COLLAGEN POLYDISPERSIONS WITH SPECIFIC PROPERTIES FOR SEEDS TREATMENT

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Collagen polydispersions obtained by associated enzymatic and chemical processes for high-yield extraction of collagen from leather waste, under mild reaction conditions, are fit for application in agriculture. The present study highlights the specific properties of collagen polydispersions for cereal seed treatment. Collagen polydispersions were characterized by chemical and instrumental analyses: gravimetric, volumetric, potentiometry, gas chromatography and HPLC, IR spectroscopy, tensiometer methods, Dynamic Light Scattering (DLS). Analytical investigation has shown that the collagen polydispersions have bioactive properties due to the content of free amino acids, with a total of approximately 10 g/100 ml solution and very small sized particles composition, in the 1-10 nm range, able to penetrate the seed coating; the larger sized particles, situated in 100-1000 nm and 1000-10000 nm ranges, ensure the bioactive deposit in the film matrix on the surface; the wetting ability of collagen polydispersions, which is lower than that of water, ensures film matrix formation on seed surface, with long term releasing ability, leading to seed nutrition and stimulating germination. The synergy of collagen hydrophilicity, its known biodegradability, bio-active potential and film-forming properties recommend collagen polydispersions for applications in mixtures for seed treatment.

Keywords: collagen, chromium-tanned waste, seed treatment.

INTRODUCTION

Development of concepts for collagen-based biomaterials began many years ago and new elements are constantly revealed (Ramshaw *et al.*, 2001; Trandafir *et al.*, 2007). In present, the materials based on protein extracted from collagen and collagen matrix materials, mainly used in medical, pharmaceutical and cosmetics fields, are made using primary collagen resources (Santos *et al.*, 2013; Jayathilakan *et al.*, 2012). In order to use proteins in agriculture, secondary collagen resources such as by-products from natural leather processing industry were identified. Most of the research in recent years has focused on extracting collagen from leather waste (Jian *et al.*, 2008; Zainescu *et al.*, 2011) and using these extracts in crop fertilization (Lacatus *et al.*, 2009; Gaidau *et al.*, 2009), as an alternative for the synthesis amino acids used in fertilizer formulas (Chitu *et al.*, 2010; Light *et al.*, 2005) or as an amendment to agricultural soils (Zainescu *et al.*, 2010), but the aim has been to recover collagen from untanned hide waste. However, the largest amount of leather waste resulting from tanneries is chrome tanned leather waste (over 20% of the preserved hides that are processed), which is on the one hand, an environmental problem, and on the other hand, an untapped resource of protein. More recent research focuses on the exploitation of bio-active properties of these collagen extracts in seed treatment to increase germination potential and protect against pests (Gaidau *et al.*, 2013; Lomate *et al.*, 2011).

This paper is focused on presented the collagen extracts recovered, from chromium(III) tanned leather waste, a systematically avoided resource, due to the psychological impact generated by the term “chrome”. Only a small segment of the population knows that modern leather industry, which applies the best practices, neither

uses nor directly discharges hexavalent chromium, generator of extreme toxic effects. Our own previous research (Niculescu *et al.*, 2009; Gaidau *et al.*, 2010) has shown that combined processes for extracting collagen from these by-products may have a very high efficiency in separation of chromium, so that its content in collagen polydispersions would be within the strict limits allowed for drinking water (max. 50 ppb). In this research the collagen polydispersions are obtained by associated chemical-enzymatic processes with high yields extraction, under mild reaction conditions. The biological potential and film-forming properties of collagen polydispersions, recommend this as a valuable bioactive additive in mixtures for seeds treatment.

EXPERIMENTAL

Experimental Techniques

The collagen hydrolysates were obtained from tanned leather by-products, by chemical-enzymatic processing, at atmospheric pressure and temperature ≤ 80°C, for a total duration of max. 6 hours, followed by processing the resulting product be decanting and filtering, mixing batches and conditioning by adjusting pH at max. 7.

Methods of Analysis

The collagen hydrolysates obtained were analysed using: STAS 8574-92, Finished hides and fur finished hides; SR ISO 5397-96 for total nitrogen determination; SR EN ISO 4045-02 for pH determination; SR EN ISO 13903-05 for determination of amino acids content; Sorensen method to determine aminic nitrogen; STAS 8602-90 for chrome oxide determination; chromatography, with a gas chromatograph coupled with a mass spectrometer, AGILENT 7000 GC/MS TRIPLE QUAD Gas Chromatograph, to identify dipeptide, tripeptide and amino acid sequences and HPLC (Thermo Electron, Finningen Surveier) was used for the qualitative and quantitative determination of aminoacids (according to SR EN ISO 13903); IR spectral analysis by FT/IR-4200 (Jasco) with ATR device equipped; Dynamic Light Scattering (DLS), to determine particle size and distribution with ZetaSizer device Nano ZS (Malvern, UK); the CAM 200 optical system from KSV Instruments was used to measure contact angles under static conditions.

RESULTS AND DISCUSSIONS

The intermediary and final collagen hydrolysates, described in Table 1, were assessed by chemical and instrumental analyses, to establish chemical and physical characteristics and bioactive properties induced by the free amino acid content.

Table 1. Description of collagen hydrolysates samples

Sample code	L1C	L2C	MC	CMC
Description	Collagen hydrolysate from lot no. 1	Collagen hydrolysate from lot no. 2	Mixture of collagen hydrolysates from lots no. 1 and no. 2	Conditioned mixture of collagen hydrolysates, at pH = 7

Results of analyses performed in order to establish the chemical composition of collagen hydrolysates are presented in Table 2.

Table 2. Physical-chemical characteristics for collagen hydrolysates

No.	Characteristics, MU	L1C	L2C	MC	CMC
1	Dry substance, %	8.00	8.16	8.24	8.77
2	Total ash, %	8.50	8.95	7.89	7.87
3	Organic substance, %	91.50	91.05	92.11	92.13
4	Total nitrogen, %	16.13	15.69	15.78	15.17
5	Amino nitrogen, %	1.12	1.20	1.04	1.04
6	Average molecular weights, Da	10800	8800	12900	12900
7	Protein substance, %	90.63	88,11	88.71	85.18
8	pH	9.38	9.35	8.88	6.90

Aminic nitrogen content over 1%, corresponding to an average molecular mass below 13000 Da, indicates the possibility of a very high polydispersion of protein fragments. The presence of dipeptide, tripeptide and free amino acid fragments in collagen hydrolysates was investigated by chromatography, using a gas chromatograph coupled with a mass spectrometer.

Figure 1 presents the chromatogram of a collagen polydispersion (L1C), with molecular masses of components marked on each peak. The chromatogram reflects the fact that dipeptide and tripeptide fragments were separated, as well as fragments with mass values close to the mass of amino acids, in accordance with the results of previous research and confirming the reproducibility of the framework model for developing collagen hydrolysates (Niculescu *et al.*, 2009).

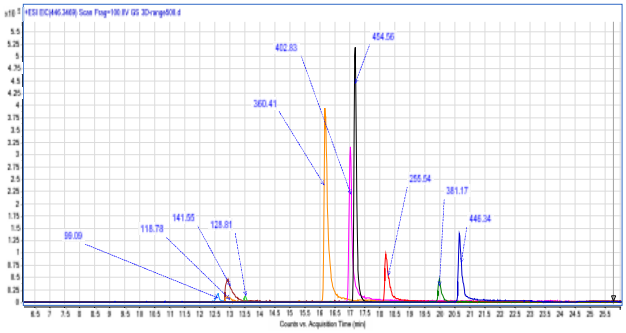

Figure 1. Chromatography of collagen hydrolysates

The IR-ATR spectroscopy method was used for the structural analysis of intermediary collagen hydrolysates and the experimental batch of collagen hydrolysate obtained by their combination. Corrections of ATR, CO_2 and H_20, baseline, were performed for each sample.

Figure 2 comparatively presents IR spectra of collagen hydrolysate batches collected from processes of hydrolytical disintegration of leather waste (collagen hydrolysates LC.1 and LC.2), of the final batch (MC.3) developed by combining batches of extracted hydrolysate and experimental batch obtained after pH correction (CMC.4).

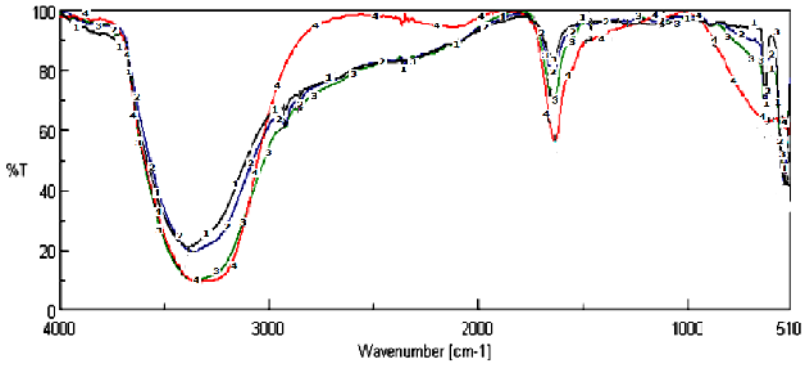

Figure 2. IR spectral analysis of collagen polydispersions: 1–L1C, 2–L2C, 3–MC, 4–CMC

Noteworthy is the presence of wave numbers in the 3100-2600 cm^{-1} and 1660-1610 cm^{-1} spectral ranges, specific to free amino acids, as well as in 1400-1465 cm^{-1} and 1264-1450 cm^{-1} spectral ranges, characteristics to proline amino acids, aspartic acid and glutamic acid (Balaban *et al.*, 1983; Barth, 2000). The spectral analysis provides information in accordance with chromatographic analysis, which signalled the dipeptide, tripeptide and amino acid content of collagen hydrolysates, extracted from semi-processed hide fragments.

Using the high-performance liquid chromatography method, it was established that collagen hydrolysates obtained by hydrolytical disintegration of residual semi-processed hide fragments have a total amino acid content of approximately 10 g/100 ml solution, with an average concentration of amino acids, presented in Figure 3.

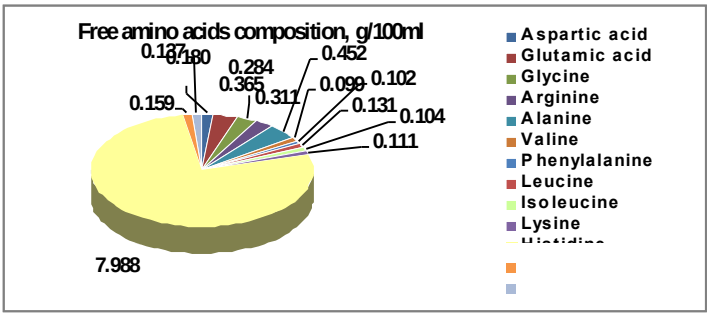

Figure 3. Average concentration of free amino acid in collagen hydrolysates

Particle size and distribution in the representative batches of collagen hydrolysates, L1C and L2C, obtained in this study, were analysed by DLS (Dynamic Light Scattering). Results of analyses are presented in table 3.

Table 3. Distribution of particle size in collagen hydrolysates

No.	Sample	Distribution of particle size, %		
		1-10 nm	100-1000 nm	1000-5000 nm
1	L1C	88.0	7.3	4.7
2	L2C	93.7	4.2	1.1

Another important feature of surface treatment products is the contact angle. The contact angle of a liquid drop and a solid surface is a sensitive indicator of changes in surface energy and in the chemical and supramolecular surface structure. Knowing the contact angle allows us to estimate the type of interaction between the surface and the liquid. In this regard, we determined contact angles of collagen hydrolysates in relation to glass, as inert control surface, and contact angles of collagen hydrolysates in relation to the surface of cereal seeds.

As seen in Figure 4, the contact angle varies in the 24°-44° range, relative to glass, but has much higher values and a broader range, 65°-105°, relative to the seed surface.

The contact angle of collagen hydrolysates in relation to seeds is found to be at least double compared to the contact angle in relation to glass, a consequence of surface roughness, on the one hand, and of collagen hydrolysate characteristics, on the other hand, important in this regard being the slight decrease of contact angle upon addition of acetic acid, for conditioned mixture of collagen hydrolysates, CMC compared with the mixture of collagen hydrolysates, MC.

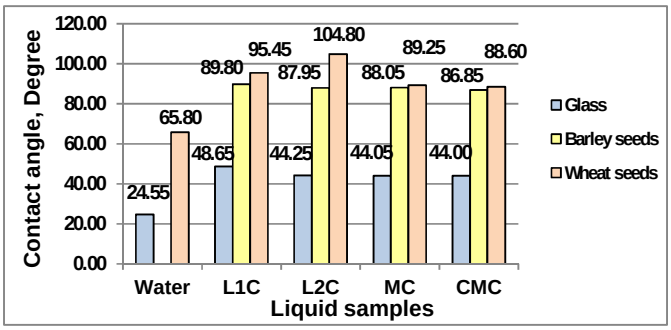

Figure 4. Comparative contact angles

It is noticed that particularly the wetting ability of collagen hydrolysates is lower than that of water, and their hydrophobicity is therefore favourable to the film matrix formation on seed surface.

CONCLUSIONS

It was proved that the collagen hydrolysate extracted from solid wastes from tanneries through chemical-enzymatic hydrolysis under atmospheric pressure conditions, at temperatures in the range of ≤ 80°C, by stirring, for maximum 6 hours, has bioactive properties, due to the diversified content of free amino acids, including essential amino acids, with a total of approximately 10 g/100 ml solution.

It was proved that the collagen hydrolysates predominantly contain very small sized particles, in the 1-10 nm range, able to penetrate the seed coating, but they also contain larger sized particles, situated in 100-1000 nm and 1000-10000 nm ranges, which will ensure the bioactive deposit in the film matrix on the surface.

The wetting ability of collagen hydrolysates is lower than that of water, property associated to the contact angle and favourable to film matrix formation on seed surface.

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