: saving resources for their manufacture, reducing waste and supporting local businesses.

CONCLUSIONS

This paper investigates the awareness of the Romanian consumers towards ethical footwear. From the research conducted it can be seen that consumers are interested for ethical footwear, but the knowledge and information they have are limited.

It is not necessary to purchase eco footwear in order to be an ethical / conscious consumer. Buying footwear from ethical brands it is the aim but it is enough if the consumers would be more educated and informed about the consequences of higher consumption of footwear.

In the end, clients have a strong aspiration to buy more consciously and the key is to continue to educate them in order to become a conscious consumers.

REFERENCES

AAFA (American Apparel & Footwear Association) reports, https://www.wewear.org/apparelstats-2014-and-shoestats-2014-reports.

APICCAPS, http://www.apiccaps.pt/web/guest.

Caniato, F., Cardi, M., Crippa, L. and Moretto, A. (2011), "Environmental sustainability in fashion supply chains: An exploratory case based research", *International Journal of Production Economics*, 135, 659-670.

Deselnicu, D.C., Vasilescu, A.M., Purcarea, A.A. and Militaru, G. (2014), "Sustainable consumption and production in the footwear sector", *Leather and Footwear Journal*, 14(3), 159-180.

Elkington, J. (1994), "Towards the Sustainable Corporations: win-win-win business strategies for sustainable development", *California Management Review*, 36(2), 90-100.

Eurostat, http://ec.europa.eu/eurostat.

Hoyer, W.D., Macinnis, D.J. and Pieters, R. (2012), *Consumer Behaviour*, Sixth edition, South-Western, USA.

Kleindorfer, P.R., Singhal, K. and Wassenhove, L.N.V. (2005), "Sustainable operation management", *Production and Operations Management*, 14(4), 482-492.

Norwegian Ministry of Environment (1994), Oslo Symposium, http://www.unep.org/resourceefficiency/Home/WhatisSCP/tabid/105574/Default.aspx.

World Footwear Yearbook (2015), http://www.worldfootwear.com/yearbook.asp?s=1&ss=91&year_sel=2015.

Zhu, Q., Sarkis, J. and Lai, K.H. (2008), "Confirmation of a measurement model for green supply chain management practices implementation", *International Journal of Production Economics*, 111(2), 261-273.

KNOWLEDGE PLATFORM FOR TRANSFERRING RESEARCH AND INNOVATION IN FOOTWEAR MANUFACTURING

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Knowledge 4Foot (K4F) is an ERASMUS+ project which is running for a period of three years (2015-2018) within the framework of Key Action 2: Cooperation for Innovation and the Exchange of Good Practices/Strategic Partnerships for Higher Education. The project will develop and demonstrate new types of cooperation between universities, research organisations and business, which is based on open innovation aimed to increase the flows of knowledge. The three sides of the 'knowledge triangle' are joined through K4F project in order to enhance the contribution of higher education to growth in EU footwear sector, as well as its attractiveness in partner countries by applying good practices and mastering the most advanced methodologies for transferring the results of research into business environment thought real project-based work focused on technological transfer. The Knowledge4Foot project introduces innovative tools to adapt and update the learning and training curricula of higher education providers for managers, designers, and engineers, in order to achieve greater creativity, innovation and high performance in European footwear manufacturing and related sectors.

Keywords: e-learning, shoe, research, higher education

RATIONALE OF AND BACKGROUND TO THE PROJECT

In the report titled *Key Data on Education in Europe 2009*, published by the Education, Audiovisual and Culture Executive Agency, the idea of involving higher education, research and industry will increase the Europe's capacity for innovation (in Key Data on Education in Europe, 2009). By creating and developing so-called Knowledge Communities through a real partnership among higher education, research and industry this idea will become sustainable.

The quality of higher educational programs should be assessed not only according to the university curricula, but taking into consideration also the students' involvement in different extracurricular activities, in order to adjust the study programs to a problem-solved approach of learning, which is more suitable to industry needs. Equally, the university curricula and the other activities (research, industrial placement, volunteering etc.), which students (undergraduates and graduates) should be motivated to participate at, contribute for graduating competent and multi-skilled engineers and managers (Mihailescu *et al*., 2008).

Starting with 1990s, in order to face the future demands of the knowledge-based society, the higher education is being asked to produce graduates who are flexible and have market-related skills and abilities. Barnett (1998), cited by Savin-Baden (2000), stated that there is a growing awareness for developing skills and abilities to research and critique information.

In terms of how the future graduate of higher education should look like, whatever is the technical and scientific field he/she graduates, an ideal profile has been defined: able to work in teams, to communicate well, to analyse and synthesise, to be self -transformative, to have reflective and critical abilities. Harvey (2000) stated that the graduates need to be flexible and able to help the organizations/ companies, in which they work after graduation, to face with all rapid and continuous changes. Therefore, the training and study programs must treat the student as intellectual performers rather than as conforming audience.

The European reports and directives, official political declarations, scientific reports upon this subject require for integrating research with teaching activities based on the numerous advantages that are reflected to the ones who are customers of higher education business, the students. Beside the incontestable truth that research enriched training, there are factors that make this process not so easy. A research about fundamental review of research policy and funding, undertaken by Higher Education Funding Council for England (HEFCE) has concluded that "new ways of managing the teaching and research relationship need to be considered" (Coate, 2001). The university – research – industry relations should function increasingly as laboratory of knowledge-intensive networks.

Europe has valuable tradition and leading recognition in footwear manufacturing. In a global economy where enterprise sustainability and employability are uncertain, the need for making the knowledge triangle (education, research and business) to work becomes obvious. It is very well known that the manufacturing sectors are very sensitive to international challenges represented by globalization and competition. In this general context, the footwear companies are forced to make important strategic changes by adding value to their products. And the added value comes from research and innovation in terms of styling and design, customization, brands, high quality, high-tech, new business models, sustainable development and environmental values.

On the other hand, the footwear companies in Europe are small and medium sized. For this reason most of the companies cannot create own research and technological transfer departments and they are very much dependent on external offers of universities and/or research centers. All these challenges require for highly qualified employees, including technicians, engineers, product & process developers, top and middle managers, who should have the right mix of skills, both professional and transversal, in order to demonstrate their competence for applied research, development and technological transfer. This goal of having right skills for transferring knowledge and innovation into the daily based business environment of the footwear companies could be possible by improving the quality and relevance of higher education addressing to these professionals. Also, because of labour costs, most of the footwear companies do not afford to have numerous staff having an academic degree or much narrowed specialized employees. Therefore, for top and middle managers, for engineers, for product and process developers having skills and competencies in research, innovation and technological transfer is more than necessary in order to complete their professional university degree, experience and knowledge background. Linking the education with the work environment is one of the goals of the EU policies and national political priorities in all EU 27 countries. Therefore, supporting active learning by creating right mix of core, transversal and professional skills emerges from the recommendations on different levels: EU, national authorities and social partners. The European Commission's report - 'New Skills for New Jobs: Action Now' (2010)- emphasizes the following directions: ‘to better link education, training and work, to develop the right mix of skills, and to better anticipate those skills needed in the future’.

AIM AND OBJECTIVES OF THE PROJECT

Making the knowledge triangle work by connecting Higher Education, Research and Business for excellence is one of the goals of the EU policies and national political priorities in all EU countries. Knowledge 4Foot (K4F) project aims to contribute at fostering the excellence in training for footwear manufacturing by connecting the three areas of education, research and business oriented innovation in order to demonstrate good practices of cooperation and to bridge the worlds of education and work. Therefore, the Knowledge4Foot project targets the following two groups:

- Students enrolled in Higher Education
- Professionals involved in top and middle management in footwear companies: mangers, designers, engineers and technicians.

In order to achieve this aim, the project has following objectives:

- to develop active collaboration among universities, business community and research centres to assess the needs of skills for innovation and technological transfer;
- to design, test and implement a common curriculum for virtual internships and the related e-learning content, which incorporates a creative thinking and problem-solving approach;
- to set-up a Knowledge Platform that facilitates the transfer of innovation in footwear manufacturing by simulating the developing stages of the research projects.

The project consortium involves eight organizations with different expertise and competences such as universities, research centres, and SMEs from Romania, Spain, Portugal, Greece and Croatia and the European Confederation of Footwear Industry (CEC) with headquarters in Brussels. The project partners are:

- TUIASI - Universitatea Tehnica Gheorghe Asachi din Iasi
- CEC - European Confederation of the Footwear Industry
- Virtual Campus Lda
- INESCOP - Instituto Tecnologico del Calzado y Conexas
- CTCP- Centro Tecnologico de Calcado de Portugal
- UNIVERSITY OF ZAGREB - Faculty of Textile Technology (TTF)
- The Research Committee of the Technical University of Crete
- INCDTP - Institutul National de Cercetare-Dezvoltare pentru Textile si Pielarie
- CRE.THI.DEV- Creative Thinking Development

Knowledge Platform for Transferring Research and Innovation in Footwear Manufacturing

Fostering the excellence in tertiary level of training and education for design, product development, engineering and management by connecting the three areas of the knowledge triangle: Education, Research and Business.

We bridge the world of Education, Research and Business!

Figure 1. The K4F project website - http://www.knowledge4foot.eu/

ENVISAGED RESULTS

The Knowledge4Foot project introduces innovative tools to adapt and update the learning and training curricula of higher education providers for managers, designers, and engineers, in order to achieve greater creativity, innovation and high performance in European footwear manufacturing and related sectors.

The envisaged project results/products are the following:

- ***Mapping the knowledge triangle for transferring research and innovation in footwear manufacturing.*** The aim of this study is to give a deep overview of the labor market needs for highly qualified professionals in footwear manufacturing with right mix of transversal and professional skills in order to boost the transfer of novelties coming from research and innovation in product, processes and services that provide added value.
- ***Training program and e-learning content for transferring research and innovation.*** The training courses will be focused on developing skills and competencies in research, innovation and technological transfer applied to footwear manufacturing. Three modular courses will be developed, with 4 units/modules each (total 12 modules), for a training program having 125 total hours.
- ***Multimedia handbook for project-based training and virtual placement*** of HE students and trainees from SMEs. The content of the multimedia handbooks addressing both to students and supervisors will be elaborated and transformed into e-booklets.

- ***Knowledge4Foot Platform for Transferring Research and Innovation in Footwear Manufacturing.*** An online knowledge-based platform will be designed, developed and released. It will integrate the developed resources within the project (curriculum, training content, guides, other resources) in order to resemble an Internet-based research and development center that will act upon virtual placement of trainees and project-based activities.
- ***Book of lectures for Entrepreneurial thinking in footwear manufacturing***. This e-book will be used and tested by the participants in the Intensive Summer Course.
- ***Final conference on Research and Innovation in Footwear Manufacturing*** for experts, researchers and other professionals from the footwear and leather industries, universities, research institutes and training centers.
- ***Intensive program for higher education learners.*** The participants (students) will follow core and optional lectures where they will receive knowledge and skills for transforming innovative ideas into startup businesses in footwear sector. They will be tutored by trainers from partner institutions and will be coached by representatives from footwear companies.

The project is sharing the vision of the European Education and Training Commission (ET 2020) stating that opening up education may lead to a situation where all individuals may learn anytime, anywhere, with the support of anyone, using any device. The project promotes and supports the open access to educational materials, reports, published scientific or advertising material. All products without any exception will be delivered in English, but some of the project deliverables will be translated in the consortium languages.

In order to maximize the applicability of the tools and to achieve the greatest impact on the sector, a common action is required for better integrating wider working conditions, business principles, knowledge on technology, knowledge on IT tools, etc. The K4F project includes activities that meet the conditions for effective generalisation, and exploitation in other European countries. Thus, by following up a virtual internship program, the HE students and trainees will be able to understand the needs for innovation and to gain competencies for transferring, applying and adapting research results to boosting the economical grow of this traditional manufacturing sector that is identified with Europe itself in terms of brands and high quality products. The virtual internship will complement the real placements in work environment, as preparatory stage of it. As multiplication, the universities and the vocational education and training schools could apply the hereby solution in order to overcome the difficulties with their study programs addressing to applied technical sciences (including education and training for Leather & Footwear sector also). By K4F project, the area of study for applied technical sciences will be adequately promoted and sustained in order to be attractive and motivating for young generation of students.

The K4F project identifies a clear rationale for a European focus and proposes viable solutions that could have a great impact on footwear manufacturing from other EU countries. There are visible benefits accruing from the collaboration at national level and across national borders in sharing case studies as well as strengthening the need for appropriate training opportunities in transferring the results of research from technological centres to businesses through skilled graduates of HE study and training programs. The translations of the outputs in 7 project languages ensure a significant distribution of training materials, books, reports and guides across Europe. Also, the project considers the cultural differences based on geographical composition of the consortium, a special attention being given by all partners to supporting the cross-cultural dialogue. The inclusion of a large number of stakeholders is based on own networks and partnerships that each partner brings from previously implemented projects at national and EU/international levels.

Acknowledgements

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REFERENCES

Coate, K., Barnett, R. and Williams, G. (2001), "Relationships between Teaching and Research in Higher Education in England", *Higher Education Quarterly*, ISSN: 1468-2273, 55, 158–174.

Harvey, L., (2000), "New Realities: The Relationship between Higher Education and Employment", *Tertiary Education and Management*, ISSN: 1573-1936, 6, 3–17.

Mihailescu, C., Suteu, D. *et al*. (2008), "The Involvement of Students in Research Activities", *Proceeding of The 5th International Seminar on Quality Management in Higher Education*, Rusu C., (Ed), ISBN 978-973-730-496-4, Sinaia, Romania, Publisher Technical Univ. Iasi, 547-552.

Savin-Baden, M. (2000), *Problem-Based Learning in Higher Education: Untold Stories*, Published by SRHE and Open University Press, ISBN 9780335203376

*** Key Data on Education in Europe (2009), published by the Education, Audiovisual and Culture Executive Agency (EACEA P9 Eurydice), ISBN 978-92-9201-033-1, p.3.

HIGH SPEED SHOE FACTORY – A NEW FOOTWEAR FACTORY MODEL

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The footwear industry is traditionally characterized by production units consisting of sections physically separated from each other and with autonomous organizational and management processes. This type of organization creates areas of intermediate stocks, large production lead times, excess of materials and product handling, thus long delivery times. High Speed Shoe Factory project developed and implemented a new model of footwear factory for quick response, able to produce pair-to-pair, in order to satisfy internet sales, small orders and quick replacements. The shoe production was designed in a logic of a “single section” with high flexibility and materialised by developing and implementing new agile and reliable technological solutions, including an interactive online shop, a new multi tool cutting system and an integrated logistics flexible production system, which crosses in a single step, the traditional productive sections of cutting, sewing, assembling and finishing.

Keywords: footwear, production systems, consumer tools.

INTRODUCTION

The Portuguese footwear industry has registered a strong performance over the last five years, with exports growing by 54% from 700 to 1850 million euros. Portugal is highly specialized in leather footwear which it actually exports to 152 countries. Despite the global constrains, during this period, footwear continued to growth also in terms of the number of jobs created (2534), the result of a concerted effort on the part of the manufacturers themselves, the industry association APICCAPS, the political authorities, national and international research and development (R&D) partners and CTCP.

Worldwide production of shoes is typically organized in three main phases: the cutting of upper and lining materials, the stitching of the pieces in the shoe’s upper part and the assembly and finishing of the final product. These production stages are, in general, physically separated from each other and managed in an independent way within different departments or production units under specific organizational processes supported by different enterprise software management systems.

This type of organization may create, namely: important levels of stock of intermediate products along the manufacturing chain; large lead times of production; and excessive handling of raw materials, components and products; with significant impact in the delivery time of customer orders, that generally are not less than about 3-4 weeks. This has become increasingly important once European footwear industry has focused on the manufacturing of small series of differentiated and fashion products.

STATE-OF-THE-ART

During the last decades, footwear manufacturing companies have promoted and adopted innovative beyond state-of-the-art technologies and automated solutions on their manufacturing and distribution processes, targeting increased flexibility as a way to promptly respond and face the market challenges.

As a first step, the Portuguese footwear industry committed itself to radically renew the cutting processes, by replacing the manual and mechanical cutting of the leather. In parallel, the industry also made a commitment to radically renew the internal distribution processes of the work-in-progress items (materials, components, and products), by replacing the manual distribution or distribution based on mechanical conveyors with dynamic and automated distribution systems (internal logistic systems) capable of transporting the work-in-progress items between the manufacturing operations within each independent production unit.

In this context, the Portuguese footwear industry is now a worldwide highly competitive industry whose success is closely based on this continuous capacity of innovating the manufacturing and distribution processes, along with a strong strategy of internationalization, own brands and technological innovation in the footwear product itself. This has been and intends to remain the strategy to respond and to anticipate the needs of the market thus guaranteeing the competitive positioning in the worldwide market.

It is in this context of a mature industry in terms of technological processes and management of own footwear brands that was developed the Research and Technological Development project, with acronym HighSpeedShoeFactory, led by KYAIA as footwear manufacturer and with the active involvement of CEI by ZIPOR as cutting and automated solutions developer and provider, CREATIVE SYSTEMS as expert in RFID, FLOWMAT and SILVA FERREIRA as logistic systems providers, INESCTEC as R&D in logistics and software, FEUP faculty as expert in engineering and CTCP as R&D expert in footwear and project management. This project resulted in a new footwear production model and state-of-the-art prototype solutions and systems for the footwear industry, installed at the main KYAIA's manufacturing unit.

NEW FACTORY MODEL, TECHOLOGIES AND SYSTEMS

HighSpeedShoeFactory is a new footwear factory model for 24/48 hours of agile response in order to satisfy:

- multiple distribution channels, including sales over the Internet;
- the production of customized and very small orders;
- the rapid replenishment of the product in stores;
- the development and production of new shoe models, new collections and footwear samples;
- the simultaneous production of different footwear products with different constructive methods;
- a meaningful reduction of work-in-progress stocks;
- and to reduce human intervention in routine repetitive works and increase their versatility and empowerment in the production system.

To develop this model, different conceptual approaches were considered and the respective implementation solutions considered. The solution finally developed is consumer centered and supported by an innovative production concept based in integrating in a "single productive phase" the cutting, stitching, assembly and finishing that are traditionally performed by separate independent production units or departments. To implement the model several solutions were developed, namely: on-line interactive virtual shopping application, new cutting system and new logistic systems.

Interactive Online Shopping Tool

The tool was designed to facilitate and enhance the consumers shopping experience (Fig. 1) by embedding a range of customized products and the possibility to do some products personalization (aesthetic details enabled by the cutting system).

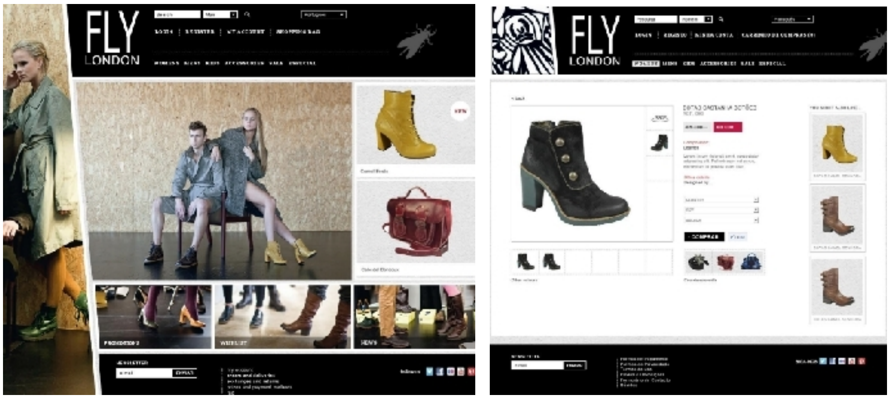

Figure 1. Interactive online shopping tool featuring products catalogue, suggestions and personalization options

Online interactivity gives insights about the consumer profile and feeds relevant characteristics to the suggestion system. Suggestion algorithms propose alternative shoes and complementary accessories. The tool is integrated with several information systems, namely the ERPs. CAD and production systems.

Innovative Cutting System

The innovative cutting system features 2 independent working areas and 2 heads that may work in the same or different areas. Each head integrates up to 12 tools, namely, several types of punches, pens, oscillating cutting knife and laser engraving possibility. These tools allow materials cutting and aesthetic personalization and customization to reply to costumers and client's desires or demands.

Figure 2. Multi tool cutting system development and final prototype

New integrated digitalization and refined nesting algorithms result in improved leather use. Proprietary software and controls allow fast replies to models changes, flexibility to cut different materials and integration with some factory processes.

Logistic Systems

The integration into a single production phase is supported by a new internal logistic system that ensures the automatic transport of items in the whole manufacturing process, assigning and controlling the work flow along the different production workstations. This solution is managed at the top level by the company ERP system and at the factory level by the logistic software integrating three different software-based systems that automate the flow of work-in-progress and do their assignment to the working posts in the cutting, stitching and assembly manufacturing phases.

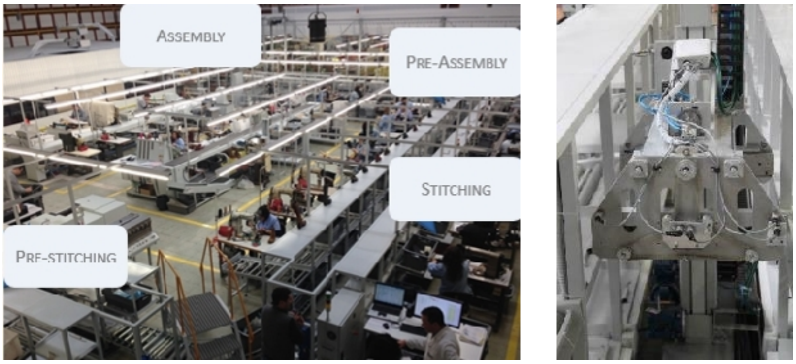

Figure 3. Internal logistic system general view and robotic manipulator details

To implement the concept new stitching and assembling logistic systems were developed. The first comprises 3 independent sub-systems, pre-stitching, stitching and pre-assembly, featuring each a robotic manipulator (developed) that moves the materials in boxes between the working posts (WP). Each WP includes 1 working location/cell, 1 waiting cell and 2 store locations. During the tests the system was able to manage simultaneously up to 70 models and do 1215 movements per day (8 hours).

The assembling system comprises 1 external internal through which the pallet trays containing the upper and components flow from WP to WP, 1 high speed internal ring and transference points to move the pallets between the rings. The systems was conceived to facilitate the simultaneous production of several shoe models of different construction systems (e.g. Glued, Pratik, Goodyear).

CONCLUSIONS AND FUTURE WORK

These solutions aim the sustainable and agile production of small series (European footwear producers important target), facilitating the production and commercialization of customized or personalized shoes, supported by innovative cutting and logistic solutions that contribute to eliminate a relevant level of stock of intermediate work-in-progress, dramatically reducing the product lead time from 3/4 weeks to 1/2 days, while simultaneously allowing the effective production of several shoe models of different construction systems.

The model will continue to be developed and improved at European and National level. In the frame of the HORIZON 2020 EU Innovation Action aiming CPS-oriented Future Internet-based machine-factory-cloud service platform *CPPS-isation* and Experimentation, Ref. 680633, BEinCPPS project, lead by POLIMI and in which KYAIA, INESCTEC and CTCP participate a Cyber Physical System-based automation approach will be implemented so that the logistic High Speed Shoe Factory systems will become connected and cooperative elements.

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The authors thank HORIZON 2020 EU Innovation Action aiming CPS-oriented Future Internet-based machine-factory-cloud service platform *CPPS-isation* and Experimentation, Project BeinCPPS, ref. 680633, the possibility of continuing this work.

REFERENCES

Ferreira, M.J., Pinto, V. (2016), “High Speed Shoe Factory” oral presentation, 19th UITIC Congress, 3-5 February 2016, Chennai, India.

Pohl, K. (2010), *Requirements Engineering – Fundamental, Principles and Techniques*, Springer, NY.

World Footwear, (2016), “Freedom of movement”, *World Footwear*, May/June, 18-19.

IDENTITY DESIGN - METHOD OF GENERATING CULTURAL SUSTAINABILITY OF CREATIVE INDUSTRIES PRODUCTS

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Creative industries, those industries structured around the cultural creativity - individuality - prototype system, are built based on the need for added value of the product. This concept of valued identity may be, when considering a creative industry, the paradigm of an identity of the product concept, of the brand or an ethnical, national identity of the trademark. Decoding product concepts at object level is very important in the context of cultural sustainability of creative industries because it enables knowledge of the semantic and graphic construction and of the general stylistic expression of the product through the analysis of visual semiotics and symbolic items. To establish the place of this type of research in design taxonomy, a synthesis of the European design knowledge map over the last four decades will be presented. In this context the paper proposes to present a method of identity design that focuses on sustainable use of cultural instruments. Identity design, as generative method of the originality of the culturally sustainable design concept, is defined by the complexity of cultural items and visual archetypes that transcend the primary message and reveal, through symbolic qualities of visual language elements and artistic techniques, an entire characteristic universe. Theoretical and experimental data of the method applied in fashion design are presented.

Keywords: identity design, method, cultural sustainability

INTRODUCTION

Creative industries are economic activities whose main ingredient is cultural creativity. Creative products are objects, actions or services through which artists generate new meanings and experiences, making the connection between man and man-dominated world, object-based and symbolic. There is only one keyword for all creative industries, from architecture and design, to performing arts, fashion, film and media, music and IT games and that is: ARTIST.

The economic and industrial mechanisms of today comprise technical and cultural innovation in the rhythms imposed by consumption. If manufacturing industries use either technical or marketing innovation as a sales growth element, in creative industries, the cultural innovation that the product embeds is psycho-philosophical because it is designed to meet the requirements of novelty, change, originality or artistic-cultural identity of the consumer.

The economic dynamics of the creative industries is influenced by four exogenous factors:

- market demand,
- public policies in the field,
- advanced digital and cultural technologies,
- globalization.

These socio-economic components are found in the concept of creative product. Given the societal coordinates, design was developed both in philosophical terms and through practices based on knowledge and skills gained through research, throughout the twentieth century. Research into product design has already been practiced in Europe for over 36 years, leading to the shaping and domination of the object world of to what today is called human civilization. A team of British researchers from the

University of Birmingham mapped the European design knowledge over the last four decades to highlight the structure and level of knowledge in this field. The basis to trace the history and development of design ideas and design writing over the following 36 years through classifying the issues that are central to the papers published in Design Studies, and quantifying the changes of emphasis in design topics over that period (Burns *et al*., 2016), figure 1.

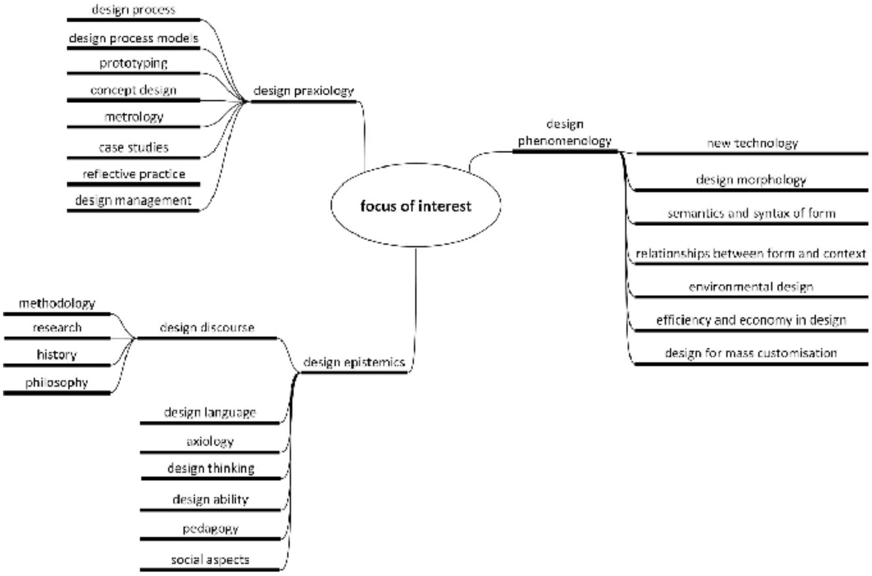

Figure 1. Mapping design knowledge: 36 years of Design Studies, 2016 (with permission)

The field of fashion design comprises most types of product design knowledge. From the perspective of fashion design knowledge, therefore fashion, identity design can be approached from the perspective of design phenomenology, as well as epistemic design based on design practice. In our reasoning, the phenomenological approach will be presented, as the interest is focused on product sustainability.

In the field of fashion and life-style industries, Ethical Fashion Forum defines sustainability as the approach to fashion that maximizes benefits for people and minimizes environmental impact. In this context cultural sustainability of the fashion product and processes consists in achieving, by means of the dominant culture, subculture and alternative culture in a system, a diversity of creation that is in opposition to the universal monoculture imposed by globalization.

THEORETICAL FOUNDATIONS OF IDENTITY DESIGN METHOD

We know from practice that, in the textile and leather processing industries, there is a system of fashion, and a practice of using the notion of fashion over the entire value chain of the product, a practice more or less coherent and assumed by clothing manufacturers. All activities pertaining to product conceptualization, design and communication are components of immaterial production, a production dominated by the creativity and artistic expression of the product, brand or trademark. All this can be seen in the aesthetics of a product through its style, through the cultural items that a consumer notices and identifies with, leading to the decision to purchase the product, in most cases. However immaterial production in fashion is less defined in manufacturing or processing of raw materials, as the division of labor is very high and the whole concept of a product dissipates into its components, converted into parts which no longer carry the entire philosophy of the product. Unlike the industrial manufacturing of a fashion product, which requires a technological process divided into several operations, where the immaterial fashion production dissipates, in creative companies, based on prototype and short series, where handmade processes are preponderant, and the ability of keeping the philosophy, aesthetics and artistic message of the product and of the author are recognizable. Thus, we identify a mass fashion and designer fashion, this time not by encodings of international standards, but by the artistic codes of immaterial production. Designer production is one based on identity because it is focused on the identity of the creative individual. In the industrial fashion production there is an identity of the brand and one of the trademark, that may be oriented towards an ethnic identity or a corporate one. This type of identity is not limited to just graphics that define the visual identity of the brand or trademark, but also encompasses the cultural identity of the product. As corporate identity is best evidenced by the graphic design practices of a globalized culture, ethnic identity design analysis may reveal mechanisms of creativity and can emphasize the dominant cultural items.

The sustainable identity-based product development method we propose was developed in the field of fashion and textile arts and design applied to textiles and is based on two pillars of understanding the creative processes:

- the use of cultural instruments;
- conceptual hybridization actions.

Cultural sustainability of the product of creative industries is expressed by:

- the authenticity of creation;
- the uniqueness of innovation;
- the stylistic concept.

Sustainability is created using cultural instruments:

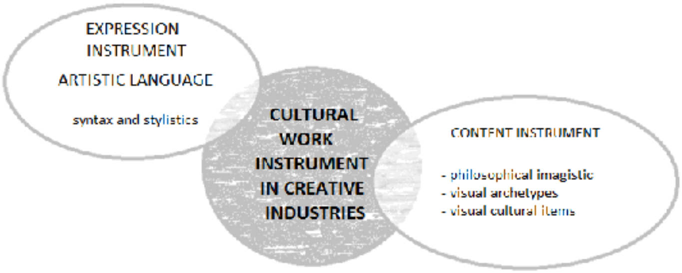

Figure 2. Cultural instruments

In fashion, the most common cultural work instruments range from images or visual elements, to cultural items of: trends, stylistic themes, motifs, mythological, philosophical and artistic images, archetypes and visual cultural elements.

The cultural instruments of the creative individual have two components: semiotic codes and aesthetic material resources. These are two keys to understanding the cultural dimensions of creative products that are subject to permanent change.

Semiotic codes emphasize the symbolic nature of the product and are an expression of artistic language that gives meaning to the work of the creative individual (Hirsch, 1972; 2000). Semiotic codes vary depending on the conventions of artistic systems they are a part of. Symbolic codes change much more slowly in the area of classical music or museum structures, focusing on refining the field, while the cultural system of fashion is very dynamic and permanent.

The aesthetic material resources include not only the materials that shape creative products, but also technologies and socio-technical systems that enable the production and consumption of creative products (Miller, 2010).

The material resources of creative products entail distinct ecosystems of knowledge, types of suppliers, artists and consumers that allow development through co-creation, proactive design, and collaborative design.

The so-called hybrid combinations of changing the semiotic codes and aesthetic material resources, which occur in all types of creative thinking, lead to an increase in creativity, and therefore, a permanent cultural and economic development of such industries. These transversal actions are processes of Conservation, Ideation, Transformation and Recreation.

Conservation, used in museums, architecture, design, fashion, music, media, cinema refers to the collection and preservation of a semiotic code pertaining to a cultural instrument such as the style of an art object, the ornamental motif of a fabric, the expression of a character, etc., and its recurrence in a new product.

Ideation processes, specific to areas such as fashion, music, digital games, cinema, media, visual arts, design and architecture, refer to original and innovative processes of creation that provide new aesthetic elements through both innovation and creative diversity of aesthetic concepts;

Transformation is a kind of thinking in design, and therefore creation, applied in creative industries, by means of which the aesthetic material resources are acted upon using cultural instruments, in order to create a greater diversity of cultural objects and creative products, transforming matter.

Recreation is a creative process that occurs in all creative industries and refers to shaping the semiotic codes and aesthetic material resources recognized as valuable and transforming them into new creative ideas, objects and artistic processes that are subject to economic niches.

EXPERIMENT

During the development of a complex work instrument regarding the Romanian cultural identity, called the Romanian Imagistic Repertoire, the identity design method, whose instrumental elements were listed above, was attempted together with a team of Master's students of the National University of Arts (UNA), Bucharest. Thus, during work sessions, each Master's student has chosen a Romanian landmark, that they have used to develop the scope and structure of their personal imaginary, and then to create their own sketches of ideas and products (Figures 3 and 4).

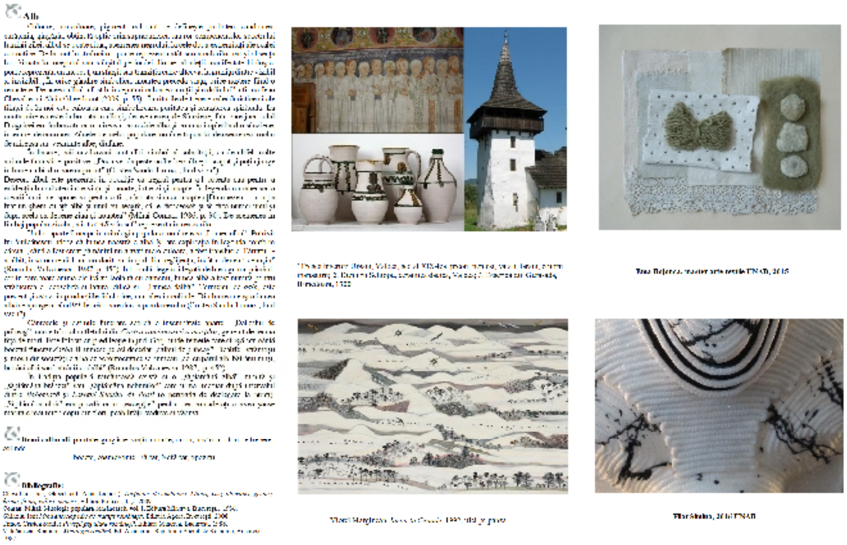

Figure 3. Imagistic instrument in the Romanian Imagistic Repertoire

Figure 4. Fashion design works of UNA students, 2014 and 2016

CONCLUSIONS

It was noted during experiments, that any additional visual knowledge increases the value of ideas and concepts. The knowledge base of students or any creative individual can be developed, on the recommendation of the study or work environment of each person that implements the identity design method.

It was also noted that collaborative design is more semantically, cognitively and emotionally charged than the average individual design.

In addition, experiments have shown that the cultural sustainability of creative industries products will exist as long as the appropriate cultural instruments are used, both in terms of semiotic and semantic code, and aesthetic material resources.

In this context, in the Romanian identity design, the Romanian Imagistic Repertoire developed by INCDTP-ICPI in cooperation with UNA Bucharest in the experimental part, within the AAICREA project, is a response to the need to map the Romanian identity cultural instruments. The repertoire constitutes an aesthetic material resource that comprises aesthetic identity codes, crucial for a creative individual, artist or designer in the creative industries.

REFERENCES

Bruter, M. (2001), “Understanding identity realignments: The emergence of a mass European identity”. Doctoral dissertation, University of Houston, Texas.

Burns, K., Ingram, J. and Annable, L. (2016), “Mapping design knowledge: 36 years of Design Studies”, *50th Anniversary Conference*, 27-30 June 2016, Brigthon, UK.

Carlile, R.P. (2002), “A pragmatic view of knowledge and boundaries: boundary objects in new product development”, *Organisational Science*, 13(4), 442-455.

Chai, K. and Xiao, X. (2012), “Understanding design research: a biliometric anlysis of Design Studies (1996 – 2010)”, *Design Studies*, 33, 24-43.

Flew, T. (2013), *Global Creative Industries*, Polity Press Cambridge, UK.

Hirsh, D. (2010), “Researching vocabulary”, in Brian Paltridge, Aek Phakiti (Eds.), *Continuum Companion to Research Methods in Applied Linguistics*, 222-239, London: Continuum.

Holmes, D.R. and Marcus, G.E. (2004), “Cultures of expertise and the management of globalization: Toward the re-functioning of ethnography”, in Ong and Collier (eds), *Global Assemblages: Technology, Politics and Ethics as Anthropological Problems*, London, Blackwell.

Ingram, J., Shove, E. and Watson, M. (2007), “Products and Practices: Selected Concepts from Science and Technology Studies and from Social Theories of Consumption and Practice”, *Design Issues*, 23(2), 3-16.

Kenton, S.B. and Valentine, D. (1997), *CrossTalk: Communicating in a multicultural workplace*, Upper Saddle River, NJ: Prentice Hall.

Low, S.M. and Lawrence-Zúñiga, D. (eds) (2003), *The Anthropology of Space and Place: Locating Culture*, Oxford: Blackwell.

Marcus, G.E. (1995), “Ethnography in/of the world system: The emergence of multi-sited ethnography”, *Annual Review of Anthropology*, 24, 95–117.

Maxwell, D., Edwards, L., Pillatt, T. and Niamh, D. (2016), “Stories in a Beespoon: Exploring Future Folklore through Design”, *50th Anniversary Conference*, 27-30 June 2016, Brigthon, UK.

Miller, H. (2010), *Brand Guidelines*, Herman Miller Inc, 2010.

Thornton, R. (1988), “The Rhetoric of Ethnographic Holism”, *Cultural Anthropology*, 3, 285–303.

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WITHIN INNOVATION
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