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Physicochemical and emulsion properties of edible protein concentrate from coconut (*Cocos nucifera* L.) processing by-products and the influence of heat treatment

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ABSTRACT

The physicochemical and emulsion properties of the edible coconut protein concentrate (CPC) obtained from coconut wet processing by-products were evaluated in order to characterize the protein. Native CPC and heat-treated CPC (CPCH) were used as an oil-in-water emulsifier at a constant protein concentration of 0.2% (w/v) in systems containing 10% (v/v) virgin olive oil or sunflower oil at pH 6.9. Nutritionally, CPC (80.3% protein) contains all the essential amino acids and predominantly leucine, lysine and valine. The coconut proteins ranged in molecular weight from 15 to 220 kDa, with both 11S and 7S globulin present. The presence of CPC as an oil-in-water emulsifier enhanced the stability of the system, and the virgin olive oil-in-water emulsions with CPC showed a higher stability than the corresponding sunflower oil-in-water emulsions. Furthermore, CPCH showed a greater solubility, interfacial tension and emulsifying activity than CPC but with a reduced emulsifying stability, particle size distribution ($D_{3,2}$) and flocculation. However, the protein secondary structures, zeta potential and Z-average diameter ($D_{4,3}$) of CPC were not significantly different from CPCH. Thus, coconut wet processing by-products can be used as a source of protein for humans, and it is possible to use the functional ingredients in oil-in-water emulsion foods.

1. Introduction

Proteins are one of the essential nutrients for the human body, and they are usually obtained from animal sources. However, storage proteins from plant origin have recently received great interest and extensive research work due to their comparable low cost, health benefits and good functional properties (Iqbal, Khalil, Ateeq, & Khan, 2006; Molina, Papadopoulou, & Ledward, 2001; Palazolo, Mitidieri, & Wangner, 2003; Shao & Tang, 2014; Wang, Wang, Li, Adhikari, & Shi, 2011). Moreover, the demand for plant proteins as an alternative to animal proteins due to dietary restrictions from religious or moral beliefs has increased (Karaca, Low, & Nickerson, 2015). Generally, soy protein has been applied in a wide range of food formulations due to its widespread availability, low cost and good functional properties (Molina et al., 2001; Palazolo et al., 2003; Shao & Tang, 2014; Wang et al., 2011). However, many people do not want to eat foods containing soy protein due to their beany flavor, allergies to soy, objections to using genetic

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modified organisms (which forms an increasing proportion of cultivated soy), and other dietary restrictions. For these reasons, the development of new sources of protein from low cost materials, such as coconut oil by-products, is a new and interesting alternative.

Although the protein content of fresh coconut meat (*Cocos nucifera* L.) is only 2.6%–4.4% on a wet basis (w/w) (Kwon, Bae, Park, & Rhee, 1996; Tangsuphoom & Coupland, 2005), the large mass of coconuts grown globally makes recovery of the protein desirable. Coconut is grown in 92 countries worldwide on, typically, very large plantations, especially in Indonesia, the Philippines, South Asia, East Africa and the Caribbean, with a world production level of coconuts estimated to be 50–60 million tons per year (Chambal, Bergenståhl, & Dejmek, 2012; FAO & APCC, 2013). Virgin coconut oil (VCO) is one of the valuable products from coconut used in food, medical and cosmetic products. The VCO demand is still increasing due to demand from both the local and international markets, especially in the Philippines, Thailand, Indonesia and Malaysia (Marina, Che Man, & Amin, 2009). Typically VCO is extracted from fresh coconut meat without chemical processes and without the use of heat, in the so called wet extraction process (Hamid, Sarmidi, Mokhtar, Sulaiman, & Aziz, 2011; Marina et al., 2009; Xu et al., 2015). Conventionally, VCO processing involves the three main steps of (i) extraction of coconut milk from fresh and mature coconuts, (ii) separation of the coconut milk into cream and skim milk, and (iii) separation of the cream into oil and protein (Gunetileke & Laurentius, 1974; Hagenmaier, Cater, & Matti, 1972a; Marina et al., 2009; Rosenthal, Pyle, & Niranjan, 1996). During VCO production, the by-products of coconut skim milk and solid substances are obtained, which are underutilized and often released as waste into the environment. However, coconut skim milk and solid residues contain 29.9 and 16.0% of the total protein content, respectively. Therefore, efforts have been undertaken to utilize the protein that remains (45.9%) in VCO by-products (Mepba & Achnewhu, 2003; Smith et al., 2009). As recently reported by the Food and Agriculture Organization of the United Nations (FAO) and Pacific and Asia Pacific Coconut Community (APCC), VCO production in the Asia Pacific region has increased by 5% (FAO & APCC, 2013) and it is predicted that demand for VCO will increase further leading to an increased production of coconut by-products (Sangamithra et al., 2013). Previous research on the characterization of coconut skim milk has been aimed at its potential as a value-added product, medical food (Remya, Chikku, Renjith, Arunima, & Rajamohan, 2013), protein nutrient (Ng, Mohammad, Ng, & Jahim, 2015) and *Spirulina platensis* growth medium (Nur, Irawan, & Hadiyanto, 2015). Moreover, coconut protein has also been found to have potential in health promotion and disease prevention. It has a large proportion of arginine and a potent anti-diabetic activity (DebMandal & Mandal, 2011; Salil, Nevina, & Rajamohan, 2011). Consequently, it would be beneficial to make a coconut protein concentrate (CPC) with a very low fiber content that could then be more easily incorporated as food ingredients.

One of the alternative processes for CPC preparation often considered is a conventional isoelectric precipitation and centrifugation approach. Previous studies have shown that coconut proteins have a maximum precipitation at pH 3.9–4.0 (Capulso, Gonzales, & Celestino, 1981; Samson, Khaund, Cater, & Mattil, 1971a; Thaiphanit & Anprung, 2013), and that this was suitable for efficient extraction of coconut protein from coconut by-products using isoelectric precipitation (Thaiphanit & Anprung, 2014).

The functional properties of proteins are determined by their physicochemical properties, can affect their processing and influence food systems by contributing to the desirable characteristics of food (Kinsella, 1976; Soares de Castro, Bagagli, & Sato, 2015), such as colloid and emulsion formation and stabilization. Indeed, proteins are of particular interest in terms of their emulsifying properties, due to their amphiphilic nature and film-forming abilities (Lam & Nickerson, 2013). However, the solubility of a given protein is one of the key factors in determining its emulsifying efficiency (Kinsella, 1976), and protein emulsifiers must be sufficiently hydrophobic in spite of their solubility profile (Klemaszewski, Das, & Kinsella, 1992). These properties can be modified by chemical, thermal or enzymatic treatments (Palazolo, Sobral, & Wagner, 2011). Heating is one of the most important and frequently used methods for food processing. Since it was found that thermal treatment induces structural modification and denaturation of 11S and 7S globulin (Palazolo et al., 2011), heating of proteins also have influence on the stability of oil in water emulsions as was reported in isolate soy proteins (Mitidieri & Wagner, 2002; Palazolo et al., 2011), whey soy proteins (Palazolo, Sorgentini, & Wagner, 2005; Ray & Rousseau, 2013) and whey protein (Dybowska, 2011; Kim, Decker, & McClements, 2006). Several methods have been used to determine the effectiveness of a protein as an emulsifier, such as interfacial tension, emulsifying activity, emulsifying stability, zeta potential, droplet size and size distribution and the flocculation degree. The coconut protein from fresh coconut meat has been reported to have good emulsifying properties (Onsaard, Vittayanont, Sringam, & McClements, 2006). As protein can lower the interfacial tension at the oil-water interface of the droplets, concentration of the protein at the oil-water interface is one factor in the stability of the emulsions (McClements, 2004). As previously reported, the sufficient protein concentration to cover the surfaces of oil droplets for the coconut protein is 0.2% (w/v) (Onsaard et al., 2006).

The emulsion properties of the protein also depend on other physicochemical properties, such as their molecular weight, protein structure, protein flexibility and surface activity (Karaca et al., 2015; Papalamprou, Doxastakis, & Kiosseoglou, 2010). These protein properties are very important because their ability to form stable emulsions is determined by the ability of the protein to adsorb and to unfold at the oil-water interface during emulsion formation (Munk, Erichsen, & Andersen, 2014). Additionally, emulsifying properties also depend upon a suitable balance between the hydrophiles and lipophiles of the protein (Yust, Pedroche, Millán-Linares, Alcaide-Hidalgo, & Millán, 2010). Conventional methods for characterizing the molecular weight of proteins have been based on polyacrylamide gel electrophoresis (PAGE), especially denaturing sodium dodecyl sulphate (SDS)-PAGE (Chambal et al., 2012; Ng et al., 2014). Fourier transform infrared spectroscopy (FT-IR) is well suited to detect relative changes in protein secondary structure due to external factors by analyzing the amide I band of proteins between 1700 and 1600 cm^{-1} (Jackson & Mantsch, 1995). This band is influenced by hydrogen bonds, which are mainly affected during conformational changes in the protein secondary structure.

Nowadays, consumer awareness about dietary fat is increasing, and vegetable oils can be used as an alternative to solid fats to produce oil-in-water emulsions with a healthier fatty acid profile. At this point, emulsions formulated with “olive oil” or “sunflower oil” instead of saturated fat can include a claim of higher amounts of monounsaturated and polyunsaturated fatty acids. The effectiveness of protein fractions extracted from coconut cream protein to act as emulsifiers in oil-in-water emulsions has been reported by Onsaard et al. (2006). Their study was conducted to compare the properties of corn oil-in-water emulsions stabilized by coconut cream proteins from conventional isoelectric precipitation and freeze–thaw treatment with those stabilized by whey protein isolate. However, they did not investigate the effect of heat treatment on emulsion properties of CPC. The purpose of the current

work was to report the physicochemical and emulsion properties of CPC from coconut by-products and to investigate the possibility to use native CPC and heat-treated CPC (CPCH) as emulsifiers to stabilize virgin olive oil and sunflower oil-in-water emulsions.

2. Materials and methods

2.1. Materials

Fresh coconut wet processing by-products were supplied by the 100 Phan Ma-phrau Thai group at Prachuapkhirikhan Province of Thailand, and then stored at −20 °C until used. All chemicals were of analytical grade and were purchased from Scientific Equipment Co. Ltd., Roongsarp- Chemical (Partnerships), CTI & Science Co. Ltd. and Gibthai Co. Ltd., Bangkok, Thailand. Commercial virgin olive oil and sunflower oil were both purchased from a local supermarket.

2.2. Preparation of CPC

Coconut skim milk (~8% total solids) and solid by-product substances (~19% total solids), were mixed before the coconut cream proteins were extracted by conventional isoelectric precipitation with slight modification of a previously reported method (Thaiphanit & Anprung, 2013, 2014). The mixture (initial pH = 5.47) was gently stirred at room temperature for 30 min, and then centrifuged (7,440 g 4 °C for 20 min). The pellet was discarded. The supernatant and the precipitate were mixed together and homogenized. The mixture (initial pH = 5.47) was adjusted to pH 4 with 1 N HCl and then centrifuged (5,700 g, 4 °C for 15 min). After decanting the supernatant layer, the obtained precipitate was washed in distilled water, and the protein separation procedure was twice repeated as above. HCl was used to precipitate coconut protein because it was reported as the most effective acid among HNO_3, H_2SO_4, H_3PO_4 and HOAc (Samson, Khaund, Cater, & Mattil, 1971b). The protein content was determined by the Kjeldahl method using a nitrogen conversion factor of 6.25. The coconut cream proteins were freeze dried in a Labconco FreeZone® Freeze Dryer (Floor-model, USA) to yield the CPC. CPCH was prepared by dissolving 10 g of CPC in 100 ml distilled water and heating at 85 °C for 15 min (the heating-up time was about 5 min), after which the dispersion was cooled rapidly in an ice bath and freeze dried. The native CPC, and subsequently the CPCH, were prepared with a protein content of at least 80%. Both the CPC and CPCH powders had particle sizes of <30 mesh.

2.3. Chemical analysis

The chemical composition of CPC was evaluated according to AOAC standard methods 962, 09–992.06 (AOAC, 1990). The moisture content was assayed by loss in weight on heating at 60 °C in a hot air circulating oven (BINDER GmbH, Germany) to constant weight. The ash content was determined by incineration of known weights of samples in a muffle furnace (Carbolite CWF11/13/201, UK). The crude fat content was determined by exhaustively extracting a known weight of sample in petroleum ether in a Soxhlet extractor. The ether was volatilized and the dried residue quantified gravimetrically and reported as percentage (w/w) of fat. Protein content was determined by the Kjeldahl method using a nitrogen conversion factor of 6.25. Crude fiber content was determined after digesting a known weight of a fat-free sample in refluxing 1.25% (v/v) sulfuric acid and 1.25% (w/v) sodium hydroxide. The available carbohydrate content was determined by difference. All measurements were performed in triplicate.

2.4. Amino acid analysis

Amino acid analysis was performed by the Central Lab Thai (Thailand) utilizing high performance liquid chromatography with 20 standard amino acids, as previously reported (González-Castro, López-Hernández, Simal-Lozano, & Oruña-Concha, 1997). The internal standard method was used to quantitate the amino acids in the CPC according to Eq. (1);

$$K = (A_i \times W_x)/(A_x \times W_i), \quad (1)$$

where W_x is the weight of component x, K the relative response factor for the particular amino acid, A_i the area under the peak of the internal standard, A_x the area under the peak of component x, and W_i the weight of the internal standard. The relative retention time (R_t; the ratio of the retention time of the standard amino acids to that of the internal standard) was used to identify the amino acids that were present in the sample.

2.5. SDS-PAGE

The SDS-PAGE was performed on a discontinuous buffered system according to the method of Laemmli (1970), using a 10% (w/v) acrylamide resolving gel. In brief, 0.50 g of protein sample was dissolved in 20 ml of 5% (w/v) SDS at 80 °C for 60 min, cooled to room temperature and centrifuged (4,190 g, 4 °C for 20 min). The protein containing solution (12 μg protein) was diluted 1:1 (v/v) with sample buffer and 6 μl of diluted protein solution was loaded per lane. After the electrophoresis, the gel was dyed in Comassie brilliant blue (R-250) staining solution for one hour and then destained in 250 ml ethanol/100 ml acetic acid to remove the background color of the gel. The molecular weight of CPC was estimated by comparing the relative mobility (R_f) of the protein bands to that of the coresolved standard marker proteins (Bench-Mark™ Protein Ladder, Cat. No.10747-012). The total protein in the solution was determined using the Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as the standard.

2.6. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

The ATR-FTIR spectra were obtained in the wave number range from 400 to 4000 cm^{-1} during 64 scans with 4 cm^{-1} resolution using a Fourier transform infrared spectrometer (PerkinElmer Spectrum One, USA). The CPC and CPCH spectra were collected in absorbance mode for samples placed directly onto the ATR crystal. The collected spectra were corrected against air as background.

2.7. Protein solubility

Protein solubility profiles of CPC and CPCH were obtained by traditional isoelectric precipitation, where the residual protein in solution was considered as soluble. Dilute protein solutions (0.7% (w/v)) were prepared by dispersing the samples into distilled water and adjusting to the desired pH (pH 2–9). The protein solutions were stirred at room temperature for at least 30 min, centrifuged (8,400 g, 25 min at 4 °C), and the supernatant harvested and filtered through Whatman No. 1 filter paper to remove flocculent materials. The protein content of the filtered supernatant was determined as in Section 2.5. The protein solubility (PS) was calculated from Eq. (2);

$$PS = 100 \times (P/P_T), \quad (2)$$

where P is the protein content remaining in the supernatant after

centrifugation and filtration, and P_T is the total protein content present in the original solution.

2.8. Surface and interfacial tension

Protein dispersions were prepared by mixing CPC and CPCH into PBA (0.1 M phosphate buffer/0.01% (w/v) sodium azide, pH 6.9) with stirring at 100 rpm for 2 h at room temperature. The dispersions were left overnight at room temperature to fully hydrate, and then mixed by using an Ultra-Turrax T25 with a dispersing tool (Model S 25 KR-18 G, IKA Instruments, Germany) at 2000 rpm for 2 min.

The interfacial tension of CPC and CPCH at the air–water interface and the oil-water interface were measured according to the Wilhelmy plate method (Krüss Force Tensiometer K20, Germany) at 20 °C. Each sample was made up to a constant volume (50 ml) for surface and interfacial tension determinations, and the oils at interface were virgin olive oil and sunflower oil.

2.9. Emulsion properties

2.9.1. Emulsion preparation

The emulsions were prepared in PBA. Coconut protein (CPC and CPCH) dispersions were prepared as in Section 2.8. Oil-in-water emulsions were prepared by blending the protein dispersions and 10% (v/v) virgin olive oil or sunflower oil (0.2% protein (w/v) following Onsaard et al. (2006)) using an Ultra-Turrax T25 with a dispersing tool (Model S 25 KR-18 G) at 16,000 rpm for 2 min at 20 °C to make 50 ml of macroemulsions as previously reported (Thaiphanit & Anprung, 2014). This condition preliminarily tested the sufficiency of coverage of the surfaces of oil droplets using centrifugation (Hunt & Dalgleish, 1994).

2.9.2. Emulsifying activity and stability

The emulsifying activity index (EAI) and emulsifying stability index (ESI) of the CPC and CPCH emulsions were determined by turbidimetric methods according to Pearce and Kinsella (1978) with slight modification. One hundred μl of the freshly prepared emulsions (Section 2.9.1) were harvested at 0 and 10 min after homogenization and added to 3.9 ml of 0.1% (w/v) SDS giving a final 40-fold dilution. The absorbance of the final dilutions was measured at 500 nm with a Spectronic 20 Genesys Spectrometer (USA). The EAI and ESI were determined from Eqs. (3) and (4), respectively:

$$EAI = (2.303 \times 2 \times A_0 \times \text{Dilution})/(\phi \times C \times 10000), \quad (3)$$

$$ESI = (A_0/\Delta A)(t), \quad (4)$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenization, C is the weight of protein per unit volume (g/ml) in the aqueous phase before emulsion formation, ϕ is the oil volume fraction of the emulsion, ΔA is the change in absorbance between 0 and 10 min and t is the time interval (10 min).

2.9.3. Zeta potential

A Zetasizer Nano Series Nano-ZS from Malvern Instruments (UK) was used to determine the electrical charge (ζ-potential) of oil droplets in the emulsions by determining their electrophoretic mobility and then applying the Henry equation. Ten minutes after homogenization, the emulsions were diluted 1:100 (v/v) with PB (0.1 M phosphate buffer pH 6.9) to avoid multiple scattering effects. The diluted emulsions were poured into a disposable capillary cell (DTS1070), and then equilibrated at 25 °C in the instrument for 60 s prior to measurement.

2.9.4. Droplet size, droplet size distribution and degree of flocculation (DF)

Particle size was measured using a Zetasizer Nano Series Nano-ZS (Malvern Instruments Ltd., Malvern, UK) within 10 min after homogenization. To prevent multiple scattering effects, two samples of each emulsion were separately diluted 1:100 (v/v) with PB with and without 1.0% (w/v) SDS. The diluted emulsions were poured into disposable capillary cells (DTS1070) after gently stirring them for 60 s, and then equilibrated at 25 °C in the instrument for 60 s prior to measurement. A refractive index of 1.463 was used in the calculations of the particle size, and a continuous phase refractive index of 1.333 (water) was used. The Z-average diameter ($D_{4,3}$) and particle size distribution ($D_{3,2}$) of the emulsion droplets were determined.

The degree of flocculation (DF) was calculated from the particle size data using Eq. (5);

$$DF\% = \left[(D_{4,3} - D_{4,3\ SDS})/D_{4,3\ SDS}\right] \times 100, \quad (5)$$

where $D_{4,3}$ and $D_{4,3\ SDS}$ are the Z-average diameter, measured in the absence and presence of 1% (w/v) SDS, respectively (Palazolo et al., 2005).

2.10. Statistics

The obtained data were analyzed using one way analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) was used to compare means. Significance was accepted at $p < 0.05$. All tests were performed using SPSS, Version 21 (IBM, USA) statistical software package.

3. Results and discussion

3.1. Chemical analysis

Using the conventional isoelectric precipitation for protein extraction, the yield of CPC was 4.56% on a wet basis (w/w), and the major constituents (as (w/w)) of CPC were protein (80.3%) and fat (18.9%), with crude fiber (0.64%), ash (0.09%) and carbohydrate (0.06%) as minor components. The fat was not completely removed during the protein extraction by the isoelectric precipitation due to lower solubility of fat in water. Seow and Gwee (1997) reported that the main carbohydrates present in coconut skim milk were sugars (primarily sucrose) and some starch, while the major minerals in raw coconut milk were reported to be phosphorus, calcium and potassium.

3.2. Amino acid analysis

The amino acid composition of CPC is shown in Table 1. CPC had

Table 1
Amino acid composition of the obtained coconut protein concentrate (CPC).

Essential Amino acid	g/100 g[a]	Nonessential Amino acid	g/100 g[a]
Histidine	2.2925	Alanine	3.6468
Isoleucine	2.0545	Arginine	12.2219
Leucine	4.7699	Aspartic acid	8.6481
Lysine	3.3476	Cysteine	1.6299
Methionine	1.5512	Glutamic acid	21.6721
Phenylalanine	4.3078	Glycine	3.9061
Threonine	2.7611	Proline	3.3710
Tryptophan	1.3807	Serine	4.1111
Valine	3.3253	Tyrosine	2.1073

[a] Average result from two independent samples.

a relatively favorable amino acid profile and contained 18 amino acids of which nine are essential to humans (predominantly leucine, lysine and valine at 4.77, 3.35 and 3.33 g/100 g, respectively) with the nine nonessential amino acids (predominantly glutamic acid, arginine and aspartic acid at 21.67, 12.22 and 8.65 g/100 g, respectively). The composition of this CPC is in agreement with that from previous reports (Hagenmaier, Cater, & Matti, 1972b; Onsaard et al., 2006; Samson et al., 1971b). In addition, all the essential amino acids in CPC were sufficient for adult humans, based on the FAO/WHO guidelines (World Health Organization, 2007). These data suggest that the coconut protein from the coconut wet processing by-products is of a suitable nutritional quality as a food protein. In addition, the side chains of most amino acids in the CPC are charged, being either acidic (glutamic and aspartic acid) or basic (arginine), providing the hydrophilic (polar) component and giving an energetically favorable contact with water (Ludescher, 1996).

3.3. Molecular weight profile of CPC and CPCH

The major (by mass) proteins in CPC ranged in molecular weight between 15 and 220 kDa, as derived from SDS–PAGE analysis (Fig. 1). The major coconut globulin, cocosin or the 11S globulin, has a native state molecular weight of 326 kDa and accounts for 86% of the total globulins, but resolves on reducing SDS-PAGE in two main sets of electrophoretic subunits at 24 and 34 kDa (Chambal et al., 2012; Garcia, Arocena, & Laurena, 2005), plus a 55 kDa band that is a recombination product of both subunits. The other main protein is the 7S globulin with a native molecular weight of 156 kDa that resolves on SDS-PAGE as 16, 22 and 24 kDa sub-units. In addition, the pH used for the protein extraction is likely to have influenced the electrophoretic behavior of the protein (Balasubramaniam & Sihotang, 1979). The molecular weight profile of CPC from the present study is in agreement with these results, and so both the 11S and 7S globulins are likely present in the CPC obtained from VCO by-products of coconut skim milk and solid wastes. In addition, the molecular weight of the major protein in the native CPC was more than 50 kDa, and there were nine major subunits distributed in CPC. The relative molecular weights of the major protein component were estimated from their relative mobilities and found to be 14, 18, 22, 32, 62, 85, 100, 111 and 138 kDa. Likewise, the molecular weight profile of CPCH had a similar protein composition present in CPC. However, protein band intensity at molecular weight between 50 and 60 kDa of CPC (lane B) was larger than the corresponding band intensity of CPCH (lane C). These data suggest that conjugating proteins with polysaccharides by electrostatic attraction between oppositely charged portions or hydrophobic interactions in CPC (Corredig, Sharafbafi, & Kristo, 2011; Tapeinou et al., 2015). According to the results, protein–polysaccharide interactions in CPC maybe play a role in the formation of structure and texture as a thickening agent in emulsion products. Marked heat-induced change in the molecular weight of the coconut proteins occurred. Therefore, CPCH has potential application as a stabilizer in food emulsions that are fairly viscous.

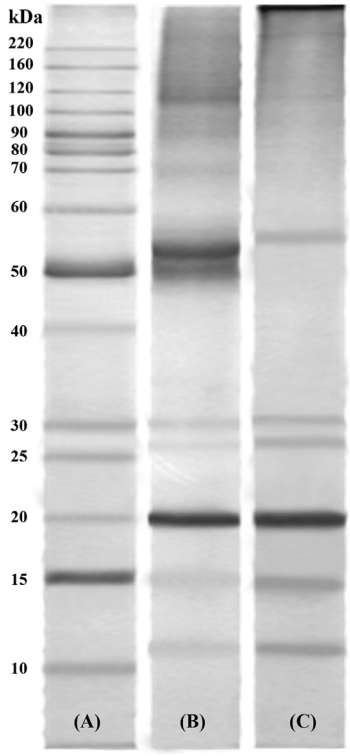

Fig. 1. SDS-PAGE resolved and Coomasie blue stained (A) molecular weight markers (B) coconut protein concentrate and (C) heat-treated CPC.

3.4. ATR-FT-IR spectra of CPC and CPCH

The secondary structure of native CPC obtained by isoelectric precipitation, and the effect of heat treatment at 85 °C for 15 min (CPCH) were evaluated by ATR-FT-IR analysis. Representative spectra are shown in Fig. 2. The major absorption peaks in the FT-IR spectra of CPC and CPCH were the O–H and N–H group stretching vibrations at 3278 cm^{-1}, CH_2 asymmetrical stretching at 2922 cm^{-1}, protein C=O stretch (amide I) at 1636 cm^{-1} and protein N–H bending and C–N stretching (amide II) at 1521 cm^{-1}. In addition, the characteristic absorption bands ranging from 1500 to 850 cm^{-1} were the fingerprint region of CPC and CPCH. The ATR-FT-IR spectra of CPCH had a similar pattern to that of CPC suggesting that no marked heat-induced change in the conformation of the coconut proteins had occurred (Lefevre & Subirade, 2001). However, heat-treatment of CPC significantly increased the intensity of the characteristic absorption band of the O–H and N–H group stretching vibrations and amide II band (1521 cm^{-1}) due to change of geometric shape of a protein tertiary structure. Knowing the effect of heating on the secondary structure of coconut proteins would be helpful to optimize the production process and maintain the biological functions of coconut protein for use in functional food and pharmaceutics.

3.5. pH-dependent solubility of CPC and CPCH

The influence of pH on the solubility of CPC and CPCH is shown in Fig. 3. The pH-solubility profile of CPC had a U-shaped curve with a minimum solubility at pH 4, which is close to the reported isoelectric point (pI) of the principal coconut proteins (Onsaard et al., 2006). Likewise, CPCH showed a similar pH-solubility profile over the acidic pH range (pH 1–4) with a minimum solubility also at pH 4.0, but CPCH was more soluble than CPC at pH 5–8. The solubility of a protein is affected by its amino acid composition, where the relative amounts of acidic and basic amino acids determine the net charge of a protein at a given pH. The relative amounts of hydrophilic and hydrophobic amino acids control its solubility characteristics (Damodaran, 1996). Although this is not likely to be different between CPCH and CPC, the surface charge of the protein also influences the solubility of the proteins within the aqueous phase (Karaca, Low, & Nickerson, 2011). At the isoelectric point, proteins are less charged and so the electrostatic repulsive forces

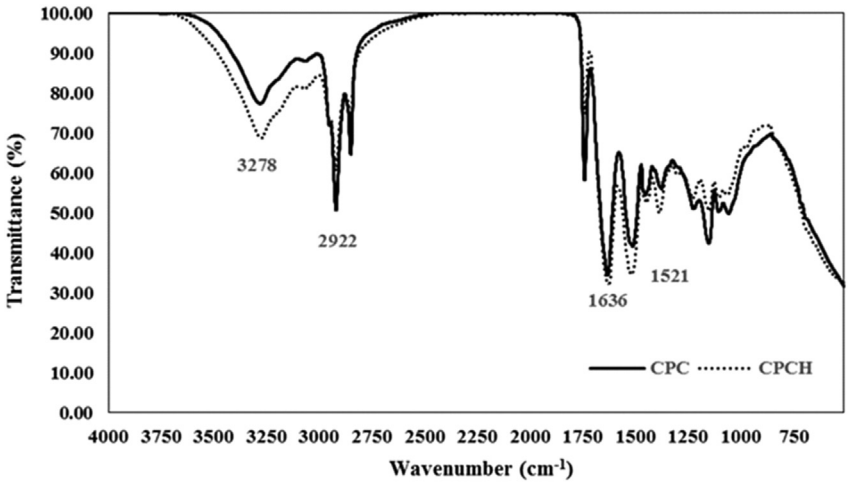

Fig. 2. Representative ATR-FTIR spectra of native (CPC) and heat treated (CPCH) coconut protein concentrate. Spectra shown are representative of those seen from three independent replicates.

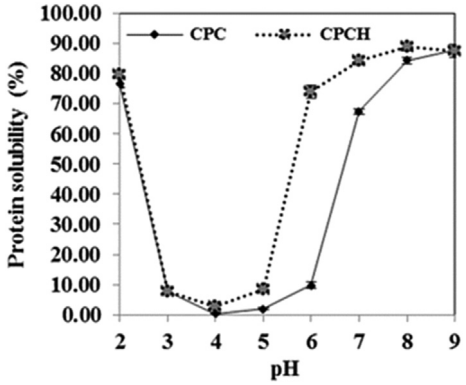

Fig. 3. pH-solubility profile of native (CPC) and heat treated (CPCH) coconut protein concentrate. Data are shown as the mean ±1 SD, derived from three independent replicates.

are weaker, which facilitates the agglutination of the proteins and results in a lower solubility. The higher protein solubility of CPCH than CPC at pH 5–8 is probably due to an increased reactivity of the free hydrophilic group. These data demonstrate that the CPC obtained from the coconut wet processing by-products is still limited to use within food systems outside of pH 3–6. Modification of CPC by heat treatment improved this slightly, reducing the unsuitable pH range to 3–5.

3.6. Surface and interfacial behavior

Interfacial tension plays an important role in emulsification. If two immiscible liquids are in contact with each other, they will tend to maintain as small an interface as possible. Consequently, it is very difficult to mix the two liquids. One way to assist the formation of an emulsion is to reduce the liquid–liquid interfacial tension to a point where the immiscible liquids will form a stable mixture. An emulsifier, or a surfactant, reduces the interfacial tension and can be added to the oil or water fraction (Barnes & Gentle, 2005). With respect to the ability of CPC or CPCH to act as an oil-in-water emulsifier, the results are summarized in Table 2. The interfacial tension was dependent upon the solvent (higher in water than in PB), but the presence of the CPC or CPCH is also an important factor that influenced the interfacial tension. Both CPC and CPCH significantly decreased the surface and interfacial tension in both virgin olive oil-water and sunflower oil-water mixes, and CPC was slightly better at reducing the interfacial tension than CPCH. Proteins are amphiphilic in nature (i.e., having both hydrophobic and hydrophilic groups) and have film-forming abilities (Foegeding & Davis, 2011; Ye & Singh, 2006). Since coconut proteins have been shown to denature and coagulate on heating to 80 °C (Tangsuphoom & Coupland, 2008), the proteins in CPCH were denatured by the heat treatment, with a likely partially unfolded structure that may have been less optimally distributed at the protein surface than those side chains that are effective at the oil and water interfaces. In addition, the interfacial tensions of the CPC and the CPCH in the virgin olive oil-water interface exhibited a lower interfacial tension than that of sunflower oil-water interface. Thus, at least under the current experimental conditions, virgin olive oil is more suitable than sunflower oil for the preparation of oil-in-water emulsions with aqueous dispersions of CPC. Moreover, these properties could influence the oil droplets size and other emulsifying properties such as EAI and ESI (Gu, Campbell, & Euston, 2009).

3.7. Emulsion properties

3.7.1. Emulsifying activity (EAI) and stability (ESI) indices of CPC and CPCH

The effects of coconut protein on the emulsifying activity and stability were analyzed in terms of the EAI and ESI, where those for virgin olive oil- and sunflower oil-in-water emulsions with CPC and CPCH at pH 6.9 are shown in Fig. 4. The EAI reflects the ability of protein to form and stabilize an emulsion, whereas the ESI refers to the ability of an emulsion to keep its properties unchanged over a certain period of time. However, as emulsions are thermodynamically unstable, changes in the emulsion properties will occur but the more slowly they change, the more stable the emulsion. Hence, the ability of discrete emulsion droplets to remain dispersed without creaming, flocculating, coalescing or oiling off is defined as the ESI. The CPC had a clear emulsifying activity and its ESI was significantly higher than that for CPCH. This could be because CPC was slightly better at reducing the interfacial tension than CPCH. Thus, interfacial tensions influence the emulsifying properties of the emulsions. However, the EAI of sunflower oil-in-water emulsions with CPCH was significantly higher than that for virgin olive oil-in-water emulsions with CPC, whilst the CPCH emulsions had a

Table 2
Surface and interfacial tensions of coconut protein concentrate (CPC) and coconut protein concentrate with heat treatment (CPCH).

Solution	Interfacial tension (mN/m)		
	Air–water[a]	Virgin olive oil–water interface[a]	Sunflower oil–water interface[a]
Distilled water	$72.6^{d} \pm 0.2$	$18.7^{d} \pm 0.5$	$23.9^{c} \pm 0.3$
PB[b]	$53.4^{c} \pm 0.4$	$16.2^{c} \pm 0.4$	$21.6^{b} \pm 0.5$
CPC in PB	$35.8^{a} \pm 0.6$	$10.7^{a} \pm 0.6$	$13.3^{a} \pm 0.7$
CPCH in PB	$38.0^{b} \pm 0.5$	$12.1^{b} \pm 0.8$	$13.7^{a} \pm 0.7$

[a] Data are shown as the mean ±1 SD and are derived from three independent replicates. Means within a column with different subscripts are significantly different ($p < 0.05$; DMRT).
[b] PB = 0.1 M phosphate buffer pH 6.9.

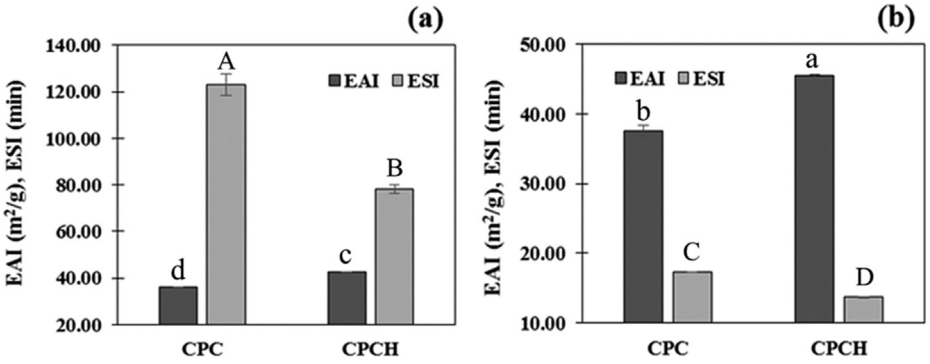

Fig. 4. The emulsifying activity index (EAI) and emulsion stability index (ESI) of (a) virgin olive oil- and (b) sunflower oil-in-water emulsions prepared with native (CPC) and heat treated (CPCH) coconut protein concentrate at pH 6.9. Data are shown as the mean ± 1 SD, derived from three independent replicates. Means with a different superscript letter are significantly different ($p < 0.05$; DMRT).

significantly higher EAI than the corresponding CPC ones. For both the CPC and CPCH emulsifiers, the virgin olive oil-in-water emulsions were more stable than the sunflower oil-in-water emulsions. The presence of the protein within the continuous phase also acts to increase the emulsion viscosity, reduce the mobility and diffusion of the oil droplets within the emulsion and leads to an increased emulsion stability (Jafari, Beheshti, & Assadpoor, 2012). However, the types of bonds formed (i.e., hydrophobic, van der Waals, etc.) within the protein film will also influence their emulsion stability. Proteins in CPCH were likely to be partially denatured by the heat treatment with an increased level of hydrophobic bonding and so a reduced emulsion stability. Thus, heat treatment to modify CPC seemed to have no beneficial effect on the emulsion properties, at least under the current experimental conditions. Moreover, CPC and CPCH were more suitable for virgin olive oil-in-water emulsions than for sunflower oil-in-water emulsions.

3.7.2. Zeta potential of CPC and CPCH emulsified oil-in-water emulsions

The zeta potential is a physical property exhibited by any particle in suspension and describes the nature of the electrostatic potential near the surface of the particle. A higher zeta potential results in greater electrostatic repulsion forces between the particles and so gives a greater separation distance between particles in the suspension and a reduced aggregation/flocculation caused by Van der Waals interactions (Malhotra & Coupland, 2004). The electrostatic charge (ζ-potential) of virgin olive oil- and sunflower oil-in-water emulsions prepared with aqueous CPC and CPCH are summarized in Table 3. The electrostatic charge on the emulsion droplets was negative, indicating that negatively charged (anionic) protein molecules were absorbed onto the oil droplet surface, consistent with the assay pH (6.9) being above the protein isoelectric point (4.0) and so the number of anionic groups dominates the cationic ones (Surh, Decker, & McClements, 2006). However, numerically the CPCH emulsions had a slightly lower electrostatic charge (and so emulsion formation activity) than native CPC, but these differences were not statistically significant. The magnitude of the net electrostatic charge of the CPC or CPCH oil-in-water droplets was relatively small and remained below −30 mV. Since a zeta potential of > ±30 mV is often used as an approximate threshold for stability, then these CPC or CPCH oil-in-water emulsions will tend to aggregate over time (Mirhosseini & Tan, 2010; Wiącek & Chibowski, 2002). However, the change in the zeta potential reflected the change in protein and surface composition (Tangsuphoom & Coupland, 2009). In addition, there are many mechanisms by which proteins can stabilize emulsions. For example, during emulsion formation, proteins are typically considered to adsorb to the interface, and thereby stabilize the emulsion against coalescence. After formation, the emulsion stability against flocculation is determined by the charge of the adsorbed protein layer (Delahaije, Gruppen, Giuseppin, & Wierenga, 2015).

3.7.3. Z-average diameter and particle size distribution of CPC and CPCH emulsified oil-in-water emulsions

The Z-average diameter ($D_{4,3}$) of the virgin olive oil- and

Table 3
The electrostatic charge (ζ-potential) of oil droplets in virgin olive oil- and sunflower oil-in-water emulsions prepared with aqueous dispersions of native (CPC) and heat-treated (CPCH) coconut protein concentrate at pH 6.9.

	Zeta potential (mV)	
	Virgin olive oil-in-water emulsion	Sunflower oil-in-water emulsion
CPC	−13.7 ± 0.9	−13.3 ± 0.8
CPCH	−12.1 ± 0.9	−12.9 ± 0.7

Data are shown as the mean ±1 SD and are derived from three independent replicates. None of the means were significantly different ($p > 0.05$; DMRT).

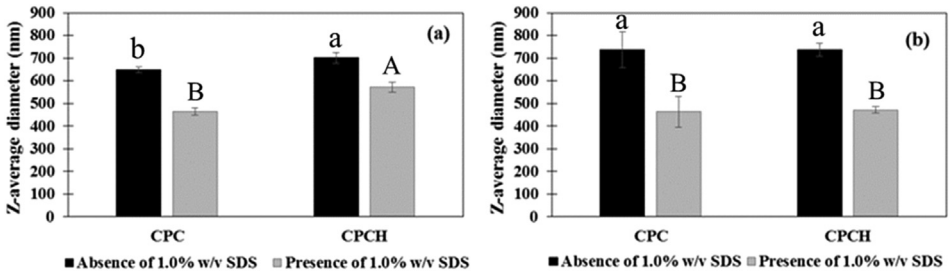

Fig. 5. Z-average diameter ($D_{4,3}$) of (a) virgin olive oil- and (b) sunflower oil-in-water emulsions prepared with aqueous dispersions of native (CPC) or heat treated (CPCH) coconut protein at pH 6.9. Measurements were performed in the absence or presence of 1.0% (w/v) SDS. Data are shown as the mean ±1 SD, derived from three independent replicates. Means with a different superscript letter are significantly different ($p < 0.05$; DMRT).

sunflower oil-in-water emulsions prepared with aqueous dispersions of CPC and CPCH are shown in Fig. 5. In virgin olive oil-in-water emulsions, the CPC emulsions had a significantly smaller Z-average diameter than the corresponding emulsions prepared with CPCH. These results are in agreement with the ESI as shown in Fig. 4. However, these results are not in agreement with the pH-dependent solubility of CPC and CPCH in the Section 3.5. Since emulsifying properties ultimately depend upon a suitable balance between the hydrophiles and lipophiles, they do not necessarily increase as a protein becomes more soluble (Yust et al., 2010). Sunflower oil-in-water emulsions prepared with aqueous dispersions of the CPCH emulsions had a slightly lower Z-average diameter than the native CPC, but these differences were not statistically significant. These results conflict with the ESI results in the Section 3.7.1. Flocculation did not affect droplet size since no droplets merged (Palazolo et al., 2005), Flocculation is one factor in the physicochemical destabilizing processes to break down emulsions (Sakai et al., 2001).

The particle size distribution (PSD) of the different emulsions is shown in Fig. 6. The emulsion formed with CPCH had a slightly smaller size distribution than those formed with CPC for both virgin oil-in-water emulsions (623 vs. 674 nm diameter, respectively) and sunflower oil-in-water emulsions (555 and 706 nm, respectively). From the size of the oil droplets, these are macroemulsions (droplet size > 400 nm). However, whilst a monomodal droplet size distribution was observed in the virgin olive oil-in-water emulsions prepared with either CPC or CPCH (Fig. 6a), the sunflower oil-in-water emulsions showed a bimodal size distribution under the same conditions (Fig. 6b). In general, the oil droplet size is the key parameter to characterize the stability of emulsions because instability either affects or is affected by droplet size. Coalescence and Ostwald ripening tend to increase the average droplet size, while flocculation does not affect the droplet size because droplets do not merge.

3.7.4. Degree of flocculation (DF) of the CPC and CPCH emulsified oil-in-water emulsions

Droplet size measurements can be used to determine if flocculation is present in the emulsion. In the absence of any flocculation, droplet size will not change. However, if there is flocculation, droplet size will decrease. When the virgin olive oil- and sunflower oil-in-water emulsions were mixed gently with 1.0% (w/v) SDS to break up the aggregates, the Z-average diameter ($D_{4,3}$) of these emulsions decreased (Fig. 5), indicating that the oil droplets in both the CPC and CPCH emulsions had flocculated to some extent under these current experimental conditions. The DF of the virgin olive oil- and sunflower oil-in-water emulsions prepared with CPC was 39.7 and 59.2%, respectively, and these values were higher in those prepared with CPCH (22.7 and 56.1%, respectively). In other words, the CPCH emulsions exhibited a lower DF in the two different oil-in-water emulsions, whilst the virgin olive oil-in-water emulsions stabilized by CPC or CPCH were more resistant to flocculation than the corresponding sunflower oil-in-water emulsions. This result conflicts with previous findings (Tangsuphoom & Coupland, 2005). These data emphasize that the quality of the coconut protein can be adversely affected by the protein extraction conditions.

4. Conclusions

Edible CPC, obtained as wet processing by-products from VCO production, is a good source of protein for humans, containing all the essential amino acids and especially leucine, lysine and valine. Both 11S and 7S globulin were present in the CPC. The solubility of CPC and CPCH were minimal at pH 4 and increased rapidly at values pH below 3 and above pH 5 and 6 for CPCH and CPC, respectively. The stability of the virgin olive oil- and sunflower oil-in-water emulsions was enhanced when CPC was used as an emulsifier in the systems. Heat treatment of CPC (i.e., CPCH) significantly increased the solubility, interfacial tensions and emulsifying

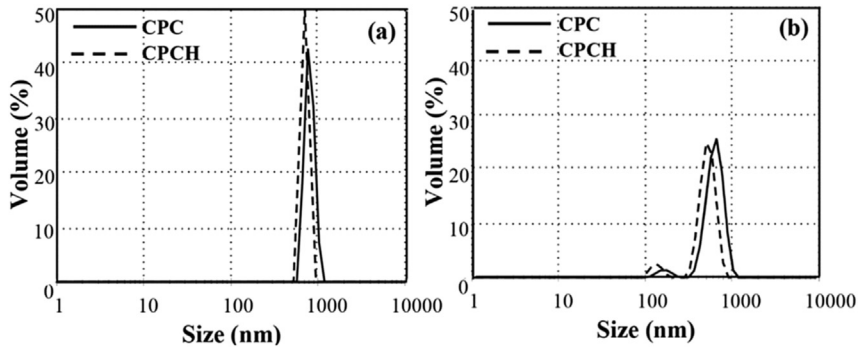

Fig. 6. Particle size distribution (PSD) by volume of the (a) virgin olive oil- and (b) sunflower oil-in-water emulsions prepared with aqueous dispersions of native (CPC) or heat treated (CPCH) coconut protein concentrate at pH 6.9. Data shown are representative of that seen from the average of three readings.

activity but reduced the emulsifying stability, particle size distribution ($D_{3,2}$) and flocculation. The protein secondary structures, zeta potential and Z-average diameter ($D_{4,3}$) of CPC were not significantly affected by heat treatment. CPC was a more effective emulsifier for the oil-in-water emulsions than CPCH because CPC produced a lower interfacial tension between the oil and water phases and a higher ESI than that CPCH. In addition, the virgin olive oil emulsions showed a higher stability than the sunflower oil emulsions. Overall, CPC has potential application as a nutritional supplement and to stabilize oil-in-water food emulsions that are fairly viscous. The use of limited enzymatic hydrolysis or the addition of hydrocolloids to enhance the emulsifying properties of the CPC may be worth evaluating.

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