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Bioactive coconut protein concentrate films incorporated with antioxidant extract of mature coconut water

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ABSTRACT

Coconut is a very important fruit worldwide. Coconut milk cake and mature coconut water (CW) are the by-products from coconut processing. Therefore, it is of interest to exploit coconut milk cake and CW as a source of protein and antioxidant, respectively. In particular, antioxidant compounds from CW were extracted using a solvent (acetone and diethyl ether) and the evaporation compared with coconut water concentrate (CWC) which was extracted using evaporation. CWC had a 4-times higher total phenolic content than that of CW. Even though the solvent extract had a higher total phenolic content than the evaporation-only extract, the antioxidant capacity of the solvent extract of CWC and the evaporation-only (CWC) was not significantly different. Moreover, using CW as a solvent also reduced the amount of glycerol needed to form a satisfactory coconut protein film. The use of CW as a solvent and the incorporation of CWC significantly increased the film solubility and water vapor permeability but improved the film mechanical properties. The total phenolic content and antioxidant capacity increased in protein films incorporated with CWC and using CW as a solvent. Fourier transform infrared spectroscopy showed interactions among the antioxidant compounds from CW, CWC and protein. The formation of a polyphenol-protein complex doubled the film elongation. Thus, the coconut protein film produced using CW as a direct solvent and incorporated with CWC can provide an environmentally friendly active packaging material for agricultural and food product applications.

1. Introduction

Coconut (*Cocos nucifera* L.) is an important fruit tree in the world, especially in tropical and subtropical regions (DebMandal & Mandal, 2011). Each year, approximately 1 million t of coconut is produced in Thailand in both immature and mature stages (Siriphanich et al., 2011; FAO, 2014). In mature fruit, the fresh kernel is usually used for coconut milk production, while the dry kernel (copra) is mainly used as a raw material for oil extraction. Coconut milk production involves manually or mechanically shredding the coconut kernel and squeezing it with or without the addition of water (de Leon & Delores, 2004). Coconut milk cake and coconut water are the major by-products from the coconut milk industry. Due to the problems of disposal, attention has been focused on the development of value-added products from these by-products. Although coconut milk cake contains only 4–7% protein content (Chambal, Bergenståhl, & Dejmek, 2013; Rodsamran & Sothornvit, 2018), the huge amount of coconut production makes this a potential source of protein. Recently, plant protein has been of interest not only for a wide range of applications in the food industry such as food supplements, as an emulsifier in salad dressing, soups, confectionery and frozen desserts and as a water and oil binding additive in meats, sausages and bakery (Espino-Sevilla et al., 2013; Wu, Wang, Ma, & Ren, 2009), but also as a useful material for forming biodegradable films. Environmentally friendly biopolymer-based films for packaging have been developed from polysaccharides, proteins and lipids or their combinations. Among them, protein-based films provide a better gas barrier and mechanical properties compared to lipid and polysaccharide films (Ou, Wang, Tang, Huang, & Jackson, 2005). The by-products from food industries have been investigated as possible plant protein sources

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of biomaterial packaging, including sunflower protein (Salgado, López-Caballero, Gómez-Guillén, Mauri, & Montero, 2012, 2013), peanut protein (Reddy, Jiang, & Yang, 2012), pumpkin oil cake protein (Popović, Peričin, Vaštag, Lazić, & Popović, 2012), hazelnut meal protein (Aydemir, Gökbulut, Baran, & Yemenicioğlu, 2014) and castor bean cake protein (Chambi et al., 2014). The inexpensive protein source of by-products such as coconut cake from the coconut milk industry makes it necessary to investigate adding value to the by-products and reducing the cost of biopolymer films simultaneously.

Nowadays, natural antimicrobials or antioxidant additives have been extracted from agricultural waste and underutilized by-product from the food industry. Phenolic compounds are a common antioxidant which has been incorporated to enhance the bioactive properties of biopolymer films. For example, antioxidant extracts from murta leaves (Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007) and borage seeds (Gómez-Estaca, Giménez, Gómez-Guillén, & Montero, 2009) were incorporated and improved the antioxidant properties of gelatin films. Furthermore, the total phenolic content and antioxidant activity of chitosan film increased with the incorporation of *Zataria multiflora* Boiss essential oil and grape seed extract (Moradi et al., 2012). Likewise, the main component of mature coconut water is approximately 2–3% sugar, 1% sorbitol, 0.0007–0.005% ascorbic acid and small quantities of several vitamins (Janick & Paull, 2008). In addition, mature coconut water possessed approximately 42.59 mg/L total phenolic content (Tan, Cheng, Bhat, Rusul, & Easa, 2014). Moreover, the coconut water extracted by the hydrodistillation process had higher scavenger activity than solvent extraction (da Fonseca et al., 2009). Thus, coconut water is of interest as a potential natural antioxidant food additive incorporated into biopolymer films.

From the available literature, there has been no report on the combination of coconut milk cake protein and coconut water to prepare biodegradable films. Therefore, the objectives of this work were: i) to evaluate the antioxidant properties of the extract from mature coconut water and ii) to assess the physicochemical, mechanical and antioxidant properties of protein film prepared from coconut milk cake protein, mature coconut water and the extract of mature coconut water.

2. Materials and methods

2.1. Materials

Coconut milk cake was obtained from a local market (Nonthaburi, Thailand), dried at 50 °C for 10 h in a hot air dryer (RedLINE RF 115, Deutschland, Germany) and ground using a hammer mill (Roter grinder, Retsch GmbH, Germany) with a 1 mm screen. The mature coconut water (CW) was donated by Theppadungporn Coconut Co., Ltd. (Nakhonpathom, Thailand). For film forming preparation, CW was used to fill a 200 mL glass bottle, sterilized at 121 °C for 5 min in an autoclave (HIRAYAMA HA-300 MIT, Tokyo, Japan) and stored at room temperature (RT, 28 ± 2 °C) for further use.

All chemical reagents were laboratory grade. Absolute ethanol (99.8% v/v) was purchased from Liquor Distillery Organization (Excise Department, Bangkok, Thailand). Folin-Ciocalteu reagent was purchased from Merck KGaA (Darmstadt, Germany). Glycerol and sodium carbonate (Na_2CO_3) was purchased from Ajax Finechem Pty Ltd (New South Wales, Australia). Gallic acid monohydrate (≥98.0%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%) and 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH•) were purchased from Sigma-Aldrich and 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 98%) from Alfa Aesar were supplied by U&V Holding (Thailand) CO., LTD (Nonthaburi, Thailand).

2.2. Antioxidant extraction

2.2.1. Coconut water concentration

CW (1 L) was concentrated using evaporation in a rotary evaporator (Rotavapor R-220, BÜCHI, Flawil, Switzerland) at 40 °C under vacuum for 2 h. Coconut water concentrate (CWC) and the vapor of coconut water after evaporation (CWE) were collected in plastic bottles and stored at −18 °C for further use.

2.2.2. Solvent extraction

The antioxidant substances from the three parts of coconut water (CW, CWC and CWE) were extracted using the solvent extraction technique according to the modified method of da Fonseca et al. (2009).

The concentrated CW (1 L) was extracted twice with 150 mL of solvent (acetone or diethyl ether), at RT for 10 min each time. The solvents were separated and the CW extracts were concentrated under vacuum for 2 h. The CW extracts with acetone (CWA) and diethyl ether (CWD) were stored in a plastic bottle at −18 °C for further analyses.

For the extraction of CWC and CWE, 100 mL of CWC or CWE was extracted twice with 50 mL of solvent (acetone or diethyl ether) at RT for 10 min. The solvents were separated under vacuum for 10 min and 5 min for the acetone and diethyl ether, respectively. The CWC and CWE extracts using acetone (CWCA and CWEA) and diethyl ether (CWCD and CWED) were collected in plastic bottles at −18 °C for further analyses.

2.3. Assessment of physicochemical properties of coconut water extract

The total soluble solids (SS) were expressed as % Brix using a hand refractometer (N-1α, ATAGO, Tokyo, Japan) at 25 °C. The pH was measured using a pH meter (PB-10, Sartorius AG, Goettingen, Germany). The total solid content (TS) was determined according to AOAC Official Method 925.45: Moisture in sugars (AOAC, 2000a).

The turbidity was measured using the method of Campos, Souza, Coelho, and Glória (1996) with a spectrophotometer (UV–Visible 1800, Shimadzu, Tokyo, Japan) at a wavelength of 610 nm. Absorbance (Abs) of the sample was read in relation to distilled water and turbidity was calculated using the following equation:

$$Turbidity = 100 - \left(100 \times 10^{-Abs}\right) \quad (1)$$

2.4. Determination of total phenolic content

The total phenolic content (TPC) was evaluated using the Folin-Ciocalteu method as described by Tan et al. (2014) with some modification. In brief, the sample (0.1 mL) was placed in a 10 mL volumetric flask followed by 7 mL of distilled water and 0.5 mL of 10% Folin-Ciocalteu reagent. After the sample was incubated at RT for 5 min, 1.5 mL of 7.5% (w/v) sodium carbonate was added and filled with distilled water to 10 mL. The mixture was incubated and protected from light at RT for 1 h. The absorbance was read at a wavelength of 765 nm. The TPC was expressed as gallic acid equivalents (GAE) using units of milligrams GAE per liter (mg GAE/L).

2.5. Evaluation of antioxidant capacity

2.5.1. DPPH method

The CW extract sample was dissolved in distilled water at various concentrations (% v/v) in a final volume of 2 mL prior to mixing with 2 mL of the ethanol solution of DPPH (200 μM). The absorbance (A_s) was recorded at a wavelength of 515 nm after the sample was incubated at RT for 1 h in a dark place. The absorbance of the control (A_0) was obtained from a control solution consisting of 2 mL of distilled water and 2 mL of DPPH solution. The antioxidant capacity was calculated using the following equation:

$$\text{Antioxidant capacity } (\%) = \frac{(A_0 - A_s)}{A_0} \times 100 \quad (2)$$

The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of antioxidant capacity against sample concentration.

2.5.2. ABTS method

The ABTS assay was measured using the modified method of Müller, Gnoyke, Popken, and Böhm (2010). Briefly, the mixture stock solution (1: 1, v/v) of ABTS (7.4 mM) and potassium persulfate (2.45 mM) were left to stand for 16 h at RT in a dark place to form the radical cation $ABTS^{\bullet+}$. An $ABTS^{\bullet+}$ working solution was prepared by dilution with distilled water to an absorbance of 0.70 ± 0.05 at 734 nm. One hundred mL of Trolox solution (0–750 μM/L) or sample was mixed with 3900 mL of $ABTS^{\bullet+}$ working solution. Absorbance was measured at 734 nm after 20 min. The antioxidant capacity of the sample was expressed as Trolox equivalents (TE) using units of micromoles TE per liter (μmol TE/L).

2.6. Protein concentrate preparation

Coconut protein concentrate was prepared using the method of Rodsamran and Sothornvit (2018). In brief, ground coconut milk cake was suspended with distilled water (1:12, w/w) and adjusted to pH 11 with 0.7 M sodium orthophosphate (Na_3PO_4). The mixture was stirred at 50 °C for 1 h in a water bath (Memmert WNB 7–45, Schwabach, Germany). After the suspension had been separated using cold centrifugation (Eppendorf centrifuge 5804 R, Hamburg, Germany) at 12,000×g (0 °C) for 30 min, the supernatant protein solution was collected and precipitated at pH 4 using 3 M hydrochloric acid (HCl) and stirred at RT for 30 min. The protein precipitate pellet was separated using centrifugation at 12,000×g (0 °C) for 10 min and washed with distilled water, then centrifuged again at 12,000×g (0 °C) for 10 min. The protein precipitate pellet was frozen at −50 °C for 48 h prior to lyophilization to obtain the protein precipitate powder (PPP). The PPP was defatted with hexane at RT for 3 h. After solvent evaporation, the defatted PPP was kept in a polyethylene zip lock bag and stored at RT for further use.

2.7. Coconut protein film formation

The defatted PPP (5 g) was dispersed in 100 mL of distilled water or CW and stirred at RT for 30 min. The pH of the protein solution was adjusted to 11 using 0.7 M Na_3PO_4 and then stirred for 30 min. The protein solution was homogenized using a high speed homogenizer (POLYTRON PT-MR 3100D, KINEMATICA AG, Luzern, Switzerland) at 19,000 rpm for 15 min. After adding glycerol (0–50% w/w of PPP) and the selected CW antioxidant extract (0–2 times of IC_{50}), the mixture was homogenized again at 19,000 rpm for 1 min and then centrifuged at 3500×g for 5 min to separate the insoluble particles. The air bubbles were removed under vacuum for 1 h. The film solutions were cast on a 10 × 10 cm silicone plate to obtain 1.2 ± 0.2 g of dried solid. Films were allowed to dry at 60 °C for 5 h in a hot air oven at low speed air velocity. The coconut film was peeled off from the plate and stored at 25 ± 3 °C and 50 ± 5% relative humidity (RH) for further analyses.

2.8. Characterization of films

2.8.1. Moisture content

The moisture content was determined according to AOAC methods (934.01 AOAC, 2000b). Film samples were weighed, dried at 105 °C for 24 h and then weighed again until they reached a constant weight.

2.8.2. Film solubility

Film specimens were cut into 2 × 2 cm and dried at 105 °C in a hot air oven for 24 h. After drying, the films were weighed to the nearest 0.01 mg to determine their initial dry weights. Films were individually placed into 20 mL of distilled water in glass tubes. The tubes were capped and placed in a shaking water bath at 25 ± 0.1 °C for 24 h. Film pieces were then taken out and dried at 105 °C in a hot air oven for 24 h to determine the final dry weights of films. Three replicates of each film were done. Solubility in water was calculated using the following equation:

$$\text{Solubility } (\%) = \frac{(\text{Initial dry weight} - \text{Final dry weight})}{\text{Initial dry weight}} \times 100 \quad (3)$$

2.8.3. Film opacity

The film opacity was measured according to the method described by Pereda, Amica, and Marcovich (2012) with some modification. In brief, three rectangular strips (1 × 4 cm) were directly placed in the test cell of a spectrophotometer. The film opacity was expressed as absorbance at 600 nm divided by the film thickness (A_{600}/mm).

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

The measurements of the ATR-FTIR spectra were recorded between 600 and 4000 cm^{-1} using a diamond ATR crystal with a Fourier transform infrared spectrometer (Perkin Elmer, Spectrum two, Illinois, USA). In total, 32 scans were carried out at 8 cm^{-1} resolution.

2.8.5. Water vapor permeability

The water vapor permeability (WVP) of the films was determined gravimetrically according to ASTM E96 (ASTM, 1995). Prior to the determination, three film samples without defects and pinholes were cut and stored at 25 ± 3 °C and 50 ± 5% RH for 48 h. The film samples were sealed to the cup base previously filled with 6 mL distilled water and then placed into a cabinet at 29 ± 1 °C and 50 ± 5% RH. Cups were then periodically weighed for 10 h. The WVP of the films was calculated by multiplying the water vapor transmission rate by the film thickness and dividing by the water vapor partial pressure difference across the films.

2.8.6. Mechanical properties

The coconut protein film's tensile strength (TS), elastic modulus (EM), and elongation at break (E) were determined using a Universal Testing Machine (5569, Instron, Norwood, MA, USA) with a 50 N load cell. The test was performed at 23 ± 2 °C and 50 ± 5% RH according to ASTM D882–97 (ASTM, 1997). Five dumbbell-shaped specimens (8 mm wide × 50 mm long) for each treatment were

cut and preconditioned at 25 ± 2 °C and 50 ± 5% RH for 48 h prior to the determination.

2.8.7. Antioxidant property of coconut protein film

To prepare the film extract solution, the film sample (200 mg) was dispersed in distilled water (20 mL), homogenized at 5000 rpm for 30 s, and then centrifuged at 12,000×*g* for 10 min. The clear solution was collected and used for antioxidant property determination according to the methods described in Sections 2.4 and 2.5. The TPC was expressed as gallic acid equivalents (GAE) using units of milligrams GAE per gram (mg GAE/g). The antioxidant capacity (DPPH assay and ABTS assay) of the sample were expressed as Trolox equivalents (TE) using units of micromoles TE per gram (μmol TE/g).

2.9. Statistical analyses

A completely randomized experimental design was used with analysis of variance performed to evaluate the effect of extraction technique on the antioxidant properties and the effect of solvent types, glycerol concentrations and the incorporation of coconut water extract on the film properties. Duncan's multiple range test was applied to determine the difference between mean values of the film properties. Statistical analysis was performed using the SPSS software package (Version 11.5, SPSS Inc., Chicago, IL) at a significance level of 0.05.

3. Results and discussion

3.1. Appearance of mature coconut water antioxidant extract

The mature coconut water (CW) was a slightly cloudy white color (Fig. 1). The change of color in the CW was due to the solvent extraction and the evaporation effect (CWA and CWD). Similarly, the coconut water concentrate (CWC) had a tinge of yellow and was cloudier than the CW. In addition, the extracts with acetone (CWA and CWCA) were yellower than the extracts with diethyl ether (CWD and CWCD). Increased yellowness in the extract might be explained by non-enzymatic browning reactions, especially the Maillard reaction (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005; Chen, Yang, Chen, & Liu, 2009). This might have been due to the heat treatment during evaporation (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001).

Moreover, sugars are reactants in both the Maillard reaction and caramelization (Ajandouz & Puigserver, 1999). It is known that coconut water consists of sugars, which are the main component of soluble solids, such as sucrose, sorbitol, glucose and fructose (Del Rosario, Bergonia, Flavier, Samonte, & Mendoza, 1984; Ogundiya, 1991) and some amino acids, such as alanine, arginine, cysteine and serine (Shivashankar, 1991; Sierra & Velasco, 1976), resulting in the occurrence of a Maillard reaction and caramelization enhancing the development of the brown color in the CWA, CWD, CWC, CWCA and CWCD. However, the vapor of coconut water after evaporation (CWE) and the extract of both solvents (CWEA and CWED) were colorless solutions containing a large amount of water.

3.2. Physicochemical properties of antioxidant extract from coconut water

The physicochemical properties of the antioxidant extract from CW are shown in Table 1. The pH of the samples varied slightly with the solvent extraction. Overall, the pH of the CW extracts (4.52–5.09) decreased slightly from that of the CW (5.19). Generally, the pH of the coconut water was in the range 4–6, depending on the stage of maturity and the variety of coconut (Prades, Dornier, Diop, & Pain, 2012). This meant that the extraction process condition did not affect the pH change of CW.

The level of total soluble solids (SS) relates to the sweetness of the coconut water and corresponds to the total solid content (TS), which is the remaining dry matter after the removal of all water from the sample. As expected, the overall SS and TS increased after solvent extraction and the evaporation effect. Basically, water is removed during the evaporation process resulting in an increased solid content (Wilhelm, Suter, & Brusewitz, 2004). In the current study, the extract was evaporated again after the solvent extraction was completed resulting in a more concentrated solution. In addition, the extract with acetone (CWA and CWCA) had higher levels of SS and TS than the diethyl ether (CWD and CWCD) due to the longer time required to evaporate the solvent. Unexpectedly, less SS and TS were found in the CWE, CWEA and CWED. This might be reflected in the appearance of the samples as shown in Fig. 1. This confirmed that the evaporation of solvent was sufficient. Similarly, the solvent extracts from CW and CWC were more turbid due to the increase in the levels of SS and TS of the extract (Jackson, Gordon, Wizzard, McCook, & Rolle, 2004). The lowest turbidity was represented in the transmittance of the clear solution in the CWE, CWEA and CWED samples (Fig. 1).

3.3. Antioxidant properties of extract from coconut water

As expected, there was a significant difference among the levels of TPC in all extracts from CW (Table 2). Both solvent extracts had increased TPC levels, compared with the CW or CWC. However, there was no significant difference between both solvents. The TPC of CWC was 4-times higher than that of CW. Normally, the TPC of coconut water varies with the maturity and variety of the coconut. For example, the TPC was reported to be 42.59 mg GAE/L in 'Malayan Tall' mature coconut water (Tan et al., 2014) and ranged from 58.0 to 66.8 mg GAE/L in 'Nam Hom' mature coconut water (Mahayothee et al., 2016). Clearly, there was no TPC in the CWE,

Fig. 1. Appearance of coconut water (CW), coconut water concentrate (CWC), vapor of coconut water after evaporation (CWE) and coconut water extracted using acetone (CWA, CWCA, CWEA) and diethyl ether (CWD, CWCD, CWED).

Table 1
Total soluble solid (SS), pH, turbidity and total solid content (TS) of coconut water (CW), coconut water concentrate (CWC), vapor of coconut water after evaporation (CWE) and coconut water extracted using acetone (CWA, CWCA, CWEA) and by diethyl ether (CWD, CWCD, CWED).

Sample	Solvent	pH	SS (% Brix)	TS (%)	Turbidity
CW	–	$5.19^{a} \pm 0.01$	$4.50^{f} \pm 0.12$	$2.09^{f} \pm 0.07$	$39.53^{d} \pm 1.04$
CWA	Acetone	$4.95^{b} \pm 0.03$	$17.15^{d} \pm 0.75$	$10.18^{d} \pm 0.54$	$94.37^{a} \pm 1.03$
CWD	Diethyl ether	$4.96^{b} \pm 0.04$	$15.40^{e} \pm 1.28$	$8.70^{e} \pm 0.55$	$91.33^{b} \pm 1.18$
CWC	–	$4.86^{b} \pm 0.02$	$20.65^{c} \pm 1.33$	$12.44^{c} \pm 0.89$	$86.36^{c} \pm 0.35$
CWCA	Acetone	$4.93^{b} \pm 0.04$	$30.25^{a} \pm 1.68$	$17.86^{a} \pm 1.01$	$95.17^{a} \pm 0.04$
CWCD	Diethyl ether	$4.93^{b} \pm 0.01$	$26.00^{b} \pm 1.51$	$15.51^{b} \pm 1.20$	$91.43^{b} \pm 0.09$
CWE	–	$4.52^{c} \pm 0.08$	$0.50^{g} \pm 0.12$	$0.00^{g} \pm 0.00$	$0.86^{e} \pm 0.43$
CWEA	Acetone	$5.09^{a} \pm 0.11$	$0.80^{g} \pm 0.00$	$0.00^{g} \pm 0.00$	$0.58^{e} \pm 0.40$
CWED	Diethyl ether	$4.91^{b} \pm 0.22$	$0.40^{g} \pm 0.00$	$0.00^{g} \pm 0.00$	$0.00^{e} \pm 0.00$

Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significantly different values ($p < .05$).

Table 2
Total phenolic content (TPC) and antioxidant capacity (IC_{50} and ABTS assay) of coconut water (CW), coconut water concentrate (CWC), vapor of coconut water after evaporation (CWE) and coconut water extracted using acetone (CWA, CWCA, CWEA) and by diethyl ether (CWD, CWCD, CWED).

Sample	Solvent	TPC (mg GAE/L)	Antioxidant capacity	
			IC_{50} (%v/v)	ABTS (μmol TE/L)
CW	–	$57.56^{d} \pm 0.00$	$77.22^{b} \pm 1.86$	$233.48^{c} \pm 1.64$
CWA	Acetone	$184.83^{c} \pm 38.64$	$26.08^{c} \pm 0.78$	$385.15^{b} \pm 20.84$
CWD	Diethyl ether	$184.83^{c} \pm 23.70$	$26.97^{c} \pm 1.30$	$399.67^{b} \pm 18.16$
CWC	–	$236.68^{b} \pm 24.15$	$14.03^{e} \pm 0.24$	$560.50^{a} \pm 11.01$
CWCA	Acetone	$324.84^{a} \pm 25.02$	$14.34^{e} \pm 0.98$	$547.45^{a} \pm 15.78$
CWCD	Diethyl ether	$292.55^{a} \pm 21.51$	$15.90^{d} \pm 1.01$	$531.24^{a} \pm 33.93$
CWE	–	$0.00^{e} \pm 0.00$	$>100^{a}$	$0.00^{d} \pm 0.00$
CWEA	Acetone	$0.00^{e} \pm 0.00$	$>100^{a}$	$0.00^{d} \pm 0.00$
CWED	Diethyl ether	$0.00^{e} \pm 0.00$	$>100^{a}$	$0.00^{d} \pm 0.00$

Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significantly different values ($p < .05$).

CWEA and CWED samples. This indicated that the antioxidant substances were extracted effectively using the solvent extraction technique. To the best of our knowledge, there has been no reported study on the TPC of the solvent extracts from CW and CWC. Therefore, the information obtained on the physicochemical and antioxidant properties of the extracts from CW and CWC will open up the opportunity for these to be applied as a source of antioxidant compounds for food applications.

Generally, the DPPH assay is an easy and accurate method and is commonly used to express the antioxidant capacity at the 50% inhibition concentration of DPPH (IC_{50}), where a low IC_{50} value is associated with a stronger DPPH scavenging capacity resulting in a high antioxidant capacity (Samuagam, Sia, Akowuah, Okechukwu, & Yim, 2013). The ABTS assay is a method used to test the reaction between the radical and electron/hydrogen donors to form colorless ABTS. A decrease in the $ABTS^{\bullet+}$ concentration is linearly dependent on the extract concentration, including Trolox as a calibrating standard (Berg van den, Haenen, Berg van den, & Bast, 1999; Re et al., 1999).

The DPPH and ABTS assays produced similar results for the antioxidant capacity in all antioxidant extracts from CW and CWC (Table 2). The solvent extractions of CW (CWA and CWD) had higher antioxidant capacities (lower IC_{50} and higher ABTS) than the CW extract. In contrast, differences in the antioxidant capacity were not found among all concentrates of CW (CWC, CWCA and CWCD). In addition, all concentrates of CW had higher antioxidant capacities than did CW and all the solvent extracts (CWA and CWD). Similar results were reported by da Fonseca et al. (2009) who showed that the coconut water extract obtained from hydrodistillation and solvent extraction had higher scavenger activity than the extract obtained using only solvent extraction. Unexpectedly, no antioxidant capacity was observed in CWE, CWEA and CWED. Similarly, CWE and the solvent extract of CWE did not show any antioxidant activity due to having no TS resulting in no TPC being detected. Interestingly, the evaporation of water from CW was sufficient to obtain effective antioxidant substances in CWC. It is safe to extract the antioxidant without the use of an organic solvent.

Based on the highest antioxidant capacity and acceptable TPC, CWC was selected to be incorporated into the coconut protein film. Amounts of CWC in the range 0–2 times IC_{50} were selected for incorporation in the film-forming process and the determination of the film properties including the antioxidant capacity.

3.4. Coconut protein film properties

3.4.1. Coconut protein film formation

The influence of the type of solvent (water or CW), the glycerol concentration (0–50%) and the amount of CWC (0–2 times IC_{50}, 0–28% v/v) are shown in Fig. 2. The results showed that coconut protein films with CWC added at 2 times IC_{50} (28% v/v) stayed wet and sticky and did not peel off the silicone plate in either solvent and at all glycerol concentrations. Without glycerol (0%), the films were brittle and cracked during peeling off the plate. However, using 50% glycerol concentration made the films sticky and impossible to handle. Thus, only 9 film formations were selected to determine the film properties due to the practical consideration of handling them regarding not being sticky but remaining flexible.

The films were generally dark brown in color. Moreover, using CW as a solvent and incorporated with CWC resulted in a lighter brown film. As expected, using CW and the incorporation of CWC reduced the amount of glycerol used to form the coconut protein film. It was hypothesized that using CW as a solvent for film formation might reduce the use of a plasticizer such as glycerol due to some components in the CW (for example, the sugar content) acting as the plasticizer and facilitating flexibility in the protein film structure and thus requiring less added glycerol (Sothornvit & Krochta, 2005).

3.4.2. Moisture content and film solubility

The MC and solubility in water of the coconut protein film using water as a solvent for film formation increased with increasing glycerol content (Table 3). In contrast, the amount of glycerol did not induce any significant changes in the MC and film solubility of coconut protein film using CW as a solvent. However, the addition of CWC also increased the film MC and solubility with both solvents at similar glycerol contents. These results were probably due to the

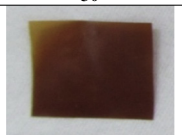
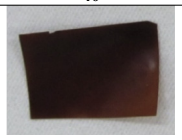
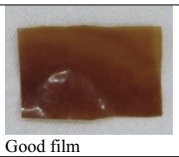
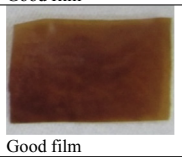
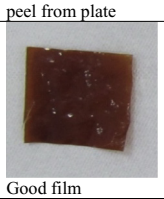
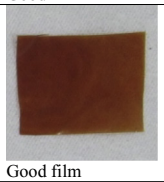
Solvent	Coconut water extract (%v/v)	Glycerol (%)					
		0	10	20	30	40	50
Water	0	Brittle and cracked during peeling from plate	Brittle and cracked during peeling from plate	Brittle	 Good film	 Good film	Sticky during storage
	14	Brittle and cracked during peeling from plate	Brittle and cracked during peeling from plate	 Good film	 Good film	Sticky and unable to peel from plate	Sticky and unable to peel from plate
	28	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate
Coconut water	0	Brittle and cracked during peeling from plate	 Good film	 Good film	 Good film	Sticky during storage	Sticky and unable to peel from plate
	14	Brittle and cracked during peeling from plate	 Good film	 Good film	Sticky during storage	Sticky and unable to peel from plate	Sticky and unable to peel from plate
	24	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate	Sticky and unable to peel from plate

Fig. 2. Photos of coconut protein films.

Table 3
Moisture content (MC), solubility in water, opacity and water vapor permeability (WVP) of coconut protein films made using different solvents and incorporating with coconut water extract.

Sample	Solvent	Glycerol (%)	MC (% wb)	Solubility (%)	Opacity (A_{600}/mm)	WVP ($\times 10^{-10}$ g m^{-1}·s^{-1}·Pa^{-1})
0% Coconut water extract (v/v)						
W30	Water	30	$20.66^{de} \pm 0.65$	$43.38^{g} \pm 2.45$	$9.72^{a} \pm 0.41$	$4.58^{c} \pm 0.50$
W40	Water	40	$25.24^{a} \pm 1.10$	$47.66^{fg} \pm 2.52$	$9.63^{a} \pm 0.51$	$5.81^{b} \pm 0.06$
CW10	Coconut water	10	$21.28^{cd} \pm 1.59$	$59.99^{cd} \pm 0.92$	$8.29^{b} \pm 0.29$	$7.58^{a} \pm 0.48$
CW20	Coconut water	20	$21.68^{cd} \pm 1.59$	$61.25^{bc} \pm 3.99$	$7.28^{c} \pm 0.58$	$7.57^{a} \pm 0.16$
CW30	Coconut water	30	$21.40^{cd} \pm 0.57$	$59.16^{cd} \pm 3.93$	$5.32^{d} \pm 0.03$	$7.05^{a} \pm 0.06$
14% Coconut water extract (v/v)						
W20C	Water	20	$19.55^{e} \pm 0.36$	$51.47^{ef} \pm 3.80$	$4.93^{d} \pm 0.03$	$7.09^{a} \pm 0.82$
W30C	Water	30	$22.74^{bc} \pm 0.47$	$55.26^{de} \pm 1.56$	$6.73^{c} \pm 0.46$	$6.48^{ab} \pm 0.75$
CW10C	Coconut water	10	$21.91^{cd} \pm 0.72$	$65.08^{ab} \pm 1.54$	$2.86^{e} \pm 0.05$	$6.38^{ab} \pm 0.13$
CW20C	Coconut water	20	$23.82^{ab} \pm 0.42$	$69.58^{a} \pm 2.01$	$3.61^{e} \pm 0.60$	$6.43^{ab} \pm 0.69$

Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significantly different values ($p < .05$).

hygroscopic properties of glycerol and the sugar content in CWC, which is able to effectively retain water molecules in the film matrix (Vieira, Silva, Santos, & Beppu, 2011). Moreover, the sugar content of CWC might be weakly linked to the protein network resulting in easier dissolution in water. Arabestani, Kadivar, Shahedi, Goli, and Porta (2016) reported a similar result in a bitter vetch protein-based film incorporated with pomegranate juice.

3.4.3. Film opacity

Using CW as a solvent and incorporating with CWC decreased the film opacity ($p < .05$, Table 3). This result correlated well with the images of films shown in Fig. 2. CW10C and CW20C were brighter and more transparent than W30 and W40. A higher opacity value indicates a good light barrier. In this study, all coconut protein films had negligible transmission of light in the range 200–400 nm (data not shown). Therefore, films produced with coconut protein may be used in UV barrier packaging due to the high content of aromatic amino acids in the structure of such protein-based films (Pérez, Piccirilli, Delorenzi, & Verdini, 2016; Ramos et al., 2013).

3.4.4. Fourier transform infrared (FTIR) spectra of the films

The Fourier transform infrared (FTIR) spectra of the coconut protein films (Fig. 3) were used to study the existence of interactions between the proteins and polyphenol compounds in CW and CWC. The broad band ranging between 3000 cm^{-1} and 3600 cm^{-1} corresponded to the free and bound −OH and −NH groups. Compared with the coconut protein films using water as a solvent and without adding CWC (W30 and W40), the clear

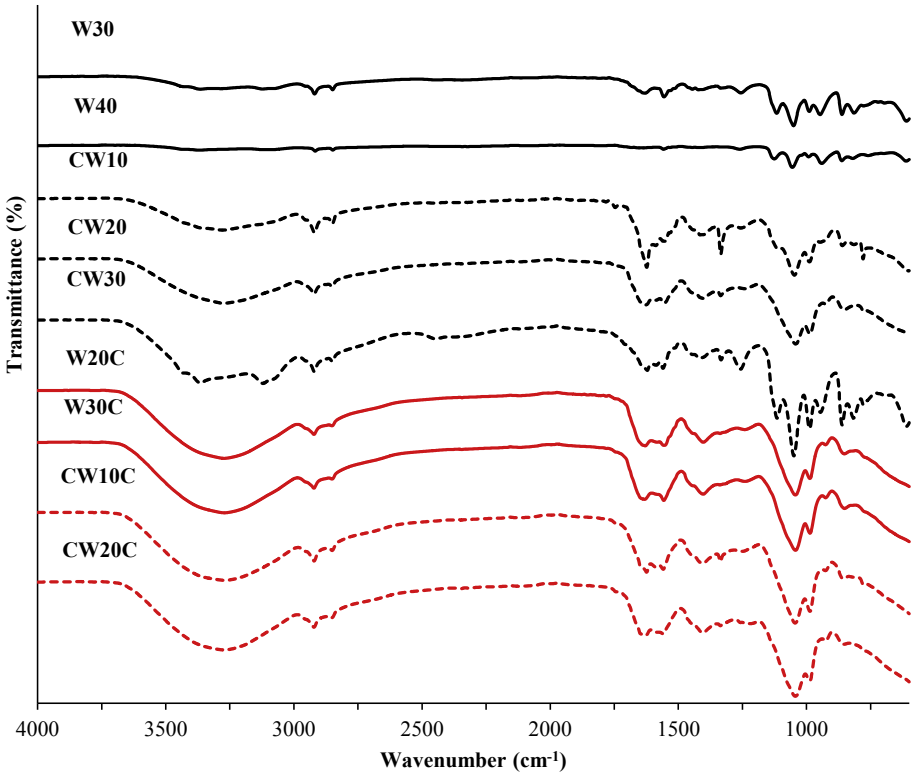


Fig. 3. Fourier transform infrared (FTIR) spectra of coconut protein films using water as a solvent incorporated without coconut water extract (W30 and W40) and with coconut water extract (W20C and W30C) and of coconut protein films using coconut water as a solvent incorporated without coconut water extract (CW10, CW20 and CW30) and with coconut water extract (CW10C and CW20C).

increase in the intensity of those peaks was associated with the free −OH groups of the polar groups of polyphenols from the CW and CWC (Ciannamea, Stefani, & Ruseckaite, 2016). The peaks at wavenumbers 2940 cm^{-1} and 2875 cm^{-1} were attributed to the −OH stretching in carboxylic acid (Solomons & Fryhle, 2011).

All coconut protein films changed the amide I and II bands to higher wavenumbers compared with coconut protein (data not shown). This might have been due to the rearrangement of the protein structure caused by the incorporation of CW and CWC. This was in agreement with Ciannamea et al. (2016), who reported that the incorporation of red grape extract in soy protein concentrate film increased the α-helix structure, corresponding to the amide I band changing to a higher wavenumber from 1620 cm^{-1} to 1651 cm^{-1}.

A new peak at wavenumber 1358 cm^{-1} was found in the coconut protein films using CW as a solvent with and without adding CWC and using water as a solvent and adding CWC (small peak). This band was attributed to the carbonyl group in polysaccharides and sugar (Kato, 2002; Li et al., 2015). The bands relating to glycerol were observed at wavenumbers between 830 cm^{-1} and 1060 cm^{-1} in all coconut protein films. However, the intensity of those peaks induced a slight increase in coconut protein films containing CW and CWC. This observation could be explained by the formation of the hydrogen bonds of -OH groups from CW and CWC with the C=O amide groups of proteins, resulting in a reduced interaction between the −NH or −SH and C=O groups of proteins; thus the flexibility and chain mobility within the film matrix increased (Ciannamea et al., 2016). This result confirmed that some compounds of CW and CWC might act as plasticizers in the films, resulting in increased mechanical properties of films, especially elongation (Table 4).

3.4.5. Water vapor permeability

As expected, the WVP of the coconut protein films using water as a solvent increased with the glycerol content ($p < .05$, Table 3). This might have been due to the increase in the free volume caused by modification of the molecular organization of the protein network resulting in a less dense network that was more permeable to the water in the films (Ciannamea, Stefani, & Ruseckaite, 2015).

The higher WVP of coconut protein films using CW as a solvent, compared to those using water as a solvent, could be explained by the high sugar content in the CW. Furthermore, the addition of CWC in coconut protein films using water as a solvent also increased their WVP values. The reduction of hydrogen bonds caused by the interaction between several hydroxyl groups of phenolic compounds contained in the CWC and carbonyl groups of coconut protein might increase the free volume of the film matrix and reduce the intermolecular-chains forces in the protein network resulting in higher film water vapor permeability (Damodaran, 1996). The WVP values of these films were the same order of magnitude as bitter vetch protein-based films incorporated with pomegranate juice (Arabestani et al., 2016) but higher than the alginate films incorporated with ginseng extracts (Norajit, Kim, & Ryu, 2010).

However, the incorporation of CWC in coconut protein films obtained using CW as a solvent slightly reduced the WVP values, although the differences were not significant.

3.4.6. Mechanical properties

The tensile strength (TS), elastic modulus (EM) and the elongation of the coconut protein films are shown in Table 4. An increase in the glycerol content reduced significantly the TS and EM values but increased the elongation in the coconut protein films

Table 4
Mechanical properties of coconut protein films using different solvents and incorporating with coconut water extract.

Sample	Solvent	Glycerol (%)	TS (MPa)	EM (MPa)	Elongation (%)
0% Coconut water extract (v/v)					
W30	Water	30	$1.01^{cd} \pm 0.33$	$4.21^{cd} \pm 1.10$	$40.13^{d} \pm 5.36$
W40	Water	40	$0.82^{d} \pm 0.17$	$4.38^{c} \pm 0.68$	$64.99^{b} \pm 1.54$
CW10	Coconut water	10	$1.89^{a} \pm 0.42$	$7.66^{a} \pm 0.89$	$27.90^{e} \pm 3.25$
CW20	Coconut water	20	$1.69^{ab} \pm 0.16$	$6.47^{b} \pm 1.01$	$37.54^{d} \pm 5.13$
CW30	Coconut water	30	$0.92^{d} \pm 0.29$	$4.83^{c} \pm 0.74$	$60.94^{bc} \pm 5.43$
14% Coconut water extract (v/v)					
W20C	Water	20	$1.66^{ab} \pm 0.09$	$4.72^{c} \pm 0.31$	$64.33^{b} \pm 3.93$
W30C	Water	30	$1.34^{bc} \pm 0.21$	$4.43^{c} \pm 0.40$	$67.07^{b} \pm 4.20$
CW10C	Coconut water	10	$1.20^{cd} \pm 0.16$	$4.20^{cd} \pm 0.33$	$56.84^{c} \pm 4.73$
CW20C	Coconut water	20	$1.05^{cd} \pm 0.23$	$3.08^{d} \pm 0.27$	$75.67^{a} \pm 4.80$

Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significantly different values ($p < .05$).
TS, tensile strength; EM, elastic modulus.

with both solvents ($p < .5$). In fact, plasticizer molecules reduced the hydrogen bonding between the protein chains resulting in increased film flexibility and spacing between molecules (Liu, Tellez-Garay, & Castell-Perez, 2004; Popović et al., 2012).

The incorporation of CWC significantly increased the elongation of both solvent coconut protein films, compared with the same glycerol content. This result could be explained by the increase in the free volume inside the film structure caused by the binding of the phenolic compounds in the CWC and coconut protein matrix. Similar results were reported from the addition of propolis extract into gelatin films (Bodini, Sobral, Favaro-Trindade, & Carvalho, 2013), the incorporation of red grape extract into soy protein concentrate films (Ciannamea et al., 2016) and the addition of pomegranate juice into bitter vetch protein-based films (Arabestani et al., 2016). Therefore, a higher glycerol content incorporated with CWC resulted in the highest elongation values in the coconut protein film using CW as a solvent (CW20C).

3.4.7. Antioxidant properties of coconut protein film

As expected, using CW as a solvent or the incorporation of CWC clearly increased the total phenolic content of the coconut protein films (Table 5). Moreover, the coconut protein films obtained using a combination of CW and CWC (CW10C and CW20C films) significantly had the highest total phenolic content because of the presence of phenolic compounds in CW and CWC as seen in Table 2. Generally, the amount of phenolic compounds also influenced the antioxidant capacity. The higher total phenolic content in the CW10C and CW20C films resulted in them having the highest antioxidant capacity in both the ABTS and DPPH assays (Table 5). Similar results were observed using pomegranate juice to improve the antioxidant capacity of bitter vetch protein-based films (Arabestani et al., 2016).

Recently, bioactive films incorporated with fruit extracts have received attention as a means of reducing the use of synthetic films in food applications. Using CW as a solvent and incorporating with CWC to form an edible film not only reduced the amount of glycerol but also increased the elongation and antioxidant properties of those films. Therefore, coconut protein film produced from the combination of CW and CWC could be beneficial for the reduction of plastic waste and to extend the shelf-life of food products.

4. Conclusion

By-products from coconut processing (coconut milk cake and mature coconut water) showed potential as an alternative renewable source from which to extract protein and antioxidant compounds. Evaporation was a better method of extracting the antioxidant from mature coconut water. Coconut water concentrate (CWC) had the highest antioxidant capacity and an acceptable total phenolic content and was selected for incorporation in the coconut protein film. Mature coconut water can be used as a solvent to produce coconut protein film to reduce the amount of glycerol used. Moreover, the incorporation of CWC was an effective antioxidant compound that improved the mechanical and antioxidant properties of the coconut protein film. Fourier transform infrared (FTIR) spectra confirmed the interactions between the proteins and polyphenol compounds from the CW and CWC. Interestingly, the films had a dark brown color; thus, they may be used in UV barrier packaging for food products.

Table 5
Antioxidant properties of coconut protein films using different solvents and incorporating with coconut water extract.

Sample	Solvent	Glycerol (%)	Total phenolic content (mg GAE/g)	ABTS (mM Trolox/g)	DPPH (mM Trolox/g)
0% Coconut water extract (v/v)					
W30	Water	30	$163.08^{c} \pm 10.88$	$308.79^{d} \pm 33.73$	$5.70^{d} \pm 1.12$
W40	Water	40	$171.46^{c} \pm 27.45$	$398.69^{c} \pm 10.56$	$5.00^{d} \pm 0.50$
CW10	Coconut water	10	$308.06^{b} \pm 71.91$	$472.42^{b} \pm 19.02$	$10.74^{c} \pm 1.15$
CW20	Coconut water	20	$316.62^{b} \pm 53.65$	$475.38^{b} \pm 21.22$	$11.44^{c} \pm 0.50$
CW30	Coconut water	30	$293.79^{b} \pm 27.19$	$475.33^{b} \pm 33.00$	$9.75^{c} \pm 1.10$
14% Coconut water extract (v/v)					
W20C	Water	20	$334.32^{b} \pm 22.68$	$487.42^{b} \pm 24.49$	$11.89^{c} \pm 0.65$
W30C	Water	30	$297.81^{b} \pm 21.21$	$501.99^{b} \pm 25.50$	$12.07^{c} \pm 0.37$
CW10C	Coconut water	10	$403.58^{a} \pm 51.12$	$606.71^{a} \pm 16.21$	$30.18^{b} \pm 2.94$
CW20C	Coconut water	20	$395.70^{a} \pm 34.33$	$616.44^{a} \pm 14.71$	$36.53^{a} \pm 3.61$

Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significantly different values ($p < .05$).

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