Quality Characteristics of Body Cream with the Addition of Gelatin from Tilapia (Oreochromis niloticus) Scales as an Emulsifier

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Abstract. Gelatin is a type of protein extracted from the skin collagen tissue, bone or connective tissue of animals. Gelatin is commonly used as a thickener, an edible film, and an emulsifier whether in food, cosmetic or pharmacy. This research aimed to examine the effect of adding gelatin as an emulsifier in cosmetic products, namely body cream. The treatment has tilapia scales gelatin with different concentrations, 0% as a control, 5%, 7%, and 9%. The difference in gelatin concentration had significantly different on the hedonic test, pH value, and specific gravity of body cream. The difference of the result shown in total bacteria and irritation test were significant. Overall, body cream characteristics with 9% gelatin had better values than the control, 5% and 7% gelatin. The body cream with 9% gelatin was pleasant for the panelists with hedonic value of 8.30 < µ < 8.48, pH 4.86, viscosity 98.13 Poise, specific gravity 1.03 g/mL, no bacteria, and non-irritation symptoms. Thus, tilapia scale gelatin has great potential to be developed as an emulsifier in body creams.

1.Introduction

Tilapia (Oreochromis niloticus) is one of freshwater fish commodities in Indonesia. According to data from the Ministry of Maritime Affairs Indonesia (KKP) [1], in 2016 the amount of tilapia production in Indonesia was approximately 1,114,156 tons and in 2017 it reached 1,265,201 tons or in other words, the amount of tilapia production in Indonesia has an increase of 13.5%. The increasing of fish production not only caused the increasing of fish based processing products but also caused the increasing of waste from processing products such as bones, skin and scales. Fish scales contain collagen which can be used as raw material for making gelatin. According to Elgadir et al. [2], currently most of gelatins produced from porcine skin, bovine bone and bovine skin. Porcine gelatin is prohibited for muslim community and bovine gelatin because of the fear of BSE (bovine spongiform encephalopathy) or mad cow disease which can be contracted through cow-based proteins. Fish gelatin has received considerable attention as the alternative to bovine and porcine gelatin.

Gelatin is a type of protein, which is extracted from collagen tissue in animals. Collagen in animals can be found in bones, skin, and connective tissue. Gelatin has long been used in the food, pharmaceutical, cosmetic, and photography industries. This is consistent with the research of Tan et al. [3], that gelatin is one of the biopolymers used as a thickener, emulsifier, gelling agent, and foaming agent. Gelatin is used in the manufacture of glues, lipsticks, shampoos, soaps, creams, and lotions. Body cream is one of the cosmetic industry products as a result of oil and water emulsion. Therefore, in the production process, it requires an emulsifier. According to Kalkurni and Shaw [4], creams typically...
contain >20% water. Creams come in the form of either oil-in-water emulsions (in which the oil phase is dispersed as droplets in the water phase; “O/W”) or water-in-oil emulsions (in which the water phase is dispersed as droplets in the oil phase; “W/O”).

The physical properties of cosmetic samples must be considered. Good cream is a sample that has acceptable limit for customer use. Parameters of the body cream were tested including hedonic test, pH, viscosity, specific gravity, irritation test and total bacteria. According to Satheeshan et al. [5], the prepared body cream formulations were light, non-greasy and liquid dosage form and appears as soft, free flowing with uniform consistency and adhere over the skin as a film. Therefore, this research is to determine the best formulation in body cream.

2. Research Methods

2.1. Materials

Tilapia scales were freshly collected from PT. Aquafarm Nusantara (Semarang, Indonesia). Chemicals in the production of gelatin were NaOH, HCl (CV. Indrasari, Semarang, Central Java). The ingredients for producing body cream were beeswax, olive oil, vaseline, aloevera extract, and cucumber extract (Syah-house, Jakarta).

2.2. Methods

2.2.1. Production of Gelatin

Production of gelatin was based on modification the research of Sghayyara et al. [6], the fish scales were rinsed with running water so that the remaining flesh and dirt could dissolve in the water. The next step was immersion in 1% NaOH solution with 1:3 ratio of b/v for three hours. Tilapia scales were then rinsed with running water to a neutral pH, immersed again in 4% NaOH solution with 1:10 ratio of b/v for 24 hours, and rinsed until the pH was neutral. Extraction was carried out in a waterbath using distilled water with 1:2 ratio of b/v at 80°C for four hours. The next process was filtering using calico cloth and drying using an oven at 60°C.

2.2.2. Production of Body Cream

Production of body cream was based on modification the research of Chen et al. [7]. The oil phase was prepared by melting the beeswax, olive oil, and vaselin at 75°C and mixing the ingredients uniformly. The water phase was prepared which were aloevera extract and cucumber. The water phase was warmed to 75–80°C until all ingredients were dissolved. When the water and oil phase were at the same temperature, the water phase and gelatin was slowly added to the oil phase with moderate agitation and was kept stirred until the temperature dropped to 40°C.

2.2.3. Gel Strength Test

Based on GMIA [8], the sample was weighed as much as 7.5 grams and placed in a bloom bottle, then added 112.5 mL of distilled water and the bottle was closed with aluminum foil. Samples were dissolved in a bloom bottle in a water bath at 50°C until dissolved. The dissolved solution was then put on a cold bath with a temperature of 10°C for 17 hours. Bloom or gel sample strength is measured using a gelometer.

2.2.4. pH test

The pH test according to AOAC [9] was carried out using a pH meter that has previously been calibrated with the buffer condensation. Measurements were made directly by dipping the pH’s eye in the body cream sample until the pH value shown on the pH meter screen was stable.

2.2.5. Viscosity

Viscosity test used the Brookfield Viscometer [10]. The spindle’s size was 64, with a speed of 0.3 rpm at 25°C. The sample viscosity measurement was by pressing the motor on up to two spindles. Finishing the measurement by pressing back the motor on until it appears off next to the speed number on the display.
2.2.6. Hedonict Test
The hedonic test was carried out on body cream products using 30 panelists who were in the age 20 to 25. The hedonic test used was the preference test for the parameters of color, smell, homogeneity, and moisture impression on a scale of 1 to 9 (strongly unpleasent to strongly pleasent).

2.2.7. Specific gravity
According to Buhse et al. [11] specific gravity measurements were carried out using a pycnometer. The mass of the pycnometer was weighed and recorded first (a). Then the body cream sample was inserted into the pycnometer until it is full and then closed, weighed and recorded its mass (b).

The specific gravity of body cream is determined by the formula (1).

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\text{Specific Gravity (g/mL)} = \frac{b-a}{Pycnometer \ Volume \ (10 \ mL)}
\] (1)

2.2.8. Total Bacteria
According to Elmorsy and Hafez [12] the total bacteria measurement was aseptically weighed as much as 1 g of body cream sample and put in 1 mL diluent condensation to be homogenized. The 1 mL sample inoculated on a sterile petri dish. Plate Count Agar (PCA) Media which was sterilized at 45° to 55°C was poured into 20 mL petri dishes. The petri dish was shaken and set aside to solidify. Incubation was carried out at room temperature for 24 hours. The number of colonies that grew was reported as the total bacteria.

2.2.9. Irritation Test
According to Jaiswal et al. [13], the irritation and sensitivity test on normal skin was carried out for 10 minutes after being applied to 30 respondents who has normal skin with the respondent's consent. The sample was smeared on the backs of the left and right hands. Furthermore, the color change that occurs on the back of the respondent's hand was observed. When there was no reaction (not red and not swollen) it was marked (-), when there was a reaction (reddening of the skin) then it was marked (+), and when there was swelling then it was marked (++).

2.2.10. Statistical Analysis
The design of this study used a completely randomized design. The parametric data were carried out by one-way analysis of variance (ANOVA), then using Tukey's test to comparison of means and non parametric data were out by Kruskal Wallis test using SPSS version 24.0 for windows (SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1. Gel Strength of Gelatin
The results of the gel strength test of tilapia scales gelatin were 215 grams bloom. The value of gelatin strength from the research that has been carried out meets the gelatin quality standard, which is in the range of 50-300 gram bloom values based on GMIA [8]. Factors that influence the value of gel strength are the molecular weight of gelatin and amino acids. According to Kittiphattanabawon et al. [14], the molecular weight of gelatin is related to the long chain of amino acid bonds that form the gelatin. The larger and longer the chain, the greater the molecular weight and the higher the value of the strength of the gel, besides that the long chain of amino acids in gelatin is determined by the temperature used during extraction.

3.2. pH of Gelatin
Based on the results of pH testing on gelatin made from tilapia (Oreochromis niloticus) scales, it is 5.026. The pH value is one of the important chemical properties of gelatin, because the pH value can affect the other properties of gelatin, thus determining the next gelatin application. The pH value is in
accordance with the requirements according to the GMIA [8], the pH of gelatin that meets the requirements is pH 3.8-5.5.

3.3. Viscosity of Gelatin
The results of the viscosity test of tilapia scales gelatin were 0.0221 Poise (2.21 cP). This viscosity value is included in the criteria for gelatin type A. According to the GMIA [8], the requirements for good gelatin viscosity for type A are 15-75 mPs (1.5 cP to 7.5 cP) while for type B is 20-75 mPs (2 cP to 7.5 cP).

3.4. Hedonic

| Gelatin Concentration (%) | Specification | Color       | Smell        | Homogenity   | Moist Impression | Average    |
|---------------------------|---------------|-------------|--------------|--------------|------------------|------------|
| 0                         |               | 8.43±0.50a  | 8.10±0.76b  | 7.13±0.73a   | 6.87±0.86c       | 7.47<µ<7.79|
| 5                         |               | 8.37±0.49b  | 8.03±0.72ab | 7.57±0.57b   | 7.37±0.76b       | 7.73<µ<7.93|
| 7                         |               | 8.70±0.47b  | 8.40±0.56b  | 8.03±0.67c   | 7.70±0.60b       | 8.10<µ<8.32|
| 9                         |               | 8.83±0.38d  | 7.77±0.77a  | 8.67±0.55d   | 8.30±0.53c       | 8.30<µ<8.48|

Note:
- The data is average yield of three replications ± standard deviation
- Different superscripts show significantly different (p<0.05)

3.4.1. Color
Based on data, the highest value of hedonic body cream color with a value range of 1 to 9, is obtained in 9% tilapia gelatin body cream of 8.83, while the lowest value is 5% tilapia gelatin body cream gelatin of 8.37. This shows that the color of 9% tilapia scales gelatin body cream is the most preferred by panelists compared to other tilapia gelatin body cream. Tilapia gelatin body cream which is more attractive to panelists because it has more attractive brightness level. Body cream with 9% gelatin formula has color between cream and yellow, while body cream with 5% gelatin formula has more dull color and is not bright enough so that panelists do not like it. The higher the gelatin concentration, the more homogeneous and stable the body cream sample is. According to Hiola et al. [15], a homogeneous sample will give an even dosage color, while product instability can be indicated by discoloration and odor.

3.4.2. Smell
Based on the hedonic odor of body cream with a value range of 1 to 9, the highest score is obtained for body cream with the 7% gelatin addition at 8.40, body cream without the gelatin addition is 8.10, the 5% gelatin addition is 8.03, while the lowest score is in body cream with the 9% gelatin addition at 7.77. It can be concluded that the smell of body cream with the 7% gelatin addition is the most preferred by panelists compared to other body creams. The smell of the body cream has a lavender scent influenced by the fragrance addition to the sample. The odor caused by the gelatin addition can be masked by this fragrance addition. Gelatin also has characteristic that can bind water, which is the basic ingredient of this fragrance. According to Syahida et al. [16], gelatin has the ability to bind water. Gelatin easily absorbs and binds water by forming a stable matrix in the gel formation process so that water is retained in the matrix arrangement. This was confirmed by Tan et al. [17], that gelatin is hydrophilic and lipophilic, so it can bind water on one side and increase the oil phase on the other.

3.4.3. Homogeneity
Based on the hedonic homogeneity of body cream with a value range of 1 to 9, the highest score is obtained in body cream with the addition of 9% tilapia gelatin, which is 8.67, while the lowest score is found in body cream without gelatin addition, which is 7.13. It can be concluded that the homogeneity
of body cream with 9% concentration of tilapia fish scales gelatin addition is the most preferred by panelists compared to other body creams. The body cream sample consists of water and oil phases which need an emulsifier so that the sample becomes homogeneous. Gelatin has function as an emulsifier, so the higher the gelatin concentration is added, the more homogeneous the body cream sample will be. According to Nguyen et al. [18], gelatin is amphiphilic because it contains amino acids that are hydrophilic and lipophilic so that trigger some activities on the surface of the molecule. These characteristics indicate that gelatin can function as an emulsion forming and stabilizer because it can be absorbed on the oil and water interface in the emulsion system.

3.4.4. Moist impression
Body cream itself is one of the cosmetics which is essential to everyone that has a function as a skin moisturizer. The addition of gelatin concentration in the body cream can affect the panelists' assessment of the moist impression. The higher the gelatin concentration added, the higher the panelist's assessment is. This is in accordance with the function of gelatin as a emulsifier. According to Fan et al. [19], the quality of fish gelatin is mostly affected by its amino acid composition. Its unique amino acid composition, particularly the content of amino acids (hydroxyproline and hydroxylysine). The composition of these amino acids causes gelatin to be a multi-purpose material that functions as a filler, emulsifier, binder, as a preservative, humectant and stabilizer.

3.5. pH
Based on the pH test (Fig. 1), the results obtain the pH range between 4.86 to 6.86 which means that body cream with the tilapia gelatin addition is acidic. The pH test is carried out to see the acidity level of the gel sample and to ensure that the gel sample does not cause irritation to the skin. The pH value is in the range of pH values according Chen et al. [7] as a condition for the quality of a skin moisturizer (4.0 to 6.0) so that the resulting body cream is relatively safe to use. This is in line with the results of research conducted by Susilowati et al. [20], pH 4.5 to 6.5 which is owned by the cream is not too far from the physiological pH of the skin, 4.5 to 8.0 so that it is acceptable for the skin used. This is confirmed by Setyawaty et al. [21], skin irritation that can be caused such as the skin becomes dry, cracked, and easily develops infections. If the pH is too acidic it will cause skin irritation and if it is too alkaline it will cause itching on the skin.

![Figure 1. The pH of Body Cream with Differences of Gelatin Concentration](image)

3.6. Viscosity
Based on the viscosity test (Fig. 2), the results of body cream viscosity are between 36.13 to 98.13 Poise (3613-9813 cP). The viscosity test is carried out to see the thickness and flow rate of particles in the
body cream product. The viscosity value is in the range of viscosity values according to Chen et al [7], namely 2000-60,000 cPs so that the resulting body cream is relatively safe to use. According to Sutaryono et al. [22], a good cream must have a thickness level that is not too low and not too high. The thickness of the lotion that is too high will result in the more difficult drug to be released from the lotion preparation, whereas if the thickness is too low it will reduce the length of time the lotion stays on the skin when used.

The stability of the body cream emulsion is also affected by its viscosity. Body creams with high viscosity values will be more stable but tend to be difficult to apply because they have low spreadability, but have high adhesion. On the other hand, the lower the viscosity of a body cream, the greater the spreadability yet the lower the adhesion. This is consistent with the statement of Saptarini and Hadisoebroto [23], the stability and application behavior of lotions and creams are important factors for subject acceptance. That the spreadability is inversely proportional to the viscosity of semisolid samples, the greater the spreadability of cream, the smaller the viscosity. According to Venter et al. [24], a viscosity that is too low will make it easier for the internal phase (oil phase) to move out of the dispersion medium. This results in an unstable dispersion system.

![Figure 2. The Viscosity of Body Cream with Differences of Gelatin Concentration](image)

### 3.7. Specific Gravity

Specific gravity is the ratio of the weight of a substance to the weight of a raw substance with the same volume at the same temperature and expressed in decimals. Specific gravity is used as one of the analytical methods that play a role in determining liquid compounds. It is also used to test the purity of compounds, especially in liquid form, as well as to determine the level of solubility of a substance.

Specific gravity test data (Fig. 3) show the highest average gelatin concentration is 9% (1.03 g/mL) and the lowest average is in the control treatment (0.92 g/mL). The specific gravity value with the gelatin addition is in the range of specific gravity, not far different from the Alamein et al research [25] which shows specific gravity of cosmetics is between 1.005 and 1.013 g/mL. Specific gravity of a material is related to its thickness. The higher the thickness of the material, the higher the specific gravity value. Body cream with the gelatin addition has a higher thickness because gelatin has the ability to become a thickener. According to Shimokawa et al. [26], gelatin has the ability to improve product thickness compared to other thickening agents.
3.8. Total Bacteria

Total bacteria analysis is important to determine whether there are bacteria in body cream products. This analysis is important because contamination from bacteria can cause separation, shrinkage of product weight and the development of unpleasant odors. Damage to the product can be caused by bacteria, yeast, or fungus. The characteristics of microorganisms that have a high adaptability to the environment cause various natural organic components to be easily damaged or degraded.

Based on Figure 4, the log results of the total bacteria test obtained are namely $3.63 \times 10^3$ colonies/g in the control body cream, $3.06 \times 10^3$ colonies/g in body cream with the 5% gelatin addition, and $2.8 \times 10^3$ colonies/g in the 7% gelatin addition, the total bacteria did not fill the requirements while body cream with the 9% gelatin addition was not found bacteria so it fills the requirements based on National Agency of Drug and Food Control of Republic of Indonesia (BPOM RI) [27] no 17 tahun 2014 regarding the requirements for bacteria and heavy metal contamination in cosmetics, namely total bacteria ≤ $10^3$ colonies/g.

Based on the results of the study, the negative or blank controls were not overgrown by bacterial colonies indicates that the media and solvents used in the study were sterile. The absence of bacteria in the body cream with the 9% gelatin addition can be influenced by the pH of the product which tends to be more acidic. Based on research conducted by Dadashi and Dehghan Zadeh [28], bacteria commonly grown in cosmetic products include Bacillus subtilis, Esherichia coli, Bacillus, mycoides, Aerobacter aerogenes, pseudomonas, Sarcina lutae, proteus vulgaris, and Staphylococcus. These bacteria generally cannot grow and develop optimally in too acidic pH. According to Dien, et al. [29], Staphylococcus sp. had a minimum, optimum and maximum pH for growth, namely 4.2; 7.0 to 7.5 and 9.3. Vibrio parahaemolyticus has an optimum pH for growth ranging from 7.8 to 8.6, although it can grow at pH 4.8 to 11.0. Vibrio parahaemolyticus and Esherichia coli can grow at pH of 6.0 to 8.1. The results of total bacteria body cream testing with the addition of tilapia scales gelatin are presented in Figure 4.
3.9. Irritation Test
Based on the results of the irritation test on 30 panelists, there were no irritation symptoms caused by the body cream samples. Skin irritation test is performed to determine the occurrence of side effects on the skin. This is characterized by the absence of red spots, itching, hot skin and pain on the panelists’ skin smeared with lotion samples. According to Ministry of Health of Republic Indonesia (DepKes) [30], an open test for irritation is carried out by applying the sample to the skin and observing the skin reactions that occur. If there is no irritation such as itching and redness, the body cream sample is declared to fulfill the testing requirements.

Skin irritation is closely related to the pH level in body cream samples. The sample is still safe to use because the pH of the sample is still in the appropriate range, namely 4.5 to 8.0. pH value that is too acidic can cause irritation and dry skin. This condition can be caused by emulsifiers and solvents, acids. Once the contact with the chemical that caused the condition is stopped, the skin will heal as it was before. According to Zutshi et al. [31], the pH of cream samples must match the pH range of the skin in humans. If the pH is too alkaline, it will result in scaly skin, whereas if the skin is too acidic it can trigger skin irritation due to damage to the acid mantle in the stratum corneum layer.

4. Conclusion
Body cream with the tilapia fish scales gelatin addition has the potential to be produced, namely pH between 4.86-6.86, viscosity 36.13-98.13 Poise, specific gravity between 0.92-1.03 g/mL and total bacteria 0-3.63x10^3/g. The best body cream formulation is the tilapia scales gelatin addition with 9% concentration based on the hedonic test with a confidence interval of 8.30 <µ <8.48, which is adored by the panelists. pH value 4.86, viscosity 98.13 Poise, specific gravity 1.03 g/mL, no bacteria were found and did not cause irritation.

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