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

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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

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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

DSC (Perkin-Elmer, Norwalk, CT, USA), equipped with a water cooling system. Nitrogen was used as a purge gas at a flow rate of 20 mL/min. An empty oven, indium (T_m: 156.6 °C, ΔH: 28.7 J/g) and sapphire were used to calibrate the baseline, temperature and enthalpy, and the specific heat capacity, respectively.

Samples (3–5 mg) were placed in a 40 μL aluminum pan and allowed to stabilize at room temperature (RT = 28 °C) for 24 h. Then, the pans were hermetically sealed and an empty pan was used as a reference. DSC curves were recorded during heating at 10 °C/min from 30 to 180 °C. The denaturation temperature (T_d) and enthalpy (ΔH) were computed from the thermogram using the Pyris software (Perkin-Elmer, Norwalk, CT, USA).

2.5. Functional properties

2.5.1. Protein solubility

Protein samples (40 mg) were dissolved in 40 mL distilled water and the pH was adjusted in the range 2–11 using 3 M HCl or 0.7 M Na_3PO_4 solutions. These suspensions were stirred at room temperature for 30 min and then centrifuged at 3500 × *g* for 20 min. The protein content of supernatant ($SP_{protein}$) was determined according to the method of Bradford (1976) using bovine serum albumin as a standard. The weight of coconut cake was indicated as *WC*, whereas the percentage of protein content in the coconut cake was indicated as $C_{protein}$. Protein solubility was calculated using Eq. (2).

$$Protein\ solubility\ (\%) = \frac{SP_{protein}}{WC \times C_{protein}} \times 100 \quad (2)$$

2.5.2. Water and oil absorption capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) were determined using the method of Aydemir and Yemenicioğlu (2013). Briefly, 20 mg of protein sample and 1.5 mL of distilled water or soybean oil were mixed in a vortex in a 2 mL centrifuge tube for 20 s. After incubation at 30 °C for 30 min, the tubes were centrifuged at 15,000 × *g* for 20 min at room temperature. The free water or oil was removed using a pipette and the sample was reweighed. The WAC and OAC were expressed as grams of water or oil absorbed per gram of protein sample.

2.5.3. Foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) were determined using a modified method of Aydemir and Yemenicioğlu (2013). Twenty milliliters (*V*) of protein solution (10 mg/mL) was prepared in distilled water and its pH was adjusted to 11. The solution was stirred at 30 °C for 30 min and then homogenized at 13,500 rpm for 1 min in a high speed homogenizer (Polytron® PT-MR 3100D, Kinematica AG, Luzern, Switzerland). The whipped protein solution was transferred into a 50 mL graduated cylinder and the volume was recorded at 0 min (V_0) and 60 min (V_1). The FC and FS were calculated using Eqs. (3) and (4), respectively.

$$FC\ (\%) = \frac{(V_0 - V)}{V} \times 100 \quad (3)$$

$$FS\ (\%) = \frac{(V_1 - V)}{V} \times 100 \quad (4)$$

2.5.4. Emulsifying properties

The emulsifying activity index (EAI) and the emulsifying stability index (ESI) were determined according to the method of Pearce and Kinsella (1978). The protein solution (10 mg/mL, *WE*) was adjusted to pH 11 and stirred at 30 °C for 30 min. Then, 18 mL of protein solution was mixed with 2 mL of soybean oil and then homogenized at 13,500 rpm for 1 min. The emulsion (50 μL) sample was pipetted at 0 and 10 min from the bottom of the tube and diluted with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance of emulsion at 0 min (A_0) and 10 min (A_{10}) after homogenization was measured at 500 nm using a spectrophotometer (Shimadzu UV–Visible 1800, Tokyo, Japan). The EAI and ESI were calculated using Eqs. (5) and (6), respectively.

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.1 \times WE} \quad (5)$$

$$ESI\ (min) = \frac{A_0 \times 10}{A_0 - A_{10}} \quad (6)$$

2.6. Statistical analyses

A completely randomized design was performed for this experiment with duplication. Independent sample *t*-tests were carried out to determine significant differences among samples of the two coconut cakes ($P < 0.05$) using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Fractionation of protein extract from coconut cake

The total protein recovery from milk cake of 43.15% was significantly higher than the 4.59% protein from oil cake (Table 1). The high temperature during oil extraction probably caused protein denaturation resulting in the low extracted protein content. Albumin, globulin and glutelin were the three major proteins from coconut investigated in this study (Kwon et al., 1996). Glutelin was the most abundant protein in both coconut cakes (82.31–89.59%) with the highest protein content, while albumin and globulin were observed to have rather low protein contents. Similarly, glutelin was the major recovered protein fraction found in pumpkin seed protein (Rezig et al., 2013) and rice kernel (Agboola, Ng, & Mills, 2005). Based on the highest protein content and protein recovery of glutelin in this study, coconut protein samples derived from both coconut cakes were extracted using alkaline extraction at pH 11 and acidic precipitation at pH 4 followed by the lyophilization to determine the physicochemical and functional properties.

3.1.1. Electrophoresis patterns

Molecular sizes of albumin, globulin and glutelin from coconut milk cake and oil cake were analyzed using SDS-PAGE (Fig. 1). The protein profiles from milk cake (lanes 2–4) showed more intense bands than from oil cake (lanes 5–7) corresponding to the concentration of each extracted protein found in Table 1. The higher protein molecular weight (> 250 kDa) retained at the top of the separating gel occurred in

Table 1
Protein content and protein recovery of sequential extraction coconut cake proteins.

Protein extract	Protein content[1]	Protein recovery (%)	Ratio (%)
Coconut milk cake			
Albumin	$60.44^{aA} \pm 2.42$	$2.33^{aB} \pm 0.11$	5.40 ± 0.26
Globulin	$36.87^{aB} \pm 0.37$	$2.16^{aB} \pm 0.01$	5.01 ± 0.03
Glutelin	$63.99^{aA} \pm 2.60$	$38.66^{aA} \pm 0.42$	89.59 ± 0.97
Coconut oil cake			
Albumin	$25.12^{bC} \pm 1.59$	$0.40^{bB} \pm 0.06$	8.62 ± 1.39
Globulin	$13.67^{bB} \pm 0.09$	$0.42^{bB} \pm 0.05$	9.06 ± 1.08
Glutelin	$52.06^{bA} \pm 2.32$	$3.77^{bA} \pm 0.18$	82.31 ± 4.01

Values are mean of three replicates ± standard deviation.
[a–b]Different lowercase letters for the same protein fraction indicate significant differences ($P < 0.05$) in means.
[A–C]Different uppercase letters for the same coconut cake indicate significant differences ($P < 0.05$) in means.
[1] Protein content expressed as g/100 g dry basis.

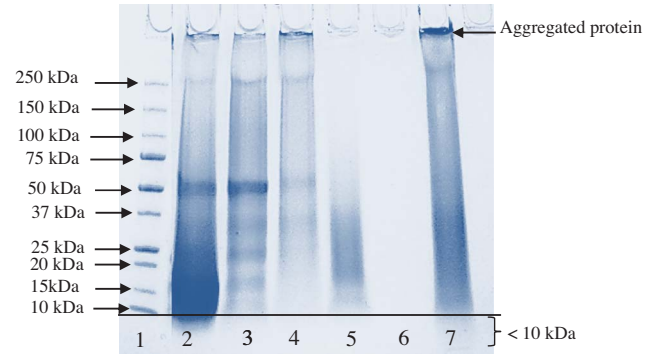

Fig. 1. SDS-PAGE of fractionation of coconut protein. Lane 1: standard protein marker; lane 2: albumin from milk cake; lane 3: globulin from milk cake; lane 4: glutelin from milk cake; lane 5: albumin from oil cake; lane 6: globulin from oil cake; lane 7: glutelin from oil cake.

lanes 2, 3, 4 and 7 due to the protein aggregation; however, the lower protein molecular weight (< 10 kDa) was observed in lanes 2, 3, 5 and 7. The patterns of protein fraction from milk cake appeared to be quite different and showed prominent bands with molecular weights from 10 to 52 kDa. For albumin, the densest bands resolved at 52, 34 and 24 kDa, with one wider and darker band between 10 and 18 kDa. A similar pattern was also observed in globulin but the clear bands were observed at 18 and 14 kDa. The similar protein patterns may have been due to sequential contamination between protein classes (Kwon et al., 1996). The glutelin pattern only showed the presence of three major bands of 52, 34 and 24 kDa, similar to those observed in albumin and globulin. In previous study, coconut protein exhibited all bands with molecular weights ranging between 17 and 55 kDa (DeMason & Chandra Sekhar, 1990). Kwon et al. (1996) showed that coconut flour had seven prominent bands with molecular weights between 14 and 52 kDa and the major bands appeared in the albumin, globulin and glutelin fractions at 52, 34 and 24 kDa. In addition, Chambal et al. (2012) reported that coconut milk cake protein obtained using alkaline extraction resolved in bands with molecular weights at 53, 42, 34, 24, 22 and 16 kDa.

There was a streaking of bands rather than clear bands in the oil cake protein patterns. This might have been due to the high heat treatment during oil extraction processing leading to protein denaturation. The albumin and glutelin fractions presented the densest bands near 15–33 kDa and 10–50 kDa, respectively. However, the protein pattern of globulin had a fainter color and narrower appearance compared to the patterns in other fractions, perhaps as a result of low quantities of globulin in the oil cake.

3.1.2. Fourier transform infrared (FT-IR) spectra

FT-IR is one of the most common techniques providing information about the protein conformation, especially its secondary structure (Zeng, Cai, Cai, Wang, & Li, 2011). Regarding the same protein fraction, the protein spectra from milk cake had a more intense magnitude than did the oil cake (Fig. 2), similar to those observed using SDS-PAGE (Fig. 1). The broad band of 3300–3100 cm^{-1} (amide A), indicating –NH stretching, was the strongest spectrum in albumin and the weakest in globulin. Similarly, the lotus seed protein had more inter-molecularly H-bonded NH groups at the strong band around 3300 cm^{-1} (Zeng et al., 2011). The bands appearing at 2860–2930 cm^{-1} (amide B) were attributed to the symmetric and asymmetric stretching vibration of $–CH_2$ and $–CH_3$ groups found in the aliphatic chain of protein and lipids (Jamin et al., 1998). The intensity of these bands corresponded to the oil content in the protein samples. Similar bands were found in protein fractions from lotus seed (Zeng et al., 2011) and camelina meal (Li et al., 2014). Similarly, the bands at 1714 and 1740–1750 cm^{-1} (C═O stretching) related to the ester absorbance which was associated with the oil content (Silverstein, Bassler, & Morrill, 1981). There were intense peaks in the spectra of albumin (Fig. 2a) and glutelin (Fig. 2c) from milk cake, while there were small peaks in the spectra of protein from oil cake (Fig. 2b, d, f). Thus, it may be concluded that the protein from oil cake had a lower oil content left than from milk cake. Similar observations were reported for pennycress press cake protein (Selling et al., 2013) and camelina meal (Li et al., 2014).

The second largest peaks appeared at 1650, 1550 and 1460 cm^{-1} and were related to amide I, II and III, respectively. The amide I and II absorptions are the most prominent vibrational bands of the protein backbone (Kong & Yu, 2007). Amide I arises mainly from C═O stretching vibrations coupled with C—N stretching vibrations, whereas amide II contains the combination of N—H bending vibrations and minor C—N stretching vibrations (Barth, 2007). Most plant proteins have similar band regions of amide I (1630–1660 cm^{-1}) and amide II (1520–1550 cm^{-1}) such as lotus seed protein (Zeng et al., 2011), pennycress press cake protein (Selling et al., 2013) and camelina meal protein (Li et al., 2014). The bands in the range 965–1260 cm^{-1} were attributed to C—O stretching vibrations in polysaccharides that referred to the presence of carbohydrates (Li et al., 2014). These bands were detected in all protein samples with different intensities. The high intensities of peaks referred to the oil and carbohydrate and might confirm a low protein content in coconut protein (Table 1). Furthermore, the weak band in all regions of globulin from oil cake (Fig. 2f) might be assumed to be due to it having the lowest protein content.

3.2. Extraction of coconut cake protein concentrates

3.2.1. Chemical composition

The proximate compositions of the two coconut cakes and the protein samples obtained from them are shown in Table 2. Lipid and carbohydrate were the major components of both coconut cakes. The amount of oil retained in the oil cake was significantly ($P < 0.05$) less than in the milk cake due to the difference in the purpose of extraction and instrument used. However, the ash, protein and carbohydrate contents of the oil cake were significantly ($P < 0.05$) more than in the milk cake. Likewise, Yalegama, Karunaratne, Sivakanesan, and Jayasekara (2013) reported that the chemical components of oil cake (protein, sugar, ash, crude fiber and carbohydrate), except fat, were higher than in milk cake. The components in the chemical composition of milk cake in this study were close to the published values in coconut milk cake reported by Chumwaengwapee et al. (2013) and Yalegama et al. (2013), which were 5% and 4.2%, respectively. A lower fat content (13.4%) and higher protein content (21.8%) of coconut defatted flour were reported by Yalegama and Chavan (2006). This information implied that there is variation in the protein content of the same plant material due to differences in the variety and processing techniques. For example, the protein content of both coconut cakes in this study was lower than in pennycress press cake (Selling et al., 2013), camelina meal (Li et al., 2014) and castor bean cake (Chambi et al., 2014).

The ash and carbohydrate contents were the major components in SPP from both coconut cakes. The SPP from milk cake contained significantly ($P < 0.05$) higher oil and protein contents than from oil cake. The lower protein content of SPP from oil cake may have been due to the denaturation of soluble protein during oil extraction at high temperature. The SPP from milk cake had a protein content close to the value previously reported in coconut press cake using the same extraction method (Chambal et al., 2013). However, the protein content in SPP corresponded to the protein content of the raw material. For example, Chambal et al. (2013) found that coconut milk cake had 6.35% protein content and that the protein content increased to 30% after extraction at 50 °C and pH 11 for 1 h followed by lyophilization. Likewise, the protein content of castor bean cake increased from 39.5% to 69.2% after protein extraction at 50 °C and pH 11 for 30 min (Chambi et al., 2014).

After the precipitation at pH 4, the PPP from both coconut cakes had a high protein content. As expected, the decrease in pH close to the

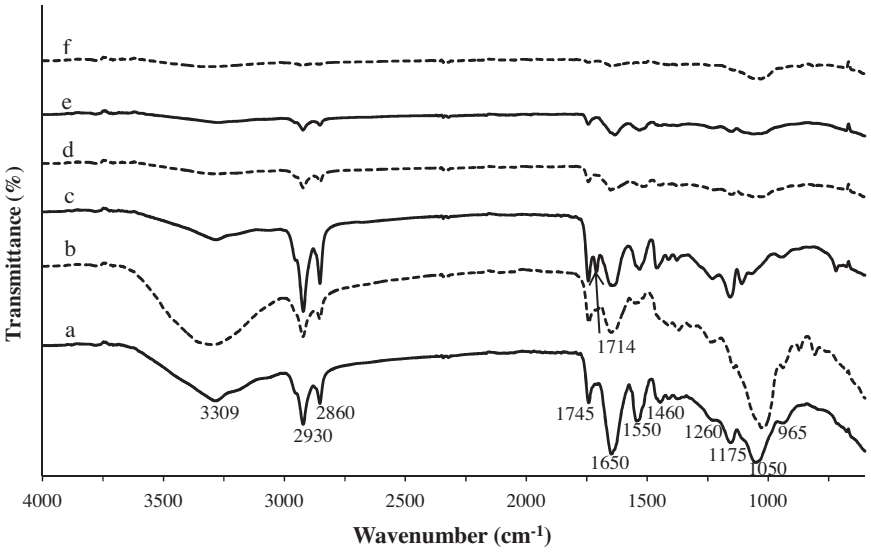

Fig. 2. Fourier transform infrared spectra of coconut protein fractions in 4000–400 cm^{-1} regions: (a) albumin from milk cake; (b) albumin from oil cake; (c) glutelin from milk cake; (d) glutelin from oil cake; (e) globulin from milk cake; (f) globulin from oil cake.

isoelectric point of protein can separate the protein from the other components, resulting in a higher protein content. The protein content of PPP in this study was lower (55.44–63.06%) than in other agricultural proteins. This may have been due to the high amount of oil and low protein content in the coconut cake itself. Generally, the retained oil in the raw material was removed using solvent extraction to increase the protein content of defatted material prior to the protein extraction as shown in peanut (Wu et al., 2009) and in chickpea, lentils and soybean (Aydemir & Yemenicioğlu, 2013). In our study, both coconut cakes were used as received without removing oil to determine the possible protein contents and characterize their properties to save time and be more practical for further utilization on an industrial scale.

3.2.2. Color

Coconut cake, SPP and PPP from milk cake had significantly ($P < 0.05$) higher L^* values than all the values from oil cake (Table 2). The original coconut oil cake had a darker visual appearance corresponding to greater a^* and b^* values than coconut milk cake. This may have been due to the substantial Maillard reaction between reducing sugars and amino groups in proteins that occurred at high temperature during the oil extraction. Similarly, the walnut cake obtained using screw pressing at 70 °C had a higher a^* value than the hydraulic pressing at 18 °C (Labuckas et al., 2014). The SPP from both coconut cakes was a fine powder. The higher L^* and b^* values of SPP from milk cake resulted in a light beige protein powder ($P < 0.05$).

The visually darker PPP protein powders from both coconut cakes had lower brightness values. However, the PPP from milk cake had higher values of all three color parameters than those from oil cake. The higher a^* and b^* values of PPP powder from milk cake were due to the red-brownish color; whereas, the lower L^*, a^* and b^* values of the PPP powder from oil cake were due to the quite black color. Similarly, Samson, Cater, and Mattil (1971) reported that the PPP from coconut meal obtained by extraction at pH 10.5 were brownish in color.

3.2.3. Thermal properties

Differential scanning calorimetry (DSC) has been used to study the

Table 2
Composition, color and thermal properties of coconut milk cake, coconut oil cake, supernatant protein powder (SPP) and precipitate protein powder (PPP).

	Coconut cake		SPP		PPP	
	Milk cake	Oil cake	Milk cake	Oil cake	Milk cake	Oil cake
Chemical composition[1]						
Dry matter	$95.20^{a} \pm 0.11$	$95.61^{a} \pm 0.32$	$90.29^{a} \pm 0.53$	$90.12^{a} \pm 0.31$	$94.25^{b} \pm 0.53$	$97.37^{a} \pm 1.59$
Ash	$0.94^{b} \pm 0.32$	$2.43^{a} \pm 0.13$	$31.53^{b} \pm 1.37$	$40.68^{a} \pm 0.56$	$2.01^{b} \pm 0.20$	$3.01^{a} \pm 0.33$
Lipid	$31.81^{a} \pm 1.36$	$16.08^{b} \pm 1.35$	$5.15^{a} \pm 1.38$	$2.55^{b} \pm 1.50$	$13.87^{a} \pm 1.11$	$8.66^{b} \pm 0.30$
Protein	$5.19^{b} \pm 0.14$	$8.58^{a} \pm 0.08$	$22.38^{a} \pm 0.94$	$7.21^{b} \pm 0.23$	$63.06^{a} \pm 1.05$	$55.44^{b} \pm 0.41$
Carbohydrate	$62.07^{b} \pm 1.67$	$72.91^{a} \pm 1.32$	$40.94^{b} \pm 1.40$	$49.56^{a} \pm 0.99$	$21.06^{b} \pm 0.94$	$32.90^{a} \pm 0.03$
Color						
L^*	$62.35^{a} \pm 0.76$	$39.95^{b} \pm 0.59$	$64.04^{a} \pm 0.49$	$44.40^{b} \pm 0.62$	$33.68^{a} \pm 0.12$	$29.56^{b} \pm 0.03$
a^*	$3.23^{b} \pm 0.13$	$4.44^{a} \pm 0.23$	$5.00^{a} \pm 0.31$	$5.34^{a} \pm 0.12$	$4.92^{a} \pm 0.14$	$0.36^{b} \pm 0.01$
b^*	$9.16^{b} \pm 0.29$	$10.08^{a} \pm 0.50$	$14.9^{a} \pm 0.34$	$11.36^{b} \pm 0.28$	$6.80^{a} \pm 0.15$	$2.58^{b} \pm 0.01$
Thermal Property						
1st Endothermic peak						
T_d (°C)	$113.57^{b} \pm 0.49$	$156.63^{b} \pm 3.37$	$88.63^{a} \pm 0.33$	$88.67^{a} \pm 0.47$	$117.06^{a} \pm 5.90$	$122.41^{a} \pm 7.82$
ΔH (J/g)	$53.47^{b} \pm 6.21$	$201.65^{a} \pm 30.26$	$9.43^{a} \pm 2.87$	$10.32^{a} \pm 3.76$	$0.70^{a} \pm 0.09$	$1.07^{a} \pm 0.24$
2nd Endothermic peak						
T_d (°C)	–	–	$151.41^{b} \pm 0.48$	$160.20^{a} \pm 3.33$	$137.51^{b} \pm 1.39$	$152.87^{a} \pm 2.67$
ΔH (J/g)	–	–	$23.24^{b} \pm 0.25$	$242.63^{a} \pm 56.94$	$31.06^{b} \pm 10.10$	$390.75^{a} \pm 42.45$

Values are mean of three replicates ± standard deviation.
[a–b]Different lowercase letters indicate significant differences ($P < 0.05$) between two types of coconut cakes.
[1] Chemical compositions expressed as g/100 g dry basis.

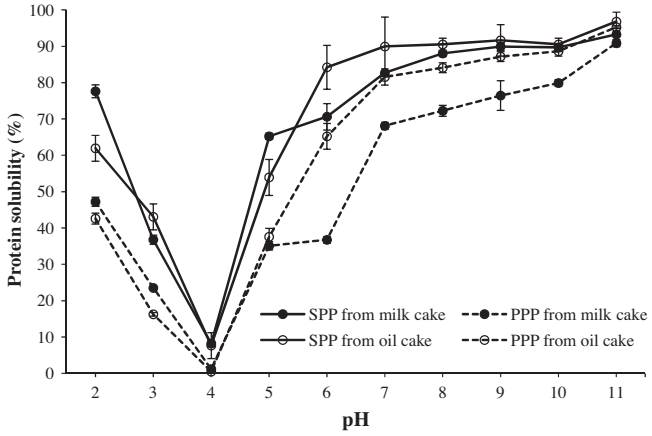

Fig. 3. Protein solubility profile of supernatant protein powder (SPP) and precipitate protein powder (PPP) from coconut milk cake and coconut oil cake.

denaturation or unfolding of the protein molecule (Ma & Harwalkar, 1991). The characteristics of thermal transitions can predict the thermal behavior of protein in the food system. Thermographs of coconut cake displayed a single, sharp endothermic peak, whereas the thermographs of SPP and PPP had two endothermic peaks (one broad peak and one sharp peak). The endothermic peak provides information on the denaturation temperature (T_d) and enthalpy (ΔH) of the major proteins in a sample. In fact, the T_d value is a reflection of the disruption of hydrogen bonds to maintain the tertiary and quaternary structures of protein, indicating the protein's thermal stability (Ma & Harwalkar, 1991; Tang & Sun, 2011). In addition, the ΔH value is correlated to the energy required to unfold or denature the protein structure and reflects the extent of the ordered secondary or tertiary structure of the protein (Tang & Sun, 2011).

From the result of protein fractionation, coconut protein included a major fraction of glutelin and minor fractions of albumin and globulin. Only the T_d of glutelin occurred in both coconut cakes (Table 2), and oil cake had a higher T_d than milk cake. Similarly, the ΔH value of oil cake was significantly ($P < 0.05$) higher than for milk cake. Chumwaengwapee et al. (2013) reported the values of T_d and ΔH of coconut milk cake as being around 82.8 °C and 95.7 J/g, respectively. The first and second endothermic peaks of SPP and PPP can be attributed to the denaturation temperature of albumin and globulin, and glutelin, respectively. For the first endothermic peak, no significant differences in T_d and ΔH between milk cake and oil cake were found in both SPP and PPP ($P > 0.05$). In contrast, the T_d and ΔH values for both SPP and PPP from milk cake were significantly ($P < 0.05$) lower than from oil cake with the second endothermic peak. The differences in T_d and ΔH from both coconut cakes may be ascribed to differences in the protein conformations of the original coconut cakes due to the distinctively different extraction processes. The native conformation in terms of tertiary or quaternary structure in coconut oil cake may be partial unfolded during oil extraction at high temperature and the intermolecular aggregation among unfolded proteins occurred during protein extraction. The higher T_d and ΔH values observed in both proteins from oil cake were due to the requirement of higher temperature and energy to unfold the aggregated or compacted protein. According to these results, it may be concluded that the protein powders from oil cake had more ordered structures and structural unfolding than from milk cake. Compared with the other plant precipitate proteins, coconut protein had a higher T_d value than pumpkin seed protein (Rezig et al., 2013) and African yam bean protein (Wani, Sogi, & Gill, 2015). The differences may be attributed to the variation in the predominant proteins in each plant.

Kwon et al. (1996) found that the albumin and globulin fraction from coconut meal had an average T_d of approximately 93 °C and in the range 82–112 °C, respectively. Moreover, Horax, Hettiarachchy, Over, Chen, and Gbur (2010) reported that the T_d value of glutelin (133.6 °C) from bitter melon seed protein was higher than for the albumin (112.0 °C) and globulin (117.3 °C) fractions. These results confirmed that the first broad peak at low temperature and the second sharp peak at high temperature corresponded to the T_d values of minor proteins (albumin or globulin) and a major protein (glutelin) of coconut protein, respectively. Based on the higher T_d and ΔH values, the coconut proteins from both coconut cakes probably are suitable for specific product applications dealing with higher temperature processing.

3.2.4. Functional characteristics

The functional properties also affect the behavior of proteins in food systems during processing, storage, preparation and consumption (Kinsella, 1982). The most interesting properties prior to food application are the water and oil absorption capacities, solubility, foaming properties and emulsifying properties. A comparison of the functional properties of different coconut cakes was provided for each type of protein (SPP and PPP).

3.2.4.1. Protein solubility. The protein solubility profiles of SPP and PPP from both coconut cakes at different pHs in the range 2–11 are shown in Fig. 3. The protein solubility patterns were the same in all samples. Minimum solubility was observed at pH 4 due to this being the isoelectric point of coconut protein. Moreover, high solubility was evident under extremely acidic (pH 2) and alkaline (pH 11) conditions. When the pH values are far away from the isoelectric region (pH 3–5), the protein possesses net positive or negative charges leading to electrostatic repulsion and ionic hydration resulting in protein solubilization (El Nasri & El Tinay, 2007). Similar results were observed in peanut protein (Wu et al., 2009), cashew nut protein (Ogunwolu et al., 2009), *Caragana korshinskii* Kom. protein (Zhong et al., 2012), coconut press cake (Chambal et al., 2013) and Indian black gram (Wani et al., 2015). The protein solubility profile provides an important guide to indicate the types of food or beverage into which the protein could be incorporated (Horax, Hettiarachchy, & Jalaluddin, 2004).

3.2.4.2. Water and oil absorption capacities. The water and oil absorption capacities refer to the ability of binding water and oil molecules under limiting water and oil conditions (Boye, Zare, & Pletch, 2010). The water absorption capacity (WAC) of protein from milk cake was higher than from oil cake ($P < 0.05$) for both SPP and PPP (Table 3). However, the oil absorption capacity (OAC) was similar. These results may be explained by the protein denaturation as confirmed by the report of Labuckas et al. (2014). They showed that the WAC of walnut cake obtained using hydraulic pressing was higher than from screw pressing at high temperature. The WAC and OAC values of coconut PPP determined in this study were higher than for cashew nut protein (Ogunwolu et al., 2009) and peanut protein (Wu et al., 2009); whereas, these values were lower than reported by Aydemir and Yemenicioğlu (2013) and in Indian black gram protein (Wani et al., 2015) The differences may have been due to the variation in the raw material obtained and the assay conditions in the WAC and OAC determinations and variation in the protein compositions of materials (Aydemir & Yemenicioğlu, 2013).

3.2.4.3. Foaming properties. Foaming properties indicate the capacity when the protein unfolds to form an interfacial skin that keeps air bubbles in suspension and prevents their collapse (Wani et al., 2015). Difference in the foaming capacity (FC) among the coconut cake samples were found for both SPP and PPP (Table 3). The protein from oil cake had a larger FC than from milk cake. During oil extraction, the denaturation of protein resulted in a greater unfolding structure leading to more interactions at the air-water interface. Moreover, the flexibility of protein to form good foam by reducing

Table 3
Functional properties of supernatant protein powder (SPP) and precipitate protein powder (PPP) from coconut milk cake and coconut oil cake.

Property	SPP		PPP	
	Milk cake	Oil cake	Milk cake	Oil cake
Water absorption capacity (g/g)	2.92[a] ± 0.04	0.78[b] ± 0.03	3.43[a] ± 0.21	2.30[b] ± 0.17
Oil absorption capacity (g/g)	3.25[a] ± 0.31	2.26[b] ± 0.40	3.40[a] ± 0.07	2.60[b] ± 0.47
Foaming capacity (%)	51.67[b] ± 7.64	85.00[a] ± 5.00	28.33[b] ± 5.77	50.00[a] ± 0.00
Foaming stability (%)	43.33[a] ± 7.64	35.00[a] ± 13.23	16.67[a] ± 5.77	17.50[a] ± 3.54
Emulsifying activity index (m^2/g)	32.85[b] ± 3.68	53.34[a] ± 1.74	29.22[b] ± 1.90	38.54[a] ± 1.61
Emulsifying stability index (min)	18.11[b] ± 0.38	23.51[a] ± 1.16	16.52[a] ± 0.79	17.23[a] ± 0.72

Values are mean of three replicates ± standard deviation.
[a–b]Different letters for the same protein types indicate significant differences ($P < 0.05$) in means.

surface tension enhanced the high FC (Damodaran, 1997).

In addition, the foaming stability (FS) indicates the percentage of foam remaining after a given period of time (Zhong et al., 2012). No differences ($P > 0.05$) in the FS of both protein powders from coconut milk cake and oil cake were observed. The FS at 60 min for SPP and PPP was similar and in the range 35–43%. The FS is dependent upon the formation of a thick cohesive layer around the air bubble (Damodaran, 1997). The FC and FS values of coconut protein were close to those reported for peanut protein (Wu et al., 2009). Foaming properties provide useful information for protein applications in food product processing such as in beverage, mousses and whipped toppings.

3.2.4.4. Emulsifying properties. The emulsifying properties are a measure of the effectiveness of proteinaceous emulsifiers in terms of the emulsifying capacity, emulsifying stability and emulsifying activity (Pearce & Kinsella, 1978). The emulsifying activity index (EAI) is a function of the interface area stabilized per unit weight of protein. Similar to the results of foaming properties, the SPP and PPP from oil cake provided EAI values higher than from milk cake (Table 3). The EAI values of coconut protein varied from 29.22 to 53.34 m^2/g and the SPP from oil cake had the highest EAI. The EAI of peanut protein (Zhang et al., 2014) and Indian black gram protein (Wani et al., 2015) were in the same range as for the coconut protein results obtained in the current study. However, our values were lower than the 80–120 m^2/g reported for *Caragana korshinskii* Kom. protein (Zhong et al., 2012).

The emulsifying stability index (ESI) indicates the ability of the emulsion to resist change to its structure over a defined time period (Boye et al., 2010). Significant ($P < 0.05$) differences were observed in the ESI of SPP from different coconut cakes. In contrast, no significant ($P > 0.05$) difference in the ESI of PPP was evident among coconut cake samples. The ESI values of all coconut proteins ranged from 16.52 to 23.51 min. Our results were close to those for peanut protein (Wu et al., 2009) and Indian black gram (Wani et al., 2015) but lower than for peanut protein isolate (Zhang et al., 2014). The emulsifying properties were correlated to the protein solubility, surface charge, surface hydrophobicity and molecular flexibility. The denatured proteins are exposed to the hydrophobic groups due to the dissociation and partial unfolding of globular proteins, producing an increase in the surface activity and adsorption at the oil and water interface (Soria & Villamiel, 2010) and this resulted in the higher EAI and ESI values of protein from oil cake.

4. Conclusion

The soluble proteins from coconut milk cake and oil cake were extracted at pH 11 for 1 h to obtain supernatant protein and with further precipitation at pH 4 to obtain precipitate protein followed by freeze drying. Protein powders from milk cake provided higher water and oil absorption capacities but protein powders from oil cake had good foaming and emulsifying properties. Protein powders from both coconut cakes can be solubilized under extremely acidic and alkaline solutions. Understanding protein properties can be useful for the integration of coconut protein powder as a food ingredient in food systems.

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