Effects of Cooking and Screw-pressing on Functional Properties of *Cuphea* PSR23 Seed Proteins

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ABSTRACT: This investigation determined the effects of oil processing conditions on some functional properties of *Cuphea* PSR23 seed proteins to evaluate their potential for value-added uses. Flaked *Cuphea* seeds were cooked at 82°C (180°F) for 30, 75, or 120 min in the seed conditioner and then screw-pressed to extract the oil. Cooked flakes and press cakes were analyzed for proximate composition and protein functional properties. Results were compared with those of unprocessed ground, defatted *Cuphea* seeds. Protein from unprocessed *Cuphea* seeds had excellent emulsifying properties, poor foaming properties, poor solubility (10%) at pH 4–7, and much greater solubility at pH 2 and 10 (57 and 88%, respectively). Solubility profiles showed that cooking the flaked seeds to 82°C for 30 min resulted in a 50–60% reduction in soluble proteins. Cooking for 120 min gave <6% soluble proteins at all pH levels. Cooking for 75 min gave good oil yields but also resulted in <10% soluble proteins at pH 2–7 and 25% soluble proteins at pH 10. Seed cooking and screw pressing during oil extraction had significant detrimental effects on the solubility of *Cuphea* seed protein but generally improved its foaming capacity and emulsifying activity.

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KEY WORDS: *Cuphea, Cuphea* protein, functional properties, oil processing.

There is great interest in developing the *Cuphea* plant as an alternative source of industrial oil. Its seed produces 16–42% oil that is rich in medium-chain FA (MCFA), such as caprylic, capric, lauric, myristic, and palmitic acids (1,2). These MCFA are used in detergents, cosmetics, lubricants, and fuels. The current commercial sources of MCFA are the tropical oilseeds (coconut and palm kernel oils). *Cuphea* oil has a strong potential to augment or replace these imported sources of MCFA (1).

Cuphea, however, has some undesirable agronomic traits that are considered as major deterrents to its domestication and commercialization, including: indeterminate growth and flowering patterns, excessive seed shattering from maturing pods, and the presence of sticky substances on leaves and stems that create harvesting problems (2,3). Domestication studies at Oregon State University (Corvallis, OR) identified *C. lanceolata*, *C. wrightii*, and *C. viscosissima* as the most promising species for cultivation (3,4). Breeding efforts at the same institution produced PSR23, a semidomesticated hybrid from *C. lanceolata* and *C. viscosissima* that exhibits partial seed retention on maturity and contains high amounts of capric acid (4). *Cuphea* PSR23 has been subjected to field testing for the past 5 yr.

If *Cuphea* oil production is successful, then it is anticipated that protein-rich meals will be also be generated because the seed contains as much as 25% crude protein (CP) (2). Current literature on *Cuphea* reports only the amount of protein in the whole seed. There is no information available on the quality and properties of *Cuphea* seed proteins. Recently, our research team completed a pioneering study that determined the SDS-PAGE profile, soluble classes, and amino acid composition of *Cuphea* seed protein. We detected six major subunits, all resolving at less than 100 kDa, comprising the reduced proteins of the whole seed. We also observed that protein solubility was greatest at pH ≥ 10 and the dominant protein classes were the alkali-soluble and water-soluble fractions (accounting for 83 and 15%, respectively, of the total protein extracted) (Evangelista, R.L., Y.V. Wu, and M.P. Hojilla-Evangelista, unpublished data). We noted that the distribution of protein soluble classes in *Cuphea* was between those of cereal proteins (8–16% CP, with 28% albumins + globulins, 40% glutelins, and 33% prolamins) and high-protein seeds (26–42% CP; with 92% albumins + globulins, 7% glutelins and <1% prolamins) reported by Nikokyris and Kandylis (5). The present study expands our previous work and was conducted to determine the functional properties of *Cuphea* seed protein, how they were affected by oil processing conditions, and what possible uses *Cuphea* meal may have in value-added applications.

EXPERIMENTAL PROCEDURES

Starting materials. This study used *Cuphea* PSR23 (*C. viscosissima* Jacq. × *C. lanceolata* W.T. Aiton) seeds from the 2003 harvest in Central Illinois that had been dried to 11.6% moisture content in a Grain Technology 245XL Grain Dryer (GT Mfg., Inc., Clay City, KS) according to the method described by Cermak *et al.* (6). Seeds were flaked to 0.25 mm (0.01 in.) thickness by using a Roskamp flaking mill (Model SP900-12; CPM Roskamp Champion, Waterloo, IA). Cooked flakes were obtained by heating the flaked seeds to 82ºC (180ºF) in the laboratory seed conditioner (Model 324; French Oil Mill Machinery Co., Piqua, OH). Residence times in the conditioner were 30 min (time needed to reach 82ºC), 75 min, and 120 min. Cooked flakes were thus designated as CF30, CF75, and

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CF120, respectively. Their corresponding press cakes (PC) were obtained after oil extraction with a heavy-duty laboratory screw-press (Model L-250; French Oil Mill Machinery Co.).

Proximate analyses. Unprocessed *Cuphea* PSR23 seeds, CF, and PC were ground into *ca*. 30-mesh particle size by using a coffee grinder (Model DCG-12BC; Cuisinart, East Windsor, NJ) for 2 min. It was not necessary to dry the samples before proximate analyses. Moisture, CP (%N × 6.25), and crude oil contents of the samples were determined by using AOCS standard methods Ba 2a-38, Ba 4e-93, and Ba 3-38, respectively (7).

SDS-PAGE. SDS-PAGE of reduced proteins was done by following the method described by Wu and Hojilla-Evangelista (8). Ground, unprocessed *Cuphea* PSR23 seeds, CF, and PC were weighed out to provide 4 mg protein/mL in 500 μL of solubilization buffer (42 nM Tris-HCl (pH 6.8), 2% SDS, 7% glycerol, 4.4% β-mercaptoethanol, and 5 M urea), and then heated in a boiling-water bath for 5 min. Protein samples (20 μL) were loaded onto 4–12% Bis-Tris NuPAGE pre-cast gradient gel (Invitrogen Corp., Carlsbad, CA). Bio-Rad (Bio-Rad Laboratories, Hercules, CA) pre-stained broad-range SDS-PAGE protein standards (6.5–196 kDa) were included in the gel. Electrophoresis was done in a Novex XCell II Mini Cell system (Novex, San Diego, CA) in the presence of NuPAGE MES-SDS running buffer (SDS, Tris, and 4-morpholinoethane sulfonic acid).

Native gel electrophoresis. Ground, unprocessed *Cuphea* PSR23 seeds, CF, and PC were weighed into centrifuge tubes to provide 4 mg protein/mL in 500 μL of commercial native sample buffer (Invitrogen Tris-glycine native sample buffer, pH 8.6, containing 100 mM Tris HCl, 10% glycerol, 0.0025% bromophenol blue) (Invitrogen Corp.). The tubes were shaken for 10 min on a platform shaker and then centrifuged at 5,000 × *g* for 5 min. Supernatants (20 μL) were loaded onto pre-cast Novex™ Tris-Glycine 6–18% gradient gel. Invitrogen NativeMark™ unstained protein standards, with M.W. ranging from 20 to 1236 kDa, were included in the gel. Electrophoresis was done in an Invitrogen XCell SureLock™ Mini-Cell system at 125 V and running time of 78 min. The running buffer was Invitrogen Tris-glycine native running buffer (25 mM Tris base, 192 mM glycine), pH 8.3, which was diluted to 10× volume with nanopure water before use.

Functionality tests. Ground unprocessed *Cuphea* seeds, CF, and PC were first defatted by hexane extraction at 25°C. In each extraction, solvent was added to the sample (10 mL: 1 g) and the mixture was stirred for 1 h with a magnetic bar. The mixture was allowed to stand until the supernatant had cleared, and then the solvent layer was pipetted out and discarded. Extraction was repeated three more times until residual oil content was ≤0.5% (dry basis). Defatted ground samples were air-dried in a fume hood until the hexane smell has dissipated completely and then stored in screw-capped vials at room temperature before use.

(i) Solubility profiles. Solubilities of samples (10 mg protein/mL) were determined at pH 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0 using the method of Balmaceda *et al.* (9), except that the yellow-green to greenish-brown supernatants were freeze-dried and their nitrogen contents were determined by the Dumas combustion method [AOCS Ba 4e-93 (7)].

(ii) Foaming properties. Foam capacity and stability of samples (10 mg protein/mL) were determined at the pH where protein solubility was greatest by following exactly the procedure described by Myers *et al.* (10). Foam capacity was the volume (mL) of foam produced in 1 min. Foam stability was expressed as the % foam remaining after standing for 15 min.

(iii) Emulsifying properties. Emulsification activity index (EAI, in m^2/g protein) and emulsion stability index (ESI, in min) were determined by using the method of Wu *et al.* (11). Emulsions were prepared by homogenizing mixtures of 6 mL of sample solutions (1 mg protein/mL) and 2 mL of corn oil with a hand-held homogenizer operated at high setting (20,000 rpm) for 1 min.

(iv) Water-holding capacity (WHC). WHC of the samples was determined according to the procedure for insoluble or partly soluble materials by Balmaceda *et al.* (9). The sample amount was reduced to 0.5 g, and the amount of distilled water added was adjusted accordingly (15 mL). All other steps and calculations were done exactly as described in the original method.

Statistical analyses. Statistical analyses were performed by using the SAS® Systems for Windows software (SAS Institute Inc., Cary, NC). ANOVA and Duncan's Multiple Range tests were performed on duplicate replications of data to determine significant differences among the treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate composition. Unprocessed *Cuphea* seed contained substantial amounts of crude oil and CP (Table 1). The oil content of *Cuphea* seed was similar to that of whole soybeans (20%) and of delinted cottonseed (21.6%) (11) but was less than the 28–35% we determined for mature, density-graded seeds we used in another study (12). The protein content of *Cuphea* seed was also similar to that of cottonseed but only half that of soybeans (13). Moisture contents of the flaked seeds decreased significantly when they were held for longer periods in the seed conditioner, as would be expected (Table 1). Additional moisture was lost during pressing. Heating the flaked seed to 82ºC (CF30) had no effect on the oil content. Oil contents of flakes cooked for 75 or 120 min were only slightly less than that of the unprocessed *Cuphea* seed (Table 1). PC of the flaked seeds all contained <10% oil after screw-pressing. CP contents of CF and PC remained essentially unchanged under the oil processing conditions used (Table 1).

Electrophoresis results. Reduced protein bands for unprocessed *Cuphea* seed were well-defined and resolved between 6 and 96 kDa (Fig. 1, lane 2). The dominant subunits, represented by the darkest bands, were estimated to have M.W. of 15, 30, 40, and 50 kDa. These protein fractions were still heavily concentrated in the cooked and screw-pressed *Cuphea* samples (lanes 3–8). There were indications, though, that heating caused some modifications, such as the faintness or disap-

TABLE 1
Partial Proximate Composition of Ground *Cuphea* Seeds, Flakes Cooked for Various Times (CF), and Corresponding Press Cakes (PC)[a]

Sample	Moisture (%)	Crude fat (% db)	Crude protein[b] (% db)	Crude protein (% db, ffb[c])
Cuphea seed	10.0[a]	21.0[a]	19.3[a,b]	24.4[a]
CF30	6.8[b]	20.3[a,b]	17.4[b]	21.8[a]
CF75	4.7[d]	19.5[b]	17.4[b]	21.6[a]
CF120	2.3[f]	19.2[b]	16.6[b]	20.6[a]
PC30	5.5[c]	8.3[c,d]	23.2[a]	25.3[a]
PC75	3.2[e]	7.4[d]	21.6[a,b]	23.3[a]
PC120	2.1[f]	9.0[c]	21.1[a,b]	23.1[a]

[a]Values are means of duplicate determinations. Means within a column followed by different letters are significantly different ($P < 0.05$).
[b]Dumas N × 6.25.
[c]Abbreviations: db, dry basis; ffb, fat-free basis.

pearance of the 6 kDa (lanes 3–6 and 8) and 90 kDa (lanes 3,4, 6, and 8) bands, loss of the minor 36 kDa fraction, and the slight narrowing of protein band widths in the CF and PC. These signs were most pronounced in protein from PC produced from flakes that were cooked the longest (PC120, lane 8).

Effects of heating on *Cuphea* proteins were more clearly evident from band patterns observed in the native gel (Fig. 2). The most prominent subunit in unprocessed *Cuphea* seed, which resolved between 242 and 480 kDa (Fig. 2, lane 2), gave narrower and less-defined corresponding bands in the CF and PC (lanes 3–7). This major subunit was no longer present in the PC120 sample (lane 8). Similarly, the subunit in unprocessed *Cuphea* seed that resolved at around 500 kDa (lane 2) became less defined in the heated samples (lanes 3–7) and was also gone in PC120 (lane 8). The high-M.W. protein fraction (approx. 1236 kDa, lane 2) was apparently not markedly affected during cooking, as indicated by the still-dark colors and similar widths of protein bands in the CF (lanes 3, 5, and 7). This high-M.W. protein still gave a dark band in PC30 (lane 4) but was noticeably fainter in PC75 and PC120 (lanes 6 and 8, respectively). These results provide additional evidence that *Cuphea* proteins were severely affected by heat during cooking and oil pressing.

It is well-established that thermal treatments of proteins result in structural changes, hydrolysis of peptide bonds, sidechain modifications, and condensation with other molecules,

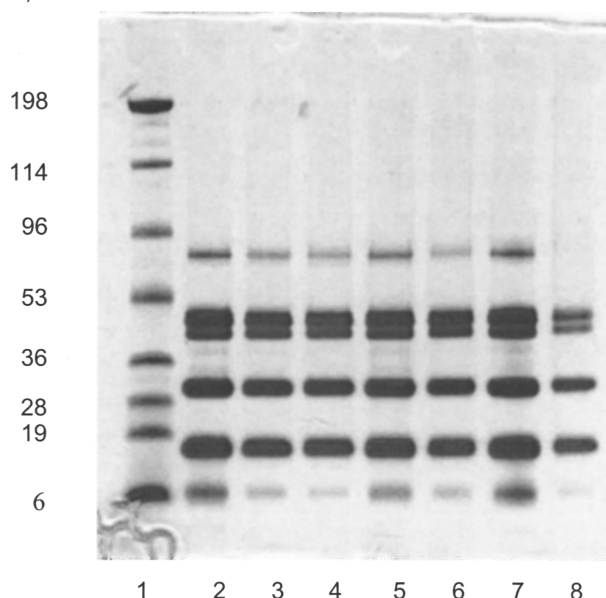

FIG. 1. SDS-PAGE profiles of *Cuphea* seed, cooked flakes (CF), and press cakes (PC): (1) M.W. standards; (2) ground, defatted *Cuphea* seed; (3) CF30; (4) PC30; (5) CF75; (6) PC75; (7) CF120; and, (8) PC120. Concentration = 4 mg protein/mL; sample load volume = 20 μL.

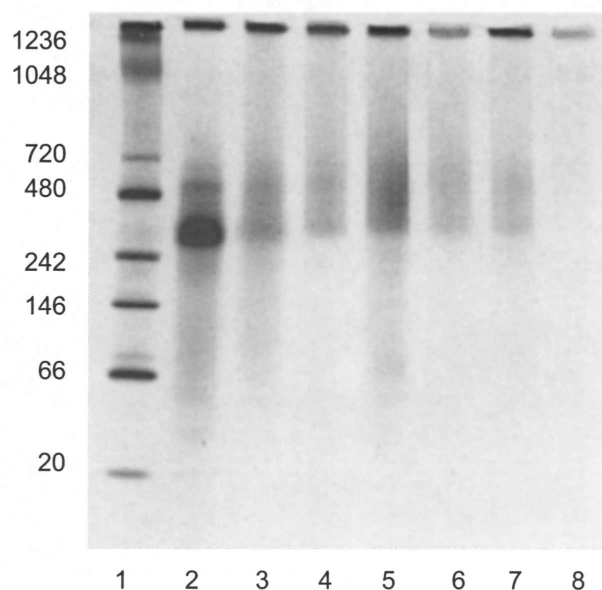

FIG. 2. Native gel protein band patterns of *Cuphea* seed, CF, and PC: (1) M.W. standards; (2) ground, defatted *Cuphea* seed; (3) CF30; (4) PC30; (5) CF75; (6) PC75; (7) CF120; and, (8) PC120. Concentration = 4 mg protein/ mL; sample load volume = 20 μL. For abbreviations see Figure 1.

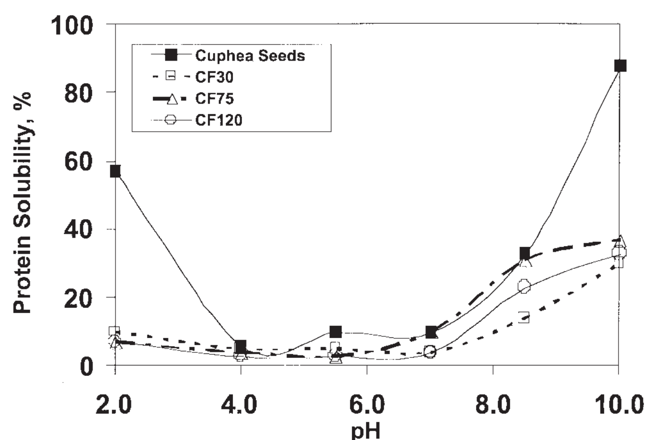

FIG. 3. Solubility profiles of ground, defatted *Cuphea* seed and cooked flakes (CF) heated for 30, 75, and 120 min in the seed conditioner.

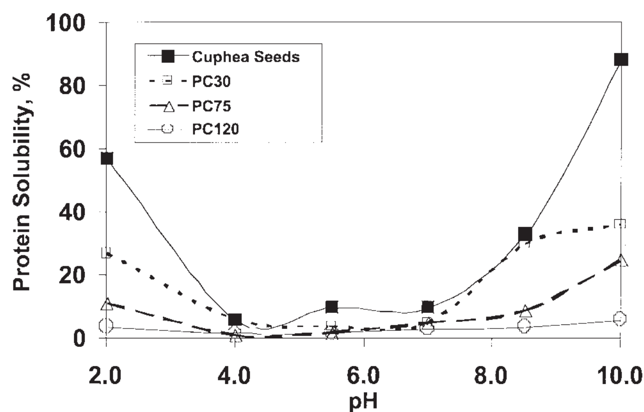

FIG. 4. Solubility profiles of ground, defatted *Cuphea* seed and press cakes (PC) from flakes cooked in the seed conditioner for 30, 75, and 120 min.

depending on the intensity and duration of heating, pH, presence of salts, water activity, and other factors. The structural changes and limited hydrolysis of peptide bonds have a significant influence on protein functionality (14).

Protein solubility. Solubility properties have practical usefulness in determining the conditions for extracting and purifying proteins from natural sources and for fractionating the protein subunits (14). Solubility behavior also serves as a good index of the potential applications of the protein, because insolubility is often a measure of denaturation and denatured or aggregated proteins have frequently shown impaired abilities to gel, emulsify, or foam effectively (14).

The protein in unprocessed *Cuphea* seeds had poor solubility (10%) at pH 4–7 (Fig. 3), which may be due to a highly cross-linked structure as indicated by the high M.W. of the native protein fractions (Fig. 2, lane 2). At pH 2 and 10, *Cuphea* protein solubility was 57 and 88%, respectively (Fig. 3). The almost 90% solubility of *Cuphea* seed proteins at alkaline pH was markedly greater than those of commercial soybean flour proteins (50–70%) that were tested for soy-based plywood glues (15). This finding also implies that *Cuphea* seed proteins may have some use in applications having alkaline environments, such as wood adhesives. Because the greatest solubility was observed at pH 10, the other functional properties were evaluated at this pH. However, we caution that the results may not be applicable to foods because the pH values of most food systems are below pH 10. The data will likely be useful for industrial nonfood applications.

Solubility profiles of proteins from CF (Fig. 3) showed that simply heating the flaked seeds to 82ºC (CF30) resulted in 50–60% reduction in soluble proteins at pH 2, 8.5, and 10. Cooking for 75 min at 82°C resulted in less than 5% soluble proteins at pH 2–7 and 37% soluble proteins at pH 10. CF120 samples gave solubilities that were only slightly less than those of CF75. Most proteins undergo significant and irreversible reduction in solubility when subjected to heat, because of the exposure of hydrophobic groups and the consequent aggregation of the unfolded protein molecule (14,16).

The solubility profiles of the PC (Fig. 4) also showed significant reductions in the amounts of soluble proteins at almost all pH levels. There was 50–65% less soluble protein from the PC30 samples at all pH values except at pH 8.5. The reductions in soluble proteins were even more pronounced in the PC75 and PC120 samples, especially for the latter, where only 2–6% soluble proteins were determined at all pH levels. These results demonstrated the deleterious effects of heat on *Cuphea* protein

TABLE 2
Crude Protein Contents and Selected Functional Properties of Ground *Cuphea* Seeds, Cooked Flakes (CF), and Press Cakes (PC)[a]

		Functional Properties[b] at pH 10				
	Crude protein	FC	FS	EAI	ESI	WHC
Sample	(% db, ffb)	(mL)	(% foam left)	(m^2/g)	(min)	(g water/g protein)
Cuphea seed	24.4 ± 4.7[a]	49 ± 2[f]	0.0 ± 0.0[a]	33.8 ± 0.6[d]	31.4 ± 1.5[a]	3.40 ± 0.00[a]
CF30	21.9 ± 0.1[a]	128 ± 0[a]	0.8 ± 1.0[a]	61.9 ± 4.2[b]	16.5 ± 3.1[b]	3.50 ± 0.00[a]
CF75	20.6 ± 0.6[a]	62 ± 1[e]	0.0 ± 0.0[a]	47.4 ± 0.7[c]	23.4 ± 1.2[a,b]	3.36 ± 0.00[a]
CF120	20.6 ± 4.8[a]	104 ± 8[b]	0.0 ± 0.0[a]	78.7 ± 2.7[a]	12.9 ± 0.0[b]	3.76 ± 0.00[a]
PC30	25.2 ± 0.8[a]	84 ± 0[c,d]	0.0 ± 0.0[a]	75.7 ± 6.3[a]	13.2 ± 0.3[b]	2.80 ± 0.10[c]
PC75	23.3 ± 0.1[a]	78 ± 6[d]	0.6 ± 0.9[a]	77.5 ± 1.6[a]	13.0 ± 0.4[b]	2.79 ± 0.14[c]
PC120	23.1 ± 0.4[a]	92 ± 0[c]	3.8 ± 3.8[a]	67.9 ± 6.3[b]	13.6 ± 0.0[b]	3.02 ± 0.03[b]

[a]Values are means ± SD of duplicate determinations. Means within a column followed by different letters are significantly different ($P < 0.05$).
[b]FC, foaming capacity; FS, foam stability; EAI, emulsion activity index; ESI, emulsion stability index; WHC, water-holding capacity.

solubility and further supported our findings from native gel electrophoresis (Fig. 2).

Foaming properties. *Cuphea* seed proteins did not produce much foam (Table 2), and the foam disintegrated quickly. The foaming behavior of *Cuphea* protein was notably inferior to that of soybean flour with a foaming capacity of 136 mL and foam stability of 96% remaining foam after 15 min standing (Hojilla-Evangelista, M., unpublished data). Kinsella (16) explained that in protein foams, the surfactant protein must perform two functions concurrently for film formation to occur: (i) reduce interfacial tension of the liquid, and (ii) form continuous, cohesive, elastic films around air droplets. The second function requires some degree of surface denaturation, which is achieved during aeration, but complete denaturation is undesirable because it results in foam collapse. *Cuphea* protein evidently does not have the capability to perform these two functions effectively, as shown by its poor foaming properties.

Cooking the flakes markedly improved the foaming capacity of the protein, especially at 30 min residence time (Table 2). Proteins from the PC still had greater foam capacity than the protein from unprocessed ground seeds, which further showed the beneficial effects of heating on foaming ability. Heat-induced unfolding of the protein, provided it is not accompanied by aggregation and loss of solubility, results in improved protein orientation at the interface and greater foam capacity (14). All the heated *Cuphea* samples, however, produced foams that collapsed immediately (Table 2). Foam instability arises when there are limited protein–protein interactions at the interface, resulting in weak films that are unable to prevent the leakage of lamellar fluid (17).

Emulsifying properties. An indicator of a protein's emulsifying capacity is the EAI, which measures the area of oil–water interface stabilized by a unit weight of protein (11). Higher EAI values indicate better emulsifying capacity. The EAI value at pH 10 for unprocessed *Cuphea* proteins (Table 2) was more than twice that of low-fat soy flour determined by Heywood *et al.* (18), but 25 and 50% less than the EAI for acid-precipitated lupin and soybean proteins, respectively, reported by Hojilla-Evangelista *et al.* (19). Heating the flaked seeds (in the conditioner and screw press) increased EAI values by at least 40% (CF75) and as much as 133% (CF120). When heat treatment can induce unfolding of protein structure without aggregation, the result is improved emulsifying capacity, which is believed to be due to the increased amphipolarity of the initially highly hydrophilic protein (14).

The ESI value for unprocessed *Cuphea* seed protein (Table 2) was double that observed for acid-precipitated soybean protein (19), which implied that emulsions formed by *Cuphea* protein were more stable than those formed by soybean protein. Cooking the flakes and screw-pressing reduced ESI values by 25–59% (Table 2), suggesting that *Cuphea* protein subjected to prior heating formed less stable emulsions. With the reduced protein solubilities in the CF and PC, there may have been insufficient soluble proteins that could adsorb at the oil/water interface to produce a strong interfacial membrane and prevent coalescence of the oil droplets, leading to unstable emulsions (14). If protein aggregation occurs because of heating, nonpolar groups also become unavailable for the hydrophobic interactions at the oil–water interface and emulsion stability is impaired (17).

WHC. The WHC of proteins is an important functional property in viscous foods, such as doughs, comminuted meats, soups, and processed cheeses. WHC varies with protein source and is influenced by pH; presence of carbohydrates, lipids, or salts; and previous processing treatment (16). Heating generally decreases protein WHC, because the subsequent denaturation and aggregation reduce protein surface area and availability of polar amino groups for hydrogen bonding with water molecules (14). This may explain why protein from *Cuphea* PC had less WHC than the control (Table 2). In some instances, heating may improve WHC, because the resulting unfolding of the protein's compact structure exposes polar side chains that can bind water. We did not observe any such increase in WHC values among our samples.

Cuphea seed proteins may find some use in nonfood industrial applications because of their very high solubility at alkaline pH. Exposure to heat during seed cooking and screw-pressing had significant detrimental effects on the solubility of *Cuphea* seed protein, which may limit the usefulness of *Cuphea* PC as a protein source in nonfood applications. Heat treatment improved foam capacity and emulsifying activity of *Cuphea* seed proteins but reduced emulsion stability and WHC.

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