Fractionation and Characterization of Proteins from Coconut (*Cocos nucifera* L.)†

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Five protein fractions (albumins, globulins, prolamines, glutelins-1, and glutelins-2) from defatted coconut flour were fractionated and then characterized by SDS−PAGE, gel filtration, differential scanning calorimetry (DSC), and amino acid analysis. The albumins and globulins were the predominant protein fractions. Native coconut proteins consisted of four major polypeptides with molecular weights ranging from about 22 to greater than 100 kDa. However, in a reduced state, there were about seven major bands with molecular weights of between 14 and 52 kDa. SDS−PAGE of protein fractions indicates that the total protein, globulins, and glutelins-1 are composed of polypeptides linked via one or more disulfide bonds. DSC analysis showed that albumins had an endothermic peak of 94 °C, while globulins had peaks at 92, 98, and 112 °C. Amino acid analysis demonstrated that the coconut proteins had a relatively high level of glutamic acid, arginine, and aspartic acid.

Keywords: *Coconut protein; albumins; globulins; prolamines; glutelins; differential scanning calorimetry*

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an important oilseed source, especially in many underdeveloped countries, including the Philippines, Ceylon, India, Malaysia, parts of West Africa, and Oceania. Apart from fat, coconuts provide a potential source of proteins with good nutritional value (Krishnamurthy et al., 1958) and a relatively well-balanced amino acid profile (Srinivasan et al., 1964), but coconut proteins are not used extensively for human consumption in the form of processed food. Dried kernel, copra, contains 15−20% protein by weight and is used for edible products from confections to milk substitutes, but as yet very little is known about the coconut proteins.

Over the years, various fractionation techniques have been used to explain the contribution of each solubility protein fraction to the total protein content and size and charge heterogeneity of the polypeptide comprising each fraction (Hu and Esen, 1981). The relative proportion of each fraction in a seed protein affects the nutritional quality and the functional properties of the total protein fraction (Johnson and Lay, 1974). Coconuts do not have well-defined solubility fractions, comparable to those in cereals and legume seeds. Sjogren and Spychalski (1930) observed a high molecular weight protein (cocosin) in coconut endosperm. They used salt and a phosphate buffer to extract the storage protein from coconut. Coconut albumins and globulins have also been extracted and characterized to determine characteristic of the storage protein in coconut and to compare these characteristics to the globulins of legumes (DeManson et al., 1990). However, there still is a lack of information on fractionation and characterization of total coconut proteins.

Therefore, this study was undertaken to fractionate coconut protein by solubility and to characterize by their electrophoretic properties, gel filtration chromatography, thermal analysis, and amino acid profiles for better understanding and potential application in various food systems.

MATERIALS AND METHODS

Materials. A batch of commercial desiccated coconut meal was purchased from Coco Cold Manufacturing Industries Inc. (The Republic of the Philippines). The coconut meal was defatted at low temperature by conventional hexane extraction. The defatted meal was then dried in a fume hood and ground in a coffee grinder to pass a 40-mesh screen. The defatted flour obtained was stored at −20 °C until further use. Electrophoretic grade sodium dodecyl sulfate and acrylamide were purchased from Bio-Rad (Richmond, CA). All other chemicals used in this study were of reagent grade.

Protein Fractionation. Extraction and fractionation of coconut proteins were carried out according to the modified method of Hu and Essen (1981). Five different solvents—deionized distilled water, 0.5 M NaCl, 70% 2-propanol (IPA), 50% glacial acetic acid, and 0.1 M NaOH—were used in sequence to extract virtually all of the proteins in the coconut flour. The protocol for this sequential extraction is outlined in Figure 1. Defatted coconut flours were extracted by simply stirring, using a meal to solvent ratio of 1:10 (w/v), for 14−16 h at 4 °C. Insoluble residue was removed by centrifugation at 20000*g* for 30 min. Extraction with each solvent was repeated three times, and all supernatants for each solvent were pooled to obtain a representative of each solubility fraction. Each fraction then was dialyzed against its own solvent followed by deionized water. Dialysates were freeze-dried, and resulting protein powders were stored in a freezer at about −20 °C. Water-, NaCl-, IPA-, acetic acid-, and NaOH-soluble proteins were designated "albumin", "globulin", "prolamine", "glutelin-1", and "glutelin-2", respectively.

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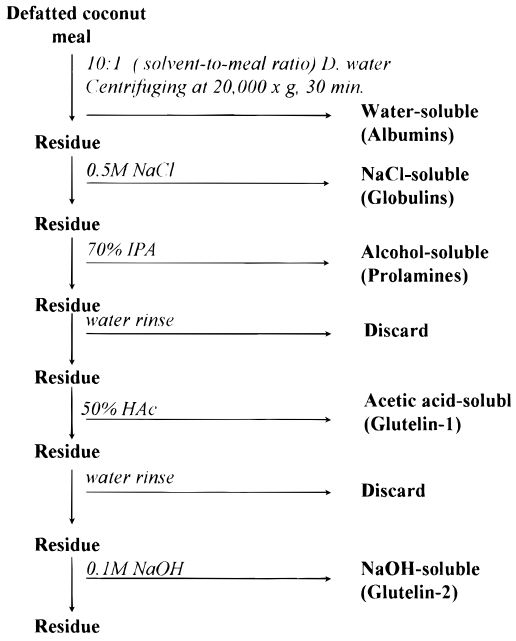

Figure 1. Flow sheet of the protocol used for fractionation of coconut proteins by different solubility.

Protein Content. Each solubility fraction was assayed for protein content according to the method of Bradford (1976) using bovine serum albumin (BSA) as the protein standard. In addition, nitrogen content of the coconut meal was determined by micro-Kjeldahl method. Nitrogen content was converted to protein by using the conversion factor of 6.25.

Gel Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) was carried out with 12% (w/v) acrylamide gels according to the procedure of Laemmli (1970). Electrophoresis was performed in a 7 × 8 × 0.75 cm PROTEAN II dual slab cell (Bio-Rad, Richmond, CA) at a constant voltage of 200 V. Protein powders were solubilized to a final concentration of 10 mg/mL in the sample buffer containing 2% SDS, 10% glycerol, and 0.05% bromophenol blue in Tris-HCl, pH 6.8, and then heated at 95 °C for 3 min prior to loading. For analyzing reduced proteins, β-mercaptoethanol (ME) was added to the sample buffer to a final concentration of 5%. Approximately 10 μg of protein was loaded into each well. The gels were stained for 30 min in a solution of Coomassie blue R-250 staining solution and destained in a 40% methanol and 10% acetic acid (HAc) solution. The destaining solution was replaced as necessary. After destaining, the gels were dried on a gel dryer for permanent storage and photography. Protein bands were compared in relation to the mobilities of the following marker proteins: phosphorylase *b* (97.4 kDa), BSA (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa).

Gel Filtration. Gel filtration chromatography of the major solubility fractions, albumin and globulin, was conducted on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden). The degassed gel was equilibrated with extraction buffer, packed in a column (2.5 × 100 cm), and allowed to settle for 48 h during equilibration with buffer. The bed volume (V_t) of the column was 500 mL, and void volume (V_0) was 142 mL. The void volume was determined using blue dextran 2000. About 40−50 mg of the samples was loaded onto the column and eluted with extraction buffer. The column was calibrated with aldolase (molecular weight 15 8000), albumin (67 000), ovalbumin (43 000), chymotrypsinogen A (25 000), and ribonuclease A (13 700). Fractions of 3.0−3.5 mL were collected in an automatic fraction collector at a flow rate of 10 mL/h, and the absorbance of the fractions was measured at 280 nm in a Beckman DU-6 spectrophotometer.

Differential Scanning Calorimetry. The thermal behavior of the protein fractions was examined with a differential scanning calorimeter (DSC) 120 (Seiko Co., Japan). Samples (2 mg each) were directly weighed onto the aluminum pan, and 20 μL of distilled water was added. The heating rate was 5 °C/min over the range of 30−130 °C. The enthalpy (ΔH) of denaturation, as J/g, was calculated from the area under the curve.

Table 1. Distribution of Proteins in Different Coconut Protein Fractions of Defatted Coconut Meal According to Solubility

fraction	extraction solvents	total protein extracted (%)
albumins	deionized water	21.0 (0.4)[a]
globulins	0.5 M NaCl	40.1 (0.3)
prolamines	70% isopropyl alcohol	3.3 (0.2)
glutelins-1	50% acetic acid	14.4 (0.4)
glutelins-2	0.1 M NaOH	4.8 (0.7)
unextractable protein	8.1 (0.9)	

[a] Values in parentheses are standard deviations obtained from triplicate experiments.

Amino Acid Analysis. Amino acid analyses were performed on three major coconut fractions (albumins, globulins, and glutelin-1) and defatted coconut flour sample according to the method of Knabe et al. (1989). Each sample (50 mg) was placed in a glass ampule to which was added 6 N HCl (6 mL). The samples were sealed with nitrogen and hydrolyzed at 110 °C for 22 h. The sample was filtered through a 0.5-μm Milipore filter and brought up to 200 mL with deionized water. Aliquots (5 mL) of the hydrolysates were mixed with 19.5 mL of HPLC water and then analyzed with ion-exchange column chromatography, using a Waters System HPLC instrument (Milipore Corp.) equipped with a Model 510 pump, temperature control module, and visible absorbance detector. The buffer used was 0.2 M borate (pH 6.5). Ninhydrin was used for the derivatization of amino acids, and detection was at wavelengths of 430 and 546 nm. Tryptophan and cysteine were not determined because tryptophan is decomposed by acid hydrolysis and the sulfhydryl group in cysteine is extremely reactive and readily cross-links to form disulfide linkage during the assay.

RESULTS AND DISCUSSION

Distribution of Protein Fractions. The protein content of the defatted coconut flour used in this study was 17.2% (w/w, dry weight basis) as determined by the micro-Kjeldahl method after defatting. The amount of protein recovered in each fraction is given as percent of the total extractable protein in Table 1. The sum of the protein in the five protein fractions amounted to 84.4% (w/w) of the total protein content. This means that the sequential extraction with five solvents did not extract all of the protein in the coconut flour. Khaund (1971) suggested vigorous grinding for recovering proteins from coconuts because most of the protein is inside the cell. The yield of all the fractions was nearly 92% of the total coconut protein. The albumin and globulin fractions were the predominant protein fractions, accounting for 21% and 40% of the total protein, respectively. The acetic acid-soluble glutelin-1 had 14.4%, but prolamine and NaOH-soluble glutelin-2 fractions were less than 5% of the total protein. Samson et al. (1971) reported that globulin fraction was the major coconut protein with 62% of the total followed by albumins with 30.6%, according to the Osborne (1924) classification. Fractionation of plant proteins on the basis of solubility is only an approximation of the actual protein composition. Therefore, differences in protein extraction conditions and determination may lead to different values.

SDS−PAGE. Electrophoretic patterns of unreduced (without ME) and reduced (with ME) protein fractions and the coconut flour are shown in Figure 2. Electro-

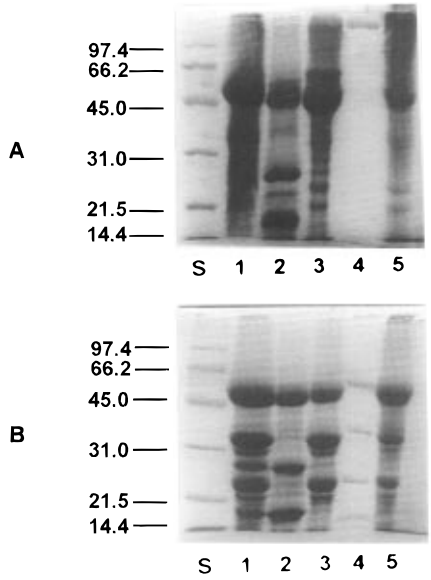

Figure 2. SDS−PAGE patterns of solubility fractions of coconut proteins in the absence (A) and presence (B) of β-mercaptoethanol: (S) low molecular weight standards, (1) coconut flour, (2) albumins, (3) globulins, (4) prolamines, and (5) acetic acid-soluble glutelins. Approximately 10 μg of protein was loaded on each well.

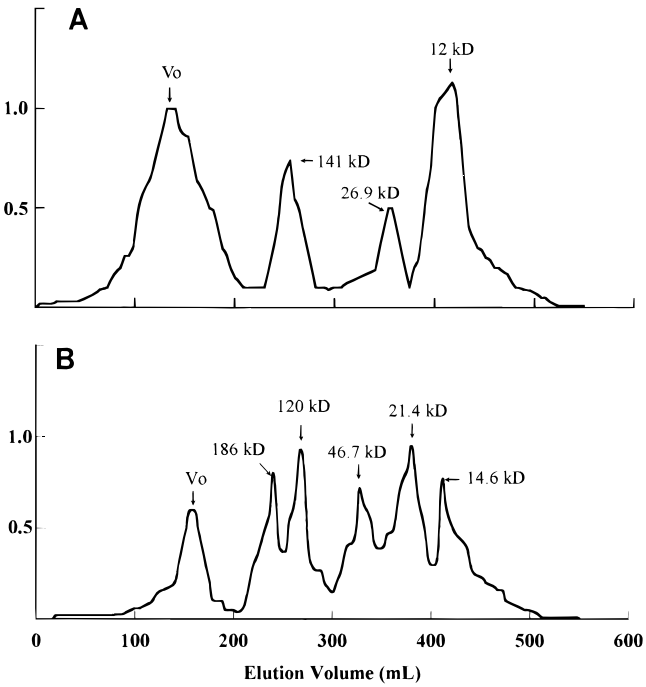

Figure 3. Gel filtration patterns of albumins (A) and globulins (B) on Sephadex G-200 in each extraction buffer.

phoretic separations revealed size heterogeneity within each of the four different solubility fractions. There is a streaking in the patterns of the unreduced native proteins of the coconut flour. Five major bands, with estimated molecular weights ranging from 22 kDa to greater than 100 kDa (Figure 2A, lane 1), dominated the total protein (coconut flour) composition in the unreduced form. However, the reduced coconut flour was shown to have seven prominent bands with molecular weights of between 14 and 52 kDa with less prominent polypeptides of molecular masses 21 and 14 kDa (Figure 2B). These results suggested that the major coconut protein may consist of polypeptides linked via one or more disulfide bonds. The albumin and globulin patterns appeared to be quite different but had a few bands in common. In the absence of ME, the albumin and globulin fractions had three (52, 28, and 18 kDa) and two (61 and 44 kDa) major polypeptides, respectively. Most bands of the albumin proteins persisted even in the presence of ME. They may represent albumin proteins without disulfide linkage. However, after reduction, the globulin proteins dissociated into five to seven major bands. The two major bands with molecular weights above 60 kDa disappeared in the reduced sample. The glutelin-1 pattern was similar to that of globulins. This may indicate the contamination of components between the protein classes. Some high molecular weight components near the origin and a major band of 55 kDa were shown in the acetic acid-soluble glutelin-1, and those subunits were dissociated into more than seven bands (14.4−100.0 kDa) upon addition of ME. There was an apparent lack of major bands above 100.0 kDa. The components of prolamine were quite faint. The unreduced prolamine fraction showed a band near the origin of application with low affinity for the dye. On reduction, the band near the origin separated into four bands (56, 37, 25, and 17 kDa) with low intensity.

In a previous study, the total coconut protein was found to have a similar pattern (Demason et al., 1990); nonreduced coconut proteins separated into four bands with molecular weights of greater than 100, 55, 25, and 17 kDa. The reduced proteins resolved into seven major and four or more minor bands ranging between 17 and 55 kDa.

Gel Filtration. Figure 3 shows the gel filtration patterns of the two major fractions (albumins and globulins) on Sephadex G-200. The albumins (Figure 3A) separated into two major peaks of about 141 and 12 kDa and one minor peak of 27 kDa. The globulins also showed a peak (200 kDa) near the void volume (Figure 3B). The globulins exhibited five poorly resolved peaks with molecular weights of 186, 120, 46.7, 21.4, and 14.6 kDa, respectively. There was little cross-contamination between the fractions. The estimated molecular weights of the globulin components were close to the reported value (208 kDa) of cocosin (Sjogren and Spychalski, 1930) and also close to the values observed in previously mentioned SDS−PAGE patterns (Figure 2A). However, the albumins displayed a slight difference among molecular weight values as observed by both gel filtration and SDS−PAGE. A major albumin component (46 kDa) observed in SDS−PAGE was not detected by gel filtration.

DSC. Thermal analysis, in particular DSC, to study thermal denaturation and conformation changes provides a relatively simple and convenient technique and has been used in various food systems. The DSC thermograms of albumins and globulins from coconuts are presented in Figure 4. The albumin fraction showed a broad endothermic peak with a denaturation temperature or peak maximum temperature (T_d) of approximately 93 °C. The globulin fraction was found to give a broad peak with two T_d values of 82 and 98°C and a sharp peak with a T_d of about 112 °C. The prolamine and glutelin fractions did not show any detectable thermal response. The reaction enthalpy (ΔH) of the albumin fraction was not calculated because of difficulty in establishing a base line, and the ΔH values of the globulins were 12.5 and 16.4 mJ/mg for a broad peak and a sharp peak, respectively. The T_d and ΔH for coconut protein fractions were similar to those reported for oat proteins prepared by the Osborne classification

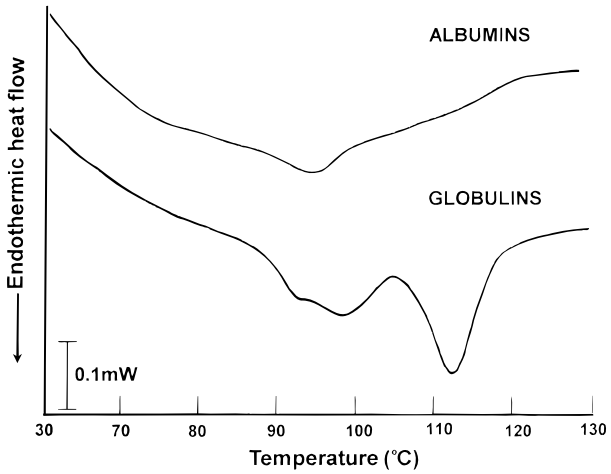

Figure 4. Differential scanning calorimetric thermograms of albumins and globulins from coconuts.

Table 2. Amino Acid Composition of the Three Major Protein Fractions and Coconut Flour from Defatted Coconut Meal (Grams/100 g of Protein)

amino acids	albumins	globulins	glutelins-1	coconut flour
isoleucine	2.8	4.1	3.7	4.2
leucine	3.9	6.5	6.5	7.4
lysine	5.1	3.5	3.5	4.7
methionine	1.2	2.9	2.1	1.8
phenylalanine	2.7	5.9	4.6	5.1
threonine	3.3	3.3	3.2	2.5
valine	3.5	7.5	6.7	5.4
histidine	1.8	1.9	1.9	1.8
tyrosine	3.0	3.7	3.1	1.8
aspartic acid	5.6	8.9	8.3	9.3
proline	2.7	3.4	3.2	3.6
serine	3.1	5.0	3.9	5.3
glutamic acid	24.9	17.5	17.0	22.4
glycine	4.0	4.9	4.5	5.1
alanine	2.9	4.1	3.9	4.8
arginine	17.9	15.0	14.2	12.3

(Ma and Harwalkar, 1984). They also reported the absence of endothermic peaks in oat prolamines and suggested the extraction procedure for the prolamines and glutelins was likely to cause protein denaturation. While the coconut albumins with a broad peak are likely to be a heterogeneous protein mixture with a multistep thermal transition, the globulins belong to a protein system with a high cooperative thermal transition, as suggested by Ma and Harwalkar (1984).

Amino Acid Analysis. Table 2 shows amino acid compositions of coconut protein fractions and defatted coconut flour. In general, coconut protein had a relatively high level of glutamic acid, arginine, and aspartic acid and was deficient in the sulfur amino acid methionine, as reported for other legumes. Similar findings were reported by Samson et al. (1971). Levels of most amino acids were lower in the albumin fraction, but the amounts of lysine, arginine, and glutamic acid were higher. The amino acid compositions of globulin and glutelin-2 are very similar. It is noteworthy that all the amino acids are higher in globulin than glutelin-1. The globulin protein contained a higher content of essential amino acids including phenylalanine and valine. In general, a characteristic amino acid profile of high glutamic acid and arginine in the three protein fractions was common in the coconut flour as mentioned above. Particularly, the amino acid profile of the globulin fraction compares most closely to that of the coconut flour since it is the major fraction of the coconut protein.

Table 3. Bigelow Parameters Calculated from the Amino Acid Composition of Coconut Protein Fractions and Coconut Flour

proteins	NPS[a]	HΦ[b]	P[c]
albmumins	0.18	765	3.46
globulins	0.29	965	1.70
glutelins-1	0.28	940	1.82
coconut flour	0.28	864	1.84

[a] The frequency of nonpolar side chains calculated according to Waugh's definition by counting the Trp, Ile, Tyr, Phe, Pro, Leu, and Val residues and expressing the sum as a fraction of the total number of residues (Waugh, 1954). [b] Average hydrophobicity. [c] Ratio of polar to nonpolar side chains.

The frequency of nonpolar side chains (NPS), the average hydrophobicity (HΦ), and the ratio of polar to nonpolar side chains (P) calculated according to Bigelow (1967) from the amino acid composition of coconut protein fractions are demonstrated in Table 3. Among these proteins, albumins showed the highest P value but the lowest NPS and HΦ values, indicating the higher proportions of polar side chains of albumins. Globulin and glutelin-1 fractions of coconut had higher levels of NPS and HΦ than albumins, which explain higher hydrophobicity.

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