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Physicochemical and functional properties of protein concentrate from by-product of coconut processing

Pattrathip Rodsamran[a], Rungsinee Sothornvit[b,c,*]

[a] School of Culinary Arts, Suan Dusit University, Bangkok 10700, Thailand
[b] Department of Food Engineering, Faculty of Engineering at Kamphaengsaen, Kasetsart University, Kamphaengsaen Campus, Nakhonpathom 73140, Thailand
[c] Center of Advanced Studies in Industrial Technology, Kasetsart University, Thailand

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ABSTRACT

Coconut cake, a by-product from milk and oil extractions, contains a high amount of protein. Protein extraction from coconut milk cake and coconut oil cake was investigated. The supernatant and precipitate protein powders from both coconut milk and oil cakes were compared based on their physicochemical and functional properties. Glutelin was the predominant protein fraction in both coconut cakes. Protein powders from milk cake presented higher water and oil absorption capacities than those from oil cake. Both protein powders from oil cake exhibited better foaming capacity and a better emulsifying activity index than those from milk cake. Coconut proteins were mostly solubilized in strong acidic and alkaline solutions. Minimum solubility was observed at pH 4, confirming the isoelectric point of coconut protein. Therefore, the coconut residues after extractions might be a potential alternative renewable plant protein source to use as a food ingredient to enhance food nutrition and quality.

1. Introduction

Interest in plant proteins as an alternative to animal protein has currently grown due to the increase in consumer demand originating from health concerns, religious restrictions and vegetarianism trends with a comparative low cost (Aydemir & Yemenicioğlu, 2013). Many plant residues from food industries are good candidates as low cost materials for plant proteins, especially from oil processing. Coconut milk press cake (Chambal, Bergenståhl, & Dejmek, 2012, 2013) and peanut cake (Zhang et al., 2014) are such residues due to their large amounts of desirable protein recovery.

Coconut (*Cocos nucifera* L.) is predominantly planted in southern Thailand. From a total coconut production of 1 million tonnes/year (FAO, 2013), approximately 60% is used for domestic consumption of coconut milk and coconut oil. Traditionally, fresh coconut kernel and dry coconut kernel (copra) are widely used for the extraction of coconut milk and coconut oil, respectively. The large amount of coconut cake considered as a by-product is reported to have protein content in the range 4–25% depending on the extraction process (Chambal et al., 2012; Chumwaengwapee, Soontornchai, & Thongprajukeaw, 2013). Generally, some coconut cake is used as a low cost animal feed ingredient. However, large amounts of coconut residues can cause an environmental as it usually ends up rotting. Therefore, effort is needed to identify potential uses to value-add to coconut processing by-products, specifically as a source of plant proteins. The extracted protein can serve as an alternative food ingredient that can be returned to food industries leading to a more sustainable environment. Generally, the alkaline method is commonly used for plant protein extraction. The coconut supernatant protein is extracted using one-step alkaline protein extraction at pH 11 to produce approximately 30% protein content (Chambal et al., 2013). The one-step alkaline method used for protein extraction produces a lower protein content; therefore, isoelectric precipitation is frequently used to separate protein from interfering compounds in the supernatant protein after extraction with alkaline solution. The precipitation technique produced a greater protein content in sunflower protein from 41.4% in supernatant protein to 70.4% in precipitate protein (Salgado, López-Caballero, Gómez'Guillén, Mauri, & Montero, 2012). However, a suitable method of protein extraction could be modified from various sources to obtain protein purity and good properties.

It is well known that the practical food application of plant protein also depends on the functional characteristics of the protein. Functional properties affect the behavior of food systems during manufacturing, processing, storage, preparation and consumption due to the physical and chemical properties and the molecular structure and size of the protein (Wu, Wang, Ma, & Ren, 2009). The important functional

* Corresponding author.
E-mail address: fengrns@ku.ac.th (R. Sothornvit).

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properties of protein include solubility, water and oil absorptions, emulsification, foaming properties and gelation (Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009; Zhong et al., 2012). Nevertheless, the variation in the protein content and functional properties is affected by the type of raw material itself, the processing history of the obtained raw material (processing steps and instruments used) and finally by the protein extraction method. For example, Aydemir and Yemenicioğlu (2013) found that the protein content of chickpea protein (73%) was lower than green lentil (88.5%) and red lentil (91.5%) when using alkaline extraction at pH 9.5 followed by precipitation at pH 4.5. Labuckas, Maestri, and Lamarque (2014) reported that the values of protein solubility and water holding capacity of walnut flour obtained from a screw press at 50 °C were higher than those from a hydraulic press. Moreover, alkaline extraction followed by precipitation at the isoelectric point was a more efficient protein extraction method than micellar precipitation to obtain pea protein isolate (Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015).

However, processing coconut by-products such as coconut milk cake and coconut oil cake has not been simultaneously explored for the functional properties of the extracted proteins. Therefore, the objective of this study was to investigate and compare the physicochemical and functional properties of protein concentrate from two different coconut processing by-products prior to their integration into food applications.

2. Materials and methods

2.1. Materials

Two types of coconut cake were studied. Coconut milk cake, a by-product from coconut milk extraction, was received from a local market (Nonthaburi, Thailand) and dried in a hot-air dryer (Redline RF 115, Tuttlingen, Germany) at 50 °C for 10 h. Coconut oil cake, a by-product from coconut oil extraction, was donated from Theppadungporn Coconut Co., Ltd. (Nakhonpathom, Thailand). Coconut milk cake and coconut oil cake were ground using a hammer mill (Roter grinder, Retsch GmbH, Haan, Germany) with a 1 mm screen prior to further studies. All chemicals of analytical grade were obtained from Merck KGaA (Darmstadt, Germany) and Ajax Finechem Pty Ltd (Taren Point, New South Wales, Australia) supplied by U & V Holding (Thailand) Co., Ltd (Nonthaburi, Thailand).

2.2. Fractionation of coconut protein

Protein fractions were determined by adapting the method of Kwon, Park, and Rhee (1996). Sequential extractions were done first to obtain albumin using distilled water, then globulin using 0.5 M sodium chloride and finally glutelin using 0.7 M tri-sodium orthophosphate (Na_3PO_4) at pH 11. The sample-solvent ratio of 1:12 (w/w) was constantly stirred at 50 °C for 1 h in a water bath (Memmert WNB 7–45, Schwabach, Germany) and the supernatant was obtained using cold centrifugation (Eppendorf centrifuge 5804 R, Hamburg, Germany) at 12,000 × *g* (0 °C) for 30 min. Based on the preliminary test, albumin, globulin and glutelin were precipitated from their supernatants by adjusting to pH levels of 4.1, 4.3 and 4.8, respectively. The resulting precipitate proteins were washed with distilled water, centrifuged and then lyophilized using a freeze dryer (Scanvac Coolsafe 100–4 Pro, Lynge, Denmark). The percentage of protein contents of both coconut cakes ($C_{protein}$) and protein fractionation ($PE_{protein}$) were first determined using the Kjeldahl method (Method 984.13, AOAC, 2000) with a conversion factor of 6.25. Weights of coconut cake and coconut protein extract were indicated as *WC* and *WE*, respectively. Finally, the protein recovery of each protein fraction was calculated using Eq. (1).

$$Protein\ recovery(\%) = \frac{WE \times PE_{protein}}{WC \times C_{protein}} \times 100 \quad (1)$$

2.2.1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was run according to the method of Laemmli (1970) in a Mini Protein II electrophoresis unit (Bio-Rad Laboratories Inc., Richmond, CA, USA). The dried precipitate proteins were dissolved in distilled water and adjusted to pH 11, mixed for 1 min using a vortex and centrifuged at 12,000 × *g* for 10 min. The supernatant protein solutions (10 μL) were mixed with 10 μL of sample buffer (containing 950 μL Laemmli buffer and 50 μL β-mercaptoethanol) and then heated at 90 °C for 10 min. Fifteen mL of each sample and marker (Precision Plus Protein All Blue standard, Bio-Rad Laboratories Inc., Richmond, CA, USA) were loaded onto 4–20% precast polyacrylamide gel (Mini-Protein® TGXTM Precast Gels). Electrophoresis was performed in an electrode buffer (containing 25 mM Tris-HCl, pH 8.3, 0.19 M glycine and 0.1% SDS) at 120 V for approximately 40 min. Protein was stained with 0.125% Coomassie brilliant blue G 250 and destained with 30% methanol and 10% acetic acid.

2.2.2. Fourier transform infrared (FT-IR)

The FT-IR spectra of samples were determined using an attenuated total reflectance-Fourier transform infrared spectrometer (Perkin Elmer, Spectrum two, Illinois, USA). Samples were ground and compressed into a disc prior to analysis within the wavenumber range 4000–600 cm^{-1}. Spectra were recorded in a transmission mode with 32 scans per spectrum at a resolution of 8 cm^{-1}.

2.3. Extraction of coconut cake protein concentrates

2.3.1. Supernatant protein powder

Coconut milk cake or oil cake was mixed with distilled water at the ratio of 1:12 (w/w). The mixture was adjusted to pH 11 using 0.7 M Na_3PO_4 and stirred at 50 °C for 1 h. After the suspension had been separated using cold centrifugation at 12,000 × *g* (0 °C) for 30 min, the supernatant protein solution was collected and lyophilized. The supernatant protein powder (SPP) was kept in a polyethylene zip lock bag and stored at room temperature (28 ± 2 °C) for further analyses.

2.3.2. Precipitate protein powder

The precipitate protein powder (PPP) was obtained by adjusting the supernatant protein solution (Section 2.3.1) to pH 4 with 3 M HCl and stirring at room temperature for 30 min. The pellet precipitate protein was separated using centrifugation at 12,000 × *g* (0 °C) for 10 min and washed with distilled water, then centrifuged again under the same conditions. Finally, the PPP was obtained using a freeze-drying method, kept in a polyethylene zip lock bag and stored at room temperature for further analyses.

2.4. Physicochemical properties

2.4.1. Proximate composition analysis

The total protein contents of coconut milk cake, oil cake, SPP and PPP samples were evaluated using the Kjeldahl method with a conversion factor of 6.25. The moisture, lipid and ash contents were determined using the AOAC standard methods 934.01, 954.02 and 942.05, respectively (AOAC, 2000). The carbohydrate content was calculated by subtracting the percentages of lipid, protein and ash contents from 100.

2.4.2. Color

The color of samples was measured on the basis of the CIE-color system (L^*, a^*, b^*) using a spectrophotometer (BYK Gardner GmbH, Geretsried, Germany). A white standard plate (L^*, 95.83; a^*, −0.78; b^*, −0.02) was used to calibrate the instrument.

2.4.3. Thermal properties

The thermal properties of samples were examined using a Diamond