Lesquerella fendleri Protein Fractionation and Characterization

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ABSTRACT: *Lesquerella fendleri* is a promising new crop whose seed contains hydroxy FA TG with potential industrial uses as well as substantial amounts of valuable gums. The defatted *L. fendleri* seeds also contain more than 30% protein. The objective of this study is to process and characterize this protein component for possible future uses in food. Hexane-defatted seed has more than 30% protein content. Defatted lesquerella meal was extracted sequentially with 0.5 M sodium chloride (2×), water, 70% ethanol, and 0.1 N sodium hydroxide (2×). Each sodium chloride extract was dialyzed against deionized water and centrifuged to separate the water-soluble fraction (albumin) from the salt-soluble fraction (globulin) before freeze-drying. The ethanol extract and the neutralized sodium hydroxide extracts (glutelin) were dialyzed against water and freeze-dried. Albumin had the highest proportion of lysine and sulfur amino acids per 16 g nitrogen among all the fractions analyzed. Glutelin and globulin accounted for the highest amount of protein nitrogen. SDS-PAGE of the reduced albumin, globulin, and glutelin showed the presence of several protein bands with M.W. ranging from 7 to 98 kDa. Nitrogen solubility of defatted lesquerella meal from pH 2 to 12 indicated a solubility minimum of 15% around pH 4.2 and a solubility of 75% at pH 11.5. Nonprotein nitrogen of defatted meal was 12% of total nitrogen. Defatted lesquerella meal has the potential for food use based on good nitrogen solubility and good amino acid composition.

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KEY WORDS: Amino acid composition, *Lesquerella*, lesquerella protein, nitrogen solubility, nonprotein nitrogen, protein fractionation, SDS-PAGE.

Lesquerella fendleri is a promising new crop whose seed contains hydroxy FA TG with potential uses in cosmetics, paint, coatings, lubricants, and grease (1). The seed also contains valuable polysaccharide gums (2) and 22% protein with favorable amino acid composition (3). The status of lesquerella as an industrial crop was reviewed (4). Forty acres (16.2 ha) of lesquerella was grown in the southwestern United States in 2003. In present practice, the lesquerella seed is being processed for oil, and it is essential to find an economic outlet for the defatted meal. There is increasing interest among vegetarians and health-conscious people to consume protein-enriched foods from plant sources, which have no cholesterol and a low saturated fat content. Protein accounts for 30% of defatted lesquerella meal (3), but no information is available on the individual proteins of lesquerella. The historical way to fractionate plant protein is to separate protein into water-soluble albumin, salt-soluble globulin, ethanol-soluble prolamin, and alkali-soluble glutelin. More basic information on lesquerella protein fractions is necessary to better utilize the protein. The objective of this paper is to study the fractionation of protein into albumin, globulin, and glutelin fractions and to determine nitrogen solubility and nonprotein nitrogen of lesquerella meal as a basis for increased utilization.

EXPERIMENTAL PROCEDURES

Lesquerella fendleri. The *L. fendleri* seed was from a 2002 crop grown in Arizona. The seed was cleaned and ground through a 420-μm screen. The ground seed (300 g) was defatted sequentially with 3,000 mL and 2,400 mL hexane at room temperature to minimize denaturation of the protein. The partially defatted lesquerella was dried in a hood at room temperature and ground through a 250-μm screen. An aliquot of 220 g was defatted successively with 2,200, 1,760, and 1,320 mL of hexane. The residual oil content of defatted lesquerella meal was 0.48%, as-is.

Nitrogen solubility. Defatted lesquerella meal (200 mg) was mixed with 30 mL of water and adjusted to various pH values with 1 N HCl or 1 N NaOH. Each mixture was stirred magnetically for 20 min, and then centrifuged at 10,690 × *g* for 20 min. The clear supernatants were analyzed for nitrogen by Kjeldahl, and the percentage of soluble nitrogen at each pH was calculated.

Nonprotein nitrogen. Defatted lesquerella meal (200 mg) was stirred with 20 mL of TCA of various molarities (0–5 M) for 20 min and centrifuged at 10,690 × *g* for 20 min. The clear supernatants were analyzed for nitrogen by Kjeldahl, and the percentage of soluble nitrogen at each TCA concentration was calculated.

Protein extraction. The defatted lesquerella meal (30 g) was sequentially extracted at room temperature with 900 mL of 0.5 M NaCl, then 450 mL of 0.5 M NaCl, water, 70% ethanol, and 0.1 N NaOH (2×) in a blender for 15 min and centrifuged at 26,385 × *g* for 20 min at 25°C in a Sorvall RC-5B refrigerated centrifuge (DuPont Instruments, Wilmington, DE). The volume of each supernatant was measured, an aliquot of each supernatant was analyzed for nitrogen by Kjeldahl, and the percent meal nitrogen of each fraction was calculated.

The sodium chloride extracts, ethanol extract, and sodium hydroxide extracts were neutralized with 1 N HCl and then dialyzed against water for 2 d with several changes of water at

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4°C. The dialysis tubing had a nominal M.W. cutoff of 3,500 (Fisher Scientific, Pittsburgh, PA). The volume of protein solution inside the dialysis tubing was 200 mL, and the volume of dialysis solution was 1,800 mL for sodium chloride and sodium hydroxide extracts and 3,800 mL for the ethanol extract. Each dialyzed sodium chloride extract was centrifuged to separate supernatants (albumin) from solids (globulin). Dialyzed ethanol extract and sodium hydroxide extracts gave prolamin and glutelin, respectively. The first outside dialysis solution from the first sodium chloride extract was freeze-dried to recover nonprotein nitrogen. Nitrogen content of each solid was determined, and percent nitrogen of each solid fraction was calculated.

SDS-PAGE was done according to the Sessa and Wolf modification (5) of the Fling and Gregerson method (6). Dialyzed and freeze-dried protein extracts were weighed out to provide 5 mg protein/mL in 500 μL of sample buffer (containing Tris-HCl, SDS, glycerol, β-mercaptoethanol, and urea), then heated in a boiling-water bath for 5 min. Protein samples (15 μL) were loaded onto 4–12% Bis-Tris NuPAGE precast gradient gel (Invitrogen Corp., Carlsbad, CA). Bio-Rad (Bio-Rad Laboratories, Hercules, CA) prestained 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) with the NuPAGE MES-SDS running buffer (SDS, Tris, and 4-morpholinoethane sulfonic acid).

Analyses. Nitrogen was determined in duplicate by AOAC Official Methods 990.03, by combustion analysis, and 976.05, by the automated Kjeldahl method (7). The amino acids were determined by cation exchange chromatography in a Beckman 6300 amino acid analyzer (Beckman Instruments, San Ramon, CA) after samples were hydrolyzed by 6 N HCl for 4 h at 145°C (8). Methionine and cystine were oxidized by performic acid before hydrolysis (9), and tryptophan was determined by a colorimetric method after enzymatic hydrolysis by pronase (10,11). Amino acids were determined for each hydrolysate in duplicate.

ANOVA (12) was used to treat the data, and Tukey's Studentized range test was used to determine significant differences from duplicate experiments ($P < 0.05$).

RESULTS AND DISCUSSION

Nitrogen solubility. Figure 1 shows the nitrogen solubility of defatted lesquerella meal from pH 2 to 12. The starting pH of the meal dispersion was 5.1, and the nitrogen solubility was 17%. The lowest nitrogen solubility was around 14.6% at or near pH 4.2. There was a large increase in solubility at pH > 8. The solubility at pH 9.3 was 43.5%, at pH 10.1, 63%, and at pH 11.5, 75%.

Nonprotein nitrogen. The amount of soluble nitrogen from defatted lesquerella meal was less than 20% at TCA concentrations of 0 to 2 M, with the lowest value of 11.7% at a TCA concentration of 0.6 M. The lowest value for percent soluble nitrogen corresponds with the nonprotein nitrogen of defatted lesquerella meal, because protein is expected to be precipitated at

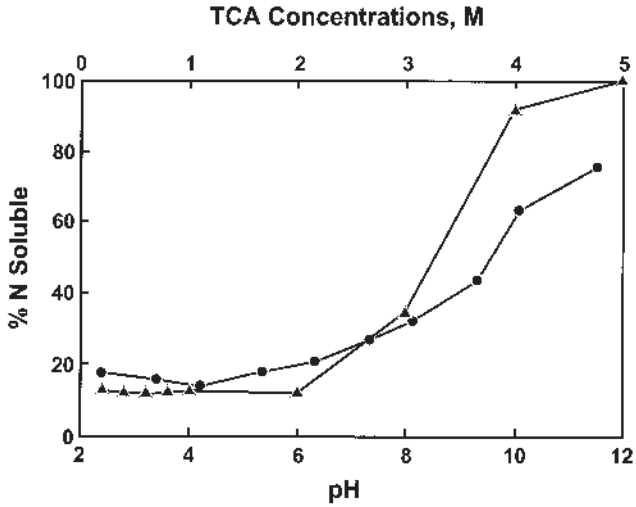

FIG. 1. Nitrogen solubility of defatted lesquerella meal as a function of pH, ●; and as a function of TCA concentration, ▲.

this TCA concentration. As the TCA concentration increased above 2 M, significantly more protein became soluble, and all nitrogen species were soluble at 5 M TCA. Carlson *et al*. (3) reported 0.7% nonprotein nitrogen for defatted lesquerella flakes. Their nonprotein nitrogen value was determined in 0.8 M TCA and translated to 14.7% soluble nitrogen, which was higher than the 12.5% soluble nitrogen at 0.8 M TCA, shown in Figure 1. The agreement between the two nonprotein nitrogen values was reasonable considering the samples were different and not processed identically.

Protein extraction. Table 1 shows that 43.1% of the total defatted lesquerella meal nitrogen was in the first 0.5 M NaCl extract. Globulin accounted for the highest amount of meal nitrogen from the first NaCl extract. Some nonprotein nitrogen and possibly some low M.W. peptide were lost from dialysis, because only the first outside dialysis solution from the first NaCl extract was collected. The second NaCl extract accounted for 7.1% of total meal nitrogen (Table 1). The globulin fractions were mostly pure protein because the nitrogen contents were more than 13%. The albumin fraction from the first and second NaCl extracts had relatively low nitrogen contents of 4% or less and probably included water-soluble gum (2), because the NaCl solution became quite viscous during extraction. The reason for water extraction after the second NaCl extraction was to reduce the concentration of NaCl solution in lesquerella meal, because prolamin from wheat is less soluble as the NaCl concentration increases (13). There was 1.3% of total meal nitrogen in 70% ethanol extract (not shown in Table 1), and the ethanol extract did not precipitate after dialysis against water and centrifugation. The recovered freeze-dried solids had a nitrogen content of only 1.6% and accounted for 0.4% of total meal nitrogen. Lesquerella meal was therefore essentially devoid of prolamin. The first and second NaOH extracts accounted for 13.5 and 6.7% of total meal nitrogen, respectively (Table 1). The residue after sequential extraction accounted for 29.1% of total meal nitrogen with a nitrogen content of 2.3%.

TABLE 1
Proteins Recovered from Defatted Lesquerella Meal by Sequential Extractions[a]

	% of Meal N					% of Meal N		
	Before dialysis	After dialysis		N content, %		Before dialysis	After dialysis	N content, %
		Albumin	Globulin	Albumin	Globulin		Glutelin	Glutelin
First NaCl extract	43.1	7.6	21.2	4.0	14.6			
Second NaCl extract	7.1	2.1	3.1	3.6	13.1			
Total NaCl extract	50.2	9.7	24.3					
First NaOH extract						13.5	12.5	9.0
Second NaOH extract						6.7	6.9	7.9
Total NaOH extract						20.2	19.4	

[a]Nonprotein nitrogen recovered from first NaCl extract was 3.6% of total meal nitrogen, and residue after sequential extraction had 29.1% of total meal nitrogen and a nitrogen content of 2.3%.

Total nitrogen recovered from all extractions and residue was close to 100% before dialysis.

Amino acid composition. Table 2 gives the amino acid composition of defatted lesquerella meal and the extracted fractions. The original amino acid composition data were reported as grams amino acid per 100 g of sample for each amino acid. The nitrogen content of the sample was used to convert the amino acid values to grams amino acid per 16 g nitrogen. The values were expressed in g/16 g N because the conversion factors for each fraction from nitrogen to protein were not known. If the customary conversion factor of 6.25 from nitrogen to protein is used, then g/16 g N is equivalent to g/100 g protein. A more meaningful comparison of the individual amino acids between the fractions can be made when g/16 g N rather than a percentage is used for amino acid composition due to the difference in nitrogen content of the fractions. All fractions have high glutamic acid content. The hydroxyproline content varied greatly, from 0.16 for globulin to 8.33 g/16 g N for residue. Albumin had the highest lysine content and can supplement cereal grains proteins, which are low in lysine. Albumin and glutelin had the highest sulfur amino acid content and can supplement soy protein, which is low in sulfur amino acids. The amino acid composition of defatted lesquerella meal agreed well with previously published data (3,14). In general, all fractions had good amino acid composition for children and adults. Lesquerella meal protein is superior to cereal proteins and soy protein because of its high lysine content as well as high sulfur amino acid content.

SDS-PAGE. The SDS-PAGE patterns of reduced lesquerella meal and protein fractions are shown in Figure 2. The estimated M.W. in kDa for dominant bands for 9 reduced albumin bands (Lane 3) are 33.2, 29.8, 22.3, 18, 10, and 7; for 6 reduced globulin bands: 43.7, 39, 32.2, 30.2, 9.7, and 6; for 10 reduced glutelin bands: 97.8, 45.8, 39.8, 31.3, 24, 18.5, 9.2, and 7.2, and

TABLE 2
Amino Acid Composition (g/16 g Nitrogen) of Defatted Lesquerella Meal and Fractions[a]

Amino acid	Meal			Albumin	Globulin	Glutelin	Residue
	Ref. 3	Ref. 14	Ours				
Hydroxyproline	ND[b]	2.46	2.43[b]	1.54[c]	0.16[e]	0.49[d]	8.33[a]
Aspartic acid	7.14	7.23	7.16[c]	7.71[a,b]	8.16[a]	7.23[b,c]	7.40[b,c]
Threonine	4.08	4.45	4.04[c]	4.36[b]	3.80[c]	4.93[a]	4.77[a]
Serine	4.43	4.64	3.59[b]	4.26[a]	3.11[c]	4.53[a]	3.62[b]
Glutamic acid	13.20	13.70	13.35[c]	13.73[c]	16.76[a]	12.81[c]	14.84[b]
Proline	6.07	6.67	6.35[b]	4.54[d]	5.68[c]	5.68[c]	10.90[a]
Glycine	5.05	5.94	5.78[b]	13.28[a]	4.80[c]	5.74[b]	5.70[b]
Alanine	3.72	4.50	4.28[c,d]	4.52[c]	4.21[d]	5.36[a]	4.88[b]
Cysteine	1.99	1.78	2.28[c]	3.00[a]	2.19[c]	2.54[b]	1.53[d]
Valine	4.52	4.78	4.85[b]	4.08[c]	4.52[b]	4.67[b]	7.45[a]
Methionine	1.77	1.34	1.55[b]	1.38[c]	1.64[a,b]	1.75[a]	1.59[b]
Isoleucine	3.37	3.55	3.45[c]	2.98[d]	3.84[b]	3.87[b]	4.22[a]
Leucine	5.45	5.81	5.64[c]	4.15[d]	7.31[a]	6.86[b]	5.97[c]
Tyrosine	3.10	2.98	2.74[b]	2.85[b]	2.73[b]	3.19[a]	3.13[a]
Phenylalanine	4.12	3.82	4.03[c]	3.37[d]	4.86[a]	4.88[a]	4.49[b]
Histidine	2.30	2.53	2.34[c]	2.48[b,c]	2.71[a]	2.67[a,b]	2.03[d]
Lysine	5.67	6.64	5.80[b]	7.78[a]	4.53[c]	4.89[c]	6.08[b]
Arginine	8.73	7.86	7.04[b]	4.59[c]	9.04[a]	9.03[a]	4.66[c]
Tryptophan	ND	ND	0.81[c]	0.76[c]	1.60[a]	1.00[b]	1.05[b]

[a]Means in each row with different letters are significantly different ($P < 0.05$).
[b]Not determined.

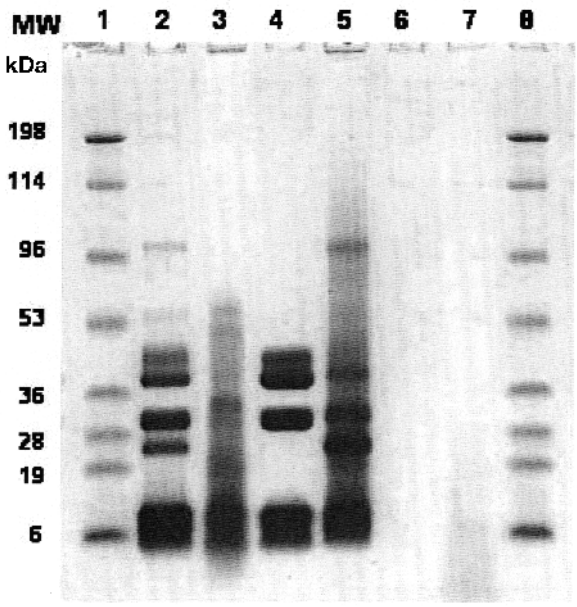

FIG. 2. SDS-PAGE of reduced defatted lesquerella meal and protein fractions. Lanes 1 and 8: M.W. standards; Lane 2: reduced defatted lesquerella meal; Lane 3: reduced albumin; Lane 4: reduced globulin; Lane 5: reduced glutelin; Lane 6: running buffer; Lane 7 reduced residue.

for 8 reduced meal bands: 43.7, 38, 30.2, 23.2, 10, and 7. The SDS-PAGE pattern of reduced globulin was similar to that of reduced meal, because globulin accounted for the most nitrogen in the meal. There was streaking for reduced albumin and reduced glutelin, which indicated some denaturation.

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