Indonesia's Potential of Natural Emulsifier from Coconut Protein Extraction in Skincare Lotion Application

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ABSTRACT

Background: The Indonesian Cosmetics Industry grew rapidly from 2017 to 2021. However, around 80% of Indonesia's cosmetic raw materials are still imported. In 2018, although Indonesia's coconut potential reaches 2.8 million tons and is estimated to be able to produce 12.9 thousand tons of coconut protein, to take advantage of this potential, in this study, an emulsifying lotion with coconut protein will be made.
Methods: The protein isolate is obtained by isoelectric precipitation of skimmed coconut milk phase. The optimization of coconut protein as emulsifier in lotion is done by several variations; type of emulsifiers, type of oil phases, and pH solubility. The evaluation of emulsion stability is done by observing its accelerated storage test, globules, and sensory evaluation.
Results: An average of 1.36 kg of fresh coconut flesh produce 4.5 g of protein isolate with 57.5% of protein content by Kjeldahl method analysis. The viscosity of the formula using protein isolate was lower (±800 cP) than the lecithin emulsifier (±2000 cP). The stability of protein isolate as an emulsifier in lotion preparations was better than commercial hydrogenated lecithin and the most stable formula against changes in globule size was Formula FP004 (protein dissolution of pH 8, olive oil).
Conclusion: The average dry protein isolate was 4.5 g from 1.36 kg of fresh coconut flesh with a protein content of 57.5%. The viscosity of the formula using protein isolate was lower (±800 cP) than the lecithin emulsifier (±2000 cP). The stability of lotion using protein was better than commercial lecithin.

Keywords: *Coconut, Protein, Isoelectric, Emulsifier, Natural*

INTRODUCTION

Indonesia is one of the three largest coconuts (*Cocos nucifera*) producers in the world. Indonesia's coconut production in 2018 reached 2.8 million tons (which expected to produce around 760 thousand tons coconut flesh), but most of it was exported in the form of unprocessed dry old coconut (copra). Coconut meat has the potential to produce higher economic value if processed first because it contains oil (35%), protein (3.8%), and water content (40.9%) [1]. Coconut has many derivative products in various industrial sectors, one of which is in the cosmetic industry. Coconut can be processed into coconut milk, which is an emulsion that is naturally stabilized by the presence of emulsifiers in the form of proteins and phospholipids. An emulsifier is an important component in an emulsion system that serves to stabilize the oil and water mixture so that separation does not occur.

Coconut protein extraction can be carried out from the skimmed coconut milk phase, which is a by-product of processing Virgin Coconut Oil from the coconut cream phase. The protein extraction process from the skimmed coconut milk phase can be carried out by the isoelectric precipitation method. In this method, protein deposition occurs when the solution reaches the isoelectric point. The isoelectric point is a state where the principle is based on the difference in ionic properties at the surface of the amino acids. The net charge will be more positive at low pH and more negative at high pH. The isoelectric point is a state with a net charge of zero when the pH of a negatively charged amino acid is the same as a negatively charged [2]. At a certain pH, the solubility of a molecule can be affected by the isoelectric point conditions. Precipitation will occur when a solution will have minimum solubility in water or salt solution at a pH corresponding to the isoelectric point of the molecule. Precipitation is caused by reduced protein solubility due to changes in chemical structure. This precipitation can occur due to disruption of colloid stability caused by a decrease in the electrostatic charge of the protein. In the end, the gravitational force will dominate compared to the repulsion between molecules so that the protein can be deposited properly and protein isolates are obtained [3].

The cosmetic market in Indonesia is very large but is still largely dominated by foreign brands, according to data from the Central Statistics Agency 2018. In imported 8.661,591 kg of cream-shaped cosmetics. Protein isolates can be applied as emulsifiers in lotion preparations because they have hydrophilic and hydrophobic sides that can act as emulsifiers

and can create layers between phases [4]. As for the raw material for making cosmetics, namely emulsifiers, Indonesia imports 23.5 thousand tons, so the presence of protein as a natural emulsifier in coconut has the potential to be extracted and used as an emulsifier for lotion preparations and is estimated to produce 12.9 thousand tons of coconut protein. It is expected to reduce by half the amount of emulsifier imports in Indonesia. to take advantage of this potential, in this study, an emulsifying lotion with coconut protein will be made.

MATERIALS AND METHODS

MATERIALS

Allantoin, castor oil, coconut, disodium EDTA, ethylhexylglycerin, glycerin, lecithin, olive oil, phenoxyethanol, protein, sodium polyacrylates, tocopheryl acetate, and water **(**All materials used in this study were in cosmetic grade).

METHODS

Protein Extraction

The research began with protein extraction from fresh coconut which is processed into coconut milk. Protein extraction from coconut milk is carried out without using a solvent. The coconut milk that has been made is left overnight to destabilize the emulsion which is then centrifuged at 5000 rpm for 30 minutes to separate into two phases, namely the skim phase and the cream phase. Protein was isolated from the skimmed coconut phase, which in the industry is a by-product of processing Virgin Coconut Oil from the coconut cream phase. Then protein isolation from the coconut skimmed phase was carried out by an isoelectric precipitation method.

Protein isolation with isoelectric precipitation was carried out by adjusting the skimmed phase at pH 4 which is the pH of the isoelectric point of coconut protein, by adding 0.1 N HCl. The solution was then stored in the refrigerator overnight for further separation of the protein from the extract. Protein separation was carried out by utilizing the principle of precipitation of protein isolate using a centrifugation speed of 5000 rpm for 30 minutes until finally a precipitate was obtained in the form of a protein isolate which was then filtered, taken, and washed with aqua DM. The protein obtained was weighed and dried and then tested for levels using the Kjeldahl method.

Application of Protein in Lotion Preparations

The protein isolate obtained was then applied as an emulsifying agent in lotion preparations with a concentration of 1%. Lotion formulations were made using various types of oil phases in the form of olive oil and castor oil, variations in the types of emulsifiers in the form of protein isolate and lecithin, which are commercial emulsifiers as comparisons, as well as variations in the pH of protein dissolution when applied to the lotion formulation at Ph 6 and 8. table and matrix of lotion emulsion formulations made.

Table 1. Lotion emulsion formulations

Phase	Material	Emulsifier Variations	
		Protein (%)	Lecithin (%)
Water	Water	93,2	93,2
	Dissodium EDTA	0,1	0,1
	Allantoin	0,1	0,1
	Glycerin	2	2
Emulsifier	Sodium Polyacrylates	0,7	0,7
	Protein	1	-
	Lechitin	-	1
Oil Variations	Castor oil	2	2
	olive oil		
Additive	Tocopheryl acetate	0,1	0,1
	Phenoxyethanol and Ethylhexylglycerin	0,8	0,8

Table 2. Lotion emulsion formulation matrix

<table>
<tr><th rowspan="2">No.</th><th rowspan="2">Formulation</th><th rowspan="2">Fixed Variable</th><th colspan="3">Independent Variable</th></tr>
<tr><th>Emulsifier Type</th><th>Oil Variations</th><th>Protein Solubility pH</th></tr>
<tr><td>1.</td><td>FP001</td><td rowspan="10">• Water Phase
• Sodium Polyacrylates
• Additives</td><td rowspan="4">Coconut Protein</td><td rowspan="2">Castor Oil</td><td>6</td></tr>
<tr><td>2.</td><td>FP002</td><td>8</td></tr>
<tr><td>3.</td><td>FP003</td><td rowspan="2">Olive Oil</td><td>6</td></tr>
<tr><td>4.</td><td>FP004</td><td>8</td></tr>
<tr><td>5.</td><td>FP005</td><td rowspan="4">Hydrogenated Lechitin</td><td rowspan="2">Castor Oil</td><td>6</td></tr>
<tr><td>6.</td><td>FP006</td><td>8</td></tr>
<tr><td>7.</td><td>FP007</td><td rowspan="2">Olive Oil</td><td>6</td></tr>
<tr><td>8.</td><td>FP008</td><td>8</td></tr>
<tr><td>9.</td><td>FP009</td><td rowspan="2">Blanko
(without emulsifier)</td><td>Castor Oil</td><td rowspan="2">-</td></tr>
<tr><td>10.</td><td>FP010</td><td>Olive Oil</td></tr>
</table>

Lotion Preparation Stability Test

All formulations that have been made are tested for stability to observe the phenomenon of instability that can occur in an emulsion preparation within a certain period. The stability test of the lotion emulsion preparation was carried out for 28 days in 4 conditions, namely room temperature, sunlight, oven 45^{o}C, and oven 50^{o}C. Tests on all formulations were carried out for 5 cycles on day 0, which is post emulsification, day 3, day 6, day 14, and day 28. At the end of each cycle, an evaluation is carried out by conducting organoleptic tests including observations of changes in color, odor, shape, pH, viscosity, and globule size to determine changes in stability in all formulations and evaluate the composition of the ingredients and formulas used. Another thing that is used as a test parameter besides stability is a sensory test (*sensory feel*) of a dosage formula. This is intended to find out the effect of each different material's variations on several sensory test parameters. One ingredient with another in a cosmetic formula will provide consistency or texture and different sensory parameters, so it is necessary to test to assess how consumers feel about this preparation. In this experiment, all the lotion formulations were tested by sensory tests on 11 panelists using 9 test parameters,

namely consistency, texture, ease to pick up, ease of spreading, time of application (playing time), after-feel of lotion application, stickiness, mattress, and moisture.

RESULTS

Coconut Protein Isolation

In this study, the protein isolate was obtained by isoelectric precipitation of skimmed coconut milk phase. An average of 1.36 kg of fresh coconut flesh was extracted to produce 792 g of coconut milk which was then separated using a centrifuge to obtain 230 g of skimmed coconut phase. The coconut skimmed was then proceeded to obtain 4.5 g of protein isolate which was then analyzed using the Kjeldahl method and the protein content of the extract was 57.5%. The following is a mass balance of protein isolation from the extract.

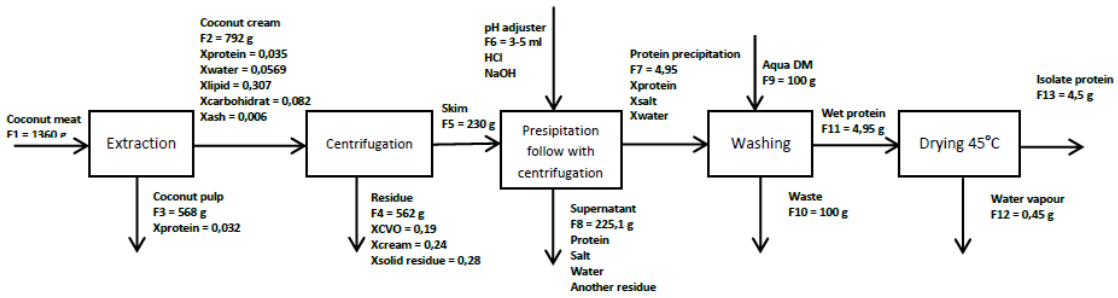

Figure 1. Mass balance of protein isolate extraction.

Figure 2. Extracted protein isolate.

Application of Coconut Protein in Lotion Preparations

The functional properties of coconut protein are strongly influenced by its solubility in solution, which also depends on pH. It can be seen in Figure 3 the solubility of proteins at various pHs.

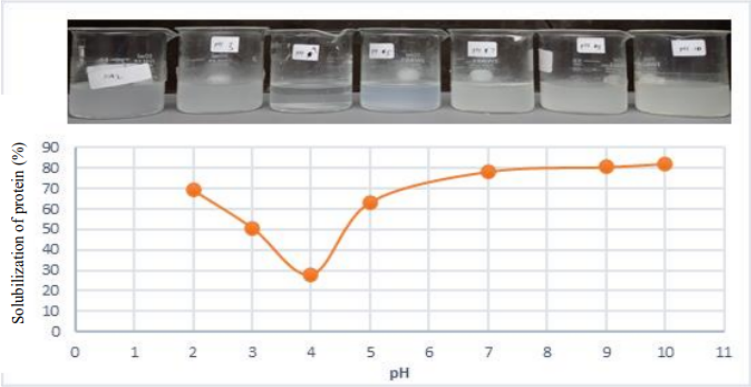

Figure 3. Effect of solution pH on protein solubility.

Stability Lotion

Analysis of Effect of Protein Dissolution pH on Lotion Stability

From the results of the study, it was found that setting the solubility of the emulsifier at pH 6 and pH 8 did not affect the final pH of all lotion formulations after they were made. It can be seen in Table 3 that the final pH of the preparation was around pH 6.

Table 3. Final pH of the lotion preparation

Formulation	FP001	FP002	FP003	FP004	FP005	FP006	FP007	FP008
pH of dissolving	6	8	6	8	6	8	6	8
final pH	6	6.09	5.99	6.16	5.99	5.96	6.01	5.96

Figure 4. Lotion with protein emulsifier (left) and lecithin (right).

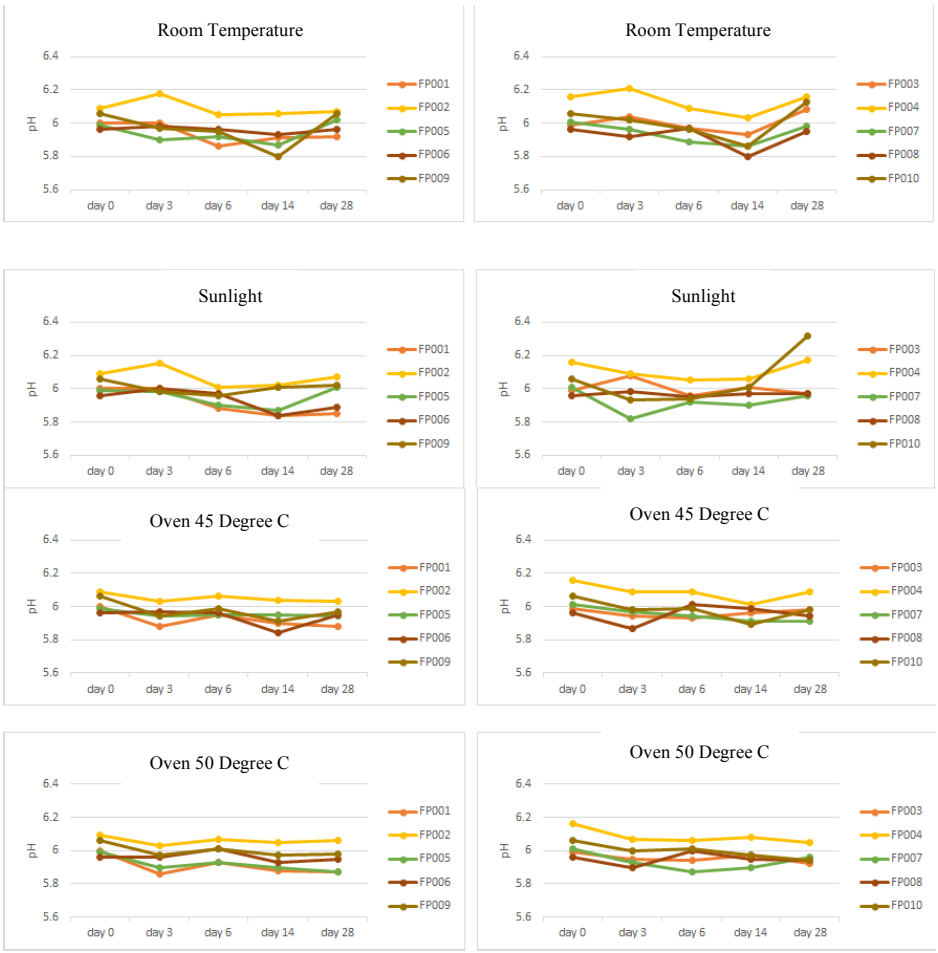

Figure 5. Graph of changes in pH with.
variations in the formulations of castor oil (left) and olive oil (right).

In addition, after the stability test was carried out with storage for 28 days, it can be seen in the graph in Figure 5. that for 28 days, all formulations under various conditions produced a pH in the range of 5.8 to 6.8 which, according to the quality standard requirements of SNI 16-4399-1996 was still included in the standard within the permissible pH range of 4.5-8.0. Thus, the stability of the lotion emulsion formula from changes in pH for the entire formulation did not change significantly.

Analysis of the Effect of Emulsifier Type on Lotion Stability

In this study, the use of different types of emulsifiers, namely coconut protein isolate and lecithin, resulted in different emulsion preparations. It can be seen in Figure 6 that the graph

of the change in viscosity with time shows different viscosity values for different types of emulsifiers.

Figure 6. Graph of changes in viscosity with.
variations in the formulations of castor oil (left) and olive oil (right).

Analysis of the Effect of Oil Type on Lotion Stability

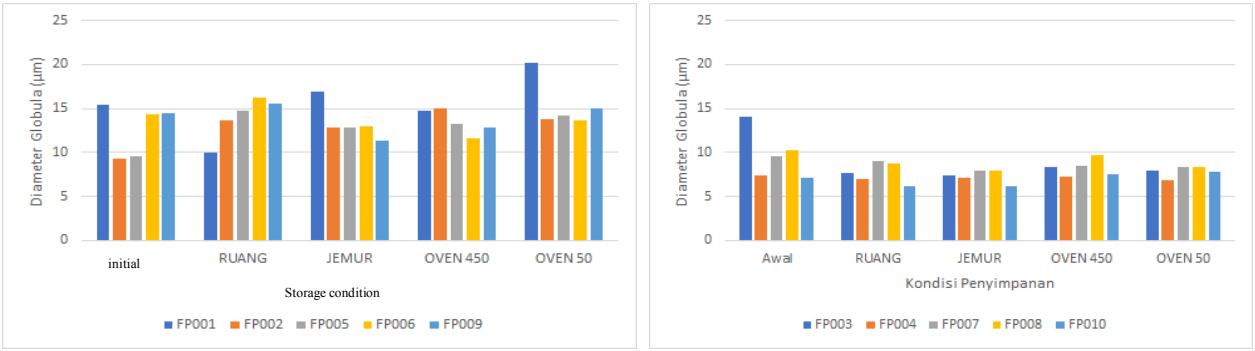

Figure 7. Changes in the globule size of castor oil formulas (left) and olive oil (right).

From the observation of the stability of the formulation organoleptic for 28 days in Figure 7 above, it is shown that the change in globule size with time under various conditions with the castor oil formula is very significant compared to the globule data on the first day of emulsification and the overall formula is relatively unstable at the end of the cycle after one month of storage from in terms of globule size. In contrast to the change in globule size with time under various conditions, the olive oil formula was not significant and the overall formula showed better globule size stability where the formula code FP004, which is a formula with olive oil at pH 8, whereas for protein emulsifier is a formula with an emulsion that has a globule size. the most stable changes with time.

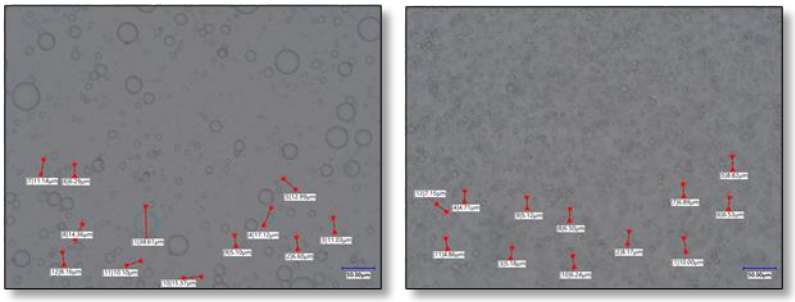

Figure 8. Average globule size of lotion with castor oil (right) and olive oil (left).

Sensory Evaluation

In Figure 9 below, it is also shown that the consistency of formulas with low codes sequentially to high formula codes has small to low consistency. Where the low formula code (FP001-FP004) is the formula code with a protein emulsifying agent which has a thinner consistency than the high code formula (FP005-FP008) with a lecithin emulsifying agent.

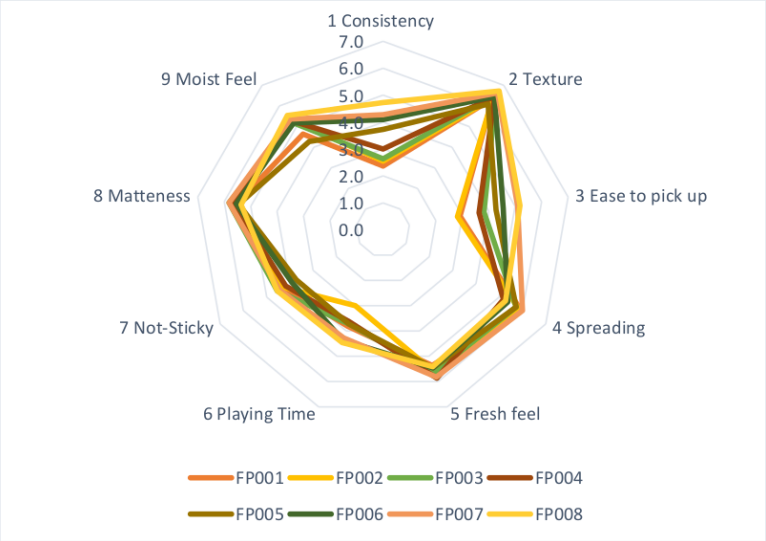

Figure 9. Sensory test of emulsion preparation.

DISCUSSION

Coconut Protein Isolation

From 1.36 kg of fresh coconut, it produced as much as 792 g of coconut milk, which, from the proximate analysis, obtained a coconut milk protein content of 3.48%, water 56.9%, fat 30.7%, carbohydrates 8.25%, and ash 0.67 %. With a protein content of 3.48% in coconut milk, ideally it contains 27.5 g of wet weight protein in coconut milk. However, the process of separating the skim phase from cream, Virgin Coconut Oil (VCO), and coconut milk solid residue causes more protein to be wasted. In addition, the skimmed phase is still going through the isoelectric precipitation stage which, after centrifugation, produces a supernatant

which may still contain a lot of protein. So that only 4.5 grams of dry protein isolate were produced from all stages of the process for protein extraction.

In addition, the processing of coconut flesh into coconut milk produces a large amount of coconut pulp, where the protein content in coconut pulp is still quite high at 3.24% and almost equals the protein content in coconut milk. This is because the extraction process carried out in this study did not involve solvents and only squeezed coconut milk made from fresh coconut flesh using a juicer. In the effectiveness of protein extraction, the use of solvents in the extraction process plays an important role. Where the more solvents used to extract protein will increase the effectiveness of protein extraction so that more protein isolates are taken [5]. The addition of water as a solvent will increase the diffusion of proteins into the water so that the remaining protein in the pulp increases.

Application of Coconut Protein in Lotion Preparations

The use of coconut protein isolate emulsifier in lotion preparations affects the overall formulation procedure, including the lotion making process must use the cold process method that does not involve heat because coconut protein is considered very sensitive to heat and can be denatured at a temperature of 50-80^{o}C [1].

The highest solubility of protein molecules occurs at the pH of alkaline solutions, namely at pH 7-10. This is indicated by the color of the solution being cloudier because the protein is well dissolved, while the clearest solution is the initial protein pH at pH 4 and has the lowest solubility. This is because the condition of coconut protein is still at the isoelectric pH point during the protein deposition process with the isoelectric precipitation method. The structure of protein molecules composed of amino acids is very easy to change due to the influence of environmental pH. When the protein environment has a low pH, the amino acids will be positively charged and vice versa, at a high pH they will be negatively charged. The isoelectric point can occur at pH when the positive charge on the base group is the same as the negative charge on the acid group, so that the isoelectric pH point indicates a neutral charge on the protein which causes the protein to be difficult to dissolve or agglomerate [6]. Therefore, it is necessary to adjust the pH in advance to change the charge on the surface of the protein and increase the electrostatic repulsion force so as to increase the elasticity of the protein on the surface of oil and water. So that the protein can dissolve in the water phase

and function as an emulsifier with variations in protein dissolution at pH 6 and 8 because, based on the graphic profile of protein solubility, alkaline pH is a pH that has good protein solubility compared to pH 4. So the variations used in this study include variations in protein dissolution at pH 6 and 8, variations in the type of emulsifier in the form of protein isolate and lecithin, as well as variations in types of oil such as olive oil and castor oil.

Stability Lotion

Analysis of Effect of Protein Dissolution pH on Lotion Stability

The pH setting was carried out before the emulsification process for all formulas with the aim of increasing the solubility in the aqueous phase so that during the emulsification process, coconut protein could form a film or thin layer between the oil and water surfaces. pH adjustment is very necessary to increase the solubility of protein in water and in the emulsification process, but after that the neutralization process occurs again due to the addition of an oil phase, additives, and sodium polyacrylate so that it does not affect the final post-emulsification pH.

Analysis of the Effect of Emulsifier Type on Lotion Stability

Lecithin is a type of commercial emulsifier that is often used as an emulsifying agent because it can reduce surface tension in emulsion systems and has often been applied in the cosmetic, pharmaceutical and food industries. Lecithin contains the main component in the form of a mixture of phospholipids (lipids) with ethanolamine, phosphatidylcholine, and inositol [7]. In addition to this content, lecithin also has a protein content of about (1338 mg/kg) with the largest fraction being the globulin fraction[8]. Similarly, coconut protein obtained from skimmed coconut contains the largest fraction in the form of globulins, which reached 61.9% [9].

The viscosity of the lotion emulsion preparation using protein isolate (FP001-FP004) tends to have a smaller viscosity value ranging from ±800 cP, so that the consistency is more watery when compared to the viscosity value of the lotion emulsion preparation using lecithin which has a larger value ranging from ±2000 cP. This can be caused by impurities from the extracted coconut protein isolate where from the test analysis results, the purity level of the extracted protein is only 57.5% which indicates the impurity of protein concentration as an emulsifier due to the presence of other components such as fat, carbohydrates, and non-protein

components which can reduce the functional properties of proteins to stabilize the water and oil phases. In addition, because protein synthesis using the isoelectric precipitation method is aimed at disrupting colloid stability by decreasing the electrostatic charge of the protein so that its solubility decreases and causes the protein to coagulate, the molecular chemical structure of the protein undergoes a change (denaturation).

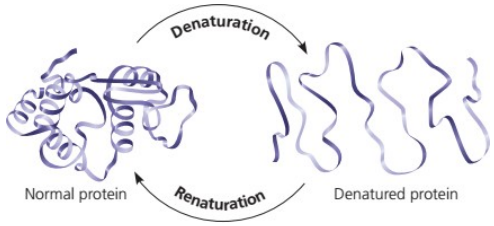

Figure 10. Denaturation and renaturation of proteins.

Proteins have hydrophobic and hydrophilic properties in their amino acid groups. Polar amino acids tend to be at the bottom of the protein molecule and can dissolve with water due to the presence of chains that have polar groups such as amino, carboxyl, carboxyl, sulfhydryl, and hydroxyl, so they can form hydrogen bonds with water [10]. Meanwhile, non-polar amino acids, which are the hydrophobic groups of proteins, are generally located on the inside of the protein molecule with the protein folded (folded, condition), so to stabilize the emulsion, the hydrophobic groups must be slightly exposed (*unfolded*) to interact with the oil phase, by denaturing. or coagulation. Protein denaturation is a condition of transformation of the folded protein structure into an unfolded one. Protein denaturation will reduce the nature of biological activities such as reduced solubility in water so that it affects the functional properties of the protein due to changes in the original conformation of the protein [11].

This denaturation process is intended to open non-polar amino acid groups, which are hydrophobic groups on the inside of the protein molecule. To stabilize the oil-water emulsion, the hydrophobic groups must be slightly opened (*unfolded*) to interact with the phase. The two states of protein folding in reaching an equilibrium condition between the folded state and the partially opened or unfolded state to interact with the water and oil phases are closely related to the change from folded to unfolded protein which goes back and forth (*reversible*) and very slowly and allows for the protein to return to its original conformation through a process of renaturation (*refolding*).

In this study, protein coagulation with isoelectric precipitation through changes in pH is intended to open the folds of proteins and can be categorized as mild protein denaturation that is reversible so that it allows the protein to be denatured by returning the protein to a suitable environment so that the chemical structure and functional properties of the protein can be returned through the re-dissolving of the protein. pH 6 and 8. This is because the denatured protein can still be re-dissolved in water with a pH beyond pH 4, which is its isoelectric point. However, protein renaturation takes place very slowly and in some cases does not even occur because the primary structure of the protein is damaged, so that during the application, the functional properties of the protein are related to surface properties related to emulsification to stabilize the interfacial tension of oil and water, because the hydrophobicity ratio is not optimal. and protein hydrophilicity was not achieved. So that the functional properties of proteins as optimal emulsifiers are needed, proteins that have good solubility in water but can also form a good hydrophobic surface and keep the conformation stable [12].

Changes in electrostatic charge affect the functional properties of proteins. Besides, the speed of diffusion of proteins towards the interface also affects the functional properties of proteins related to surface properties. Protein is a complex organic compound with a high molecular weight compared to lecithin, which has a *low molecular weight* (LMW Surfactants). This causes lecithin to be more effective in reducing the surface tension of emulsions than protein because it has a higher adsorption energy per m2 than protein. In addition, with a large molecular weight, proteins take up a large amount of space and tend to diffuse towards the interface slowly to stabilize the oil-water interface so that the volume fraction of stabilized oil is smaller than lecithin and its emulsification properties are limited. In addition, the adsorbed protein layer is mostly close to the interface in the aqueous phase rather than being evenly distributed on both sides of the interface or the oil interface, so that the adsorption of the protein interface is not as large as lecithin [13]. This can be identified by the emulsifying properties as measured by the *emulsion stability index* (ESI) and the *emulsifying activity index* (EAI), which measures the amount of oil that can be emulsified per unit of protein, where the EAI value of coconut protein is only 40 m2/g [14]. It is smaller than lecithin derived from soy protein with an EAI of 100 m2/g [15]. Protein properties such as the hydrophobicity-hydrophilicity ratio and the ease of protein folding have a major influence

on the properties of EAI and ESI [16]. Hence that the formulation of lotion preparations with protein emulsifiers has a consistency with a smaller viscosity value than lecithin.

Analysis of the Effect of Oil Type on Lotion Stability

One of the factors that determine the stability of an emulsion is the stability of the globule size (*droplet*). The instability of an emulsion is often characterized by a change in the size of the emulsion globules, causing instability in the form of flocculation when two or more droplets are in close contact with each other and coalescence when two or more droplets combine to form larger globules. High protein emulsification ability is indicated by good activity and emulsion stability. A good formula has a small and uniform globule size because it shows a high activity value and emulsion stability. The increase in the size of the oil globules in the emulsion with increasing time may indicate the occurrence of emulsion instability.

There are differences in the shape and size of the globules between the formulations using coconut protein emulsifier and olive oil or castor oil. The smaller and more uniform globule shape is seen in the lotion emulsion preparation that is used in the olive oil phase, while the preparation in the left photo, which uses the castor oil phase, looks larger and uneven. Changes in the spatial distribution of these molecules can cause changes in the properties of the emulsion itself, such as creaming, flocculation, coalescence, Ostwald ripening, and phase inversion [8]. Changes in the spatial distribution of these molecules are influenced by intermolecular interactions such as covalent interactions, electrostatic repulsion, and van der Waals interactions. The formation of more droplets can be caused by the incorporation of thin-film layers of molecules, then an exchange of material (oil) occurs and causes the formation of larger droplets. This causes a difference in density where the larger droplets of gravity will separate and migrate upwards, a process called creaming [17].

Sensory Evaluation

From the sensory test carried out, it was found that from several test parameters there were similarities between one formula and another. However, there are differences in the parameters in the form of consistency and ease of taking from several formulas. In terms of consistency, the formula with the code FP008 (lecithin, olive oil, pH 8) has a better

consistency, is not as runny as other formulas, and has a gel consistency, so that it is easier to pick up. In contrast, the formula with code FP002 (protein, castor oil, pH 8) had the lowest value of ease to pick up parameter because of the small value of consistency that is very runny, similar to the consistency of water.

CONCLUSION

From the results of the study, the average dry protein isolate was 4.5 g from 1.36 kg of fresh coconut flesh with a protein content of 57.5%. This protein isolate was applied as an emulsifier in lotion preparations. The protein isolate needs pH adjusting to improve water solubility, the pH used in this study was pH 6 and 8, and the pH adjusting did not affect the final pH of lotion preparations even after 28 days of accelerated storage test. Variations in the type of emulsifier used in the form of protein isolate and commercial lecithin affect the viscosity of the lotion emulsion preparation where the protein isolate emulsifier produces a lower viscosity value (±800 cP) than the lecithin emulsifier (±2000 cP), while the type of oil phase used is olive oil and castor oil affects the stability of the globule size of the lotion emulsion preparation where microscopically the globule size of olive oil produces more stable formula compare to castor oil. The stability of protein isolate as an emulsifier in lotion preparations was better than commercial hydrogenated lecithin and the most stable formula against changes in globule size was Formula FP004 (protein dissolution of pH 8, olive oil).

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CONFLICT OF INTEREST STATEMENT

NONE.

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