Characterization of Proteins in *Cuphea* (PSR23) Seeds

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ABSTRACT: This study characterized the proteins in *Cuphea* (PSR23) seed to provide fundamental information on their size, amino acid profile, solubility classes, and solubility behavior. The seed contained 32% (dry basis, db) oil and 21% (db) crude protein. Over 70% of the protein was extracted at pH 11.6. Nonprotein nitrogen accounted for 9% of the total N content. Compared with the Food and Agriculture Organization/World Health Organization/United Nations University suggested pattern of requirements, *Cuphea* PSR23 seed protein had sufficient amounts of methionine + cystine-cysteine, considerable amounts (90%) of valine, phenylalanine + tyrosine, but was practically devoid of tryptophan. Lysine was the second-most limiting essential amino acid at 68%. Glutelins and albumins accounted for 83.5 and 15.4%, respectively, of the total protein extracted. SDS-PAGE showed that *Cuphea* protein subunits had M.W. ranging from <6.5 to 110 kDa. Dominant protein subunits in albumins had M.W. of 30, 40, 50, and 86 kDa. Glutelins had two major protein subunits with M.W. of 15 and 30 kDa. The distribution of essential amino acids was better in the albumin and glutelin fractions than in the defatted meal.

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KEY WORDS: Amino acid composition, *Cuphea*, fractionation, protein, solubility.

The USDA conducted an extensive program in the late 1950s to search for new industrial raw materials from plants for which little or nothing was known of their composition. Such raw materials would fill a present or anticipated need and would not be in competition with presently grown crops, especially those now in surplus supply. Plant constituents with industrial significance include cellulosic fibers, proteins, oils of special composition, and polysaccharides other than starch (1).

The first report of a high concentration of capric acid in seed oil of herbaceous plants was made by Earle *et al.* (2). They discovered that the oil from seeds of *Cuphea llavea* var. *miniata* had a very low iodine value, indicating that most of the FA were saturated. Analyses of the FAME showed that 83% of the FA was capric. They realized then the potential of *Cuphea* as a domestic source of capric and lauric acid, and they re-collected *Cuphea llavea* and other species of the genus. *Cuphea* of the family Lythraceae is a large genus of over 200 species of herbs and shrubs found in the tropics and subtropics of the Americas (3). Several *Cuphea* species had been identified to contain saturated medium-chain FA (MCFA) (3–5). MCFA (C8:0–C12:0) are used in soaps, detergents, cosmetics, lubricants, and food applications. Half of the MCFA used by the U.S. soap and detergent industry is obtained from coconut and palm kernel oils, while the other half is from petroleum (6).

Efforts to domesticate *Cuphea* have progressed over the past three decades. Seed shattering and seed dormancy have been the main hurdles in its successful commercialization (7,8). A semidomesticated, high-capric acid variety with partial seed retention (PSR) was reported by Knapp in 1993 (9). *Cuphea* PSR23 is a hybrid between *C. viscosissima* (a species native to the United States) and *C. lanceolata* (a species native to Mexico). The seeds weigh 538 g/L (3.3 g/1,000 seeds) and contain up to 35% oil. The oil contains 69.6% capric, 9.4% oleic, 5.9% palmitic, 4.8% linoleic, 4.4% myristic, and 2.9% lauric acids (10). *Cuphea* PSR23 has been the subject of field studies for the past 5 yr in west-central Minnesota and central Illinois to establish the best agronomic management practices in preparation for its commercial production (11–15).

Currently, the seeds are processed mainly for oil to support research needs, as well as for product development and testing. Basic information on the seed's protein is lacking or not available in the literature. This study was conducted to determine some properties of *Cuphea* seed protein, such as solubility, solubility classes, and amino acid composition, which may be helpful in identifying value-added uses for *Cuphea*.

EXPERIMENTAL PROCEDURES

Defatted meal preparation. Cuphea PSR23 seeds used in this study were from the 2003 harvest in central Illinois. Clean seeds were ground through a 30-mesh screen by using a micromill (Glen Mills, Inc., Clifton, NJ). The ground seed meal (300 g) was extracted twice with hexane (1 g/10 mL, meal/hexane ratio) at room temperature. After air-drying, the defatted meal was ground through a 60-mesh screen and then extracted again with hexane. The defatted meal was air-dried to remove residual hexane. Crude protein (CP; % N × 6.25), oil, and moisture contents of the full-fat and defatted meals were determined.

Nitrogen solubility. Nitrogen solubility was determined as described by Wolf *et al.* (16) with minor modifications. Defatted meal (300 mg) was mixed with 25 mL distilled water. The pH was adjusted to the desired level by using 1 N HCl or 1 N NaOH and adjusted to a final volume of 30 mL by adding distilled water. The slurry was stirred for 20 min, and then the final pH was measured. The slurry was centrifuged at 10,690 × *g* for

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20 min. The supernatant was analyzed for nitrogen content, and the percentage of soluble nitrogen was calculated. Non-protein nitrogen (NPN) was determined by mixing 200 mg of defatted meal with 20 mL of TCA solution at different concentrations (0–4 M) for 20 min. The slurry was centrifuged at 10,690 × *g* for 20 min. The supernatant was analyzed for nitrogen content, and the percent soluble nitrogen was calculated.

Protein fractionation. Protein was extracted from the defatted meal by following the method of Hu and Esen (17), modified to obtain classic Osborne fractions (Fig. 1). The defatted meal was extracted sequentially with water (at 5°C to minimize possible proteolysis), 0.5 M NaCl (5°C), 70% ethanol, and 0.1 M NaOH to obtain albumin, globulin, prolamin, and glutelin fractions, respectively, which were then freeze-dried. The defatted meal, freeze-dried extracts, and the spent solids were analyzed for N content and amino acid composition.

SDS-PAGE was performed according to the Sessa and Wolf modification (18) of the Fling and Gregerson method (19). Solutions containing 5 mg protein/mL of buffer (containing Tris-HCl, SDS, glycerol, β-mercaptoethanol, and urea) were prepared using protein extracts from defatted meal. After heating the protein solutions in a boiling-water bath for 5 min, 15 μL per sample was loaded onto 4–12% Bis-Tris NuPAGE pre-cast gradient gels (Invitrogen Corp., Carlsbad, CA). Pre-stained broad-range SDS-PAGE protein standards (6.5–196 kDa) (Bio-Rad Laboratories, Hercules, CA) were also loaded onto the gel. Electrophoresis was done in a Novex Xcell II Mini Gel system (Novex, San Diego, CA) and using the NuPaAGE MES-SDS running buffer (SDS, Tris, and 4-morpholinoethane sulfonic acid).

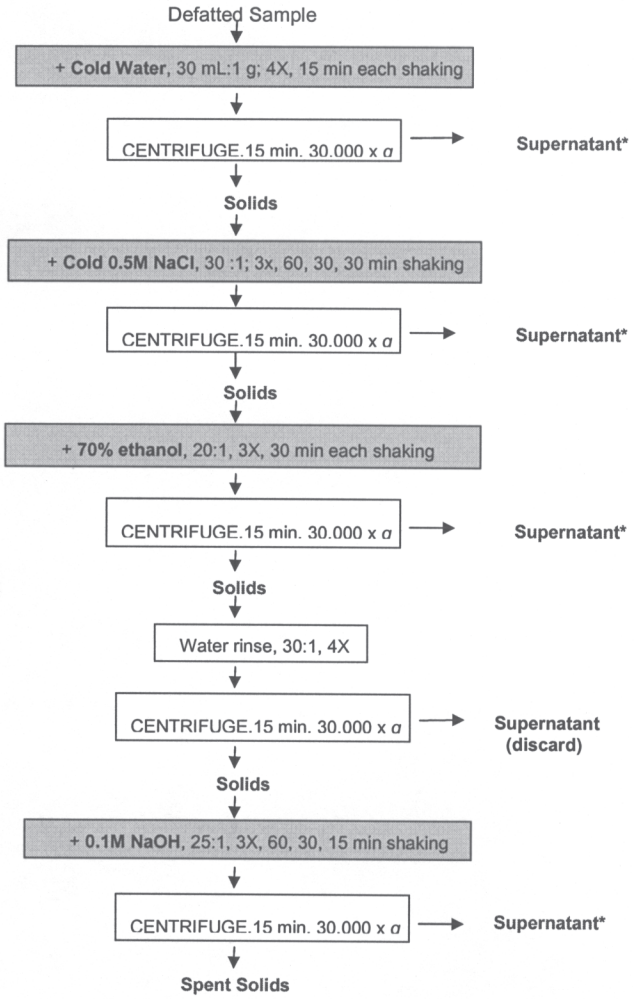

FIG. 1. Scheme for protein fractionation.

Analyses. Moisture and crude oil contents in ground seeds and defatted seeds were determined according to AOCS official methods Ba 2a-38 and Ba 3-38 (20), respectively. CP contents of the ground seeds, defatted meal, protein extracts, and spent solids were determined by the Dumas combustion method (AOCS Ba 4e-93) (20). Amino acids were determined by cation exchange chromatography (Beckman 6300 amino acid analyzer; Beckman Instruments, San Ramon, CA). The samples were first hydrolyzed in 6 N HCl for 4 h at 145°C (21). For methionine and cystine-cysteine, the samples were treated with performic acid prior to hydrolysis (22). Tryptophan was determined by a colorimetric method after enzymatic hydrolysis by pronase (23,24). All analyses were done in duplicate.

The data were analyzed using ANOVA (25), and Tukey's Studentized range test was used to determine significant differences from duplicate experiments.

RESULTS AND DISCUSSION

Cuphea PSR23 seeds used in this study contained 31.5% (dry basis: db) oil and 20.8% (db) CP. The defatted meal had a residual oil content of 0.71%. Samples of PSR23 seeds from field trials analyzed in our laboratory contained up to 35% oil and up to 22% CP.

The major amino acids of *Cuphea* PSR23 seed protein were glutamic acid, aspartic acid, and arginine (Table 1). Compared with the suggested pattern of requirements (28), *Cuphea* PSR23 protein is deficient in all essential amino acids for infants. Tryptophan, lysine, and leucine are also inadequate for children between 10 and 12 yr old, but it lacks only in tryptophan for adults. *Cuphea* PSR23 protein is practically devoid of tryptophan. Lysine is the second-most limiting essential amino acid. In comparison, protein in soybean flour is adequate in all essential amino acids except for methionine + cystine-cysteine, and leucine for infants. Coconut flour, on the other hand, has insufficient levels of threonine, leucine, histidine, and lysine for infants but meets the requirements for children and adults. No data are available for cystine-cysteine and tryptophan in coconut flour (Table 1).

Nitrogen solubility. The solubility of nitrogen extracted from defatted meal at different pH values is shown in Figure 2. About 30% of nitrogen was soluble at pH 1.2. The lowest solubility (*ca*. 8%) was at pH values ranging from 2.5 to 4.5. Above pH 4.5, the nitrogen solubility increased at an increasing rate until pH 9.5 (approximate inflection point) where 55% N solu-

TABLE 1
Comparison of Amino Acid Composition (g/16 g N) of *Cuphea* Meal, Soybean Flour, and Coconut Flour with the FAO/WHO/UNO Suggested Pattern of Requirements

				Suggested pattern of requirement[c]		
Amino acid	*Cuphea* meal	Soybean flour[a]	Coconut flour[b]	Infant	Child[d]	Adult
Aspartic acid	7.2	11.3	9.3	—	—	—
Threonine	2.8	4.2	2.5	4.3	2.8	0.9
Serine	4.0	5.0	5.3	—	—	—
Glutamic acid	12.3	17.2	22.4	—	—	—
Proline	2.8	4.7	3.6	—	—	—
Glycine	3.8	4.0	5.1	—	—	—
Alanine	3.9	4.0	4.8	—	—	—
Valine	4.3	5.3	5.4	5.5	2.5	1.3
Cystine-cysteine	2.0	1.6	N.D.	4.2[e]	2.2[e]	1.7[e]
Methionine	1.8	1.4	1.8			
Isoleucine	3.3	4.9	4.2	4.6	2.8	1.3
Leucine	5.6	8.0	7.4	9.3	6.6	1.9
Tyrosine	2.0	3.9	1.8	7.2[f]	2.2[f]	1.9[f]
Phenylalanine	3.4	5.3	5.1			
Histidine	2.1	2.7	1.8	2.6	1.9	1.6
Lysine	3.7	6.4	4.7	6.6	4.4	1.6
Arginine	8.1	7.0	12.3	—	—	—
Tryptophan	<0.04	1.2	ND	1.7	0.9	0.5

[a]Perkins (26).
[b]Kwon *et al.* (27).
[c]Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) (28).
[d]10–12 yr old.
[e]Cystine-cysteine + methionine.
[f]Tyrosine + phenylalanine. N.D., no data.

bility was obtained. The N solubility continued to increase at a decreasing rate as pH was increased to 11.6, where 72% N solubility was attained. The seed proteins can be effectively extracted at pH >10 and recovered by isoelectric precipitation between pH 2.5 and 4.5. Compared with the nitrogen solubility of other oilseed proteins, determined using similar procedures, the minimum nitrogen solubility for *Cuphea* (8%) is lower than what was reported for defatted jojoba (25%), salicornia (22%), and lesquerella (14.6%) seed meals (16,29,30).

The NPN fraction of biological materials is generally considered to consist of free amino acids, amides, peptides, polypeptides, and other low-M.W. compounds containing nitrogen, such as alkaloids and cyanoglycosides. Extraction of NPN with TCA and precipitation of protein with TCA are the two most widely used methods (31). NPN corresponds to the minimum % N solubility within 0 to 2 M of TCA where the proteins are expected to precipitate. For defatted *Cuphea* meal, we calculated an average minimum value of 9.1% between 0.2 and 1.0 M TCA (Fig. 3). The NPN content of the *Cuphea* meal is about the same as the minimum nitrogen solubility, indicating that what was extracted between pH 2.5 and 4.5 was NPN (Fig. 2). Similar results were also reported for jojoba, salicornia, and lesquerella (16,29,30). SDS-PAGE of 0.6 M TCA extracts that were dialyzed (using membrane with 3.5 kDa M.W. cutoff) against water contained polypeptides of 12 kDa for soybean meal and 28, 12, 10, and 7 kDa for almond meal (31)

Protein fractionation. The defatted meal was extracted sequentially with water, 0.5 M NaCl, 70% ethanol, and 0.1 M NaOH to obtain albumin, globulin, prolamin, and glutelin fractions, respectively. About 58% of the total nitrogen was extracted (Table 2). Glutelin was the dominant fraction, accounting for 83.4% of the total nitrogen extracted. Another 15.4% was made up of albumins. Although the SDS-PAGE of reduced proteins showed bands with M.W. of 30 to 96 kDa (Fig. 4, lane 3), most of the nitrogen extracted by water was NPN. The pH of the water extract was 5.6, about one unit higher than where the minimum N solubility was observed (Fig. 2). At pH 5.6, the nitrogen solubility was 10.2%. *Cuphea* PSR23 protein had very small amounts of globulins and prolamins, each contributing less than 1% to the total nitrogen extracted. Most of what was extracted by water, 0.5 M NaCl, and 70% ethanol was non-

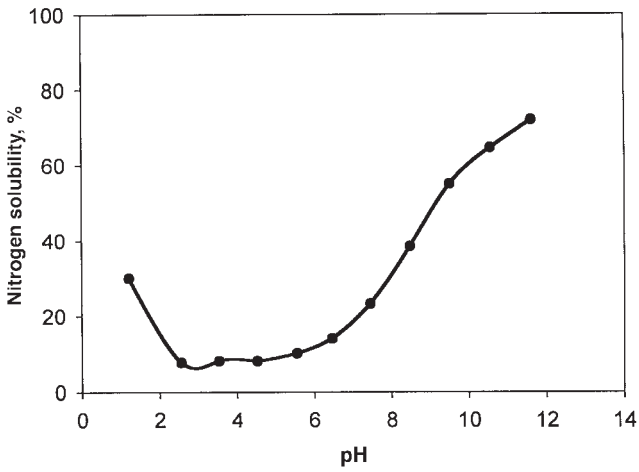

FIG. 2. Nitrogen solubility of hexane-defatted Cuphea meal as a function of pH.

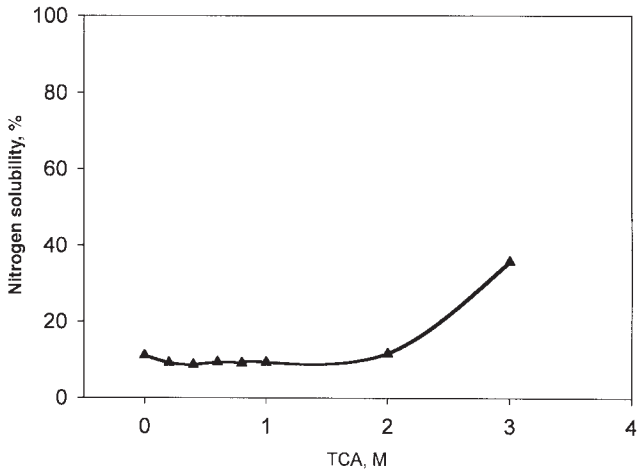

FIG. 3. Nitrogen solubility of hexane-defatted Cuphea meal as a function of TCA concentration.

protein components of the seed as indicated by their low CP contents (Table 2). Water- and dilute salt-soluble nonprotein components could be carbohydrates. Nash *et al.* (32) analyzed the nonprotein components of the ethanol (86% vol/vol) extract from soybeans and found glycerides, phospholipids, saponins, sitosterol, glycoside, and genistein.

From the protein fractionation study performed by Nikokyris and Kandylis (33) on eight high-protein seeds (26–42% CP) and 12 cereals (7.6–16.4% CP), we calculated that the protein

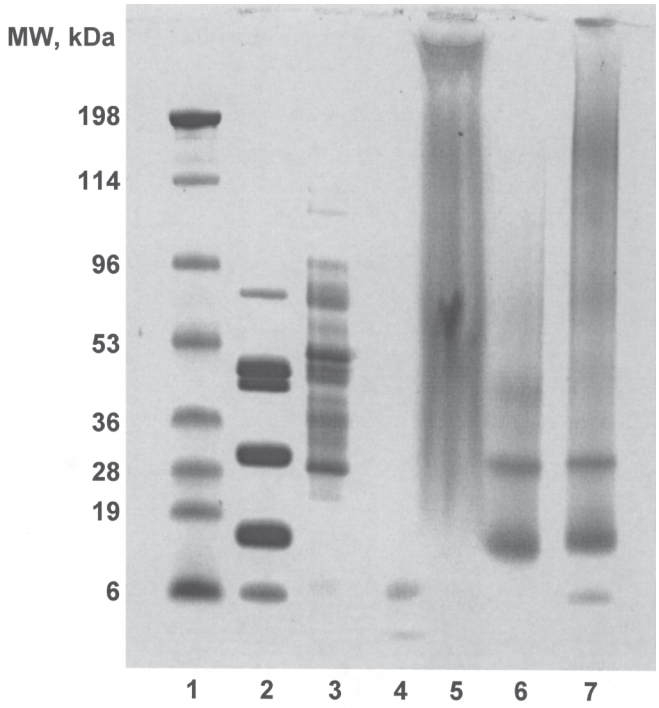

FIG. 4. SDS-PAGE of reduced defatted Cuphea meal, protein fractions and spent solids. Lane 1: MW standard; Lane 2: defatted cuphea meal; Lane 3: albumin; Lane 4: globulin; Lane 5: prolamin; Lane 6: glutelin; Lane 7: spent solids.

TABLE 2
Soluble Protein Classes in Defatted *Cuphea* Seeds

Fractions	% of total nitrogen[a]	Crude protein (%, as is)
Albumins	8.96 ± 0.72	13.20 ± 0.01
Globulins	0.41 ± 0.19	0.92 ± 0.06
Prolamins	0.24 ± 0.01	2.16 ± 0.02
Glutelins	48.56 ± 0.50	57.20 ± 2.71
Total	58.17 ± 0.90	

[a]Mean ± SD of three determinations.

extracts of high-protein seeds, on average, contained about 92% albumins and globulins, 7% glutelins, and less than 1% prolamins. Similarly, in cereals, albumins and globulins accounted for 28%, glutelins for 40%, and prolamins for 33%. Coconut flour, with CP content slightly higher than the cereals (17% CP), contained 70% albumins and globulins, 20% glutelins, and 4% prolamins (27). *Cuphea* PSR23 seed proteins, with its high glutelins and very low prolamins, is somewhere between these two types of seed.

Electrophoresis of reduced proteins from defatted meal before protein fractionation (Fig. 4, lane 2) showed bands between 6.5 and 96 kDa. The dominant protein subunits have M.W. of 15, 30, 40, and 50 kDa. The water-soluble fraction (lane 3) showed two protein subunits larger than 96 kDa that were not present in the defatted meal (lane 2). The major bands resolved around 30, 40, 50, and 86 kDa. The globulins (lane 4) consisted of two protein subunits 6.5 kDa and smaller. No distinct bands were visible in the prolamin fraction (lane 5). There was so much interference from the nonprotein components in the extract that no visible bands were observed. The glutelins (lane 6) have two major protein subunits with M.W. of 15 and 30 kDa. These protein subunits appeared to be more difficult to extract, as they were also the dominant bands in spent solids (lane7).

The amino acid composition of these soluble fractions was also determined (Table 3). The globulins and prolamins were excluded because they were present only in minute quantities. Albumin had the greatest amounts of the smaller MW amino acids (glycine, alanine and proline), as well as high amounts of polar uncharged amino acids (threonine and serine). Glutelins contained the highest quantities of the hydrophilic and hydrophobic amino acids. The albumins and glutelins have better distribution of amino acids than the defatted meal. Compared with the reference pattern (Table 1), the albumin fraction has adequate threonine and methionine + cystine-cysteine for infants; is insufficient in leucine, histidine, and tryptophan for children but is deficient only in tryptophan for adults. The glutelin fraction did not meet the amino acid requirements for infants and lacked leucine, lysine, and tryptophan for children. Tryptophan increased significantly but still remained as the first limiting amino acid in both albumin and glutelin fractions. Leucine was the second limiting amino acid in albumins, while lysine was the second limiting amino acid in glutelins.

Cuphea PSR23 proteins may have limited use in food applications because of their low solubility. The value of the defatted seed meal as feed must be evaluated to include other

TABLE 3
Amino Acid Composition of Defatted *Cuphea* Meal, g/16 g N[a]

Amino acid	Defatted meal		Albumin		Glutelin		Residue	
Hydroxyproline	1.63	c	2.63	b	0.12	d	5.71	a
Aspartic acid	7.21	c	7.75	b	7.95	a	6.58	d
Threonine	2.82	c	4.07	a	2.85	c	3.10	b
Serine	4.01	b	4.47	a	4.44	a	4.24	a,b
Glutamic acid	12.34	c	14.11	b	15.66	a	8.04	d
Proline	2.84	b	3.03	a,b	2.84	b	3.32	a
Glycine	3.79	d	4.77	a	4.08	b,c	3.91	c
Alanine	3.88	b	4.17	a	3.72	c	3.70	c
Cystine-cysteine	1.98	b	3.63	a	1.92	b	1.09	c
Valine	4.34	b	3.73	c	4.80	a	4.24	b
Methionine	1.77	b	1.19	d	2.00	a	1.63	c
Isoleucine	3.28	b	2.98	c	3.70	a	3.21	b
Leucine	5.58	c	4.47	d	6.14	a	5.87	b
Tyrosine	1.95	b	1.99	b	2.53	a	1.69	c
Phenylalanine	3.37	b	2.39	c	3.97	a	3.43	b
Histidine	2.06	c	1.59	d	2.13	b	2.28	a
Lysine	3.70	b	5.17	a	2.91	d	3.54	c
Arginine	8.14	b	3.98	d	10.78	a	4.41	c
Tryptophan	<0.04		0.60		0.66		<0.04	
Total	74.73		76.72		83.2		69.99	

[a]Means in each row with different letters are significantly different ($P < 0.05$).

components such as the carbohydrate and residual oil. Press cake from full press oil extraction conducted in our facility contains 6% residual oil. The high solubility of the protein at alkaline pH may make *Cuphea* protein useful in industrial applications such as wood adhesives, which have pH values of around 10 (34).

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