

EXTRACTION OF KERATIN FROM UNHAIRING WASTES OF GOATSKIN AND CREATING NEW EMULSION FORMULATION CONTAINING KERATIN AND CALENDULA FLOWER (*CALENDULA OFFICINALIS* L.)

by

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ABSTRACT

This study demonstrates the use of keratin extracted from unhairing wastes of goatskin for cosmetics. Keratin has been purified from unhairing wastes of goatskin according to the oxidation method for producing emulsions. The experimental process to prepare the emulsions includes Oil/Water (O/W) emulsion method with keratin and *Calendula officinalis* flower extract (*Calendula officinalis* L.). Four emulsion creams with different ratios of keratin and calendula ingredients were formulated. Fourier transform infrared spectroscopy (FTIR) analysis of the keratin purified and pH, viscosity and average particle size analyses of the emulsions prepared have been carried out. The results of the study have shown that keratin obtained from unhairing wastes of leather industry could be smoothly used in producing emulsion formulations with calendula flower ingredient.

INTRODUCTION

Unless the leather processes are planned to involve the hair or wool of the hide and skin, the removal of the hair and the wool from the hide and skin will be required. This can be realized with various procedures, while conventionally for the skins of the animals, the wool/hair is removed through the application of Na₂S and lime on the flesh surface, the destruction of the hair follicle and followed by peeling off the hair from the skin. During the hair removal process in leather production, waste hair equal to 20-30% of the goatskin arises. There is a large amount of keratin that enters the waste stream. Basing on only the leathers processed in Turkey, the elimination of 6,751 tons of hair per year is required, which indicates the importance of the situation.¹ Using hair and wool, which are waste products, and leveraging their economic value both increases the financial income of the tanners and the prevention of the disposal of these materials is highly important from the point of environment.

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Keratins are fibrous proteins found in hair, wool, feathers, nails, horns and other epithelial coverings and can be isolated from them for the another applications.^{2,3} Keratin-based products which are used for hair care products, recover the moisture content of damaged hair due to chemical operations. Due to these properties, keratin peptides are used as additives for hair care masks, serums, shampoos, care emulsions and hair shaping products.⁴ Because keratin peptides increase cell development and protein expression, they are used in skin care products as additives as they provide the best protection against oxidative stress by decreasing wrinkles and increasing the antioxidant defence system of the body. In addition, keratin peptides are also effective in providing dense moisture to dry and damaged skin and ensuring its repair.⁵

Emulsion systems are one of the important cosmetic vehicles that protect the skin. Generally, O/W emulsions (Figure 1) are typically chosen for applications requiring a relatively small amount of fatty material as hand, shaving or moisturizing emulsions. pH value of the emulsions is also important and should be compatible with skin pH. Normal skin pH is somewhat acidic and in the range of 4 to 6. It varies from one part of the body to another and, in general, the pH of a man's skin is lower (more acidic) than a woman's.⁶

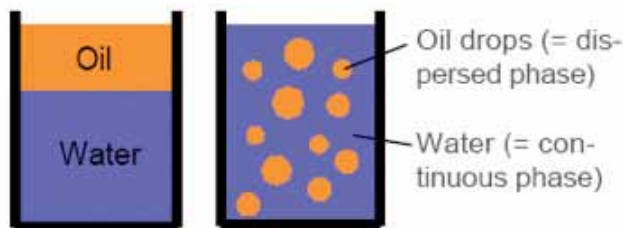


Figure 1. O/W emulsion.

In emulsion production, certain flowers are also used due to their characteristics and the features they provide. One of these flowers, *Calendula officinalis* awarded as the herb of the year by the International Herb Association in 2008.⁷ The dried flower is used as a spice and considered to be generally recognized to be safe (GRAS) by the food and drug administration (FDA) and the Flavours and Extracts Manufacturers Association (FEMA) (FEMA number 2658).⁸⁻¹⁰ *Calendula officinalis* flower extracts have been reported to possess several pharmacological activities.¹¹⁻¹⁸ *Calendula* preparations are therefore regarded as traditionally used medicines by the European Medicines Agency (EMA).¹⁹

This study aims to reduce the waste load and increase its economic value by turning it into a very precious product like keratin cream by merging two major sectors that assist the people in their passion for beauty such as leather and cosmetics. In this context, keratin was purified from hair removal wastes

and keratin hydrolysate was obtained. The use of the obtained keratin hydrolysate in cosmetics industry is commonly realized with the *Calendula officinalis* extract in different emulsion formulations and certain characteristics of the obtained emulsions have been researched.

EXPERIMENTAL

Materials

Hair wastes of Turkish domestic goatskin were used to obtain keratin. *Calendula officinalis* flower extract was used in our trials and it was obtained from Plantapharm GmbH via Guler Kimya. The product name is Plantafluid Calendula and product number is: 12244.²⁰ Cetearyl alcohol (Sigma-Aldrich), Isopropyl Myristate (Merck), Borax (Kimetsan), Lanolin (Chem. Pure), Tween 60 (Merck), Glycerine (Kimetsan), Beeswax (Doga Ilac) were used for emulsion formulation.

Extraction of Keratin

Goatskins were exposed to unhairing process with $\text{Ca}(\text{OH})_2$ and Na_2S after soaking and fleshing process. The goat hair (light brown) obtained from unhairing was washed to remove the contacted pollution and chemicals. Then the hair was dried. 40 g hair was soaked in 1 L 0.5 M NaOH and stirred from time to time for 3 hours for the hair to become swollen. Then, 80 mL H_2O_2 was added for hair hydrolysis. 5 hours later, the solution was filtered with coarse filter to remove very small amount of undissolved particles and residues. After filtration, the solution was mixed with 0.5 M HCl to precipitate the keratin and the precipitated keratin was dried at 45°C incubator for 2 days. The purified keratin hydrolysate was pulverized and used in emulsion production.

Preparing of Emulsions

The ingredients of dispersion phase, a Cetearyl alcohol, Beeswax, lanolin, Isopropyl Myristate, Tween 60, were heated to about 75-80°C. They were mixed in a vessel equipped with a sweep mixer and a homogenizer. The ingredients of continuous phase, water, glycerine and borax, were heated in a separate vessel to about 75-80°C. They were mixed until the mixture melted and became uniform. Then, the dispersion phase was added to continuous phase while mixing. As a critical point, dispersion phase must be added very slowly. This mixture was cooled to about 45-50°C by automatic temperature control system. After cooling to about 40-45°C, first keratin was added to mixture with mixing by the homogenizer and then *Calendula officinalis* while mixing. The mixture was mixed with the side-sweep agitator and homomixer. 15 minutes later, the mixture was cooled to about 30-35°C and then agitation was continued until cooling to room temperature of 25°C. 4 different emulsion combinations were prepared with this method.

TABLE I
Basic emulsion formulation.

Ingredients	%
Cetearyl alcohol	2
Beeswax	3
Lanolin	3
Isopropyl Myristate	2
Tween 60	2
Glycerine	3
Borax	1
Deionised water	84
Total	100

Emulsion No 1 (E1): Emulsion formulation given in Table I (Basic formulation)

Emulsion No 2 (E2): Basic formulation + (1%) Keratin Hydrolysate + (0.2%) *C. officinalis* flower extract

Emulsion No 3 (E3): Basic formulation + (2%) Keratin Hydrolysate

Emulsion No 4 (E4): Basic formulation + (2%) Keratin Hydrolysate + (0.2%) *C. officinalis* flower extract

FTIR

Characterization of extracted keratin was performed by FTIR spectrometer (Perkin-Elmer Spectrum BX) in the frequency of 4000-400 cm^{-1} and 4 cm^{-1} resolution using the attenuated total reflection (ATR) method under room temperature.

pH Analysis

Emulsion pH values of day 1, day 3 and day 7 were measured by using standard pH paper. pH of the emulsion was regulated with citric acid. Some pieces of emulsions were taken by means of a spatula and applied to pH papers. Then the range of pH value was noted according to the colour shown.

Particle Size Analysis

A particle size analysis was performed by laser diffraction using Malvern Mastersizer 2000 with a Hydro 2000MU dispersion unit under room temperature. The measurement range of the apparatus is 0.02-2000 μm .

Viscosimetric Analysis

Viscosities of emulsions were measured at 1 rpm constant rotation speed and by using number 4 spindle and Brookfield viscosimeter, at 20°C. Viscosity values of emulsions (cP) were given.

RESULTS AND DISCUSSION

After the emulsions were prepared, pH values of all formulations ranged from pH 5-6. In the measurement results, any changes have not been noted during the test period. It is known that in emulsion formulations, alkaline pH's cause to acne, various allergies and leather problems.⁶ During the researches, we identified an interesting issue about Calendula flower. The plant was harvested for the works related with extract acquisition, and left to dry at a cool place (Figure 3). At that moment, for 6 days, we noticed that the plants bloomed although their flowers were picked within the day, and the plant continued to produce seeds (Figure 4). In addition, when we put these seeds in the pot, a new plant developed and bloomed in 3 months (Figure 5). Here, blooming property of the plant despite lack of water and start of stem drying may be an indicator of the plant's moisture retention characteristic.

On FT-IR spectrum, 1400-4000 cm^{-1} zone is used for defining certain functional groups and this is the zone of functional groups. The zone on the 1400 cm^{-1} right is known as the fingerprint zone, and this zone causes to the absorption of both tension and bending modes. The fingerprint zone must be inspected when the left side of the spectrum is very similar.²¹ FT-IR spectra of keratin hydrolysate are presented in Figure 6 in order to explain the characteristics of transmission bands and the relative intensities (T%) of the samples.

3279, 2962, 1634, 1536 and 1449 cm^{-1} absorption peaks are noted in the functional group zone of keratin hydrolysate, and 1353, 1235 and 1043 cm^{-1} absorption peaks in the fingerprint zone. Cardamone *et al.*, during their FT-IR studies on the keratin hydrolysate that they obtained oxidatively from wool as a result of hydrolysis, identified Amid I 1680-1645 cm^{-1} , Amid II 1550-1515 cm^{-1} and Amide III 1435 cm^{-1} for Amide zones; Bunte-salt, Cystein-S-sulphonate(Cy-S-SO-3) 1012 cm^{-1} , Cystic acid (Cy-SO₃H) 1045 cm^{-1} , Cystine-S-monoxide (Cy-SO-S-Cy) 1080 cm^{-1} and cystine-S-dioxide (CySO₂-S-Cy) 1137 cm^{-1} for sulfoxide zones.²²

On the FT-IR spectra of the obtained keratin hydrolysate, in the Amide zone, absorbance peaks 1634 cm^{-1} for Amide I, 1536 cm^{-1} for Amide II, and 1449 cm^{-1} for Amide III were observed. The results obtained were compatible with the previous literatures defined as Amide peaks and show us to be derived of keratin hydrolysate successfully. In the sulfoxide zone, cystic acid (Cy-SO₃H) showed 1043 cm^{-1} absorbance peak. The adsorption zone of an amide carbonyl varies



Figure 2. *C. officinalis* in the nature.



Figure 3. *C. officinalis* - 2nd day after harvesting.



Figure 4. *C. officinalis* - 6th day after harvesting.



Figure 5. 3 months after seeding.

according to the multitude of the H-bonds between the molecules. The carbonyl peak (C=O peak) in which Amide carries the most H-bond in liquid status is called Amide I band and it is around 1650cm^{-1} . If the Amide sample is diluted with a solvent which cannot make H-bond, the number of H-bond decreases and the C=O peak shifts to a higher frequency (1700cm^{-1}). Amide II band is seen just on the right of the C=O bond, in $1515\text{-}1670\text{cm}^{-1}$ range. This adsorption is due to NH distortion. In the obtained keratin hydrolysate, Amide I peak was found as 1634cm^{-1} and Amide II peak as 1536cm^{-1} .

According to the literature data, Amide I (1650cm^{-1}) and Amide II (1547cm^{-1}) absorbance peaks keratin chain has alpha helix structure, Amide I (1638cm^{-1}) and Amide II (1515cm^{-1}) have beta layer structures. The obtained keratin hydrolysate indicates that the alpha helix structure turned into beta layer structure together with the deformation of the natural structure of keratin with NaOH and H_2O_2 .²³ The notion of average size or distribution central tendency is a tricky one since there are many ways to calculate the mean, which are not at all equivalent, particularly with asymmetrical distributions. The mean values are the mean of the distribution in volume called D(4,3) because it is the ratio of the fourth moment of the distribution in number to the third one, or the mean of the distribution in surface, so-called Sauter mean diameter SMD or D(3,2), the ratio of the third moment to the second one. Volumetric weight average D(4,3) is the most significant value to understand and discuss the size of particle.²⁴

The results of the particle size analysis of the emulsion, which contains Calendula extract and 2% keratin, are listed in Table II.

Although the study previously conducted by Yorgancioglu and Bayramoglu (2013) identified the particle size of the emulsions that contain collagen hydrolysate in the same basic formulation

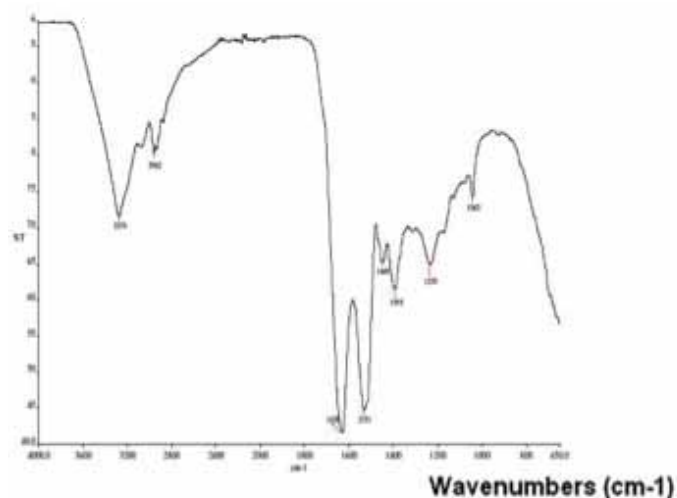


Figure 6. FTIR spectra of keratin hydrolysate.

TABLE II
Results of particle size analysis (Emulsion no: 4).

Parameter	
D(3,2)Superficial weight average(μm)	9.740
D(4,3)Volumetric weight average(μm)	199.241
D90(μm)	790.157
D50(μm)	31.243
D10(μm)	7.192
Specific surface area(m^2/g)	0.616

as 179,241 μm , among the emulsions produced in this study, the particle size of the formulation that contains Calendula extract and 2% keratin has been identified as 199,241 μm .²⁵

The distribution shape and its changes are handy information on the emulsion formation process. Figure 7 indicates different types of distribution was found in emulsion: unimodal of the log normal type produced by turbulent homogeneous stirring; narrowly shaped or highly polydispersed; separated bimodal emulsions resulting from a mixture of two emulsions, which by the way might be intentionally made to attain a low viscosity.

From an industrial point of view, viscosity and flow behavior are of big importance for the emulsions. Flow behavior defines the input energy necessary for handling, mixing, pumping or draining and also the physical aspect of emulsions such as consistency and creaminess. Viscosity values of the emulsions No 1, No 2, No 3 and No 4 have been measured as 2318 cP, 2180 cP, 1780 cP and 1720 cP. The viscosity of an emulsion

can be drastically altered by changing the shape of its particle size distribution.²⁶ The viscosity of the emulsions increased with a corresponding decrease in particle size. The increase in the viscosities of emulsions is a function of the increase of total surface area. Because the particle size of the emulsion containing Calendula extract and 2% keratin hydrolysate is high, viscosity values have been obtained lower than those of other samples. Besides, with the increase of keratin hydrolysate in the ratio of %1 decreased the viscosity of emulsion noticeably between E2 and E3.

The parameters of viscosity and particle size distribution together are the most important ones for the emulsion stability. The particle size of the formulation that contains Calendula extract and 2% keratin has been identified as 199,241 μm . As the particle size in average for emulsions increases, total surface area of the particles decreases. And, the decrease in the viscosity of the emulsions is due to this surface area decreasing. It can be mainly explained by that decreasing emulsion viscosity reduces the stability of emulsion. Consistency giving agents can be added into the emulsions to increase viscosity, while reducing emulsifiers. French (1958), in the studies performed with Brookfield viscosimeter, demonstrated that viscosity of the emulsions had changed associated with the particle size, and that decreased particle size had increased the viscosity. As the particle size in average for emulsions decreases, total surface area of the particles increases. And, the increase in the viscosity of the emulsions is due to this increase in the surface area in total.²⁷

CONCLUSIONS

Cosmetics are generally very expensive products due to their biological active ingredients. Nowadays, these ingredients obtain from living animals, plants and algae etc. and this situation sometimes leads to extinct of them and be broken the food chain of the nature. In this study, keratin hydrolysate obtained from unhairing wastes of goat skin and *Calendula*

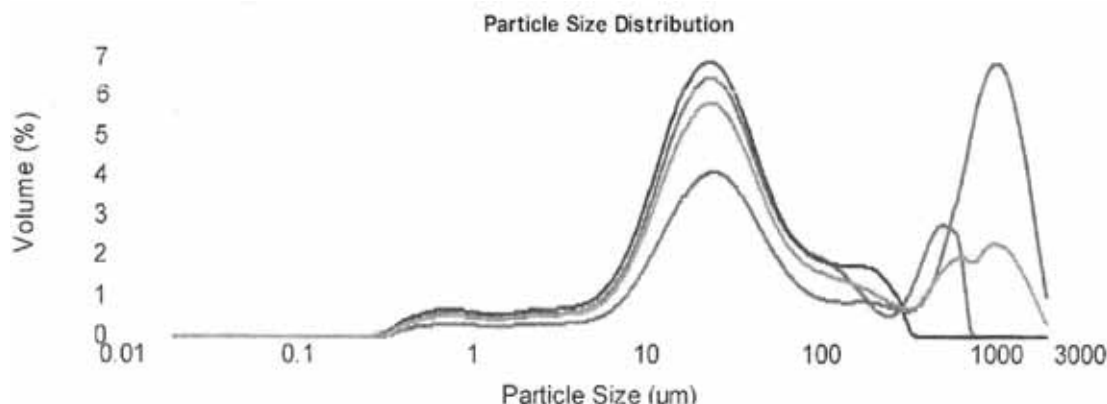


Figure 7. Particle size distribution chart of E4.

officinalis flower extract, which is very common plant in all over the world and known with its moisture retention and wound healer effects in the cosmetics industry, were used as biological active compounds instead of these ingredients.

Keratin hydrolysate and Calendula extract were formulated very well together in the emulsions. The prepared emulsions do not have a disturbing smell, and they may be provided with pleasant odours by adding different essences into the formulation. The pH's of the resulting emulsions were compatible with skin pH and not affected by the term of time period. Keratin hydrolysate dissolved very well and diffused invisibly in the emulsions. Phase separation or creaming which is one of the most common problems encountered with in emulsion impairments were not observed in any of the formulations. The emulsions were entirely absorbed by the skin and did not leave any wastes on the skin surface after the application. This study has promising results for the leather industry to remove the waste load and turn them into valuable products. We have demonstrated the utilization of keratin in the production of a value added product and we are continuing our studies for the use of leather industry waste keratin in cosmetics as a new industrial application of it.

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