Production of Coconut Oil and Protein for Food and Cosmetic Ingredients

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Abstract. Coconut (Cocos nucifera) is a tropical plant that is almost spread throughout the archipelago. Coconut is a plant of the genus Cocos widely used in various fields ranging from food to cosmetics. Most coconut parts can be utilized so that the coconut is dubbed a versatile plant and has high economic value. This tree has a fruit which is also called coconut. Coconut fruit is the most widely used and processed. Coconut meat can be directly consumed or processed into coconut milk, coconut flour (desiccated coconut), and cooking oil. Coconut oil was obtained from dried or fresh coconut flour using the Soxhlet extraction method with organic solvents. Defatted coconut flour contained a high protein of approx. 19-20%. Coconut protein has a good quality compared to other vegetable protein sources. This research investigated solvent's effect on solid ratio and oil content on coconut protein extraction yield and recovery from coconut flour. Based on experiments, the highest value of extracted protein and yield was obtained after coconut flour extraction at a solvent to flour ratio of 30 g/g. Coconut protein isolate and coconut whey protein were expected to have different functional properties which need to be further evaluated to determine their application as cosmetics or food ingredients.

Keywords: coconut oil, coconut protein, protein extraction, food ingredients, cosmetic ingredients

1. Introduction

Proteins are macromolecules composed of 20 types of amino acids linked to each other by peptide bonds to form protein molecules. Amino acids can be classified as polar, nonpolar, hydrophobic, hydrophilic, acidic, alkaline, aliphatic, and aromatic. Proteins are the main structure in cells and tissues in the body. They have essential roles in growth, maintaining, repairing damaged tissue, producing metabolite and digestive enzymes, and certain primary hormone constituents. Protein sources can be obtained from animals and plants, vegetable protein [1]. As the largest archipelago country, Indonesia has the most prominent coconut (Cocos nucifera) plantation in the world of approx. 3,745,000 ha of a smallholder plantation and income for two and a half million farmer families. The coconut plantation in Indonesia constitutes 31.2% of the total world coconut area.

Coconut has a pulp with high potential to be developed as a food raw material with high economic value. Coconut meat protein has good quality due to its high amino acid score and low content of antinutritional compounds [2]. Processing of coconut into coconut flour was one of the alternatives to using coconut meat to increase economic value, maintain its nutritional value while increasing consumer convenience [3-6]. Coconut meat can be directly consumed or processed into coconut milk, coconut flour (desiccated coconut), and cooking oil. Coconut oil can be obtained from dried or fresh coconut.
flour using the Soxhlet extraction method with organic solvents. Defatted coconut meat will have higher protein content approx. 19-20%-wt [7]. Coconut flour is coconut meat dried, milled, and processed under hygienic conditions for human consumption [5]. The use of desiccated coconut is mainly for the confectionery and bread industry. Defatted coconut flour may contains 21.6% protein, 10.4% fibre, 59.8% carbohydrates, and 8.4% fat [4]. The high protein and fiber content in coconut flour allows this flour as a raw material for food products, especially for consumers at high risk of obesity, cardiovascular disease, and diabetes [4-6]. While coconut oil is already a common ingredient in food and cosmetics formulations, Patil and Benjakul [2] also showed that coconut protein was a natural emulsion stabilizer in coconut milk. Therefore, coconut protein can be used as healthy plant-based proteins and may be potential as an emulsifier or emulsion stabilizer in food and cosmetics products.

Based on the solubility of the protein, there are four types; albumin (soluble in water), globulin (soluble in salt), prolamin (soluble in alcohol), and glutelin (soluble in dilute NaOH). The type of protein that is mainly found in defatted coconut flour/meat is 40.1% globulin, 21% albumin, 14.4% glutelin-1, 4.8% glutelin-2, and 2.2% prolamin. Each type of protein is composed of amino acids at different levels. The amino acids present in the globulin are 14.9% arginine, 19.7% glutelin/glutamic acid, 7.2% leucine, and others. Meanwhile, the amino acids in albumin are 16.5% arginine, 24.1% glutamine, 6.3% linin, and others [2]. The high content of indigestible carbohydrates can inhibit protein digestibility; therefore, the protein was extracted and isolated from coconut flour to improve its digestibility. The extraction of the protein in coconut flour can be carried out using the solid-liquid extraction method.

Solid extraction (leaching) separates a solute in a solid by bringing the solid into contact with the solvent (solvent) to dissolves solutes into the solvent. In solid-liquid extraction, there are two phases: the overflow phase (extract) and the underflow phase (raffinate/dregs) [8]. Solid-liquid extraction involves three processes: 1) solute desorption and solute dissolution in the solvent, 2) internal diffusion of the solute from the matrix to the solid matrix surface, and 3) the external diffusion of the solute from the surface of the solid matrix into the bulk phase of the solvent [9]. Protein extraction was carried out at alkaline conditions far from its isoelectric point to increase protein solubility. Proteins that have been extracted in the solution can be separated by precipitation to obtain protein isolates. Protein was precipitated from crude extract at coconut protein isoelectric pH of about 4 [10]. At isoelectric pH, the protein will form a neutral charge. The attractive forces between protein molecules in the solution are more influential than the electrostatic repulsion so that the protein molecules will aggregate, then settle, and precipitate [11].

This study aimed to investigate the effect of solvent ratios and oil content of ingredients on the extractability of protein and recovery of protein isolates from full-fat and defatted coconut flour. The high content of indigestible carbohydrates in coconut flour can inhibit protein digestibility. Therefore, protein extraction from coconut flour can improve its digestibility for better utilization to fulfill the increased protein demand and other nutrients, which will benefit food security in Indonesia.

2. Materials and methods

2.1. Materials
In this research, the materials used were commercial coconut flour, NaOH solutions, HCl; Aqua dm; hexane; Bradford assay kit reagent, and Bovine Serum Albumin protein standard.

2.2. Oil extraction from dried coconut flour
A total of 50 g of dried coconut flour was placed in the thimble, and then the oil was extracted using a hexane solvent of 300 mL in a 500 mL round flask. Oil extraction was carried out by Soxhlet extraction method for four cycles to produce oil-free coconut flour. The result of the extraction process was solids containing hexane and hexane which were rich in oil. Coconut flour containing hexane was put into an aluminum foil cup, weighed, and dried in an oven at a temperature of 80° C for 1 hour. The mass of the container and coconut flour after drying were weighed to calculate the mass of hexane-free dry flour.
The oil-rich hexane phase was evaporated using a rotary evaporator at 30-40 °C until all hexane was removed from coconut oil.

2.3. Extraction of protein from dried coconut flour
About 1 g of oil-free dried coconut flour and oil-rich dried coconut flour were weighed and put into a 50 ml Falcon tube. Then alkali solvent (0.05 M NaOH) was added with a ratio of solvent to tea of 10, 20, and 30 g of solvent/gram of coconut flour. The mass of the container and coconut flour were weighed, and then the protein extraction was done using an orbital shaker for 30 minutes at a speed of 150 rpm. After that, the mixture was centrifuged at 6000 rpm for 20 minutes. The crude extract (supernatant 1) was poured into another pre-weighted 50 mL Falcon tube. The total mass of the mixture was calculated and measured in volume. The resulting residue was transferred to a pre-weighted aluminum foil cup and dried at 105 °C overnight. The mass of the mixture after drying was weighed and used to calculate solution retention. Protein content from crude extracts was measured by the Bradford method.

2.4. Isoelectric precipitation from crude extracts
Protein crude extract was added with 0.1 M NaOH or 0.5 M HCL to adjust the pH into its isoelectric pH of 4. Then the mixture was left overnight at 4°C. The next day, the mixture was removed from the refrigerator and centrifuged at 6000 rpm for 20 minutes at room temperature to obtain a clearer supernatant. The supernatant was then filtered to another 50 mL pre-weighted Falcon tube, then the mass of the protein precipitate was weighed. The non-precipitated protein of the supernatant was measured by the Bradford method.

3. Results and discussion
3.1. Drying and oil extraction from coconut flour
The coconut flour was dried to reduce its moisture content before the oil and protein extraction process. Moisture might lessen the effectivity of the nonpolar hexane solvent to extract oil. Hexane is a nonpolar solvent, and the presence of water will interfere with the interaction between the hexane solvent and oil. The drying process was carried out at a temperature of 40°C to prevent physical damage to the flour, such as the binding of lipids to protein and carbohydrates, decomposition of carbon, and oil entry into protein. In general, the moisture content in the oil can cause rancidity and is therefore disliked. Oil extraction was carried out with dried coconut flour using Soxhlet extraction to facilitate repeated contact between the solvent and coconut flour. We obtained 30.5 g oil from 50 g coconut flour or about 61% recovery in this experiment. Oil content in defatted coconut flour obtained from the experiments was comparable to Ohtora. Table 1 shows the moisture content and oil content of coconut flour.

| Coconut flour                             | Moisture content (%-wt) | Oil content (%-wt) | Protein content (%-wt) |
|-------------------------------------------|-------------------------|--------------------|------------------------|
| Full-fat-coconut flour (FCF)              | 5.3                     | 64.5               | 6.9                    |
| Defatted-coconut flour (DCF)              | 3.6                     | 5.3                | 18.1                   |
| Defatted-coconut flour from the cold press| 4.2                     | 8.4                | 21.6                   |

3.2. Production of protein isolate from coconut flour
Protein isolate (90-95% purity) is produced by dissolving proteins from plant material or cells, followed by precipitation at its isoelectric pH. The protein extraction process was carried out on defatted-coconut flour and full-fat-coconut flour at alkaline conditions using 0.05 M NaOH solution (pH of slurry 11.0-11.4). In alkaline conditions, the OH- ion will bind H+ ions to amino acids as protein monomers, causing
interactions [14]. In opposite, protein then is recovered from the crude extract by precipitation at isoelectric pH of proteins, where the proteins have the lowest solubility. Block flow diagram of protein extraction from coconut flour is shown in Figure 1, while the mass balance calculation is presented in Table 2.

![Figure 1. Block Flow Diagram of Protein extraction from coconut flour.](image)

| Table 2. Mass Balance of protein extraction from coconut flour. |
| --- |
| **Coconut Flour** | Solvent to flour ratio (g/g) | Coconut flour (g) | NaOH solution (g) | Slurry (g) | Raffinate (g) | Crude extract (g) | HCL (g) | Coconut whey protein (g) | Wet Protein isolate (g) | Coconut protein isolate (g) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Full fat-coconut flour (FCF) | 10 | 1 | 10 | 10.9 | 6.3 | 3.5 | 0.83 | 5.3 | 0.1 | 0.017 |
| 20 | 1 | 20 | 21.1 | 6.3 | 14.7 | 3.19 | 18 | 0.9 | 0.150 |
| 30 | 1 | 30 | 31 | 7.1 | 23.2 | 4.84 | 25.9 | 0.2 | 0.033 |
| Defatted Coconut flour (DCF) | 10 | 1 | 10 | 10.9 | 7.8 | 2.74 | 0.59 | 4.3 | 0.24 | 0.024 |
| 20 | 1 | 20 | 20.8 | 8 | 10.08 | 0.53 | 7.4 | 1.07 | 0.107 |
| 30 | 1 | 30 | 30.7 | 10.9 | 20.18 | 11.80 | 30.1 | 1.08 | 0.108 |

3.3. Protein extraction from coconut flour

The effect of solvent to flour ratio on protein extractability in crude extract flow (F5 in Figure 1) is shown in Figure 2. Solvent to flour ratio of 30 g/g resulted in the highest protein extractability and yield. The higher amount of solvent increased the contact with the solid matrix of flour and increased protein dissolution from the solid matrix. Figure 2a showed that protein extractability from full fat-coconut flour (17 to 53 g protein extracted/100 g protein in flour) was about two times higher than the protein extractability from defatted-coconut flour (5 to 22 g protein extracted/100 g protein in coconut flour). The increased solvent to flour ratio from 10 to 30 g/g increased protein extractability up to three-times for both full fat or defatted coconut flour. Table 1 presented the protein content of full-fat coconut flour based on the information in the packaging (6.9 %-wt), which was well corresponded to the result of Samuel et al. [3], and the protein content of defatted-coconut flour based on calculation (18.1 %-wt.). High protein content in flour was occurred due to the removal of oil from the flour, according to the experiment of Gunathialake et al. [4]. After oil removal, coconut flour contained higher protein content and higher fiber content, which may increase the difficulties of protein extraction. Based on the
solubility, protein can be classified into four types; albumin (water-soluble), globulin (salt soluble), a prolamin (soluble in alcohol), and glutelin (soluble in dilute NaOH). The type of protein that is mostly found in defatted coconut flour/meat is 40.1% globulin, 21% albumin, 14.4% glutenin-1, 4.8% glutelin-2, and 2.2% prolamin [2]. In this experiment, the dilute alkaline solvent was expected to extract most albumin, globulin, and glutenin fractions.

Figure 2. The effect of solvent to feed ratio on (a) protein extractability and (b) protein yield from coconut flour in crude extract (stream F5 in Figure 1).

Figure 2b showed that the yield of protein extracted from full-fat coconut flour at a solvent to flour ratio of 10 and 20 g/g was slightly higher than that from defatted coconut flour, while the yield of protein extracted from defatted coconut flour at 30 g solvent/g coconut flour (40 g protein extracted/ kg flour) was slightly higher than full-fat-coconut flour (37 g protein extracted/ kg flour). Although protein extractability was much lower from the defatted coconut flour, the protein yield from flour for both full-fat and defatted coconut flour was comparable. The higher the solvent ratio to the solid, the greater the concentration gradient and caused an increase in the diffusivity of the compound from the solid material into the solvent. The contact surface area between the solvent and the material also increased with the amount of the solvent so that the amount of extracted protein also increased. These results corresponded to the research conducted by Cui et al. [15], which stated that solvent's ratio to solids affected the amount of protein extracted. Protein interaction with other compounds, such as oil and fiber, seemed to affect protein extractability. Residual oil content in coconut flour increased protein dissolution to solvent, while fiber binds protein and reduces protein diffusivity into the solvent. The higher protein yield from defatted coconut flour was caused by the higher initial amount of proteins (18 % wt.) compare to the protein content in full-fat coconut flour (7 % wt.).

3.4. Retention of solution
The solution's retention is defined as the amount of (extract) solution carried to the extraction residue/underflow (extracted coconut flour). The solvent moved to the residue contains dissolved protein so that high retention in underflow reduced the amount of recovered protein in the extract bulk phase. Solution retention analysis data are presented in Table 3. Solution retention in defatted coconut flour underflow (7-10 g crude extract/ g inert solid) was higher than full-fat coconut flour (5-6 g crude extract/ g inert solid). Residual oil in coconut flour might facilitate protein diffusion into the solvent, while fiber binds protein and reduces protein diffusivity into the solvent.

Table 3. Protein solution retention in underflow/raffinate flow (coconut flour residue).

| Coconut flour | Solvent to flour ratio (g/g) | Crude Extract Retention (g crude extract/g inert solid) |
|---------------|-----------------------------|------------------------------------------------------|
| Full-fat-coconut flour (FFCF) | 10 | 5.5 |
| | 20 | 6.1 |
| | 30 | 5.6 |
3.5. Protein Precipitation from crude extract at isoelectric pH

Coconut flour crude extract was precipitated at coconut protein isoelectric point of pH 4.00 [10] with the addition of HCl or NaOH solutions at a specific concentration to adjust the solution's pH containing coconut protein. The isoelectric point of protein is the pH value where the protein molecule has zero net charge due to the equal number of positively and negatively charged amino acid residues on the protein molecule. Therefore, the protein molecules will precipitate from solution at the isoelectric pH. The isoelectric pH of coconut protein was between pH 3.98 and 4.23 [11]. Table 4 showed that protein precipitation rate was higher in defatted coconut flour crude extracts (44-68%) than in full-fat coconut flour crude extracts (27-44%). As a result, the protein isolates yield from defatted coconut flour (4-27 g protein/kg flour) was higher than the protein isolate from full-fat coconut flour (5-11 g protein/kg flour). Protein in the crude extract was not completely precipitated at the experimental range of isoelectric pH (3.98 to 4.23). The fraction of unprecipitated proteins remained soluble in the whey fraction (stream F8 in Figure 1).

Table 4. Isoelectric precipitation of coconut protein from crude extract at pH 4 to produce coconut protein isolate (stream F7 in Figure 1).

| Coconut flour       | Solvent to flour ratio (g/g) | Crude Extract Protein concentration (mg/ml) | Whey Protein concentration (mg/ml) | Protein precipitation rate (%) | Protein isolation rate (g protein/100 g protein in flour) | The yield of Protein Isolate (g protein/kg flour) |
|---------------------|-------------------------------|---------------------------------------------|-----------------------------------|-------------------------------|----------------------------------------------------------|-----------------------------------------------|
| Full-fat coconut flour (FCF) | 10 | 2.53 | 1.42 | 43.9 | 7.3 | 5.0 |
|                     | 20 | 1.75 | 1.28 | 26.9 | 9.5 | 6.5 |
|                     | 30 | 1.5  | 1.05 | 30.0 | 16.0 | 11.0 |
| Defatted coconut flour (DCF) | 10 | 2.64 | 1.47 | 44.3 | 2.1 | 3.7 |
|                     | 20 | 2.32 | 0.36 | 84.5 | 10.4 | 18.8 |
|                     | 30 | 1.96 | 0.62 | 68.4 | 15.0 | 27.1 |

Due to the higher protein precipitation rate from defatted coconut flour crude extract, protein isolation rate from full-fat coconut flour (7-16 g protein/100 g protein in flour) was similar to that from defatted coconut flour (2-15 g protein/100 g protein in flour). Therefore, the yield of coconut protein isolates from full-fat coconut flour of approx. 5 - 11 g protein/kg coconut flour was lower the yield of coconut protein isolates from defatted coconut flour of approx. 4 - 27 g protein/kg coconut flour.

4. Conclusion

Experimental data showed that the increase of solvent to coconut flour ratio increased protein extractability and yield of protein extracted. The highest protein recovery was obtained from defatted flour at a solvent ratio of 30 g solvent/ g coconut flour. The yield of coconut protein isolates from defatted coconut flour were within the range 4 - 27 g protein/ kg coconut flour. Both coconut protein isolate and coconut whey protein were expected to have different functional properties, which need to be further evaluated to determine their application as cosmetics or food ingredients.

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