Video Article

Preparation of Keratin Hydrolysate from Chicken Feathers and Its Application in Cosmetics

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Abstract

Keratin hydrolysates (KHs) are established standard components in hair cosmetics. Understanding the moisturizing effects of KH is advantageous for skin-care cosmetics. The goals of the protocol are: (1) to process chicken feathers into KH by alkaline-enzymatic hydrolysis and purify it by dialysis, and (2) to test if adding KH into an ointment base (OB) increases hydration of the skin and improves skin barrier function by diminishing transepidermal water loss (TEWL). During alkaline-enzymatic hydrolysis feathers are first incubated at a higher temperature in an alkaline environment and then, under mild conditions, hydrolyzed with proteolytic enzyme. The solution of KH is dialyzed, vacuum dried, and milled to a fine powder. Cosmetic formulations comprising from oil in water emulsion (O/W) containing 2, 4, and 6 weight% of KH (based on the weight of the OB) are prepared. Testing the moisturizing properties of KH is carried out on 10 men and 10 women at time intervals of 1, 2, 3, 4, 24, and 48 h. Tested formulations are spread at degreased volar forearm sites. The skin hydration of stratum corneum (SC) is assessed by measuring capacitance of the skin, which is one of the most world-wide used and simple methods. TEWL is based on measuring the quantity of water transported per a defined area and period of time from the skin. Both methods are fully non-invasive. KH makes for an excellent occlusive; depending on the addition of KH into OB, it brings about a 30% reduction in TEWL after application. KH also functions as a humectant, as it binds water from the lower layers of the epidermis to the SC; at the optimum KH addition in the OB, up to 19% rise in hydration in men and 22% rise in women occurs.

Video Link

The video component of this article can be found at https://www.jove.com/video/56254/

Introduction

Slaughterhouses, the food industry, and the tanning industry annually produce immense amounts of solid keratin by-products – wool, feathers, bristles, hooves, claws, horns, and the like. According to latest statistical data, the total live weight of chickens, turkeys, ducks, and other slaughtered poultry in the USA is 62.5 billion pounds per year[1]; in the EU it is approximately 28.7 billion pounds per year. Considering that feathers make up to 8.5% of the total poultry weight, the USA alone annually produces approx. 5.3 billion pounds of waste feathers[2].

Keratin is a protein exhibiting high chemical resistance because it is strongly cross-linked with disulfide bridges that render its processing difficult. Obtaining soluble products requires cleaving cross-links and possibly carrying out hydrolysis of the peptide bonds[3]. Cleavage of the disulfide bridges may proceed through a reaction of thiol anion according to the following pattern[4,5]:

$S_a^- + –S_bS_c– \leftrightarrow –S_b^- + –S_aS_c^-$

With a very high pH level, hydrolysis of the disulfide bridges also appears, in accordance with the pattern[6]

$–SS^- + OH^- \rightarrow –S^- + –SOH$

Under mild conditions (pH approx. 8), even sulfitolysis takes place according to the following pattern:

$–SS– + HSO_3^- \rightarrow –SH + –SSO_3^-$

The most economical way of degrading keratin is microbial breakdown, which is characterized by mild reaction conditions during processing and high breakdown efficiency (approx. 90%)[7,8]. Keratinases are produced by some bacteria isolated from soil and keratin waste[9]. Microbial keratinases hydrolyze rigid and strongly cross-linked keratin structures[10] and the resulting KH prepared is rich in soluble proteins, with no loss in essential amino acids detected in it[11].

In order to incorporate a protein in cosmetic preparations (*e.g.*, emulsions, lotions, and gels), the requirements ensure that such proteins are soluble in water, the given systems are transparent, and that re-aggregation of the peptides is avoided due to hydrophobic interactions. Therefore, a common practice is to apply hydrolysates of proteins, such as hydrolyzed collagen, elastin, and keratin. When adding hydrolysates into cosmetic emulsions, steps are taken to ensure that the hydrolysate is first dissolved in water. In some cases, it is desirable that the protein (or the hydrolysate) is soluble in alcohol or other organic solvents[12].

KH is normally featured in shampoos, conditioners, lotions, and nutritive serums for hair, as well as mascaras, nail polish, and eye make-up agents. The KH effects declared usually include forming a protective film, smoothing the hair or nail structure, heightened plasticity and appearance of the treated formation, regulating the consistency of products, and encouraging the formation of foam[13,14]. It has also been shown that KH reduces surface tension, hence supplementation in cosmetics can facilitate reduction in the amount of emulsifier added to stabilize creams. KH limit the effects of irritation triggered by cleaning agents (surfactants) to the skin, eyes, and hair, thus reducing any potential side effects of cleaning agents on tissue (*e.g.*, dehydration of the skin, hardness, and decreased barrier function of the skin). The high buffering capability of hydrolysates is also exploited to stabilize the pH of cosmetics; peptides of shorter length have a greater buffering effect[15,16]. Although KHs have become established as standard components in hair and nail cosmetics as well as being utilized in products for skin care, studies on the moisturizing effects of KH do not appear in contemporary literature.

Alkaline-enzymatic technology has been developed for processing keratin by-products into KH, and active testing is in process on the effects of a number of cosmetic additives[17,18,19,20,21,22]. The advantage of two-stage alkaline-enzymatic hydrolysis using microbial proteases for chicken feathers achieves high efficiency under mild reaction conditions and the quality of KH is very high in contrast to hydrolysis employed in strong acids or alkalis. In the first stage, feathers are incubated at a higher temperature in an alkaline environment, which partially disrupts the keratin structure and swells the feathers; after adjusting the pH, the feathers are hydrolyzed with a proteolytic enzyme under mild conditions in the second stage. The dialyzed KH possesses a high content of proteins.

The purposes of the method described here are processing poultry feathers into a KH through alkaline-enzymatic hydrolysis and testing the effect of moisturizing properties of KH applied to O/W cosmetic emulsion. The moisturizing properties are investigated by instrumental non-invasive methods *in vivo*. The most frequent methods for measuring skin hydration and barrier function of SC include measuring electrical properties of the skin (conductance or capacitance). Different methods for investigating SC hydration include near infrared multispectral imagining method (NIM), nuclear magnetic resonance spectroscopy, optical coherence tomography, or transient thermal transfer[23]. Barrier function of SC correlates to the TEWL of SC and it is measured by the ventilated chamber method, unventilated chamber method, and open chamber method[24].

Properties of the model formulations are determined using the Multi Probe adapter MPA 5 with three types of probes. The first one, corneometer CM 825, measures skin hydration by assessing changes in the electrical capacity of the skin's surface; the measuring capacitor shows changes in capacitance of the skin surface in corneometric units. The corneometer gives only a relative assessment of skin hydration[25]. For TEWL, the second probe, tewameter TM 300, is used for measuring the density gradient of water evaporation (in an open chamber instrument based on Fick's diffusion law) from the skin indirectly by the two pairs of sensors (temperature and relative humidity) indicating the quantity of water being transported per a defined area and period of time ($g/m^2/h$). This method can detect even the slightest disruption of skin barrier function[26]. Skin pH is one indicator of barrier and anti-microbial function of the SC[27]. The acidity of the skin mantle was measured by a (third) skin PH 905 probe connected to the MPA 5 station. This specially designed probe consists of a flat-topped glass electrode for full skin contact, connected to a voltmeter. The system measures potential changes due to the activity of hydrogen cations surrounding the very thin layer of semi-solid forms measured at the top of the probe. The changes in voltage are displayed as pH[28].

We present experiments divided into three sections: (1) Preparation of KH from chicken feathers by two-stage alkaline-enzymatic hydrolysis and its purification by dialysis (removing salts and low-molecular fractions), (2) Preparation of cosmetic formulations containing 2, 4, and 6% KH, and (3) Testing the properties of KH by measuring skin hydration, TEWL, and skin pH. Testing was carried out on 10 women with the mean age of 27.2 years and on 10 men with the mean age of 26.2 years. The method of selecting the volunteers and the testing itself were conducted in accordance with international ethical principles of bio-medical research utilizing human subjects[29]; all persons gave their informed consent prior to inclusion in the study. Before testing commenced, the volunteers were asked to complete a questionnaire on their health status. The volunteers committed to avoid applying any cosmetic product to the test sites and surrounding regions during the 24 h prior to and during the test period; furthermore, they were only permitted brief evening washes with running water.

Protocol

Volunteers were recruited among employees and students of our university. The method of selecting was conducted according to "International Ethical Guidelines for Biomedical Research Involving Human Subjects. Council for International Organizations of Medical Sciences, Geneva (2002)." KH is a common cosmetic ingredient used in hair-care products (shampoos, conditioners, *etc.*) and hence approval from the institutional review board is not required.

1. Process Chicken Feathers into KH

1. Collect chicken feathers from a poultry farm.
2. Wash out any insoluble impurities and blood remnants from the chicken feathers with a sufficient excess of fresh running (cold) water. Place the feathers on a flat plate and dry overnight at 50 °C.
   NOTE: The protocol can be paused here.
3. Grind 50 g dried feathers in a cutting mill (suitable for soft to medium-hard sample materials, and fibrous materials) into a final fineness of 1.0 mm. Alternatively, the final fineness of grinded feathers can be higher, but not more than 3.0 mm.
   NOTE: The protocol can be paused here.
4. **Degrease feathers**
   NOTE: The most effective and economic method of degreasing poultry feather is using a commercial lipolytic enzyme.

1. In a stainless steel 27-L boiler container with temperature control, mix the feathers with water preheated up to 40 ± 2 °C in a weight ratio 1:75. Add a lipolytic enzyme (activity 100 KLU/g) in a dose of 1.5 - 2.0% (related to weighed-in dry feathers) and gently stir the contents with an overhead stirrer for 5 min.
2. Adjust the mixture pH to 9.0 ± 0.2, the value corresponding to the maximum activity of the lipolytic enzyme by adding 1% NaOH or 1% H_3PO_4 solution. Stir the mixture for 5 min with an overhead stirrer, and then check and re-adjust the pH level using a laboratory bench pH/mV meter.
3. Gently stir the mixture with an overhead stirrer for 24 h at 40 ± 0.5 °C. Alternatively, incubate the mixture at 40 ± 0.5 °C, and during the first 6 h of incubation stir the contents at 1 h intervals.
4. Filter the mixture through a fine sieve (100 µm size) and wash the degreased feathers with a stream of fresh running (cold) water. Dry the feathers on a flat plate at 50 °C in a drying chamber overnight.
   NOTE: The protocol can be paused here.

5. Perform the first stage of the chicken feather hydrolysis. Mix the feathers with 0.3% KOH water solution in a weight ratio 1:50 and gently stir with an overhead stirrer at 60 ± 0.5 °C for 24 h. The pH of the mixture decreases from approx. 12.5 at the start of incubation to approx. 11.0 at the end of incubation. After finishing the first stage of hydrolysis, adjust the pH of the mixture to the level corresponding to the maximum activity of the proteolytic enzyme with 10% H_3PO_4 (in this case, to a level of 9.0 ± 0.2) by adding 1% NaOH.
6. Perform the second stage of chicken feather hydrolysis. Add to the mixture, the proteolytic enzyme in a dose of 5.0% (related to dry matter of quantity of feathers weighed-in at the beginning of the first stage of hydrolysis). Gently stir with an overhead stirrer at 60 ± 0.5 °C for 8 h and then heat the mixture (in the same stainless steel 27-L boiler container) to a boiling point (100 °C) and boil for 10 min to inactivate the enzyme.
7. Separate the solution of KH (prepared in step 1.6) from the undissolved remnant by filtering it through low-density filter paper on a Buchner funnel with slight vacuum-pressure; alternatively, use a centrifuge.
   NOTE: The protocol can be paused here for several days if a solution of KH is stored at 5 ± 1 °C.
8. In the plastic bucket (26 cm diameter x 26 cm height) dialyze the KH using 12 K MWCO membrane to remove small peptides and salts. Pour 400 mL of the KH solution into dialysis tubing and dialyze it against 4 L of distilled water for 80 h at room temperature; change the distilled water after 18, 36, and 60 h.
9. Cast a dialyzed solution of KH on an anti-adhesive plate (*e.g.*, silicone) on a ratio of 500 mL to 1,000 cm^2 plate area, vacuum dry it on a thin film at 40 ± 0.5 °C for overnight, grind to form a fine powder, and keep it in a closed vessel in a desiccator.
   NOTE: The protocol can be paused here for several months if the KH powder is stored in a dry place.

2. Prepare Cosmetic Formulations with KH

NOTE: The OB used for testing was a commercial hydrophilic O/W cream base and comprised of aqua, paraffin, paraffin liquid, cetearyl alcohol, Laureth 4, sodium hydroxide, carbomer, methylparaben, and propylparaben.

1. Prepare formulations containing 2, 4, and 6% KH (in accordance with the base weight of the ointment). Weigh the amount of KH powder into a polyethylene vessel (7 cm diameter x 10 cm height) and add the OB at an amount that ensures the total weight of the formulation equals 50 g; see recipe in **Table 1**.

Cosmetic formulation	Weight of ointment base [g]	Weight of keratin hydrolysate [g]	Total weight [g]
Ointment base	50	0	50
Ointment base + 2 % KH	49	1	
Ointment base + 4 % KH	48	2	
Ointment base + 6 % KH	47	3	

Table 1: Weight-in quantities of ointment base and keratin hydrolysate to prepare cosmetic formulations.

2. Homogenize the mixture with a 3-bladed laboratory blender for 10 min at 134.16 x g and mix using a mechanical overhead stirrer. Maintain the prepared formulations at 5 ± 1 °C and warm them at room temperature for 2 h prior to use.
   NOTE: Homogenizing the mixture of OB with KH can be done with a non-digital stirrer as well. On a non-digital stirrer, there are scales with the approximate speed (in rpm) as well. Gentle stirring will work the best for this step.
   NOTE: The protocol can be paused here for up to 5 months if the formulations are stored at 5 ± 1 °C.

3. Test the Properties of KH by Measuring Skin Hydration, TEWL, and pH

NOTE: Perform all measurements in a conditioned room at 23 ± 2 °C and the relative humidity of 56 ± 3%.

1. Place 5 strips of filter paper (size 2 x 4 cm) into the physiological solution (0.90% NaCl) and leave them for approximately 1 min in the solution.
2. Apply two strips to the inner side of the right forearm, and three to the inner side of the left forearm, and fix them for 4 h with adhesive plasters. This step is to degrease the skin and eliminate individual characteristics of the skin at the site. After 4 h, remove the strips and demark the areas with a permanent pen, see **Figure 1**.

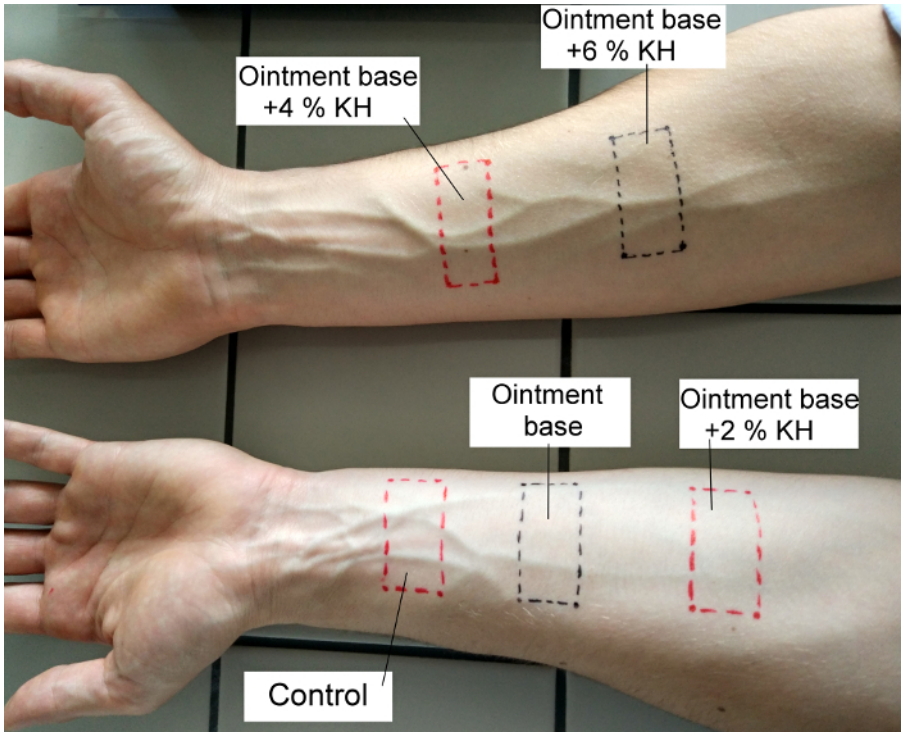

Figure 1: Method for location of test formulations on the forearm of the left and right upper limbs. Please click here to view a larger version of this figure.

3. Apply 0.1 mL of the tested formulations at each spot of the degreased forearm sites using syringes and spread it over the entire marked surface. On the left forearm, do not apply anything to the first site (it is the control), apply the OB to the second site, and the OB + 2% KH to the third. Apply the OB + 4% KH and OB + 6% KH to the right arm.
4. Measure each sample at each site and each interval (1, 2, 3, 4, 24, and 48 h) and take 5 readings with the skin hydration meter probe for skin hydration, 15 readings with the TEWL meter probe for skin TEWL, and 1 reading with the skin pH meter probe for skin pH. Do not allow volunteers to apply any cosmetic product to the test sites and surrounding areas during the test period; they are permitted brief evening washes with running water.
   NOTE: The protocol can be paused here.
5. Process the resulting readings by basic numerical characteristics of the descriptive statistics, using spreadsheet software. From the 5 hydration readings measured for each sample, ignore the lowest and the highest readings, and calculate only 3 readings for the arithmetic mean and standard deviation. From the 15 TEWL readings measured for each sample, ignore the first 5, and calculate only 10 readings for arithmetic mean and standard deviation.

Representative Results

The KH prepared according to procedure presented here (see **Figure 2**) is yellow in color, easily soluble in water with high protein content (inorganic solids represent <2.0%); the pH of the 1.0% solution of KH is 5.3, and fulfils the requirements for cosmetic-grade hydrolysates. The yield of KH from 50 g raw material is approx. 30%. The molecular weight distribution of KH was determined by SDS-PAGE and is shown in **Figure 3**.

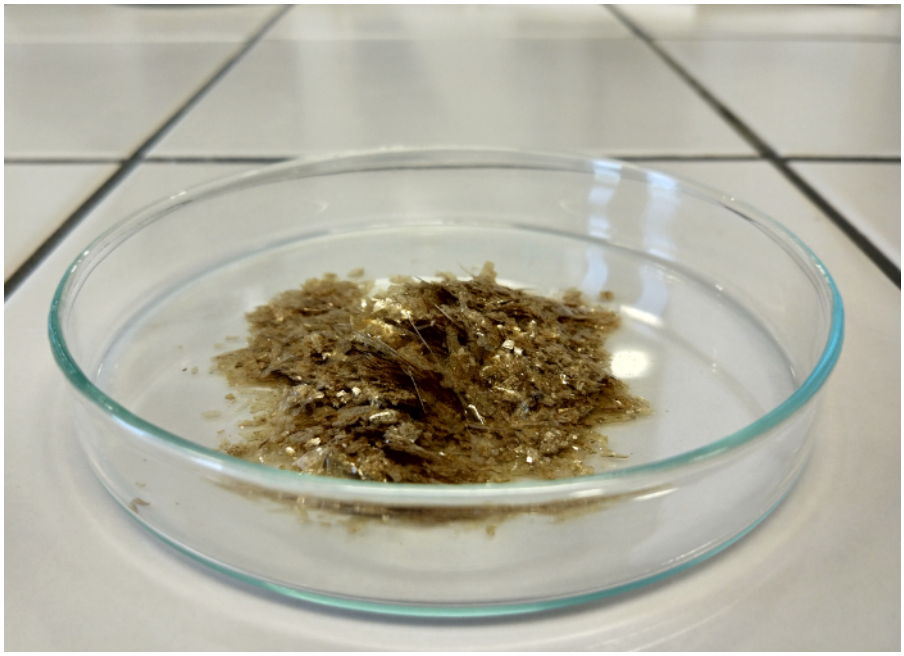

Figure 2: Representative picture of keratin hydrolysate. Please click here to view a larger version of this figure.

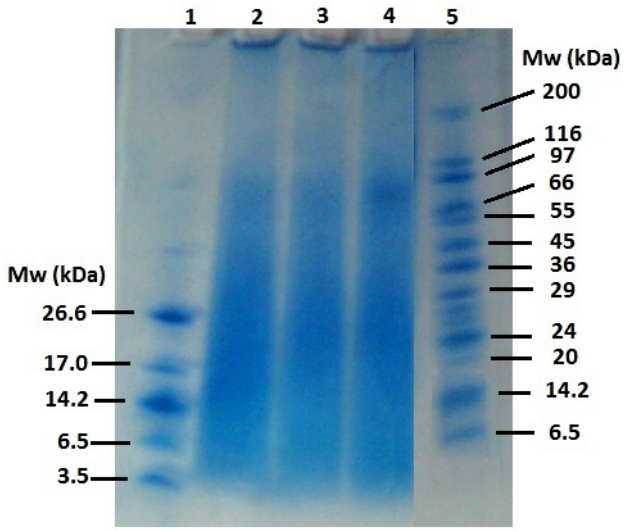

Figure 3: SDS-PAGE of keratin hydrolysate and protein standards. Lane 1: ultra-low range molecular weight marker (3.5 - 26.6 kDa). Lanes 2, 3, and 4: keratin hydrolysates prepared in 3 batches. 120 µg KH re-suspended in 20 µL loading buffer was loaded in each well. Lane 5: wide range molecular weight marker (6.5 - 200 kDa)

The hydration and TEWL values were delineated in different volunteers, and thus could not be compared to each other. Therefore, the values are expressed as change in percent in comparison with the OB on the site treated with the formulations, the latter containing 2, 4, and 6% additions of KH at intervals for measurement of 1, 2, 3, 4, 24, and 48 h. The pH values for skin are expressed as the arithmetic mean of the recorded values of skin pH for all volunteers. The results for alteration in hydration and TEWL in percent, relative to the OB, and for pH levels of the skin are given in **Table 2** for 10 men and in **Table 3** for 10 woman volunteers.

Men						
Time	**1 h**	**2 h**	**3 h**	**4 h**	**24 h**	**48 h**
	Hydration (% change vs. Oitment base) ± SD					
Ointment base + 2% KH	+16 ±15	+14 ±16	+12 ±9	+19 ±14	+11 ±18	+15 ±9
Ointment base + 4% KH	+6 ±19	+1 ±18	+5 ±10	+7 ±16	+11 ±9	+14 ±15
Ointment base + 6% KH	-3 ±25	-4 ±14	-7 ±18	-4 ±17	+11 ±14	-17 ±14
	TEWL (% change vs. Oitment base) ± SD					
Ointment base + 2% KH	-20 ±15	-20 ±22	-11 ±21	-20 ±21	-23 ±20	-21±17
Ointment base + 4% KH	-28 ±12	-29 ±20	-28 ±20	-28 ±24	-47 ±20	-36 ±20
Ointment base + 6% KH	-36 ±16	-41 ±21	-31 ±17	-36 ±17	-53 ±20	-54 ±17
	pH					
Control	4.7 ±0.5	5.1 ±0.4	4.9 ±0.4	5.1 ±0.3	4.6 ±0.5	4.8 ±0.7
Ointment base	4.8 ±0.5	5.1 ±0.3	4.9 ±0.3	5.0 ±0.4	4.6 ±0.4	5.0 ±0.6
Ointment base + 2% KH	5.0 ±0.6	4.8 ±0.4	4.9 ±0.5	4.9 ±0.5	4.7 ±0.3	5.0 ±0.6
Ointment base + 4% KH	4.8 ±0.5	4.9 ±0.3	4.8 ±0.4	4.8 ±0.3	4.7 ±0.5	4.8 ±0.5
Ointment base + 6% KH	4.7 ±0.5	5.0 ±0.2	4.9 ±0.4	4.8 ±0.4	4.8 ±0.6	5.0 ±0.6

Table 2: Results for change in hydration, TEWL, and pH of skin of 10 men volunteers at the measurement intervals of 1, 2, 3, 4, 24, and 48 h.

Women						
Time	**1 h**	**2 h**	**3 h**	**4 h**	**24 h**	**48 h**
	Hydration (% change vs. Oitment base) ± SD					
Ointment base + 2% KH	+22 ±7	+15 ±6	+15 ±8	+12 ±9	+14 ±14	+18 ±9
Ointment base + 4% KH	0 ±4	-6 ±5	-2 ±5	+1 ±7	+10 ±13	+15 ±10
Ointment base + 6% KH	-12 ±5	-14 ±2	-9 ±7	-5 ±9	+8 ±12	+10 ±9
	TEWL (% change vs. Oitment base) ± SD					
Ointment base + 2% KH	-32 ±1.6	-16 ±3.0	-12 ±1.3	-20 ±0.9	-35 ±1.9	-38 ±1.6
Ointment base + 4% KH	-41 ±1.1	-37 ±2.7	-24 ±0.8	-34 ±0.9	-44 ±1.5	-38 ±1.9
Ointment base + 6% KH	-50 ±1.4	-39 ±2.2	-29 ±0.7	-39 ±0.9	-16 ±2.4	-33 ±2.1
	pH					
Control	5.0 ±0.7	5.3 ±0.3	4.9 ±0.7	5.0 ±0.5	5.0 ±0.8	4.7 ±0.7
Ointment base	5.2 ±0.6	5.3 ±0.3	5.2 ±0.7	5.0 ±0.4	5.1 ±0.8	4.8 ±0.7
Ointment base + 2% KH	5.4 ±0.7	5.1 ±0.4	4.9 ±0.4	5.1 ±0.7	4.9 ±0.7	5.0 ±1.0
Ointment base + 4% KH	5.2 ±0.7	5.1 ±0.3	5.0 ±0.4	4.9 ±0.4	5.1 ±0.6	5.1 ±0.2
Ointment base + 6% KH	5.2 ±07	5.2 ±0.2	5.0 ±0.4	5.0 ±0.3	5.4 ±0.6	5.2 ±0.4

Table 3: Results for change in hydration, TEWL, and pH of skin of 10 women volunteers at the measurement intervals of 1, 2, 3, 4, 24, and 48 h.

Hydration of SC for Men Volunteers:

At short intervals of measurement (1 - 4 h), the highest increases in hydration of the skin (12 - 19%) were recorded for formulations supplementing the OB with 2% of KH; the addition of 4% KH showed a smaller rise (1 - 7%) in hydration. Conversely, the KH at 6% reflected negatively on values for SC hydration (a decrease of 3 - 7%). After 24 h of measurement, an increase of 11% in SC hydration was discerned for all the additions of KH to the OB tested. The same trend continued even after 48 h, following with a slight increase in hydration that still remained: a 15% increase for KH at 2%, a rise of 14% for the 4% addition of KH, and the drop of 17% for KH at 6%.

Hydration of SC for Women Volunteers:

It is observable that supplementing the OB with 2% KH causes an approximate 22% increase in SC hydration, relative to OB alone, as early as at 1 h of measurement; a 4% addition of KH to the OB has, in fact, no effect on hydration; while, conversely, adding 6% KH to the OB is reflected in an approximate 12% decrease in hydration, as compared with pure OB. Similar tendencies are seen after 2, 3, and 4 h of measurement, at which hydration increases by 12 - 15% for supplementation with 2% KH; for larger additions of KH, the hydration either stays the same or diminishes. After 24 h of measurement, the hydration was recorded as higher than for the OB for all the tested KH additions; the greatest increase (14%) in hydration occurred for the addition of 2% KH, while the lowest increase (8%) was seen for supplementation at 6% of KH. Similar results are achieved after 48 h of measurement, wherein greater hydration than for pure OB was recorded for all samples with additions of KH; the biggest rise (18%) in hydration occurred for the 2% KH addition, while the lowest increase (10%) was noted for KH supplementation at 6%.

TEWL for Men Volunteers:

The results of the TEWL make it clear that formulations supplemented with KH diminish TEWL, when applied to the skin, in comparison with pure OB. A heightened amount of KH exerted a positive effect on lower values of TEWL. After 1 h after applying the formulations, TEWL was recorded as 20% lower than for the pure OB, pertaining to a formulation with 2% KH; while KH at 4% dropped 28% in TEWL; whereas the KH at 6% resulted in a dramatic 36% decrease in TEWL. Indeed, the diminished TEWL values were also observed at 2, 3, and 4 h of measurement for formulations supplemented with KH. After 24 and 48 h, TEWL was even significantly lowered on sites treated with the KH formulations. After 24 h, TEWL observed on the skin was 23% lower for the OB with 2% addition of KH than at the site for pure OB; KH at 4% dropped by about 47% TEWL, while KH at 6% triggered TEWL to fall by 53%. A similar trend is evident even after 48 h: the TEWL for skin treated with OB containing 2% KH was 21% lower than at the site for pure OB; the KH at 4% showed a TEWL at 36% lower; and the KH at 6% brought about a drop of 54%.

TEWL for Women Volunteers:

It is evident that all the monitored additions of KH to OB shall be reflected in reduced TEWL, as seen in the men volunteers. After 1 h from application, a significant decrease in TEWL was recorded for OB samples containing all the additions of KH; about a 32% reduction in TEWL was seen for the 2% addition of KH, around a 41% decrease for 4% KH, and even a 50% drop in TEWL for 6% supplementation of KH. Following 2, 3, and 4 h of measurement intervals, the situation remains similar, *i.e.*, there is a decrease in TEWL at such time intervals; the least drop in TEWL occurs for 2% KH, while the greatest is seen for 6% KH. After 2 h, the TEWL decreases by 16% for KH at 2%, 37% for KH at 4%, and 39% for KH at 6%. At 3 h, TEWL diminishes further by 12% for KH at 2%, by 24% for KH at 4%, and 29% for KH at 6%. At 4 h, TEWL further decreases by 20% for 2% KH, 34% for 4% KH, and 39% for 6% KH. After 24 h, the least reduction in TEWL (16%) occurred for the 6% addition of KH, while the greatest (44%) was seen for 4% supplementation of KH; the KH at 2% was observed to cause a 35% decrease in TEWL. At 48 h, the results remain similar and relatively balanced, with the least decrease in TEWL (33%) for the 6% addition of KH, the greatest (38%) for 4% KH and for 2% KH.

Discussion

The advantage of alkaline-enzymatic hydrolysis is that it can be modified according to future applications of KH. For example, in hair-care cosmetics applications where a lightly brownish color of a product is not an obstacle, a higher temperature in the hydrolysis can be applied leading to a higher yield of KH. In addition, the longer processing time during both stages of the technological procedure significantly affects the overall process efficiency – yield of KH rises to 85%.

The findings for hydration measurement make it evident that, during the monitored interval of measurement (1 - 48 h), the addition of 2% KH to OB is optimum since it causes a 11 - 19% increase in SC hydration for men volunteers, and a 12 - 22% increase for woman volunteers. The KH that is added possesses a broad distribution of molecular weight. We suggest that a portion of low-molecular-weight fractions (MW <20 kDa) penetrates the epidermis following application to the skin. Increased hydration of the skin after putting on a formulation supplemented with KH is explained through the KH binding water from lower epidermis layers to the structure of the SC, leading to the formation of H-bridges between KH molecules and water. This mechanism of action is also embraced by some authors[30]. The moisturizing effect of KH is comparable with conventional moisturizers (*e.g.*, glycerol, urea, and hyaluronic acid) that were tested in emulsion and gel formulations[22].

TEWL measurements highlight that during the observed time of measurement (1 - 48 h) for men volunteers, all the formulations with KH supplementation caused TEWL to decline after application. The 2% addition of KH to the OB caused a reduction in TEWL of 11 - 23%, in comparison with pure OB. When the OB was supplemented with 4% KH, TEWL dropped by 28 - 47%; while for KH at 6% it diminished by as much as 31 - 54%. For woman volunteers, supplementing the OB with 4% KH represents the best option, as there was a 24 - 44% decrease in SC TEWL. The significantly lower TEWL for formulations supplemented with KH can be explained by the process of higher-molecular-weight fractions of KH forming a protective film once applied to the epidermis, thereby preventing the loss of epidermal water. In fact, the highly positive effect of KH on TEWL is comparable or even exceeds, for the sake of comparison, values of TEWL recorded for cosmetic gels or emulsions supplemented with 5 - 10% of glycerol and 1 - 5% of sericin. Similarly, when comparing KH with conventional mineral oils, KH diminished TEWL by approximately 25 - 30%[31]. Additionally, the barrier properties of KH are better than, for example, those for urea and hyaluronic acid[22].

pH of the Skin Surface:

For reference, pH 3.5 to 4.3 is an acidic skin surface, pH 4.4 to 5.5 is neutral in this respect, and pH 5.6 to 6.5 represents a basic skin surface[32]. We found that no significant changes were observed in the pH of the skin surface after putting on all of the tested formulations (OB + 2, 4, and 6% KH); the pH of 4.6 to 5.0 (men volunteers), and 4.9 to 5.4 (woman volunteers) corresponds to a normal skin surface. The longer-term analysis (more than 2 days) was not accomplished.

Modifications and Troubleshooting:

Processing of chicken feathers into KH is very easy, and runs under atmospheric pressure and at mild temperature. The process can be favorably transformed from a laboratory scale to a pilot plant scale and an industrial scale. In Section 2 of the protocol where the KH is homogenized with the (O/W) emulsion base, some modifications are possible. In industrial practice, O/W and W/O emulsions are prepared by mixing water (W) phase (water + cosmetic ingredients soluble in water) and oil (O) phase (oil + cosmetic ingredients soluble in oil). KH is soluble in water, so it is favorable to blend it directly into the water phase of the system.

Limitations of the Technique:

Localization of each spot for measuring hydration and TEWL and hammered pressure are measured with skin hydration meter which is expensive.

Critical steps within the protocol are mostly in Section 3. The health state, individual differences, smokers/non-smokers, gender, age differences, menstruation, and mental condition can influence measuring skin hydration and TEWL. For acquiring representative results, the same person should apply tested formulations on forearms and measure hydration and TEWL values. It is vital to perform all measurements in a conditioned room with a stable temperature and relative humidity. In case of measuring values at intervals of 24 and 48 h, acclimatization of the volunteers in a conditioned room for at least 30 min prior to measurement is necessary.

Disclosures

The authors have nothing to disclose.

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