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The Anti-Aging Face Cream Preparations Made from Collagen Membrane of Chicken Eggshell (*Gallus gallus domesticus*) Combined with Bee Honey (*Apis mellifera*)

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ABSTRACT

Handling waste such as chicken eggshells requires innovation to be able to provide added value as a sustainable product. This study aims to extract collagen from chicken eggshell membranes combined with bee honey as an anti-aging face cream. These materials hve a very high model accuracy value (97.89%) which have the potential to inhibit aging due to Matrix metalloproteinase-1 (MMP-1). This research was carried out by extracting eggshell membrane collagen, making cream combined with bee honey, analyzing product properties, and interpreting virtual data. The results showed that in 100 g of face cream formulation with active ingredients (chicken eggshell membrane collagen extract 4.1206 g and bee honey 5.0251 g) contained 77.0926% water; 0.0062% ash; 1.8690% protein; 0.0058% fat, the viscosity value was 65.94 dPas, and the pH was 7.08. Thus, this product did not irritate the skin when we use in experimental objects. The average value of attribute assessment (such as aroma, consistency, texture, stickiness, homogeneous sensation, color, appearance, itching, erythema, washing power, response after washing) was well received by the panelists with a score of 1-3,9 and the highest total achievement bacteria 25 CFU/g met the skin moisturizer quality requirement which should not exceed 10^2 CFU/g, while yeast-mold 16 CFU/g did not meet the standard of skin moisturizer based on SNI 16-4399-1996.

Keywords: collagen; Matrix metalloproteinase-1; honey; extraction; organoleptic

INTRODUCTION

Based on the data from the Central Statistics Agency in 2021, the level of egg consumption of laying hens per capita in Indonesia in 2018-2021 has increased. In 2019 the level of public egg consumption per capita increased by 65,261 tons, while in 2020 the level of community egg consumption per capita increased by 291,012 tons. This leaves eggshell waste turning into reusable food waste. In a study by Ponkham *et al.* (2011), eggshell membranes contain collagen by 8% of the total weight after going through the extraction process involving acid solvents. This amount of collagen is sufficient to be developed as collagen in the manufacture of cosmetics. In addition to its very abundant availability, its price is affordable so that cream formulation involving collagen active ingredients can bring innovation to the cosmetics industry.

The COVID-19 pandemic has led to activity restrictions. For example, it has made working from home (WFH) that requires the use of electronic media the new way of working for millions of employees. Based on the data from the

American Academy of Dermatology (2017), although UV radiation from electronic devices such as TVs, computers, and gadgets are low, the cumulative radiation can cause skin damage. UV radiation can cause photoaging in humans by stimulating MMPs (matrix metalloproteinase) as this enzyme produces collagenase (MMP-1), resulting in collagen degradation and aging. One solution to overcome this problem is the innovative application of collagen in various cosmetic and medicinal products (Alhana 2015). An anti-aging face cream solution made from eggshell membrane collagen is presented to prevent premature skin aging.

Collagen innovation in cosmetic research uses an active additive in the form of antioxidants found in honey. Cosmetics with the provision of antioxidants, especially facial cream products, have the potential to increase body resistance, especially to prevent skin aging (Safitri *et al.* 2016). Honey is a source of antioxidants because there are flavonoid compounds, phenolic acids, catalase, peroxidase, carotenoids, and nonperoxidals (Sumarlin *et al.* 2014). Creams containing antioxidants in honey have the potential to work more optimally when combined with collagen from eggshell membranes which are still considered food waste.

The output and follow-up of this research highlights the utilization and added value of chicken eggshell waste as an anti-aging ingredient made from natural ingredients which can reduce the side effects of incompatibility of other cosmetic products that are widely circulated in the market.

MATERIAL AND METHODS

The chemicals used in this experiment (details in the appendix) were of analytical grade and were purchased from Badische Anilin und Soda Fabrik (Ludwigshafen, Germany), Merck, Progo Mulyo, and Brataco Chemika. Activities related to living things have received approval from the ethics committee with reference number KE/FK/0911/EC/2021.

Identification of collagen compounds in eggshell membranes

The eggshell waste was taken from the chicken farming industry and cake shops in Sleman Regency, Yogyakarta. To identify the compounds contained in the eggshell membrane, a literature study was carried out. Literature search was done by searching related articles on the internet. Several keywords such as "Eggshell membrane", "Collagen", "Cosmetic", and "anti-aging" were entered into search-engines and the articles were used as a reference regarding the collagen content in the eggshell membrane.

Analysis of the inhibitory ability of compounds in honey on MMP1

The inhibitory ability of compounds in honey was analyzed through ligand-based virtual screening (SVBL) with KNIME software as proposed by Akgun (2020). The standard ligand targeting MMP-1 was used along with its inhibitory activity from the ChEMBL database by entering "MMP-1" as the search keyword. There were 6,871 activities in MMP-1 protein data with ID CHEMBL332 with different standard parameters. 4,089 activities were taken with the standard type of minimum inhibition at 50% concentration (IC_{50}) and downloaded in .csv format.

The inhibition prediction model was filtered using parameters such as the number of hydrogen binding acceptor (HBA), hydrogen binding donor (HBD), LogP value, and molecular weight. After the ligands were sorted, a prediction model was made using a random forest predictor and the prediction model and model performance parameters were obtained. The compound data in honey were gained from the ChEMBL database according to the phytochemicals and the SMILES code which was entered into the prediction model so that the profile of the inhibitory ability of the compound in honey was obtained. Then, the standard compound glycylhydroxamic acid, which is known to have inhibitory activity against MMP-1 was added to further validate the results.

Insulating membrane from eggshell

The membrane was insulated from eggshell in reference to the process proposed by Ponkham *et al*. 2011 with some modifications. The waste eggshells were thoroughly washed under a running tap until clean. Then, it was left until air-dried. The membrane was taken with tweezers and inserted into a falcon tube. Then, it was put into an icebox containing ice cubes.

Eggshell membrane pretreatment

The eggshell membrane was blended with cold water (1:6 w/v) at 4^{o}C for 3 min and then filtered. Then, it was mixed with 0.45 M NaCl (1:6 w/v) for 3 min with a magnetic stirrer. The retentate was homogenized with a homogenizer for 8 min with 0.45 M NaCl (1:6 w/v) at a speed of 3,000 rpm, washed with distilled water (1:6 w/v),

then centrifuged at 2000 g for 30 min at 4°C. The precipitate was collected and then stirred for 4 min in a mix of 0.2% NaOH (w/v), 0.2% H_2SO_4 (w/v), and 0.7% (w/v) citric acid. The precipitation ratio of each solution was 1:7 (w/v). Then, it was washed with neutral distilled water and filtered. Furthermore, the retentate was soaked for 24 hours at room temperature using 10% NaCl (w/v), filtered, and bleached at room temperature for 24 hours with 1% (w/v) H_2O_2 in 0.01 M NaOH with a ratio of 1:6 (w/v). Finally, it was neutralized and washed with distilled water (Ponkham *et al*. 2011).

Collagen extraction

Extraction was carried out using citric acid. The membranes resulting from the pretreatment process were mixed in a shaker water bath for 2 hours at 4°C with citric acid in a ratio of 1:8 (w/v) and then centrifuged for 8 min at a speed of 3,000 g. After that, it was mixed again with a shaker water bath (4°C, 24 h) 3 times, homogenized (6,000 rpm, 4°C) for 2 min, then centrifuged (2,500 g) at 10°C for 40 min. This precipitate was extracted once (Ponkham *et al*. 2011).

Making a facial cream formulation from eggshell membrane collagen combined with honey

Face cream making process begins with weighing the ingredients used with the following formulation.

Table I. Face cream formulation (Quantity for 100g)

Ingredients	Cream with active ingredients	Base Cream
Stearic acid	3.0002 g	3.0063 g
Cetyl Alcohol	5.0022 g	5.0083 g
Triethanolamine (TEA)	2.2084 g	2.2828 g
Glycerin	5.0018 g	5.2180 g
Propyl Paraben	0.0888 g	0.0883 g
Methyl Paraben	0.1106 g	0.1156 g
Liquid Paraffin	5.0158 g	5.0486 g
Aquades	70%	70%
Collagen Extract	4.1206 g	-
Honey	5.0251 g	-

The cream was made in reference to the process proposed by Yumas (2016) with some modifications. The cream formulation consisted of an aqueous phase (aquadest, TEA, propyl paraben, methyl paraben, and glycerin) and an oil phase (stearic acid, cetyl alcohol, and liquid paraffin). Meanwhile, collagen and honey extracts served as the active components. The oil phase ingredients were melted on a hot plate until they were dissolved and mixed consistently. At the same time, propyl paraben, methyl paraben, and glycerin were mixed in a porcelain dish and heated using a hot plate until dissolved and evenly mixed. In addition, distilled water that had been mixed with TEA in a beaker was also heated on a hot plate while being homogenized with a homogenizer at a speed of 250 rpm. When the propyl paraben, methyl paraben, and glycerin had dissolved and reached a temperature of 70 ºC, the solution was immediately poured into a beaker containing distilled water and TEA. Furthermore, when it had dissolved and reached a temperature of 70 °C, the oil phase ingredients were immediately poured into the water phase in the beaker by continuously homogenizing and accelerating to 1050 rpm to form a soft cream phase. In the manufacture of cream with active ingredients, cooling is carried out first until it reaches a temperature of 30 °C. Next, the collagen extract was put into the cream phase while being homogenized until evenly mixed. The cream was cooled again until the temperature dropped to 25 °C and then the honey was mixed into the cream phase while being homogenized.

Proximate test

Moisture test

A total of 2 g of cream samples were dried in an oven at 105 ºC for 3 hours. Then, the sample was cooled in a desiccator and then weighed. After that, it was heated again in the oven for 30 min and cooled in a desiccator and weighed. This step was repeated until a constant weight or weighing difference was < 0.2 mg. The reduction in weight determines the water content of the sample (AOAC, 1970).

Ash level test

The ashing cup was prepared and then burned in a kiln. After burned, it was cooled in a desiccator, and weighed. A total of 3-5 g of the sample was weighed in an ashing dish, then placed in an ashing sow to be burned until it became gray ash or until the weight was constant. The ashing process was carried out in 2 stages, at a temperature of 400 ºC and a temperature of 550 ºC. After that, it was cooled in a desiccator and weighed (AOAC, 1970).

Lipid level test

A sample of 4-5 g was weighed in an extraction tube. Then, it was mixed with 1.5 mL of 35% (v/v) ammonia and 7 mL of warm water until evenly distributed. Next, it was heated at a temperature of 60-70 ºC for 15 min. 10 mL of

ethanol was added and then shaken until cold. A total of 25 mL of diethyl ether was added, then the tube was closed with a tube cover and then shaken again for 1 minute until evenly distributed. The temperature was subsequently let to cool down before the cap was opened. After that, 25 mL of petroleum ether was added. Then, the tube was closed with a tube cap that had been moistened with water and then shaken evenly for 30 min. The tube was held flat on the bottom, left for 30 min until the ether layer got clear and completely separated from the aqueous layer (AOAC, 1970).

Protein content

A sample of 0.2 g that had been mashed was weighed, and then put into a Kjeldhal flask. Then, 0.7 g of N catalyst was added (250 g of Na_2SO_4 + 5 g of $CuSO_4$ + 0.7 g of Selenium/TiO_2), and 4 mL of concentrated H_2SO_4 was added. Destruction was carried out in the fume hood until the color turned clear green. After the color changed, it was cooled, and 10 mL of distilled water was added. Then, it was distilled with 20 mL of NaOH-Tio (NaOH 40% + $Na_2S_2O_3$ 5%) and the distillate was accommodated in 4% H_3BO_3 which had been given the Mr-BCG indicator. The distillation was carried out until the distillate volume reached 60 mL (the color changed from red to blue). After the volume reached 60 mL, the distillation was stopped, and the distillate was titrated with a standard solution of 0.02 N HCl to the end point of the titration (the color changed from blue to pink). The titration volume was then recorded, and the protein content was calculated (AOAC, 1970).

Viscosity and pH test

In viscosity testing, facial cream preparations were tested using a Brookfield Viscometer with the appropriate spindle number at speeds of 3, 6, and 12 rpm. Observations on the scale were conducted after 3 rounds. Measurement of the degree of acidity used the pH meter (SNI 06-6989.11-2004). A total of 10 g of the sample was dissolved in 50 mL of distilled water until homogeneous, then the pH meter was dipped into it. The pH tolerance range for the cream was between 4.0-7.5.

Microbiological testing

The test sample which had been diluted up to 10^{-2}, in each dilution 1 mL was expelled with a sterile pipette and then inoculated on the Plate Count Agar media with the pour plate method. Then, it was incubated at 30 °C for 72 hours. Next, the number of colonies on the plate was counted. Meanwhile, for the analysis of yeast-mold numbers, the test samples which had been diluted up to 10^{-2} were inoculated on Sabouraud Dextrose Agar media using the pour plate method. The next step was to incubate the test samples at 20 ºC and observe them for 7 days. After that, the fungal colonies that grew were counted.

Irritation test on animal skin

The experimental animals in this study were white rats (*Rattus norvegicus*) wistar strain from the Animal House, Faculty of Biology UGM. The rats were between 2 and 3 months and had good health conditions and healthy skin. Bedding made from sawdust was added into a cage measuring 40 cm x 40 cm. In addition, regular food supplies and drinking water were placed ad libitum in each cage. Six rats were placed in each cage that had been provided with bedding and then the cages were placed in the Animal Physiology Laboratory, Faculty of Biology, UGM for 7 days. Adaptation was done before testing. The rats were fed as much as 7.5 g/head/day. On the eighth day, prior to the cream application, the six experimental animals were anesthetized, and their blood was taken from the orbital sinus vein of the eye using a microhematocrit pipette tested with a hemocytometer. The area of 5 cm x 2 cm on the back hair of the rats was shaved with a razor and then cleaned using an alcohol swab. The rats were left for 24 hours to give them time to adapt with any changes on their skin and to be observed. A facial cream made from collagen eggshell and bee honey was applied to the area as much as 0.2 g and then covered with hypoallergenic gauze, cellophane paper, and a bandage. After 24 h, the bandage was removed and the skin area was observed at intervals of 24, 48, and 72 h after applying the cream. After the cream was applied, the blood of the mice was tested again. The primary irritation index is calculated using the following formula.

$$\text{Primary Irritation Index} = \frac{A - B}{C}$$

A: The total score of erythema and edema of all sample observation points at 24, 48 and 72 h divided by the number of observations.
B: Total score of erythema and edema of all control observation points at 24, 48 and 72 h divided by the number of observations.
C: Number of animals. (BPOM, 2020)

Organoleptic testing (Panelist test)

Panelist tests consisted of 11 men and 19 women ranging in age from 19 to 40 years were

performed. The panelist inclusion criteria would be young adults with normal hands and backs because these parts of the body were easily visible and had no history of allergies. All panelists first washed their hands before 0.5 g face cream sample was applied on their body and left for 15 min. The test criteria included aroma, consistency, texture, stickiness, sensation, color, itching, washability, and response after washing.

RESULT AND DISCUSSION

Collagen content in eggshell membrane

In the study by Ponkham *et al.* (2011), it was found that there was 8% eggshell membrane from 500 g per 15 L of sample solution. The results of HPLC (High Performance Liquid Chromatography) data analysis also revealed that type I collagen weighed 495 to 507 mg per 100 g of dry weight of the sample. Meanwhile, Bayraktar *et al.* (2021) revealed that eggshell membranes were composed of 80-85% organic components comprising 10% collagen type I, V, or type X and other protein components consisting of 70-75% osteopontin, fibronectin, glycoproteins, and proteoglycans, and 15–20% inorganic components, namely $CaCO_3$.

In a study by Kalman *et al.* (2020), eggshell membranes that are soluble in water and hydrolyzed as a source of glycosaminoglycan (GAG) were useful for hydrating the skin by increasing collagen levels, reducing matrix metalloproteinase, inhibiting collagenase and elastase, and reducing inflammation in the skin. Eggshell membrane was chosen as a source of collagen rather than other active ingredients because it is safe and it rarely causes allergic reaction when used in food products or supplements (Turck *et al.*, 2018). Anti-aging products that are widely circulated in the market today still use retinoid which can cause irritation to the skin and have teratogenic potential. Therefore, they should be avoided, especially by pregnant women and nursing mothers. The collagen content in this cream can be used before the age of 40 because at this age humans cannot produce collagen in the body, so they need external intake either orally or topically from skin care products. According to Alhana *et al.* (2015), at the age of 40, humans cannot produce collagen in the body, so they need external intake, either orally or from skin care products. Thus, this collagen cream is good for humans before the age of 40 years.

Compound inhibition ability of honey in MMP-1

The addition of honey as an active ingredient in the cream is able to inhibit the aging-causing protein, namely collagenase (MMP-1) obtained from an inhibition model with a random forest prediction. From the analysis, the model accuracy value was very high (97.89%) with error 2.1%, the true positive rate was very good (1.0), and the agreement value (Cohen's kappa) was good with value 0.698. These results indicate an accurate and optimal prediction model, so it is qualified to be used for inhibition (Figure 1).

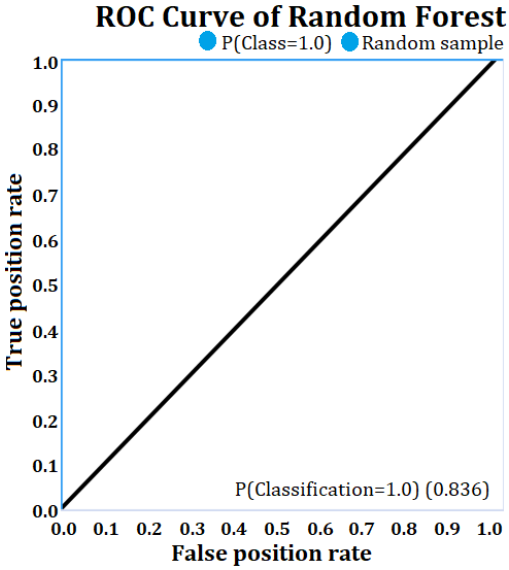


Figure 1. ROC curve of Random Forest prediction model

From the prediction model above, it is possible to compute the predicted activity of 78 phytochemicals in honey from the ChEMBL database (Table II). There were four material compounds in honey with the highest predicted inhibition of MMP-1, including benzylpenicillin with a predictive value of 1.0 (the best inhibition of MMP-1), cefuroxime, ampicillin, and amoxicillin with a value of 0.98. These four compounds have 1.0 for predictive value and close to 1.0 to show their effectiveness in preventing aging.

Proximate face cream level

The water content was 77.0926 ± 0.0401%, the ash content was 0.0062 ± 0.0005%, the protein content was 1.8690 ± 0.0085% and the fat content was 0.0058 ± 0.0002% (Table III). According to the Directorate General of POM (1979), the water content in the external cream preparation should not be less than 60% so that the water content in this cream preparation has met the requirements and is able to properly hydrate the skin.

Table II. List of predicted photochemical inhibitory activity in honey on MMP-1

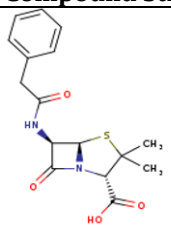
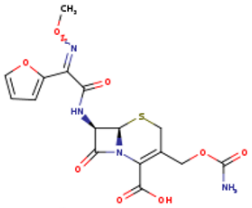
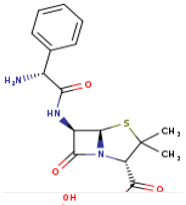
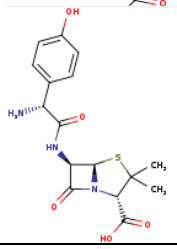
Compound Name	Compound Structure	SMILES code	Score
Benzylpenicillin		CC1(C)S[C@@H]2[C@H](NC(=O)Cc3ccccc3)C(=O)N2[C@H]1C(=O)O	1.0
Cefuroxime		CO/N=C(C(=O)N[C@@H]1C(=O)N2C(C(=O)O)=C(COC(N)=O)CS[C@H]12)c1ccco1	0.98
Ampicillin		CC1(C)S[C@@H]2[C@H](NC(=O)[C@H](N)c3ccccc3)C(=O)N2[C@H]1C(=O)O	0.98
Amoxicilin		CC1(C)S[C@@H]2[C@H](NC(=O)[C@H](N)c3ccc(O)cc3)C(=O)N2[C@H]1C(=O)O	0.98

Table III. The proximate levels of facial cream

Test Parameters	Results (%) $\bar{x} \pm \sigma$
Water content	77.0926 ± 0.0401
Ash content	0.0062 ± 0.0005
Protein content	1.8690 ± 0.0085
Fat level	0.0058 ± 0.0002

Table IV. Observations of erythema and oedema

Group	Individual	Observations after applying the cream at certain intervals (hours)							
		0		24		48		72	
		E	O	E	O	E	O	E	O
Control	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Treatment	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0

Information:E = Erythema O = Oedema

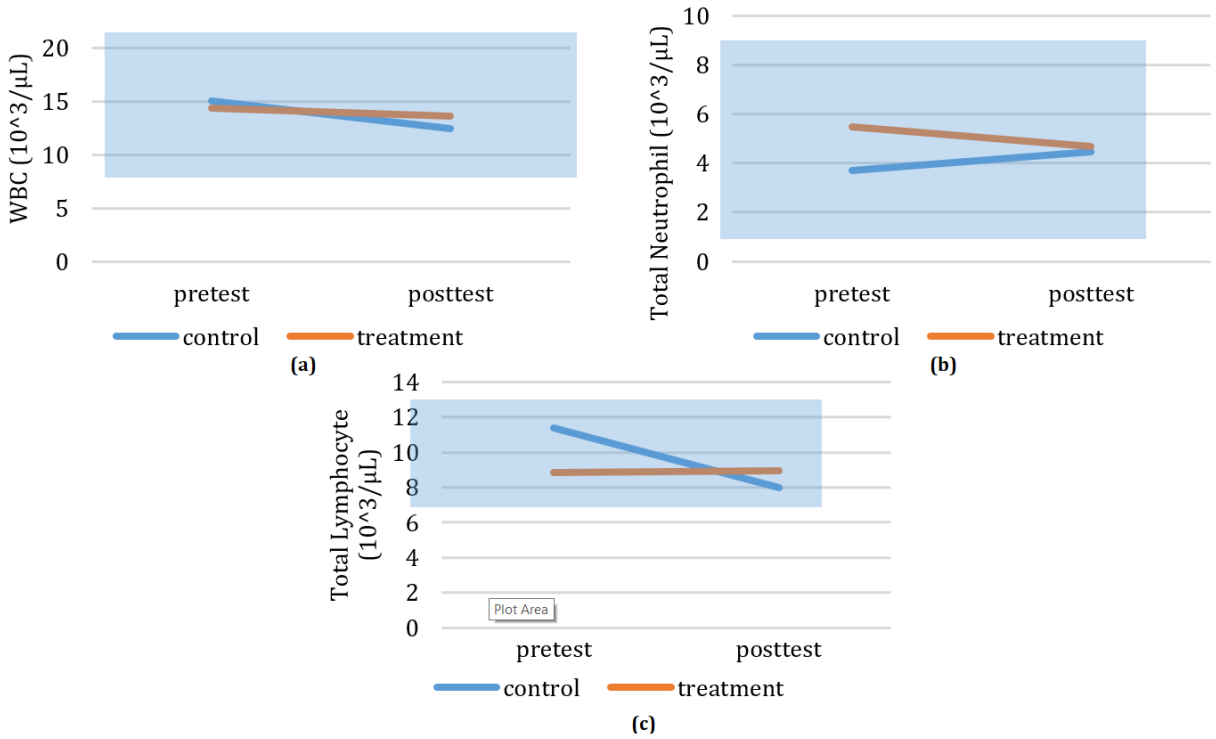


Figure 2. (a) White Blood Cells (WBC) pretest and posttest in experimental animals in the control and treatment groups, (b) The number of pretest and posttest neutrophils in experimental animals in the control and treatment group, (c) The number of pretest and posttest lymphocytes in experimental animals in the control and treatment groups

Associated with the ash, protein, and fat content is the accumulated use of ingredients in the cream which indicates that this cream consists mostly of protein and a small amount of fat.

Face cream viscosity

The viscosity value of a cosmetic ingredient shows the stability of the resulting product. The viscosity value of facial cream creams from three repetitions were 66.32 dPas (3 rpm), 68.25 dPas (6 rpm), and 63.25 dPas (12 rpm) respectively with average value was 65.94 dPas. Determination of thickness and viscosity in cream preparations is important, especially for materials classified into the oil phase such as stearic acid and cetyl alcohol because they generally have solid characteristics at room temperature. Changes in the viscosity of a product are strongly influenced by various external factors, such as concentration, temperature, metal ions, electrolytes and non-electrolytes, mechanical stirring shear, concentration, temperature, and pH (Zhenhua *et al.* 2020). The results of the facial cream viscosity analysis show that the average cream viscosity value was 65.94 dPas. According to Puspitasari *et al.* (2018), the acceptable viscosity of topical preparations is 50-1000 dPas. This viscosity value is thought to be influenced by the addition of a solution of collagen and bee honey which has high water content. The water content in the cream sample increases causing a decrease in the surface tension of the cream, so that the oil phase with the water phase is balanced.

Degree of acidity (pH)

The pH value for the experimental face creams from three repetitions were 7.08, 7.05, and 7.11 respectively with average value was 7.08. The pH value was in the range of pH values contained in SNI 16-4399-1996 as a condition of skin moisturizing quality (4.5-8.0) and the normal skin pH range is 4-6 (Ali and Yosipovitch 2013). Therefore, the facial cream was relatively safe to use. It is important to know the pH value to prevent skin damage or irritation. According to Faradiba (2013), cosmetic preparations must have a pH that is in accordance with the pH of the skin, which is

between 4.5-7.5. This is because a pH that is too acidic can cause skin irritation, while a pH that is too alkaline can lead to scaly skin.

Face cream microbiological test

There were two checking types of facial cream microbiological test that were total plate counts (TPC) and total yeast-mold counts (TYMC). The result of TPC from three repetitions were 22, 25, and 20 CFU/g respectively with average value was 22.3333 CFU/g. While the result of TYMC from three repetitions were 13, 11, and 16 CFU/g respectively with average value was 13.3333 CFU/g.

The results of TPC and TYMC indicates the presence of bacterial and fungal overgrowth in the cream preparations. This explains that the concentration of active ingredients of bee honey can only prevent the growth of certain types of bacteria and fungi but does not hinder the total growth of microbes in the facial cream. However, the amount of bacterial contamination is still in accordance with the quality requirements of skin moisturizer (SNI 16-4399-1996) which is below 10^2 CFU/g, while the amount of yeast-mold contamination is does not still in accordance with the quality requirements of skin moisturizer (SNI 16-4399-1996) which is negative.

Irritation test on animal skin

The results of skin irritation testing which include blood tests and observation of erythema-oedema after cream application on 5 months old male experimental animals Rattus norvegicus Wistar strain weighing 300-450 g (Table IV and Figure 2).

Control and treatment group of mice, at 3 times observation, at intervals of 24, 48, and 72 h, show an erythema value of 0 (no erythema) and an edema value of 0 (no edema) (Table IV). The erythema score scale of 1 indicates slight erythema, 2 indicates clear erythema, 3 signifies moderate-strong erythema, and 4 means severe erythema. Meanwhile the Edema score scale of 1 indicates mild edema, 2 signifies mild edema (obvious margins and enlargement), 3 means moderate edema (1 mm thickness), and 4 indicates severe edema (>1 mm thickness).

The irritation score (primary irritation index) was obtained at 0. Skin irritation test of experimental animals shows that the cream sample did not cause irritation. These results were confirmed by the leukocyte profile from the blood test. Regarding the number of white blood cells (WBC), lymphocytes, and neutrophils in the pretest (before applying cream) and posttest (after applying cream) (Figure 2), the values of both control and treatment groups were still in the normal range or within the baseline block (blue area) which showed that the limit range for the WBC, neutrophils, and lymphocytes respectively are 10.4-22.1 (103/µL), 2.2-9.1 (103/µL), and 5.9-13 (103/µL). Although on Figure 2a and 2c, the control group decrease while the treatment group increase. On the Figure 2b, the control group increases while the treatment group decreases. Those difference can occur due to changes in temperature or other effects and the value cannot be generalized because it is very specific. This indicates normal total leukocytes, neutrophil counts, and lymphocyte counts on the rats and there was no significant effect of applying the cream. So, it can be said that the cream did not cause irritation in experimental animals. The profile of leukocytes, especially lymphocytes and neutrophils can be used as parameters for the occurrence of irritation because white blood cells function to fight foreign substances and to heal irritating wounds. Neutrophils are cells that function as the main defense before there is an increase in lymphocytes which will produce antibodies (Sherwood, 2007). The WBC graph also shows that the two lines tend to coincide so that it can be said that the mean of total leukocyte count of rats before and after applying cream is not significantly different. The results of irritation testing and animal blood tests show that the cream formulations, both basic cream and cream with the active ingredients of collagen and honey extract, were safe and did not cause skin irritation, so the test was followed up with organoleptic tests on the panelists.

Face cream panelist test

The results of panelist tests with 19 women and 11 men ranging in age from 19 to 40 years responding to the use of facial cream products (Table V). The test criteria include aroma, consistency, texture, stickiness, sensation, color, taste. Itching, erythema, easy washing, and response after washing.

The test parameters given the assessment were favored by the panelists (19 women and 11 men, ranging in age from 19-40 years) (Table V). This shows that the face cream was positively perceived by panelists from young to Middle Ages, both males and females.

Table V. Panelist test results on facial cream

Rating parameters			Score	Conclusion
Scent	1	Not fragrant	2.1	Slightly fragrant
	2	Slightly fragrant		
	3	Fragrant		
	4	Very fragrant		
Consistency	1	Very hard	2.9	Mushy
	2	Hard		
	3	Mushy		
	4	Very mushy		
Sensation	1	Very hot	2.7	A bit cool
	2	Hot		
	3	A bit cool		
	4	Cool		
Texture	1	Very rough	3.7	Very soft
	2	Rough		
	3	Soft		
	4	Very soft		
Itchy feeling	1	So itchy	3.7	It doesn't itch
	2	Itchy		
	3	A bit itchy		
	4	It doesn't itch		
Sticky	1	Very sticky	2.7	Slightly sticky
	2	Sticky		
	3	Slightly sticky		
	4	Not sticky		
Color	1	Do not like	3.6	Really like
	2	Fairly like		
	3	Like		
	4	Really like		
Easy to wash	1	Very difficult	3.1	Easy
	2	Difficult		
	3	Easy		
	4	Very easy		
Response after washing	1	Very dry	3.6	Humid
	2	Dry		
	3	Slightly humid		
	4	Humid		
Erythema	1	Awfully	3.9	There is not any
	2	Severe		
	3	Somewhat bad		
	4	There is not any		

In addition, the face cream offered to the panelists is preferred because it has high moisturizing power and does not cause irritation to the skin. This is because all the concentrations of the ingredients used in the formulation of facial creams are still below the threshold value or are safe to use.

CONCLUSION

From the data collected, eggshell membranes were useful in hydrating the skin by increasing collagen levels, reducing matrix metalloproteinase, slowing down the production of collagenase and elastase, and shooting inflamed

skin. The addition of honey in anti-aging face cream had a very high model accuracy value (97.89%) in inhibiting MMP-1 so that it had the potential to be further developed. The results show that in 100 grams of face cream formulation with active ingredients of chicken eggshell membrane collagen extract 4.1206% and bee honey 5.0251%; 77.0926% water; 0.0062% ash; 1.8690% protein; 00058% fat, the viscosity value was 65.94 dPas, and the pH was 7.08. With these components and characteristics, this product did not irritate the skin of the experimental animals. Furthermore, the average value of the assessment of attributes (such as aroma, consistency, texture, stickiness, homogeneous sensation, color, appearance, itching, erythema, washability, response after washing) was positively perceived by the panelists with a score of 1–3.9 and the highest total achievement bacteria 25 CFU/g met the skin moisturizer quality requirement which should not exceed 10^2 CFU/g, while yeast-mold 16 CFU/g did not meet the standard of skin moisturizer based on SNI 16-4399-1996. These results cotribute as reference for other researcher in developing studies on chicken eggshell membrane collagen combined with honey for anti-aging cream.

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