UNIVERSITY POLITEHNICA OF BUCHAREST

Faculty of Applied Chemistry and Materials Science

Department of Organic Chemistry "Costin Nenitescu"

PhD Thesis Summary

Hybrid bioproducts based on fish collagen and natural extracts

Author: chem. Elena DĂNILĂ

PhD supervisor: Prof.dr.Ing. Raluca Liane STAN

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Keywords: hydrolyzed fish collagen, biocompatibility, essential oils, antimicrobial activity, hybrid bioproducts

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II. ORIGINAL CONTRIBUTIONS

The purpose and objectives of the doctoral thesis

The main objective of the thesis was to obtain fish collagen and natural extracts (essential oils), as well as their characterization, in order to obtain hybrid bioproducts. In order to achieve this main objective, the following specific objectives have been met:

1) Extraction and characterization of collagen from fish skin (carp).
2) Obtaining and characterizing plant extracts (essential oils of lavender, lemon and pink pepper).
3) Obtaining and characterizing bioproducts based on fish collagen and natural extracts: serum with collagen and AHA acid for the treatment of pigment spots, oleogels with cosmetic applications based on vegetable oils with SPF and lavender essential oil, emulsions based on hydrolyzed collagen and natural extracts with potential anti-cellulite action, emulsions based on hydrolyzed collagen and natural extracts with potential anti-acne action, emulsions based on hydrolyzed collagen and natural extracts used as cc-cream or foundation.

RESULTS AND DISCUSSIONS

5. OBTAINING AND CHARACTERIZING COLLAGEN FROM FISH SKIN

5.1. COLLAGEN EXTRACTION TECHNOLOGY FROM FISH SKIN

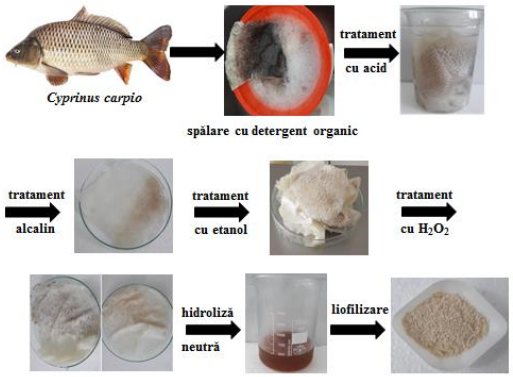

Figure 13. Steps for obtaining collagen hydrolysates from fish skin

Tabel 5. Acid and alkaline treatment of carp skin samples, collagen hydrolyzate extraction yield

Fish skin sample	Acid treatment Ratio fish skin: acid solution (m/V)	Alkaline treatment Ratio fish skin: alkaline solution (m/V)	Collagen hydrolyzate sample	Extraction yield
HO4P1s	3% mixture of citric and lactic acid solution 1:1 1:3	NaOH 1 M 1:10	HO4P1	35.5
HO4P2s	3% lactic acid solution 1:3	NaCl 1 M 1:5	HO4P2	28.1
HO4P3s	3% lactic acid solution 1:3	$NaHCO_3$ 1 M 1:10	HO4P3	24.6
HO4P4s	3% citric acid solution 1:3	NaOH 1 M 1:10	HO4P4	29.3
HO4P5s	3% lactic acid solution 1:3	NaOH 1 M 1:10	gelatină	-

The extraction yields shown in Table 5 of the collagen hydrolysates obtained according to the processing conditions were: 35.5% for HO4P1 (3% lactic and citric acid, 1M NaOH), 28.1% for HO4P2 (3% lactic acid, 1M NaCl), 24.6% for HO4P3 (3% lactic acid, 1M NaHCO3) and 29.3% for HO4P4 (3% citric acid, 1M NaOH). Mixing organic acids and pre-hydrolysis treatment with 1M NaOH (HO4P1 sample) proved to be the most effective process. The yields obtained by extraction are in accordance with the acid-soluble collagen obtained from carp skin, *Cyprinus carpio*-41.3% and *Ctenopharyngodon idellus* -25.5% [1]. Data from the literature report different extraction yields for acid-soluble fish skin collagen (Snapper red -5.71%, milk fish -4.00%, nautilus -55.2%, Japanese perch -51.4% , black mackerel -49.8%, shark -50.1%) explained as a difference in solubility in acidic solutions due to the degree of crosslinking of collagen molecules.

5.2. CHARACTERIZATION OF COLAGEN FROM FISH SKIN

5.2.1. Physico-chemical characterization

The results of the analyzes are presented in Table 6:

Tabel 6. Physico-chemical characteristics of fish skin collagen hydrolysates obtained by different methods

No. crt.	**Characteristics**	**Determined values**				**The standard of method**
		HO_4P_1	**HO_4P_2**	**HO_4P_3**	**HO_4P_4**	
1	Dry substance, %	92,89 ± 0.39	91,18 ± 0.38	92,13± 0.38	91,06± 0.38	S EN ISO 4684:2006
2	Ash, %	nedetectabil	nedetectabil	nedetectabil	nedetectabil	SR EN ISO 4047:2002
3	Total nitrogen, %	17,41± 0.11	17,38± 0.11	17,44± 0.11	17,55± 0.12	SR ISO 5397:1996
4	Protein substance, %	97,84± 2.61	97,68± 2.59	97,99± 2.61	98,62± 2.62	SR ISO 5397:1996
5	pHof 10% solution, unit. of pH	6,94± 0.1	6,24± 0.1	6,15± 0.1	6,18± 0.1	STAS 8619/3:1990
6	Aminic nitrogen, %	0,71	0,55	0.96	0.77	
7	DH, %	4.08	3.16	5.5	4.38	

Examining the data in Table 6 we can conclude that all the samples of fish collagen hydrolyzate obtained have a high degree of purity due to the high protein content (approximately 98%) and undetected values for ash content, being an important advantage of the method. extraction used. By comparison, the data in the literature reported for the acid-soluble cyprinus carpio skin collagen hydrolyzate show a protein content of 27-27.9% and an ash content of 0.22-1.21% [2]. The proposed procedure for obtaining collagen hydrolysates has led to samples with pH values in the range of 6-7, which indicates that the hydrolysates obtained are suitable for use in food supplements or cosmetics. Further information was obtained by examining the values of total nitrogen, Nt and amine nitrogen (NH2) to obtain an estimate of the degree of hydrolysis, DH [3]. As the degree of hydrolysis increases, the molecular weight of the hydrolyzate decreases, an estimation of the latter based on the amine nitrogen content being possible according to the previously reported results [4]. Thus, HO4P2 is the hydrolysate with the highest

molecular weight (DH value 3.16%, estimated average molecular weight> 25,000 Da), while the estimated molecular weight for the other samples varies between 13,000–20,000 Da. This can be explained by the differences in the treatment applied, the pKa value of the acid involved in the pretreatment step and the nature of the non-collagen protein remover: the HO4P2 sample was obtained after lactic acid treatment (pKa = 3.85, it was shown to favors hydrolysis to gelatin) and a mild saline treatment with 1 M NaCl to remove non-collagenous proteins. Acid and alkaline pretreatment of skin samples (HO4P1, HO4P3 and HO4P4, Table 12) resulted in collagen hydrolysates with a higher DH probably due to a more efficient swelling process that allows the cleavage of non-covalent bonds and intra-molecular bonds. followed by partial hydrolysis [5].

5.2.2. Infrared spectroscopy analysis (FTIR)

The IR spectra obtained by the FT-IR ATR technique (IR spectrometry with total attenuated reflection) allowed the identification of the functional groups in the collagen composition for the collagen hydrolyzate samples (Figure 15):

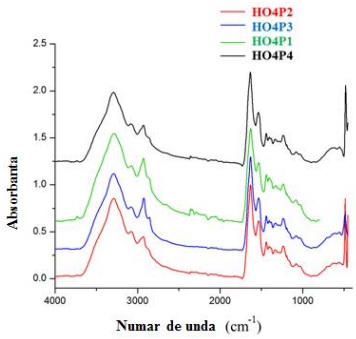

Figure 15. FT-IR spectrum for fish collagen hydrolysates

Collagen samples show the spectral bands characteristic of collagen-specific functional groups: amide A - secondary amine stretching vibrations of the NH group, amide B - asymmetric stretching vibrations of the methylene group, amide I - stretching vibrations of the carbonyl group, amide II and amide III deformation vibrations of the N - H group coupled with the tensile vibrations of the CN and CH groups, no significant differences were observed between the samples.

Amide I deconvolution (Figure 16) provided interesting information about the conformational changes of proteins in the fish skin hydrolysates obtained.

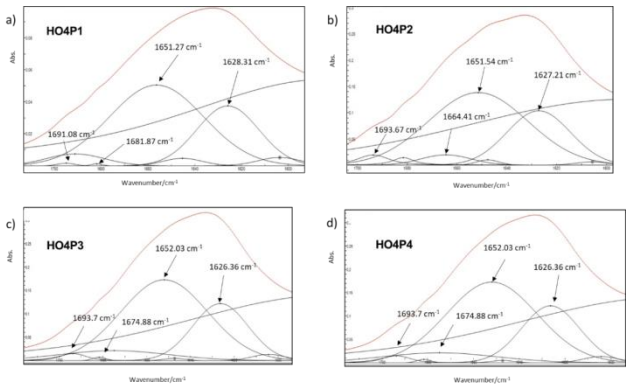

Figure 16. Deconvolution of Amide I band for fish collagen hydrolysates with fixed band components

According to literature data [6, 7], the component peaks obtained after the deconvolution of amide band I for the examined collagen hydrolyzate samples can be assigned as follows:

- Peak 1, 1625-1628 cm^{-1} - intramolecular beta sheets;
- Peak 2, 1651-1656 cm^{-1} - random coil-type conformations, imide residues and α helical conformations;
- Peak 3, 1665-1681 cm^{-1} - β-turns of telopeptides C and N in collagen;
- Peak 4, 1691-1694 cm^{-1} - gelatin and spirals of aggregated peptides.

Intense absorption at 1660 cm-1 is generally attributed to the triple helix state of collagen, a decrease in band intensity accompanied by intensification of the bands around 1630 cm^{-1} being generally associated with thermal denaturation of collagen. Examining the peaks of the deconvolution component of amide I for fish collagen hydrolyzate samples, no characteristic absorption of the triple helix structure is observed, the most intense component being due to random coil conformations, imide residues and α-like helical conformations (peak 2 to 1651-1656 cm^{-1}).

5.2.3. Circular dichroism (CD)

The CD spectra of the analyzed fish collagen samples are shown in Fig. 17:

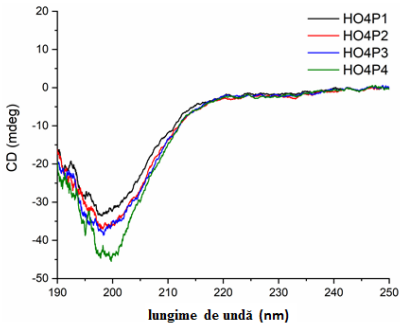

Figure 17. CD spectrum of fish collagen hydrolysates analyzed

All fish collagen hydrolyzate samples analyzed showed a pronounced negative band around 196 to 200 nm, typical of a coiled (coil) random structure and no positive maximum at 220 nm wavelength, characteristic of triple helix. Thus, a specific structure characteristic of denatured collagen (hydrolyzed collagen) has been assigned to all HO4P1-HO4P4 fish collagen extracts. [8].

5.2.4. X-ray diffraction analysis (XRD)

The X-ray spectra of the lyophilized fish collagen hydrolyzate samples shown in Fig. 18 does not show any sharp peak associated with the triple helical structure, due to the loss of native conformations of collagen during the hydrolysis process, also confirmed by the FT-IR spectrum through amide I and the circular dichroism spectra.

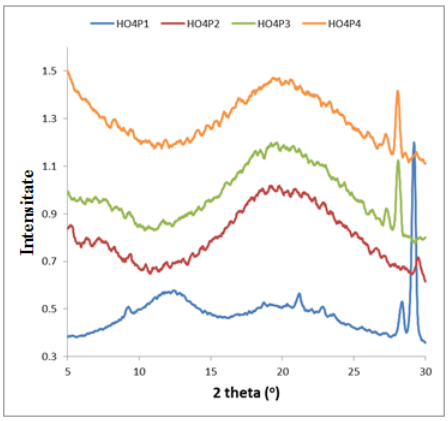

Figure 18. X-ray diffraction spectrum (XRD) for collagen hydrolysates from fish

The second peak is present at 21.19º (HO4P1), 19.18º (HO4P2), 19.28º (HO4P3) and 19.84º (HO4P4), respectively. Bragg's equation d (Å) = λ / 2sin θ (where λ is the X-ray wavelength (1.54 Å) and θ is the Bragg diffraction angle), was used to calculate the

minimum value of repeated distances. The distance between the skeletons was 4.18 Å (HO4P1), 4.62 Å (HO4P2), 4.59 Å (HO4P3) and 4.47 Å (HO4P4), respectively, according to the reported values for collagen extracted from other species. of fish [9].

5.2.5. Determination of the isoelectric point (pI)

Zeta potential measurements were performed for each fish pH collagen hydrolyzate sample at variable pH (Fig. 19):

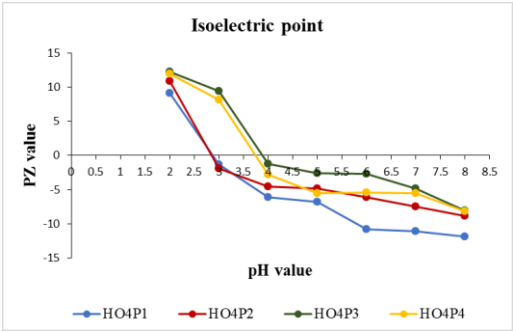

Figure 19. Isoelectric point (pI) for collagen hydrolysates in fish

The values calculated for pI vary between 3.9 and 2.9 as follows: pI = 2.9 for HO4P1 sample, pI = 2.9 for HO4P2 sample; pI = 3.7 for HO4P3 sample and pI = 3.9 for HO4P4 sample, respectively. Similar values of pI for acid-soluble collagen and pepsin have been reported in the literature on the extraction of collagen from fish skin and bones.

5.2.6 Determination of morphology by optical microscopy and reflection electron microscopy (SEM)

Figure 20 shows optical microscopy images for fish collagen hydrolysates:

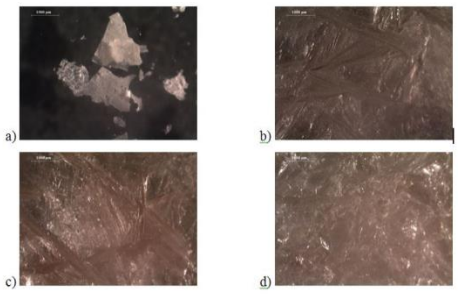

Figure 20. Optical microscopy for collagen hydrolysates form fish:
(a: HO4P1, b: HO4P2, c: HO4P3, d: HO4P4)

The images provided by light microscopy show a uniform structure of collagen hydrolysates in the form of fibers arranged in the same direction.

More detailed morphological information was obtained from SEM microscopy (Fig. 21), for all collagen hydrolyzate samples a characteristic three-dimensional, interconnected structure was identified, consisting mainly of folded and wrinkled sheets.

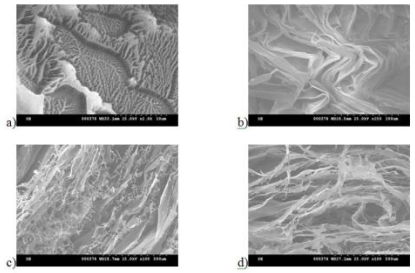

Figure 21. SEM images for fish collagen hydrolysates (a: HO4P1, b: HO4P2, c: HO4P3, d: HO4P4)

5.2.7. Evaluation of the biocompatibility of fish collagen hydrolyzate on human keratinocytes

HaCaT cell proliferation experiments (adult human keratinocytes) cultured for 6 days on medium supplemented with 1% fish collagen hydrolysates and investigated using phase contrast microscopy (Fig. 22a) did not show obvious differences in cell morphology for hydrolyzing and controlling fish collagen.

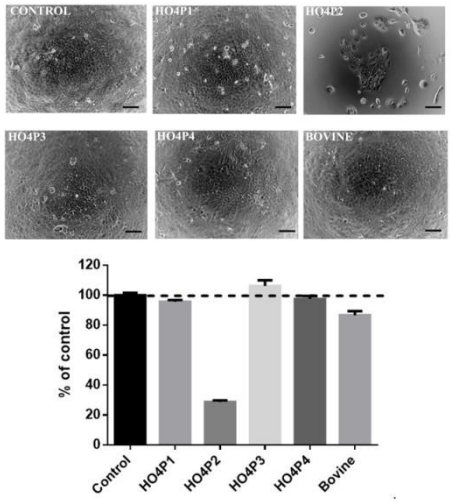

Figure 22. a) Optical microscopy images for keratinocytes cultured in the presence of HO4P1-HO4P4 fish collagen hydrolysates. The empty ladder is 100m.
b) Viability of keratinocytes cultured in the presence of collagen hydrolysates from fish skin

N = 3 per group. * P <0.05 compared to the control group, NS- insignificant

The percentage of viable cells for fish collagen hydrolysates was over 80%, except for the HO4P2 sample. Moreover, all 3 samples - HO4P1, HO4P3 and HO4P4, showed a higher viability compared to the bovine control hydrolyzate, suggesting a superior effect for biomedical applications. An explanation for the low biocompatibility of the HO4P2 fish sample hydrolyzate may be a higher molecular weight of its component polypeptides compared to other samples (according to estimates of amine nitrogen content), supported by other reports in the literature.

6. OBTAINING AND CHARACTERIZATION OF ESSENTIAL OILS

6.1. OBTAINING OF ESSENTIAL OILS

The experimental results regarding the extraction yields for the essential oils obtained from the investigated vegetable sources obtained by both methods are presented in Table 9 and 10 and correspond to the data in the literature, which shows a high purity of the essential oils obtained..

Table 9. Experimental data obtained from the extraction of essential oils by classical hydrolistillation

Raw material	Weight of raw material, g	Volume of distilled water, L	Extraction yield, %
Fresh lemon	530	500	0,67 [10]
Lavender	403,18	2000	0,124 [11]
Pink pepper	600	1000	1,135 [12]

Table 10. Experimental data obtained from the extraction of essential oils in the microwave field

Raw material	Weight of raw material, g	Volume of distilled water, mL	Extraction yield, %
Fresh lemon	500	-	2,36 [13]
Lavender	1344	1200	0,089 [14]

6.2. CHARACTERIZATION OF ESSENTIAL OILS

6.2.1. Density at 20°C

The results obtained for the density of essential oils are presented in table 11:

Table 11. Density for lavender, lemon and pink pepper essential oils

Essential oil	Coding	Extraction method	Oil volume, mL	Oil weight, g	Density, g/mL
Lemon	**ULem CL**	classical hydrodistillation	1,5	1,31	0,873
	ULem MW	microwave extraction	5,3	4,6	0,867
Lavender	**ULav CL**	classical hydrodistillation	0,5	0,44	0.88
	ULav MW	microwave extraction	1,2	1,104	0.92
Pink pepper	**UPr**	classical hydrodistillation	6,81	5.78	0.85

6.2.2. Refractive index

The results for the refractive index are presented in Table 12 and are in accordance with the standards in force, which demonstrates a high purity for all oils obtained.

Table 12. Refractive index for essential oils

Essential oil	Coding	Extraction method	Refractive index	Standard
Lemon	ULem CL	hidrodistilare clasică	1,475	ISO no. 8899/2003 [**Error! Bookmark not defined.**]
	ULem MW	microunde asistate	1,474	
Lavender	ULav CL	hidrodistilare clasică	1,468	ISO no. 3515/ 2002 [**Error! Bookmark not defined.**]
	ULav MW	microunde asistate	1,470	
Pink pepper	UPr	hidrodistilare clasică	1,478	ISO no. 16385/2014 [15]

6.2.3. Characterization by GC-MS

Gas chromatographic analysis of essential oils ULem CL, ULem MW, ULav CL, Ulav MW, UPr revealed a composition similar to those presented in the current standards.

The major components of lemon oil are Limonen (70.95% CL, 71.57% MW), β-Pinen (11.93% CL, 13.46% MW), γ-Terpinen (10.82% CL, 11% MW) and α-Pinen (1.98% CL, 2.26% MW), managing to identify a percentage of over 99% of the chemical composition, in both cases. According to the literature, the microwave extraction technique is less efficient for compounds with higher polarity, such as monoterpenes-alcohols (eg linalool) and more efficient in the extraction of hydrocarbons [16]. In this regard, it is observed that terpinen-4-ol and linalool are extracted by classical hydrodistillation (being compounds with high polarity) while α-Pinen, β-Pinen, Limonen, γ-Terpinen, with lower polarity, are extracted in a higher percentage by the microwave method.

The major components of lavender oil are the same for both oils, differing only in percentage: linalool (46.27% CL, 40.17% MW), linalyl acetate (10.77% CL, 12.31% MW), eucalyptol (8.12% CL, 7.09% MW) , terpinen-4-ol (7.07% CL, 6.78% MW), neryl-acetate (4.40% CL, 6.25% MW), endo-borneol (5.36% CL, 5.48% MW), α-terpineol (3.31% CL, 4.41% MW), managing to identify a percentage of over 99% of the chemical composition, in both cases. It is observed that some components are extracted in a higher percentage by hydrodistillation, and others by microwave technique. It is observed that linalool is extracted in a higher percentage by conventional hydrodistillation (being a high polarity compound) while acetates (Linalyl acetate and Neryl acetate), with lower polarity, are extracted in a higher percentage by the microwave method.

The main components of pepper essential oil are: α-felandren (35.84%), limonene (17.31%), β-felandren (13.04%), o-cimen (4.65%), δ-cadinen (3.27%), trans-caryophyllene (3.04%), bicyclogermacren (2.13%), α-pinen (1.98%). In the literature the main components are presented as: α-felandren (20.6%), β-felandren (10.8%), α-pinene (8.7%), β-pinene (5.1%), β-myrcene (6.9%), β -element (5.0%), copaen (6.5%), germacren (5.8%), γ-caden (6.3%) and α-humulene (5.4%). These differences can be

generated by a number of factors, such as: pedo-climatic conditions, harvest period, different subspecies, method of extraction applied.

6.2.4. Microbiological characterization

Figures 31 and 32 show the results of antimicrobial tests: determination of the minimum inhibitory concentration (MIC) and the antibiofilm effect for the two essential oils of lavender (ULav CL and ULav MW) and lemon (ULem CL and ULem MW) as well as for the mixture of oils (ULav CL: ULem CL, ULav MW: ULem MW).

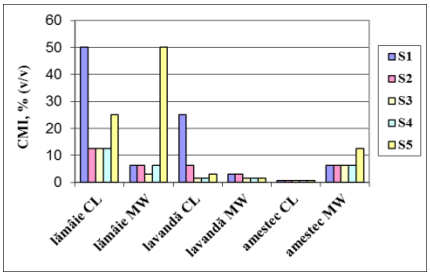

Figure 31. Minimum inhibitory concentration (MIC) against 5 microbial strains of *Staphylococcus epidermidis*, lavender oils (ULav CL and ULav MW), lemon (ULem CL and ULem MW) and their mixture (ULav CL: ULem CL, ULav MW: ULem MW)
*S1, S2, S3, S4, S5 – clinically isolated strains of *Staphylococcus epidermidis*

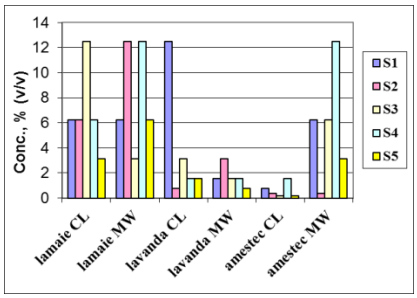

Figure 32. Antibiofim effect on 5 microbial strains of Staphylococcus epidermidis, lavender oil (ULav CL and ULav MW) and lemon (ULem CL and ULem MW) and their mixture (ULem CL and ULem MW)
*S1, S2, S3, S4, S5 – clinically isolated strains of *Staphylococcus epidermidis*

In both cases it is observed that lavender oil has better antimicrobial activity than lemon oil against the bacterial strain of *S. epidermidis*, but the mixture of oils has better activity than each essential oil when the hydrodistillation process is used for extraction, which demonstrates the synergistic effect of the two essential oils.

7. BIOPRODUCTS BASED ON FISH COLLAGEN AND NATURAL EXTRACTS

7.1. Collagen and AHA serum for the treatment of pigment spots – MELACOLL

Figure 33 shows the optimal variant selected for serum with collagen and AHA:

Figure 33. Selected optimal variant for serum with collagen and AHA

Serum with collagen and AHA complies with the rules imposed by European legislation on cosmetics, the ingredients in the composition respecting the concentration required by these rules [17].

The conclusions of the clinical study and safety assessment were as follows: the product can be sold freely on the Romanian market, provided that the declared composition, instructions for use and scope are applied, according to law 178/2000 with subsequent amendments. Moreover, the recommendations are as follows: the product can also be notified through the Cosmetics Notification Portal (CPNP).

7.2. Formulation and characterization of oleogels with cosmetic applications based on vegetable oils with SPF and lavender essential oil

Were obtained stable samples of OG 1-6 oleogels which have the characteristic color conferred by the caronoids present in carrot, marigold and sea buckthorn oils with good stability as can be seen in Figure 34:

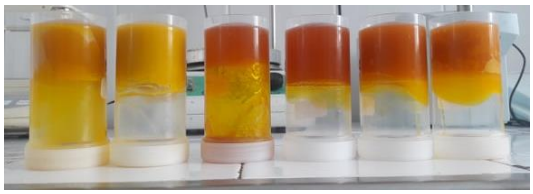

Figure 34. Vegetable oil oils with SPF and lavender essential oil

The characterization of OG 1-6 formulations followed the influence of the natural organogelling agent on the stability, the SPF value, the identification of the relevant components by IR spectroscopy, the morphology and the rheological properties.

The oil binding capacity (OBC) of the oleogel samples was evaluated and it was observed that the best oil binding capacity has the samples prepared with beeswax, followed by the samples prepared with olive wax, and the lowest values for OBC were obtained for samples prepared using glyceryl stearate as an organogeliant. The study of the structure of oleogels was performed by infrared spectroscopy and optical microscopy, being able to observe a relatively uniform and three-dimensional structure, with functional groups of type -C-H and -C = O, groups due to vegetable oils in the composition. All these physical tests demonstrated the gel structure of the formulations. Finally, a rheological study was performed to elucidate their specific properties and their stability during storage. Rheological tests confirmed the shearing behavior of the analyzed cream compositions, which means that the viscosity is not independent of the shear rate.

7.3. Obtaining and characterizing emulsions based on hydrolyzed collagen and natural extracts with potential anti-cellulite action

The obtained O / A emulsion type cellulite formulations, Eac1-6, obtained are very stable (Figure 40) and were characterized by the determination of organoleptic properties, pH, determination of stability over time, morphology and rheological analysis.

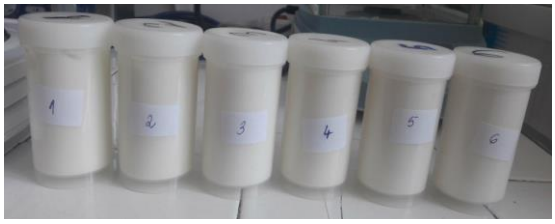

Figure 40. Oil-in-water (O / A) emulsions with anti-cellulite role Eac1-6

The 6 emulsions obtained are stable at different temperatures and the value obtained for the pH of the emulsions corresponds to the natural pH of the skin, indicating that the emulsions can be safely applied to the skin. The results of the analysis by light microscopy show that the S1-S3 emulsions (obtained with the glyceryl stearate

emulsifier) have a creamier appearance and the S4-S6 emulsions (obtained with the emulsifier cetearyl olivate and sorbitan olivate) have a slightly aerated appearance "like foam". Rheological tests confirmed the shearing behavior of the studied cream compositions, which means that the viscosity is not independent of the shear rate, but the creams became less viscous when applying higher shear rates. It can be concluded that all the prepared emulsions are stable, safe for the skin and have adequate rheological properties, so they can be used as a natural alternative for the treatment of cellulite.

7.4. Obtaining and characterizing emulsions based on hydrolyzed collagen and natural extracts with potential anti-acne action

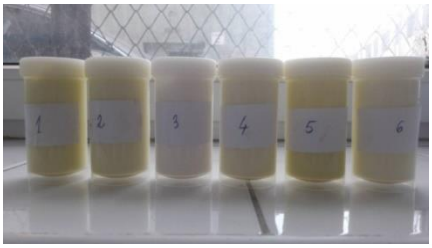

Figure 45. Dermatocosmetic emulsions Eaa1-Eaa6 obtained

The pH values of the formulations obtained were found to be consistent with the pH of human skin. They also present the two quality parameters usually estimated for topical cosmetic emulsions, namely pseudoplastic behavior (quantified by applying the rheological model of the law of power - viscosity according to
shear rate) and thixotropic behavior (quantified by thixotropic area and thixotropy index). The tested cosmetic formulations showed a non-Newtonian pseudoplastic character with shear thinning at both selected temperatures (24 ° C and 33 ° C). Flow parameters were determined and the characteristics of thixotropy were significantly influenced by both the composition of the cosmetic formulations (especially the concentration of the emulsifier based on glyceryl stearate and potassium stearate) and the viscosity-modifying agent, namely xanthan gum) and and working temperature. The cosmetic formulations proved the initial characteristics (appearance, consistency, color, smell and pH) for 6 months (at room temperature). No phase separation was observed, the formulations being stable during the mentioned time period.

7.5 Obtaining and characterizing emulsions based on hydrolyzed collagen and natural extracts used as cc-cream or foundation

Figura 55. CC cream (CC1, CC2) and foundation (FF1, FF2**)**

Both types of emulsions obtained (CC-cream and foundation) are stable at different temperatures and the value obtained for the pH of the emulsions corresponds to the natural pH of the skin, thus indicating that the emulsions can be safely applied to the skin. Optical microscopy results show that all emulsions look like foam and the foundation is slightly aerated.

The tested emulsions show a non-Newtonian pseudoplastic behavior with thin shear at both working temperatures. Thixotropy analysis was performed using specific descriptors such as thixotropic area and thixotropy index. Both the flow parameters and the determined thixotropy characteristics are strongly influenced by the composition of the cosmetic formulations and the working temperature. It can be concluded that all the prepared emulsions are stable, safe for the skin and have appropriate rheological properties, so it can be used as a natural alternative to a CC-cream or foundation.

The original results obtained in this PhD thesis were capitalized by publication and communication as follows:

List of scientific papers

Articles

1. E. Dănilă , D. A. Kaya, M. V. Ghica, M. G. Albu Kaya, Cristina Negrea, Lăcrămioara Popa, Cornelia Nitipir, Rheological properties and stability of dermatocosmetic emulsions with collagen and natural ingredients used as color correcting cream and cream foundation, Revista de chimie, vol. 70, nr. 6/2019. IF= 1.755

2. E. Danila, D. A. Kaya, M. Patrascu, M. Albu Kaya , S. Kumbakisaka, Comparative Study of Lavandula angustifolia Essential Oils Obtained by Microwave and Classical Hydrodistillation, Revista de chimie, vol. 69, nr. 8/2018 , IF- 1.755

3. E. Dănilă, Z. Moldovanu, M. Popa, M. C. Chifiriuc, A. Durmuş Kayaf, M. Albu Kaya, Chemical composition, antimicrobial and antibiofilm efficacy of *C. limon* and *L. angustifolia* EOs and of their mixtures against *Staphylococcus epidermidis* clinical strains, Industrial Crops & Products, 2018, 122, pp. 483–492., IF-=5.645

4. E. Danila, Z. Moldovan, M.G. Albu Kaya, M.V. Ghica, Formulation and characterization of some oil in water cosmetic emulsions based on collagen hydrolysate and vegetable oils mixtures, Pure and Applied Chemistry, July 2019, Conference Paper, DOI: 10.1515/pac-2018-0911., IF= 2.453

5. E. Dănilă, R. Stan, A. E. Enache, M. Türkmen, D. A. Kaya, M. Albu Kaya, A. Serafim, Obtaining and characterization of some emulsions based on collagen hydrolysate and natural extracts with a potential anticellulitic action, U.P.B. Sci. Bull., Series B, Vol. 81, Iss. 4, 2019.

6. E. Dănilă, R. Stan, M. Albu Kaya, G. Voicu, M. M. Marin, A. Moroșan, I. Titorencu, R. Ţuţuianu,Valorization of Cyprinus Carpio skin for biocompatible collagen hydrolysates with potential application in foods, cosmetics and pharmaceuticals, Waste and Biomass Valorization, 2022, 13, p.917–928, https://doi.org/10.1007/s12649-021-01569-w, IF 3.703.

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2. E. Danila, R. Stan, A. Serafim, Z. Moldovan, C. Chelaru, Formulation and characterization of oleogels for cosmetic applications based on sun protection vegetable oils and lavender essential oil, 21st Romanian International Conference on Chemistry and Chemical Engineering Constanta- Mamaia, ROMANIA - September 4 – 7, 2019.

Patent application

1. E. Danila, M. G. Albu Kaya, S. Marin, M. M. Marin, G. Bumbeneci, A. Corina, G. Coara, F. L. Albu, The process for obtaining collagen hydrolyzate from fish skin for medical and cosmetic applications, Patent application no. OSIM nr. A00841/26.10.2018.

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