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Extraction, Fractionation and Characterization of Bitter Melon Seed Proteins

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Protein fractions (water-soluble/albumin, salt-soluble/globulin, alkali-soluble/glutelin, and alcohol-soluble/prolamin) were extracted from defatted ripe bitter melon seed (*Momordica charantia*) using water, 1 M sodium chloride solution, alkali/pH 11.0, and 70% ethanol, sequentially. The main protein fraction was albumin (49.3%), followed by globulin (29.3%) and glutelin (3.1%). No prolamin was detected, and 18.3% of the protein was nonextractable. The surface hydrophobicities of albumin, globulin, and glutelin were 757, 1,034, and 292, respectively. The molecular sizes of all the fractions were mostly about 45 and 55 kDa. The denaturation temperatures of albumin, globulin, and glutelin were 111.9, 117.3, and 133.6 °C, respectively. The levels of all essential amino acids in the bitter melon protein fractions met the minimum requirements for preschool children (FAO/WHO/UNU) with the exception of Thr. Bitter melon protein fractions with unique protein profiles and higher denaturation temperatures could impart novel characteristics when used as food ingredients.

KEYWORDS: Bitter melon; albumin; globulin; glutelin; fractionation

INTRODUCTION

A systematic approach to extract proteins was first developed to classify proteins according to their solubility in various solvents by Osborne (*1*). The proteins were grouped into albumin (water-soluble), globulin (salt soluble), glutelin (alkali soluble), and prolamin (alcohol soluble). This classification is considered as the framework for modern studies of storage proteins (*2*).

Plant seeds rich in albumin include Brazil nut, rapeseed, castor bean, and sunflower (*3*−*6*). Globulin storage proteins that mainly exist in soybeans include 7S globulin (vicillin) and 11S globulin (legumin) (*2*). Prolamins are the major storage proteins in all cereal endosperms with the exception of oats and rice. In addition to its solubility in alcohol, prolamins are distinguished by their high contents of proline and amide nitrogen (glutamine and its derivates) (*2*). Glutelins present in rice, wheat, and other cereals and have a close relationship to prolamins in their structures but are not soluble in alcohol (*7*, *8*). Glutelins are also rich in proline and glutamine and sometimes are considered as prolamins (*9*).

In general, seeds are ground and passed through sieves, and the fine flour obtained is defatted by hexane followed by water extraction to obtain albumin fraction. The residue after soluble albumin removal is then extracted sequentially with dilute salt (usually NaCl) solution, alkali (sodium hydroxide) solution, and 70% ethanol to obtain globulin, glutelin, and prolamin, respectively. The albumin, globulin, and glutelin fractions are precipitated by adjusting the pH of the solutions to their isoelectric points, while prolamin is precipitated by acetone.

Information on basic physicochemical characteristics of proteins is essential for their application as functional ingredients in a food system. These characteristics include surface hydrophobicity (*10*), molecular size (*11*), and thermal properties (*12*) of the proteins.

Some studies have shown that bitter melon seeds are a rich source of protein, especially glycoproteins (*13*−*15*). The protein content in the seeds of ripe bitter melon is about 30%. However, information on fractionation and characterization of the bitter melon seed proteins is lacking in the literature. The objectives of this study were to fractionate proteins from bitter melon seeds based on solubility following the procedure of Osborne, determine isoelectric point (IP) of each of fractions, and determine surface hydrophobicity, molecular size, and thermal properties of each fraction.

MATERIALS AND METHODS

Materials. Bitter melon (*Momordica charantia*) var. Sri Lanka (Thinneyville White) was planted at the Arkansas Agricultural Experiment Station (Fayetteville, AR). Plants were irrigated daily during the dry season and were otherwise irrigated daily when conditions were dry. No fertilizers or pesticides were applied. Ripe bitter melons from 3 years of crops (2004, 2005, and 2006) were harvested when pericarp (fleshy portin) turned yellow and seed coat tissue/aril turned red (~4−5 weeks post flowering). All chemicals for protein extraction and fractionation, isoelectric point determination, protein content determination, and surface

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hydrophobicity determination were purchased from VWR International, Inc. (Suwanee, GA), and Sigma Chemical Co. (St. Louis, MO). Other chemicals for electrophoresis were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA).

Separation of Seeds from Ripe Bitter Melon. Seeds (20−30 per melon) of ripe bitter melon were manually separated from pericarp and aril, and dried on a stainless steel tray at 40 °C and 20% relative humidity in a dehydrator (Harvest Saver model #R-4, commercial Dehydrator System, Inc., Eugene, OR). The dried seeds were ground using a sample grinder (IKA WERKE grinder model M20, Ika Works, Inc., Wilmington, NC) and then passed through a 60-mesh sieve (W.S. Tyler Inc., Mentor, OH) to obtain a uniform particle size. The bitter melon seed product was defatted 3 times using hexane (ratio 1:2, w/v) by shaking using a Lab Line shaker (Lab Line Inc., Fullerton, CA) at room temperature for 8 h and vacuum-filtered. The defatted seed product was dried under a hood at ambient temperature overnight to remove the trace of the remaining hexane.

Extraction and Fractionation of Proteins from Bitter Melon Seed Flour. Proteins were extracted and fractionated from the defatted seed product based on the Osborne method as described by Ju et al. (*16*). Water-soluble protein (albumin) was extracted by stirring the dispersion of the defatted seed product (20 g) in deionized (DI) water (100 mL) for 2 h at ambient temperature. The dispersion was then centrifuged at 10000*g* (model J2-21, Beckman, Fullerton, CA) for 20 min to separate supernatant containing the extracted albumin from residue. After water extraction, salt-soluble protein (globulin) was extracted from the residue using 1 M sodium chloride solution (100 mL) by stirring the dispersion for 2 h at ambient temperature, followed by centrifugation at 10000*g* for 20 min to obtain supernatant, which contained the globular protein fraction. Alkali-soluble protein (glutelin) was extracted by adding 100 mL of DI water into the residue, followed by adjusting the pH to 11.0, and stirring for 2 h. The extracted glutelin in the supernatant was separated by centrifugation at 10000*g* for 20 min. Alcohol-soluble protein (prolamin) was extracted from the residue using 70% ethanol at 20 °C for 2 h, followed by centrifugation. The residue after extraction from each solvent was washed twice using a small portion of DI water to collect the residual protein fractions from the residues. The washings and the first extract were combined for each fraction. Albumin, globulin, and glutelin were precipitated for isolation by adjusting the pH of the obtained supernatant at their isoelectric points (IPs), while prolamin was precipitated by adding acetone as described by Tecson et al. (*17*). After centrifugation at 10000*g* for 15 min, the isolated proteins were washed twice using DI water at their respective IPs, followed by centrifugation, resolubilized by adjusting to pH 7.0, freeze-dried, and stored at 4 °C for further analysis.

Isoelectric Points (IP) Determination of Albumin, Globulin, and Glutelin. Isoelectric pHs of albumin, globulin, and glutelin were determined by measuring the turbidity (absorbance at 320 nm) of each supernatant obtained from protein extractions over a range of pHs (2.0 to 11.0). Ten milliliters of the supernatants were diluted to reach a readable concentration of turbidity and the pH of the solution was adjusted to obtain the pHs ranging from 2.0 to 11.0 at 1.0 pH increment and smaller increment near the IP using either 1 N NaOH or 1 N HCl. The turbidity was read at 320 nm with a spectrophotometer (Shimadzu model UV-1601, Kyoto, Japan). The IP for each protein fraction was the pH that gave the maximum turbidity.

Protein Determination of Albumin, Globulin, Glutelin, and Prolamin. Protein content was determined by an Automatic Kjeldahl method (*18*). Kjeldahl 2006 digester (Foss Tecator, Hoganas, Sweden) was used for digesting the protein fractions in concentrated sulfuric acid with a Kjeldahl tablet as catalyst for 1 h at 420 °C. Kjeltec 2300 analyzer unit (Foss Tecator, Hoganas, Sweden) was used to determine nitrogen content, which was automatically computed to give the protein content using a factor of 6.25. Based on the amount and protein content of the obtained fractions, yields of each fraction were calculated as follows: yield (g/100 g) = weight of fraction × % protein of fraction × 100/weight of bitter melon seed product ×x % protein of bitter melon seed product.

Molecular Size Determination. Molecular size was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) according to a procedure of Laemmli (*19*). The SDS−PAGE was carried out on a slab gel (4% stacking gel, 15% separating gel) in an SDS−Tris-Glycine discontinuous buffer system. Protein solutions (2 μg protein/μL) were prepared in reducing (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, and 0.05% bromophenol blue) and nonreducing buffer solutions (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 0.05% bromophenol blue, and 0.05% 2-β-mercaptoethanol). Ten microliters of the solution was loaded onto the gel. Electrophoresis was performed at a constant current of 45 mA per gel for approximately 45 min. The gel was stained by 0.1% Coomassie brilliant blue in acetic acid/ethanol/water solution (10/40/50, v/v/v) and destained in acetic acid/ethanol/water solution (10/20/70, v/v/v). Approximate molecular sizes of the proteins were determined by Bio-Rad molecular size standards (Bio-Rad Laboratories, Hercules, CA).

Thermal Property Determination. Thermal properties of protein fractions were determined using a differential scanning calorimeter model Pyris-1 (Perkin-Elmer Corp., Norwalk, CT) equipped with thermal analysis software (Version 4.00, Pyris-1-DSC, Perkin-Elmer Corp., Norwalk, CT). Protein−water slurries were prepared in DI water to give 20% of protein content. The slurries were left for 30 min for equilibrium before analysis. Fifty microliters of the protein slurry were accurately weighed into a stainless steel pan (large volume capsule) and hermetically sealed. The sealed pan was scanned with the calorimeter during temperature increased from 25 to 140 °C at a rate of 10 °C/min. An empty pan was used as a reference. The instrument was calibrated using indium and zinc. Peak temperature and enthalpy were computed from thermograms by the data processing software.

Surface Hydrophobicity Determination. Surface hydrophobicity determination was conducted using a method of Hayakawa and Nakai (*20*). This method is based on the hydrophobic fluorescence measurement in the present of 1-anilino-8-naphthalene sulfonate (ANS). Solutions of the obtained protein fractions were made to get concentrations ranging from 0.0002 to 0.0015% w/v in 0.01 M phosphate buffer (pH 7). Ten microliters of 8 mM ANS in 0.01 M phosphate buffer (pH 7.0) was added into 4 mL of each protein solution, and fluorescence intensities of these solutions were read at 390 nm of excitation and 470 nm of emission with a spectrofluorophotometer (Shimadzu model RF-1501, Kyoto, Japan). The surface hydrophobicities, expressed as a slope of fluorescence intensity vs protein concentration, were calculated by linear regression.

Amino Acid Determination. A performic acid oxidation with acid hydrolysis−sodium metabisulfite method of AOAC official method 994.12 (*21*) was used to hydrolyze the bitter melon seed protein fractions for amino acid determination. The protein fractions (equivalent to 10 mg nitrogen content) were pretreated with 5 mL of performic acid in an ice bath (0 °C) for 16 h to oxidize cysteine and methionine into cysteic acid and methionine sulfone, respectively, prior to acid hydrolysis. After decomposition of the performic acid by adding sodium metabisulfite, the oxidized proteins were hydrolyzed under reflux with 50 mL of 6 M HCl−phenol solution for 24 h at 110−120 °C. The hydrolyzed proteins were then cooled to room temperature, added with 20 mL of norleucine standard solution, and filtered into an evaporating flask. The norleucine standard is used as an external standard to aid in identification of amino acids based on retention time. The hydrolysates were evaporated under vacuum at 40 °C to reach about 5.0 mL of evaporates, adjusted to pH 2.2 using 2 M NaOH and made up to 50 mL with sodium citrate buffer pH 2.2. The solution was passed through a 0.2 μL membrane filter (Pall Corp., Ann Arbor, MI) into a 1.0 mL amber vial. The filtrate (20 μL) was automatically injected using 507 Autosampler (Beckman Instruments, Inc., Palo Alto, CA) and run in Spherogel IEX high performance sodium column (Beckman Instruments, Inc., Palo Alto, CA) with 126AA solvent module equipped with 166 detector (Beckman Instruments, Inc., Palo Alto, CA) at a flow rate of 0.67 mL/min (0.44 mL/min for buffer solutions and 0.23 mL/min for ninhydrin solution). Sodium citrate buffers (pH 3.3, 4.3 and 6.3) were used as eluents and absorbance of amino acids was detected at 570 nm. The amino acids were quantified by comparing their profiles with amino acid profiles from external amino acid standards.

Statistical Analysis. JMP 7 software package (SAS Institute Inc., Cary, NC) was used to obtain statistical analysis of data. Analysis of variance (ANOVA) was conducted using a Tukey HSD procedure for the significance of differences among protein fractions at a 5% significance level. All values were expressed as means of three determinations from 3 years of crop (2004, 2005, and 2006).

RESULTS AND DISCUSSION

Extraction and Fractionation of Bitter Melon Seed Proteins. Protein content of the bitter melon seed product was 30.4%,

and after defatting with hexane, the protein content of the defatted seed product was 43.5%. Albumin, globulin, and glutelin had the IP pHs of 3.6, 4.8, and 2.6, respectively (**Figure 1**). Based on these pHs, these protein fractions were isoelectrically precipitated to separate the maximum amount of proteins from supernatants. Prolamin was not detected in the 70% ethanol extract indicating that bitter melon seeds did not contain this protein fraction.

The IP precipitated fractions were freeze-dried to obtain proteins for quantification of the extractability of these fractions,

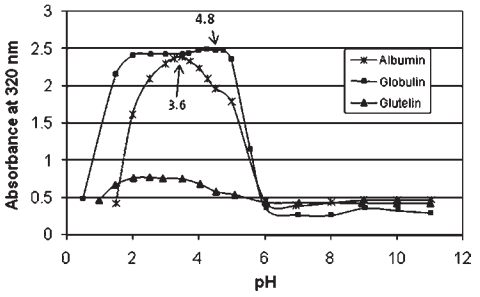

Figure 1. Absorbance of bitter melon seed protein fractions (albumin, globulin, and glutelin) at varying pHs. Isoelectric point pHs of albumin and globulin 3.6 and 4.8, respectively.

Table 1. Protein Contents and Yields of Extracted Bitter Melon Seed Protein Fractions (Albumin, Globulin, Glutelin, and Prolamin)[a]

fraction	protein content (%)	yield of extracted protein (g/100 g)
albumin	86.1 ± 1.1 ab	49.2 ± 2.0 a
globulin	87.1 ± 0.5 a	29.4 ± 1.1 b
glutelin	82.0 ± 2.9 b	3.1 ± 0.2 c
prolamin		nd
P value	0.0332	<0.0001

[a] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006). Yield of each fraction was calculated as follows: yield (g/100 g) = weight of fraction × % protein of fraction × 100/weight of bitter melon sample flour × % protein of bitter melon sample flour. Mean values with different letters in the same column are significantly different (*P*-value <0.05).

and investigation of their physicochemical properties. The protein contents were 86.1%, 87.1%, and 82.0% for albumin, globulin, and glutelin, respectively. Ripe bitter melon seeds contained 49.2% of albumin, 29.4% of globulin, and 3.1% of glutelin, while prolamin was not present in the bitter melon seeds (**Table 1**). A total of 81.7% of protein was extracted from the defatted bitter melon seed product, while 18.3% of the protein was not recovered. This protein fraction composition was completely different in comparison to the protein fractions from other seed proteins. The protein fractions from legumes that also have no prolamin range from 15 to 21%, 10 to 15%, and 60 to 70% for albumin, glutelin, and globulin, respectively for broad bean, pea, and peanut; while soybean only has albumin (10%) and globulin (90%) (*22*). In cereals, such as rice kernel, glutelin is predominant (75−79%), followed by globulin (13−15%), albumin (4.5−6%), and prolamin (less than 3%) (*12*, *23*), while wheat germ contains 34.5, 15.6, 10.6, and 4.6% of albumin, globulin, glutelin, and prolamin, respectively (*24*). Prolamin, that is present in the proteins of growing starchy endosperms of seeds, is usually not found in the seeds of any plant family including cucurbitaceae but cereals (*1*, *7*, *8*).

Molecular Size of Albumin, Globulin, and Glutelin. **Figure 2** shows electrophoretograms of albumin, globulin, and glutelin in nonreducing and reducing buffers. These electrophoresis patterns showed simplicity in the structure of all these protein fractions. The electrophoretograms of the fractions in nonreducing buffer (**Figure 2A**) showed that all three fractions in native form had the main protein with dense band of about 55 kDa. In addition to that main protein, albumin also contained two minor proteins with the molecular sizes of about 7 and 40 kDa, while globulin and glutelin had only one minor protein with the molecular size of 40 kDa. These molecular size profiles have fewer bands and are different in comparison to the molecular size profiles of rice or wheat protein fractions for example. Albumin, globulin, and glutelin fractions of rice protein contain 6 (15 to 56 kDa), 6 (14 to 55 kDa), and 4 (16 to 39 kDa) polypeptide bands, respectively (*23*); while wheat protein fractions have 19 (14 to 84 kDa), more than 7 (14 to 55 kDa), and 5 (range from 14 to 39 kDa) bands, for albumin, globulin, and glutelin, respectively (*25*). In

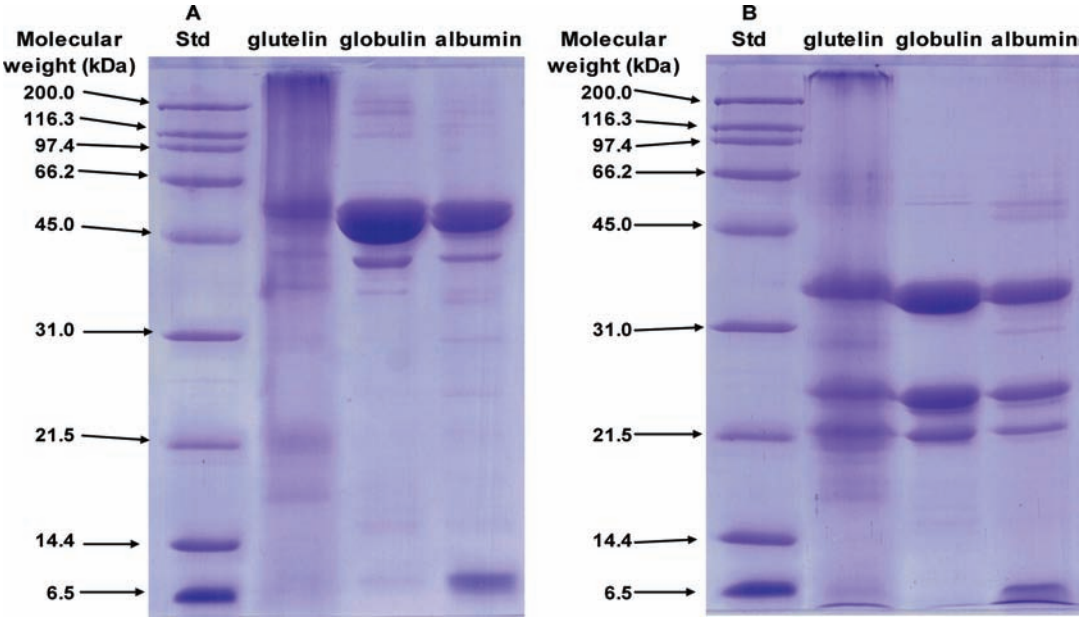

Figure 2. Electrophoretograms of bitter melon seed protein fractions (glutelin, globulin, and albumin) using nonreducing (**A**) and reducing buffers (**B**). Molecular sizes of the protein standards range from 6.5 to 200 kDa (mysosin 200 kDa, β-galactosidase 116.25 kDa, phosphorylase B 97.4 kDa, serum albumin 66.2 kDa, ovalbumin 45 kDa, carbonic anhydrase 31 kDa, trypsin inhibitor 21.5 kDa, lysozyme 14.4 kDa, and aprotinin 6.5 kDa).

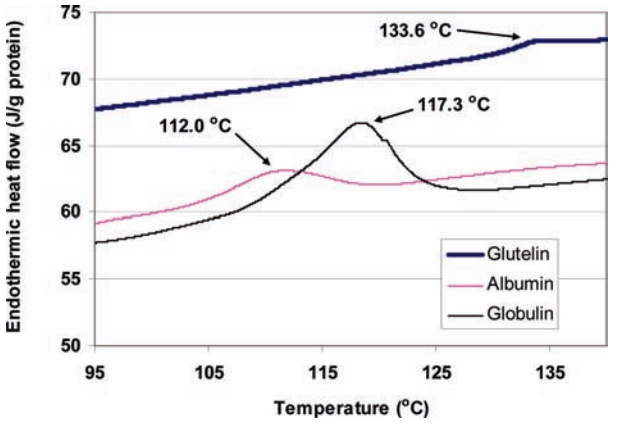

Figure 3. Thermograms of glutelin, albumin, and globulin of bitter melon seed protein.

Table 2. Thermal Properties and Surface Hydrophobicity of Albumin, Globulin, and Glutelin of Bitter Melon Seed Protein[a]

	protein fractions			
physical properties	albumin	globulin	glutelin	*P*-value
thermal properties				
onset (°C)	104.7 ± 0.4 c	109.9 ± 0.3 b	130.2 ± 1.2 a	<0.0001
end (°C)	118.7 ± 1.3 b	122.7 ± 0.9 b	136.3 ± 5.9 a	0.0020
peak (°C)	112.0 ± 1.7 c	117.3 ± 0.8 b	133.6 ± 0.6 a	<0.0001
ΔH (J/g)	9.2 ± 1.2 b	27.6 ± 5.0 a	1.9 ± 0.8 c	<0.0001
surface hydrophobicity	757 ± 85 b	1,034 ± 112 a	292 ± 71 c	0.0002

[a] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006). Mean values with different letters in the same row are significantly different (*P*-value <0.05).

reducing buffer solution that breaks down intermolecular disulfide bonds in the quaternary structure of protein, all three fractions of bitter melon seed protein had major bands of about 20, 25, and 35 kDa (**Figure 2B**). These results indicate that all the protein fractions contain disulfide bonds in their protein structure. Unlike bitter melon seed protein fractions, wheat protein fractions lack disulfide bonds and the proteins give similar bands under reduced and nonreduced conditions (*24*).

Thermal Properties. Thermal properties of the protein fractions from bitter melon seeds provide information about their behavioral changes during heat processing, and are useful for food processing strategies and designs. For proteins, a thermally induced process, detected by DSC, is the denaturation or unfolding of the protein molecule (*25*). Thermograms of bitter melon seed protein fractions on endothermic heat scanning from 95 to 140 °C are shown in **Figure 3**. There was only a single peak for each of the fractions. **Table 2** shows thermal properties of the fractions. This includes onset, end, and peak temperatures (°C), and ΔH (J/g) of each of the fractions. The onset and end temperatures indicate the protein starts to denature or unfold and completely denatures, respectively, while the peak is considered as the denaturation temperature of the protein. There were significant differences in the denaturation temperatures of albumin, globulin, and glutelin, which were 112.0, 117.3, and 133.6 °C, respectively (*P*-value < 0.0001) (**Table 2**). Higher denaturation temperature of glutelin in comparison to other fractions may be caused by its amino acid composition that contributes to the stability of its protein interior core (*26*, *27*). These denaturation temperatures were relatively higher than those for proteins from many other plant sources, such as legumes and cereals which are mostly lower than 100 °C. For instance, soybean and cowpea proteins have thermal denaturation temperatures of 83 (7S globulin) and 96 °C (11S globulin), and 85−88 °C, respectively (*10*). Ju et al. (*12*) reported that the denaturation temperatures of albumin, globulin, and glutelin of rice kernel protein were 73.3, 78.9, and 82.2 °C, respectively, much lower than those from the bitter melon seed protein fractions; whereas the denaturation temperatures of albumin and globulin of other cereals ranged from 50 to 70, and 77 to 96 °C (*24*, *28*−*30*). ΔH or enthalpy values give information about the energy required to unfold or denature the protein structure. The enthalpy values of the bitter melon seed protein fractions also showed that these protein fractions were thermodynamically more stable than the fractions from other sources. The enthalpy values of albumin, globulin, and glutelin were 9.2, 27.6, and 1.9 J/g protein, respectively, and they were not statistically the same (*P*-value < 0.01), while the reported enthalpies of albumin, globulin, and glutelin in rice were 2.9, 3.1, and 3.8 J/g protein (*12*) and 0.5−2 J/g protein (albumin) and 1.4 J/g protein (globulin) in wheat protein (*24*, *30*, *31*). Based on their higher denaturation temperature, these protein fractions probably are suitable for an application in specific products where native forms are needed to be maintained under higher temperatures during processing.

Surface Hydrophobicity. Hydrophobicity is a term related to the excess free energy of a solute in a solvent. Hydrophobicity of proteins is influenced by amino acid composition of the proteins on the surface. Surface hydrophobicities (SH) of bitter melon seed protein fractions are shown in **Table 2**. There were significant differences in their SHs among these three fractions (*P*-value < 0.05). Globulin had the highest SH, followed by albumin and glutelin, which were 1,034, 757, and 292, respectively (**Table 2**). These results indicate that bitter melon protein fractions have large variation of their hydrophobic amino acid residues, exposed to the surface of their protein structure. These SHs were higher than those of globulin (235) and glutelin (189) from rice protein (*12*). The SHs of the bitter melon seed protein fractions, particularly globulin and albumin fractions, were higher in comparison to the SHs of legume proteins, like soybean or cowpea, which ranged from 443 to 640 and 390 to 570, respectively (*11*).

Amino Acid Composition. Amino acid compositions of albumin, globulin, and glutelin from bitter melon seed are given in **Table 3**. Among all the fractions, only Thr and Lys were not significantly different (*P*-value > 0.05), while others statistically varied in the amount of their amino acid (*P*-value < 0.05). This variation may contribute to variability of their physicochemical properties. Information on these amino acid profiles of bitter melon fractions may provide a better understanding on physicochemical properties of these protein fractions. Some studies have shown that thermal stability of proteins is related to their amino acid profiles (*27*, *32*, *33*). Higher levels in hydrophobic amino acids, especially Val (38.7−45.5 mg/g protein), Ile (29.5−35.6 mg/g protein), Leu (64.5−70.1 mg/g protein), and Phe (38.7−47.7 mg/g protein) could contribute to a thermodynamically more stable protein (*27*). As shown in their thermal properties, bitter melon seed glutelin had the highest denaturation temperature and thermodynamically the most stable protein, followed by globulin and albumin. The total amount of Val, Ile, Leu, and Phe for glutelin, globulin, and albumin were 18.9, 18.0, and 17.6% mole fraction, respectively. Asn and Gln residues, and Cys and Met residues are easily deaminated and oxidized, respectively at high temperature, leading to thermodynamically less stable proteins containing high amount of these amino acid residues (*33*). The amino acid profiles showed that total amount of these amino acids were largest in albumin (27.5% mole fraction), followed by globulin (25.4% mole fraction) and glutelin (22.5% mole fraction). The other amino acids that are probably responsible

Table 3. Amino Acid Composition[a] of Albumin, Globulin, and Glutelin of Bitter Melon Seed Protein[b]

amino acids	albumin	globulin	glutelin	*P*-value
Cys	18.8 ± 0.2 (2.1) a	8.4 ± 0.2 (0.9) c	11.7 ± 0.2 (1.3) b	<0.0001
Met	32.9 ± 1.1 (2.9) b	39.5 ± 3.4 (3.5) a	27.7 ± 0.9 (2.4) b	0.0014
Thr	18.1 ± 3.9 (2.0)	13.6 ± 0.3 (1.5)	17.7 ± 0.4 (1.9)	0.0862
Ser	38.6 ± 0.9 (4.9) b	40.9 ± 0.1 (5.2) a	41.5 ± 0.7 (5.2) a	0.0038
Glx	163.9 ± 4.2 (14.9) a	143.1 ± 3.2 (12.9) b	128.7 ± 3.1 (11.5) c	<0.0001
Asx	75.8 ± 1.4 (7.6) b	80.9 ± 2.4 (8.1) a	74.1 ± 1.4 (7.3) b	0.0096
Gly	39.3 ± 0.6 (7.0) b	39.4 ± 0.9 (7.0) b	42.6 ± 1.3 (7.4) a	0.0081
Ala	38.3 ± 0.6 (5.8) c	41.2 ± 1.0 (6.2) b	44.2 ± 0.7 (6.5) a	0.0003
Val	38.7 ± 1.1 (4.4) b	41.2 ± 1.6 (4.7) b	45.5 ± 1.4 (5.1) a	0.0027
Ile	33.1 ± 1.1 (3.4) b	29.5 ± 0.8 (3.0) c	35.6 ± 1.0 (3.6) a	0.0008
Leu	65.4 ± 0.3 (6.7) b	64.5 ± 1.4 (6.5) b	70.1 ± 1.8 (7.0) a	0.0044
Phe	38.7 ± 0.4 (3.1) b	47.7 ± 0.5 (3.8) a	39.7 ± 1.1 (3.2) b	<0.0001
Tyr	41.1 ± 1.7 (3.0) c	45.5 ± 1.1 (3.3) ab	48.2 ± 2.7 (3.5) a	0.0113
Pro	49.7 ± 2.8 (5.8) b	56.0 ± 3.0 (6.5) ab	58.2 ± 2.2 (6.6) a	0.0200
His	36.5 ± 0.2 (2.5) b	33.3 ± 1.1 (2.3) c	38.8 ± 1.1 (2.7) a	0.0008
Lys	101.2 ± 3.8 (9.3)	96.5 ± 2.5 (8.8)	101.1 ± 2.7 (9.1)	0.1752
Arg	111.5 ± 2.4 (8.6) a	106.2 ± 4.2 (8.1) a	89.4 ± 0.9 (6.7) b	0.0002
NH_3	2.9 ± 0.3 (2.2) c	3.8 ± 0.2 (3.0) b	4.7 ± 0.2 (3.6) a	<0.0001
total	944.4 ± 6.3	931.2 ± 13.5	919.5 ± 15.7	

[a] In mg/g protein and % mole fraction in parentheses. [b] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006). Mean values with different letters in the same row are significantly different (*P*-value <0.05).

for thermo stability of the protein fractions are Ile (29.5−35.6 mg/g protein) and Pro (49.7−58.2 mg/g protein) residues. A greater amount of these amino acids, that are responsible for a better packing of the interior core of proteins, would lead to a more stable protein (*32*,*33*). The total amount of Pro and Ile residues in albumin, globulin, and glutelin were 9.2, 9.5, and 10.2% mole fraction, respectively.

Amino acid profiles may also provide useful information on their nutritive value. The nutritive value of a protein is based on its essential amino acid contents. FAO/WHO/UNU (*34*) recommended preschool children (2−5 years, a safe level for all age groups) to have protein diets containing at least 34, 35, 25, 28, 66, 63, and 58 mg/g protein for Thr, Val, (Met + Cys), Ile, Leu, (Phe + Tyr), and Lys, respectively. All essential amino acids of the bitter melon fractions with the exception of Thr met the minimum requirements. Thr, Val, (Met + Cys), Ile, Leu, (Phe + Tyr), and Lys of the protein fractions ranged from 13.6 to 18.1, 38.7 to 45.5, 39.4 to 51.7, 29.5 to 35.6, 64.5 to 70.1, 79.8 to 93.2, and 96.5 to 101.2 mg/g protein, respectively. These essential amino acid contents are comparable to those from proteins of cereal grains that contain 7−15, 29−44, 33−39, 65−136, 75−95, and 24−42 mg/g protein for Thr, Val, (Met + Cys), Ile, Leu, (Phe + Tyr), and Lys, respectively (*17*), or soy protein that contains 18.3−21.0, 36.4−37.1, 2.7−9.3, 29.7 −32.8, 45.8−51.7, 50.9−65.1, and 41.9−43.4 mg/g protein for Thr, Val, (Met + Cys), Ile, Leu, (Phe + Tyr), and Lys, respectively (*10*).

Conclusions. This is the first time that evaluation of protein fractions from bitter melon seeds based on their solubilities has been studied. The seeds contained albumin (49.3%), followed by globulin (29.3%) and glutelin (3.1%). No prolamin was detected, while 18.3% of the protein was nonextractable. All the protein fractions had only 3 bands demonstrating possible simplicity of their protein molecules. The denaturation temperatures, which were 112.0, 117.3, and 133.6 °C for albumin, globulin, and glutelin, respectively, are relatively higher than those from other plant proteins which are mostly lower than 100 °C. The hydrophobicity of globulin fraction was higher on the surface than other fractions, followed by albumin, and glutelin. All the essential amino acids of the bitter melon protein fractions with exception of Thr met the minimum requirements for preschool children by FAO/WHO/UNU. Based on their higher denaturation temperature, these protein fractions probably are suitable in specific products where the native form is needed, because they can resist higher temperature during processing. In addition, this information is probably useful to provide a basis for functional and structural studies of protein from bitter melon seed.

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