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Characteristics of albumin and globulin from coconut meat and their role in emulsion stability without and with proteolysis

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ABSTRACT

Albumin and globulin were fractionated from defatted coconut meat. Characteristics and emulsifying properties of these protein fractions were comparatively studied. Both fractions had protein with MW of 55 kDa as predominant and glutamine/glutamic acid were the major amino acids. However, differences in the protein pattern and amino acid composition were observed between two fractions. Higher average hydrophobicity was found in globulin fraction, compared with albumin. Additionally, globulin fraction was more hydrolyzed by Alcalase, in comparison with albumin. Coconut milk oil-in-water model emulsion was prepared using albumin and globulin protein fractions and stability of these emulsions was evaluated. Oil droplets with larger size caused by coalescence along with higher polydispersity were observed in albumin stabilized emulsion after 24 h of storage time. Conversely, globulin stabilized emulsion showed smaller oil droplet with low coalescence index and flocculation factor. Thus, emulsion stabilized by globulin fraction was more stable than that containing albumin fraction. However, the higher oil recovery was found in the globulin stabilized emulsion when treated with 1% Alcalase for 90 min, compared with albumin counterpart. This was caused by the higher susceptibility towards hydrolysis of globulin fraction. Therefore, globulin fraction mainly determined coconut milk stability and must be hydrolyzed by protease to release oil for virgin coconut oil production.

1. Introduction

Coconuts (*Cocos nucifera* L.) are economically important and extensively used in many traditional foods of Asian and Pacific regions (DebMandal & Mandal, 2011). Coconut milk, which is a milky white oil-in-water emulsion, is generally obtained from grated coconut meat (Tansakul & Chaisawang, 2006). Apart from using as food ingredient, coconut milk has been used for virgin coconut oil (VCO) production. Coconut oil is different from other vegetable oils due to high content of medium chain fatty acids, mainly lauric acid (Dayrit, 2014). VCO is rapidly gaining an immense importance due to various health benefits and high stability (Carandang, 2008). Apart from oil, coconuts also provide a potential source of proteins with good nutritive value and relatively well balanced profile. Globulin and albumin, which are soluble in NaCl and water, respectively, were major proteins in coconut meat (Kwon, Park, & Rhee, 1996). Peamprasart and Chiewchan (2006) reported that some proteins present in the aqueous phase of the coconut milk emulsion could act as emulsifier to stabilize fat globules. Basically, coconut milk emulsion can be disrupted via enzymatic treatment, heating and freeze–thawing, etc. (Gunetileke & Laurentius, 1974). Nevertheless, no information on the role of particular proteins in stabilization of coconut milk exists. Also, the collapse of coconut milk model emulsion stabilized by particular proteins caused by enzymatic treatment via protein hydrolysis, has not been studied. The information on coconut proteins and their role in emulsion stabilization could provide the better understanding on enhancing or destabilizing the emulsion of coconut milk. Thus, coconut milk emulsion can be stabilized or collapsed to obtain the desired products. The objectives of this study were to comparatively investigate the impact of albumin and globulin from coconut meat on characteristics of oil-in-water emulsion and to study the effect of proteolysis on the instability of coconut milk model emulsion, in which coconut oil could be released.

2. Materials and methods

2.1. Chemicals

2,4,6-Trinitrobenzenesulphonic acid (TNBS), sodium azide, boric

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acid and Nile blue A were purchased from Sigma (St. Louis. MO, USA). Sodium dodecyl sulfate, sodium chloride and isooctane were obtained from Merck (Darmstadt, Germany). Methanol, ethanol, acetic acid, propanol, hydrochloric acid, sulfuric acid and *n*-hexane were procured from Lab-Scan (Bangkok, Thailand). Chemicals for electrophoresis were obtained from Biorad (Richmond, VA, USA). Protein molecular weight marker was procured from GE healthcare (Buckinghamshire, UK). Alcalase (2.4L FG) with the activity of 2.4 AU/g was obtained from novozymes (Bagsvaerd, Denmark).

2.2. Preparation of defatted coconut meat

Coconuts (matured stage) were purchased from a plantation site in Yaring district, Pattani province, Thailand and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla. Coconuts were subjected to deshelling, paring and removal of water. Coconut kernel was collected manually and grated using a rotary wedge cutting machine. Grated meat was frozen in liquid nitrogen and then ground into a fine power with a grinder. The powder was sieved through a siever (0.4 mm mesh). The powder defatted with hexane, using a matter/solvent ratio of 1:10 (w/v) for 1 h. The defatting was performed twice. Defatted coconut meat was freeze-dried using a SCANVAC Cool Safe™ freeze-dryer (Cool-Safe 55, Scan Laf A/S, Lynge, Denmark). Dried powder had protein content of 13% (dry weight basis), as determined by the Kjeldahl method (AOAC, 2000).

2.3. Fractionation of proteins

The coconut proteins were sequentially fractionated from defatted coconut meat using five different solvents, including deionized water, 0.5 M NaCl, 70% 2-propanol, 50% glacial acetic acid, and 0.1 M NaOH following the method of Kwon et al. (1996). Sample was mixed with solvent, using a powder to solvent ratio of 1:10 (w/v) and the mixture was stirred at 4 °C for 14–16 h. Insoluble residue was removed by centrifugation at 20,000×*g* for 30 min using a centrifuge (Beckman Coulter, Allegra™ centrifuge, Brea, CA, USA). Extraction with each solvent was repeated three times, and all supernatants for each solvent were pooled together. Each fraction was then dialyzed against 20 volume of deionized water. Dialysates were then freeze-dried, and the resulting protein fractions were placed in a zip-lock bag and stored at −20 °C. Water, NaCl, IPA, acetic acid, and NaOH soluble fractions were designated as albumin, globulin, prolamin, glutelin-1, and glutelin-2 fractions, respectively. Protein content of each fraction was determined by the Kjeldahl method using the conversion factor of 6.25. Protein of each fraction was calculated and expressed as the percentage of total proteins in the defatted coconut powder.

2.4. SDS- polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of albumin and globulin fractions were determined by SDS-PAGE according to the method of Laemmli (1970) using 4% stacking gel and 12% separating gel. Samples (0.5 g) were dissolved in 10 mL of 5% SDS and heated at 95 °C for 1 h, followed by centrifugation at 7000×*g* for 10 min at 25 °C using a centrifuge (Beckman Coulter, Allegra™ centrifuge, CA, USA). The protein concentration of the supernatant was determined by the Biuret method (Robinson & Hogden, 1940), using bovine serum albumin (BSA) as a standard. Samples were mixed with the sample buffer containing 2% SDS, 10% glycerol and 0.05% bromophenol blue in 0.5 M Tris-HCl, pH 6.8. β-mercaptoethanol was added to sample buffer to obtain a final concentration of 5%. The mixtures were heated at 95 °C for 3 min prior to loading. The prepared samples (12 μg protein) were loaded onto the gel. Electrophoresis was performed using a vertical gel electrophoresis unit (Mini-protein II; Bio-Rad Laboratories, Richmond, VA, USA) at a constant voltage of 200 V/plate. The gels were stained with 0.125% Coomassie blue R125 in 25% methanol and 10% acetic acid. The gels were destained with 40% methanol and 10% acetic acid. Relative mobility (R_f) of proteins was calculated and the molecular weight of the proteins was estimated from the plot between R_f and log (MW) of standards.

2.5. Amino acid analysis

Amino acid compositions of albumin and globulin fractions were determined using an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan). Samples were hydrolyzed under the reduced pressure in 4 M methane sulfonic acid containing 0.2% 3-2(2-aminoethyl) indole at 115 °C for 24 h. The hydrolysates were neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 mL was applied to an amino acid analyzer.

2.6. Effect of Alcalase on hydrolysis of coconut protein fractions

Albumin and globulin fractions were dissolved in deionized water and adjusted to pH 8 using 1 M NaOH. The solution was added with Alcalase at a level of 1% (v/v). The hydrolysis was performed at 60 °C in a shaking water bath (W350, Memmert, Schwabach, Germany). The samples were taken at different times from 0 to 90 min. The enzymatic reaction was terminated by adding 1 volume of hot SDS solution (85 °C) and the mixture was placed in a water bath at 85 °C for 15 min. All samples were determined for protein pattern by SDS-PAGE.

Degree of hydrolysis (DH) was also determined according to the method of Benjakul and Morrissey (1997). The hydrolyzed samples with the appropriate dilution (125 μL) were added with 2.0 mL of 0.2 M phosphate buffer (pH 8.2) and 1.0 mL of 0.01% TNBS solution. The solution was mixed thoroughly and placed in a temperature controlled water bath at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was read at 420 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) and α-amino group was expressed in terms of L-leucine. The DH was calculated as follows:

$$DH = [(L - L0)/(L\max - L0)] \times 100$$

where L is the amount of α-amino groups of hydrolyzed sample. $L0$ is the amount of α-amino groups in the initial sample. $L\max$ is the total α-amino groups obtained after acid hydrolysis (6 M HCl at 100 °C for 24 h).

2.7. Preparation of coconut milk model oil-in-water emulsions

Albumin and globulin fractions were firstly dissolved in 5 mM phosphate buffer containing 0.02% sodium azide to obtain 1% protein. The mixture was stirred overnight at 4 °C. Emulsions were prepared by mixing virgin coconut oil and the prepared protein solutions at a ratio of 1:9 (w/w). The mixtures were homogenized using a high-speed homogenizer at 11,000 rpm for 2 min. These coarse emulsions were passed through high-pressure homogenizer (Microfluidics, Model HC 5000, Stanwood, WA, USA) at 3000psi for two times. The emulsions prepared using albumin and globulin fractions were designated as albumin stabilized emulsion (ASE) and globulin stabilized emulsion (GSE), respectively. These two emulsions were immediately used for further study.