THE INFLUENCE OF MARINE ALGAE AND NATURAL PLANT OILS ON COLLAGEN-BASED CREAM PROPERTIES

DURMUŞ ALPASLAN KAYA[1], SELIN SAYIN[2], MARIANA FERDES[3], MADALINA GEORGIANA ALBU[4], NURDAN ÜNAL[2], MUSA TÜRKMEN[1], ŞEVKET ÖZTÜRK[1], RODICA-ROXANA CONSTANTINESCU[4], MIHAELA VIOLETA GHICA[5]

[1] *Mustafa Kemal University, Faculty of Agriculture, 31030, Antakya-Hatay, Turkey*

[2] *Mustafa Kemal University, Fisheries Faculty, 31200, Iskenderun, Turkey*

[3] *"Politehnica" University of Bucharest, Faculty of Biotechnical Engineering Systems, Bucharest, Romania*

[4] *The National Research & Development Institute for Textiles and Leather, Division Leather and Footwear Research Institute, Collagen Department, 93 Ion Minulescu Str., 031215, Bucharest, Romania, albu_mada@yahoo.com*

[5] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Physical and Colloidal Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania*

The aim of this paper was to investigate the effect of various marine algae, natural plant oils and Laurus nobilis L. essential oil on stability, rheological and antimicrobial properties of collagen-based cosmetic cream. For this purpose, 3 types of algae [*Spirulina platensis (Cyanophyta), Haematococcus pluvialis (Chlorophyta) and Laminaria dictyota (Phaeophyta)],* 3 types of natural plant oils (olive, nut and laurel) and one essential oil were incorporated in an comercial collagen-based cosmetic cream which was used as a control. The rheological assessment was carried out by analyzing pseudoplastic flow and thixotropic behaviour, stability was performed by centrifugation during time and microbiological tests were carried out against germs, yeasts and molds, *S. aureus*, coliforms and *E. coli* and *P. aeruginosa*. This work showed that all the tested collagen creams showed a pseudoplastic flow and a thixotropic behaviour which promotes the flow formulation and a corresponding application on the skin and they were stable. Among the all formulations, the best properties were given using the following ingredients: Spirulina platensis, olive oil and laurel essential oil.

Keywords: cream, algae, natural plant oil

INTRODUCTION

Cosmetic industry focuses its attention on natural ingredients for cosmetics uses in order to limitate the toxic solvents (Chaudhari *et al.*, 2011; Conde *et al.*, 2014). The algae found in marine environments exhibit anticoagulant, antiviral, antioxidative, anticancer, antiinflammatory and immunomodulatory actions, and could have potential for the development of nutraceutical, pharmaceutical and cosmetic products (Senevirathne and Kim, 2013). The dominating species of marine micro and macroalgae in commercial production includes *Haematococcus pluvialis*, *Padina pavonica*, *Sargassum* sp., *Coralina* sp., *Chlorella vulgaris*, *Spirulina platensis* and *Laminaria* sp. There are many studies carried out in the *in vitro* conditions, which evidence antimicrobial activity of essential oils (Kunicka-Styczyn´ska *et al.*, 2013). Therefore, it appears that these natural compounds can successfully be used in the cosmetic industry as a preservative. Creams are semisolid emulsions which are widely used as a means of altering the physical properties of the skin (particularly the hydration state). The structure of these creams has been the subject of considerable study optimising their physical properties depenting on the ingredients (Peramal *et al.*, 1997). For the semisolid systems generally, for the creams particularly, the rheological characteristics knowledge represents an important aspect in their formulation process because the flow parameters can determine adequate consistency, extrusion capacity from the recipient, quality and stability control during

storage, ease of application, adhesion on the skin (Ghica *et al.*, 2012b; Gilbert *et al.*, 2013; Savary *et al.*, 2013). The aim of this research was to study the influence of some algae, natural plant oils and essential oils on a known comercial cream (Reference CPNP number 1388019) in order to obtain new cream formulations.

MATERIALS AND METHODS

Materials

Three types of algae, Spirulina platensis (SP), Haematococcus pluvialis (HP) and Laminaria dictyota (LD), were obtained as powder.

Spirulina platensis is a microalga whose composition is suitable for use as food supplement and can be used to combat malnutrition (Fox, 1996). It's composition includes high levels of protein (64-74%), polyunsaturated fatty acids and vitamins (Cohen, 1997), and antioxidant compounds (Collar *et al.*, 2007). This microalgae is classified as GRAS (Generally Recognized as Safe) by FDA (Food and Drug Administration), which ensures its use as food without risk to health.

Haematococcus pluvialis is a freshwater species of Chlorophyta from the family Haematococcaceae. This species is well known for its high content of the strong antioxidant astaxanthin, which is important in aquaculture, and cosmetics (Lorentz and Cysewski, 2000).

Laminaria digitata is a large brown alga in the family Laminariaceae. L. digitata was traditionally used as a fertiliser and spread on the land. It is not only used as an organic fertiliser but also for the extraction of alginic acid, the manufacture of toothpastes and cosmetics, and in the food industry for binding, thickening and moulding (URL, 1).

Leaves of Laurel (Laurus nobilis L.) were collected from Amanos Mountain (Anatolian region) in blooming period and dried at room temperature. Essential oils were obtained from dried leaves. These samples were subjected to hydro distillation for 3 h using a Clevenger type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at – 20ºC until GC-MS analysis. Yield of obtained oils was 3.8% and main component of laurel essential oil was 1,8 cineol (eucalyptol) with 62% ratio.

"The cream with collagen and vitamins" with Reference CPNP number 1388019 were obtained by the currently used technology in Collagen Department of Division Leather and Footwear Research Institute and was used as reference collagen cream in this study.

Preparation of Creams

Starting from reference collagen cream, 10 new formulations of creams were prepared having the composition presented in Table 1.

Flow Behaviour Evaluation

The rheological properties of the tested creams were conducted with a rotational viscometer Multi-visc Rheometer-Fungilab equipped with standard spindle TR 8 and ultrathermostat ThermoHaake P5 maintained at 33^{o}C, keeping the same experimental conditions as previously described (Ghica *et al.*, 2012b). The viscometer allows, for each rotational speed or shear rate specific to the spindle used, the determination of the shear stress and the viscosity of the creams. For each formulation the ascending and descending rheograms were recorded. The rheological measurements were performed in triplicate and the average results were reported. The Power law model, expressed as

viscosity as a function of shear rate (eq. 1), was applied for the assessment of creams flow behaviour:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

where m and n are parameters correlated with the tested creams formulation factors and determined through the linearization of eq. (1) by double logarithmic method (Ghica *et al.*, 2012a).

Table 1. Composition of algae-based creams

New cream formulation	Reference cream (g)	Algae type	Solvent
C1	92.5	-	Water
C2	92.5	SP	Olive oil
C3	92.5	SP	Nut oil
C4	92.5	SP	Laurel oil
C5	92.5	SP	Water
C6	92.5	HP	Olive oil
C7	92.5	HP	Nut oil
C8	92.5	HP	Laurel oil
C9	92.5	HP	Water
C10	92.5	LD	Nut oil

The ratio between algae and solvent was 2:5. All the samples were treated with laurel essential oils 4 % (v/v).

Stability Determination by Centrifugation

5 g sample of every creams were put into vials and were centrifugated at 4500 rot/min during 30 min for 3 times using centrifuge (Hettich Micro 200 mode). The sample was considered stable if it was not separated into two phases.

Microbiological Analysis

Standard plate count method was used for the determination of total aerobic microbial count (TAMC) on Plate Count Agar culture medium, at 30 ± 1°C. The selective enumeration of yeasts and molds was carried out on Chloramphenicol Yeast Glucose Agar medium at 25 ± 1°C after 5 days of incubation. *Staphilococcus aureus* was analysed on Baird Parker Agar medium. Coliforms and *Escherichia coli* were determined using Violet Red Bile Agar (VRBL) medium. The detection of *Pseudomonas aeruginosa* was made on Cetrimide Agar medium. Reference strains were used for all these analyses.

RESULTS AND DISCUSSION

The flow profiles for the creams analyzed at 33°C recorded as viscosity versus shear rate are presented in Figure 1a-d. The rheological profiles illustrated in Figure 1a-d indicated for all tested collagen creams a typical non-newtonian pseudoplastic behaviour, the viscosity decreasing for the shear stress increase. This behaviour facilitates the creams flow. Similar flow patterns were obtained for the creams with the same type of algae (Figure 1a-b). Figure 1c shows that the reference collagen cream is more viscous than cream with HP and SP. From Figure 1d it can be noticed that the presence of LD in cream formulation induced a significant increase of viscosity.

The quantification of rheological behaviour of tested creams was realized by means of the Power law model (eq. 1). The values of the parameters m and n as well as of the correlation coefficient characteristics to this model are listed in Table 2.

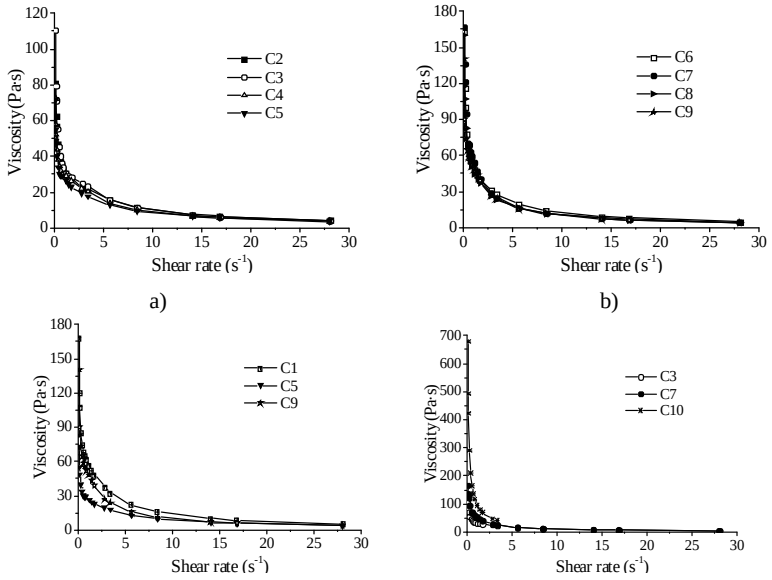

Figure 1. The rheograms recorded at 33°C for: a) Creams with SP and different types of oils; b) Creams with HP and different oils; c) reference cream and creams with different algae in water; d) creams with different algae and nut oil

Table 2. The values of the m and n parameters and the correlation coefficients specific to the Power law model applied to collagen creams

Cream	m	n	R
C1	52.564	0.440	0.9905
C2	29.879	0.381	0.9926
C3	31.802	0.474	0.9939
C4	27.780	0.374	0.9908
C5	25.263	0.397	0.9924
C6	46.176	0.477	0.9931
C7	50.168	0.487	0.9950
C8	44.089	0.505	0.9979
C9	42.834	0.438	0.9819
C10	110.210	0.730	0.9982

Analyzing the data presented in Table 2 it can be observed that the values recorded for parameter m (associated with the viscosity obtained for the shear rate of $1 \cdot s^{-1}$) (Ghica et al., 2012a) decreased by adding the SP in the reference collagen cream composition by 1.65-2.08 times and 1.04-1.22 times by adding HP respectivelly, while the presence of LD algae determines an increase of about 2.09 times.

The creams were also tested in terms of thixotropic behaviour. In Figure 2 the ascending and descending flow curves for the creams C1, C4, C8 and C10 are shown for exemplification.

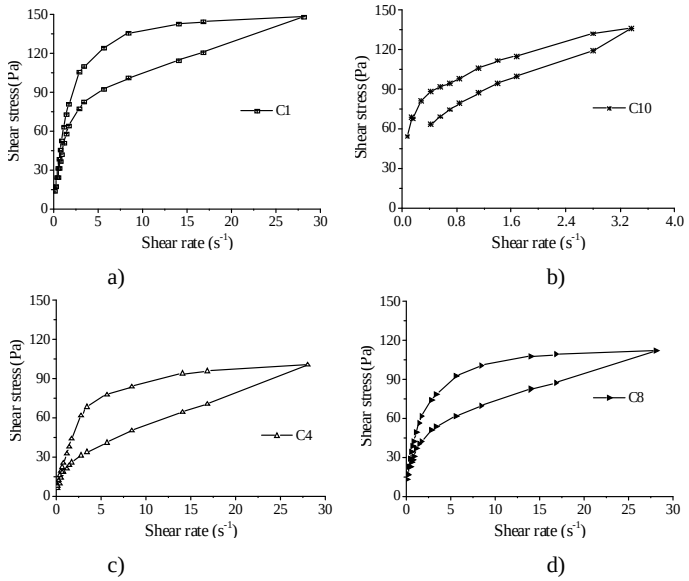

Figure 2. Flow curves (up/down) obtained at 33°C for collagen creams: C1; b) C10; c) C4; d) C8

For the same shear rate, the point on the descending curve corresponds to lower shear stress compared to the ascending curves, obtaining the hysteresis thixotropy area (Ghica et al., 2012b). All the formulation presented a thixotropic behaviour, which cause easily to display on skin.

All the creams were studied by stability point of view and all of the were very stable. Although, we noticed some differences between them as following: the best stability was observed for samples 6 and 1 and lower stability was for samples 7 and 9.

The microbiological analysis showed that *S. aureus*, coliforms and *E. coli* and *P. aeruginosa*, yeasts and molds were absent for all the samples. Some of samples contain aerobic microorganisms as we presented in the Table 3.

Table 3. Total aerobic microbial count (TAMC), (CFU g-1) developed by creams at 30°C

Cream	TAMC	Cream with LO	TAMC
C1	0	C1*	0
C2	0	C2*	0
C3	0	C3*	0
C4	0	C4*	0
C5	45	C5*	40
C6	15	C6*	0
C7	145	C7*	10
C8	120	C8*	90
C9	95	C9*	30
C10	200	C10*	10

As we can notice from Table 3, the ingredients influence the microbiological properties of creams. Comparing the microbiological activity of algae into creams we can see that *S. platensis* is the most efficient one comparing with *H. pluvialis* and *L. digitata*. *L. digitata* is not recommended to be added in cream as such because developed germs 2 times more than admitted limit. It could be added only with essesntial oil. From natural plant oil the most efficient were olive oil followed by laurel and than nut oil. The laurel essential oil decreased significantly the microbial contamination level for all the samples.

CONCLUSIONS

All the tested collagen creams showed a pseudoplastic flow and a thixotropic behaviour which promotes the flow formulation and a corresponding application on the skin. All the tested creams were stable. Among the all formulations, the best properties were given using the following ingredients: *Spirulina platensis*, olive oil and laurel essential oil.

Acknowledgements

The financial support offered by UEFISCDI through PN-II-PT-PCCA-2013-4-0415 (acronym GREENVET) projects is gratefully acknowledged.

REFERENCES

Albu, M.G., Ferdeş, M., Kaya, D.A. *et al.* (2012), "Collagen Wound Dressings with Anti-inflammatory Activity", *Mol. Cryst. Liq. Cryst.*, 555 (1), 271-279.

Balboa, E.M., Soto, M.L., Nogueira, D.R. *et al.* (2014), "Potential of Antioxidant Extracts Produced by Aqueous Processing of Renewable Resources for the Formulation of Cosmetics", *Ind. Crops Prod.*, 58, 104-110.

Chaudhari, P.M., Kawade P.V. and Funne, S.M. (2011), "Cosmeceuticals: a review", *Int. J. Pharm. Tech.*, 3, 774–798.

Cohen, Z. The chemicals of Spirulina (1997) In: Vonshak, "A. Spirulina Platensis (Arthrospira) Physiology, Cell-Biology and Biotechnology. London: Taylor & Francis.

Collar, L.M., Reinehr, C.O., Reichert, C. *et al.* (2007), "Production of Biomass and Nutraceutical Compounds by Spirulina Platensis under Different Temperature and Nitrogen Regimes", *Bioresour. Technol.*, 98(7), 1489-1493.

Fox, R.D. (1996), "Spirulina Production & Potential". Paris: Edisud.

Ghica, M.V., Albu, M.G., Coară, Gh. *et al.* (2012a), "The Influence of Crosslinking Agent on Kinetic Release and Rheological Behaviour of Some Collagen-Niflumic Acid Hydrogels", *Proceedings of the 4th Conference on Advanced Materials and Systems (ICAMS), Bucharest, Romania*, 267-272.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C. *et al.* (2012b), "*In Vitro* Kinetic Release and Flow Behaviour of Some Collagen-Minocycline Topical hydrogels", *Rev. Chim.- Bucharest*, 63(9), 929-935.

Gilbert, L., Picard, C., Savary, G. *et al.* (2013), "Rheological and Textural Characterization of Cosmetic Emulsions Containing Natural and Synthetic Polymers: Relationships Between Both Data", *Colloid Surface A* , 421, 150-163.

Kunicka-Styczyn´ska A., Sikora M. and Kalemba D. (2009) "Antimicrobial Activity of Lavender, Tea Tree and Lemon Oils in Cosmetic Preservative Systems", *J. Appl. Microbiol.*, 107, 1903-1911.

Lorentz, R. T. and Cysewski, G.R. (2000), "Commercial Potential for *Haematococcus* Microalgae as a Natural Source of Astaxanthin", *Trends. Biotechnol*. 18, 160-167.

Peramal, V.L., Tamburic, S. and Craig, D.Q.M. (1997), "Characterisation of the Variation in the Physical Properties of Commercial Creams using Thermogravimetric Analysis and Rheology", *Int. J. Pharm.*, 155, 91-98.

Savary, G., Grisel M. and Picard, C. (2013), "Impact of Emollients on the Spreading Properties of Cosmetic Products: A Combined Sensory and Instrumental Characterization", *Colloid Surface B*, 102, 371-378.

Senevirathne, W.S.M., Kim, S.-K. (2013), "Cosmeceuticals from Algae", *Funct. Ingredients Algae Foods Nutrac.*, 694-713.

URL 1, *Laminaria digitata* (Hudson) J.V. Lamouroux http://seaweed.ie/descriptions/laminaria_digitata.php. retrieved. 27.08.2014.