Protein Fractionation and Properties of Salicornia Meal

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ABSTRACT: *Salicornia bigelovii* Torr. is an annual salt-marsh oilseed plant. Hexane-defatted salicornia 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 a water-soluble fraction (albumin) from a salt-soluble fraction (globulin) before freeze-drying. Ethanol extracts and neutralized sodium hydroxide extracts (glutelin) were dialyzed against water and freeze-dried. Globulin accounted for the highest amount of protein nitrogen, followed by glutelin and albumin. SDS-PAGE of reduced albumin, globulin, and glutelin showed a number of protein bands. Nitrogen solubility of defatted salicornia meal from pH 2 to 11 indicated a minimum solubility of 22%, around pH 4.5. Nonprotein nitrogen of defatted meal was 23% of total nitrogen, higher than defatted soybean, sunflower, and rapeseed meals. Albumin had the highest proportion of lysine and sulfur amino acids per 16 g nitrogen among all the fractions analyzed.

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

Salicornia bigelovii Torr. is an annual salt-marsh oilseed plant. Previous investigators found the seed to contain 26 to 33% oil, 31% protein, 5 to 7% fiber, and 5 to 7% ash (1). Attia *et al.* (2) reported the nutrient composition and feeding value of defatted salicornia meal in broiler diets. Belal and Al-Dosari (3) replaced fish meal with defatted salicornia meal in feeds for Nile tilapia. Wu and Abbott (4) reported that protein enrichment of defatted salicornia meal can be achieved by fine grinding and air classification. Protein accounts for a significant percentage of the salicornia seed, but little is known about the individual proteins of salicornia. More basic information on protein fractions is necessary to better utilize the protein. This paper studies the fractionation of protein and properties of salicornia meal as a basis for increased utilization.

EXPERIMENTAL PROCEDURES

Nitrogen solubility. The hexane-defatted salicornia meal was prepared as described earlier (4). Defatted salicornia meal (300 mg) was mixed with 12 mL water, adjusted to various pH values with HCl or NaOH, and enough water was added to bring each mixture to 15 mL. Each mixture was stirred magnetically for 1 h, centrifuged at 16,450 × *g*, and filtered through glass wool. The supernatants were analyzed for nitrogen by Kjeldahl (5).

Nonprotein nitrogen. Defatted salicornia meal (200 mg) and 10 mL of TCA of various molarities (0–5 M) were stirred for 1 h, centrifuged at 16,450 × *g*, and filtered through glass wool. The supernatants were analyzed for nitrogen by Kjeldahl (5).

Protein extraction. The defatted salicornia meal (40 g) was sequentially extracted with 600 mL of 0.5 M NaCl (2×), water, 70% ethanol, and 0.1 N NaOH (2×). Each mixture was stirred magnetically for 20 min and centrifuged at 26,385 × *g* for 20 min at 25°C in a Sorvall RC-5B refrigerated centrifuge (DuPont Instruments, Wilmington, DE). For NaCl and water extracts, 38,200 × *g* was needed to obtain clear supernatants. The volume of each supernatant was measured, and an aliquot of each supernatant and freeze-dried residue after the second sodium hydroxide extraction was analyzed for nitrogen by Kjeldahl.

Each sodium chloride extract, ethanol extract, and sodium hydroxide extract after neutralization with HCl was dialyzed against water at 4°C for 2 d with several changes of water. The volume of protein solution 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. The dialysis tubing used has a nominal M.W. cutoff of 3,500 (Fisher Scientific, Pittsburgh, PA). Each of the dialyzed sodium chloride extracts was centrifuged to separate solids (globulin) from supernatants (albumin). Dialyzed ethanol extract and sodium hydroxide extracts gave prolamin and glutelin, respectively. Each fraction was freeze-dried and analyzed for nitrogen. The first outside dialysis solution from the first sodium chloride extract was freeze-dried to recover nonprotein nitrogen and analyzed for nitrogen. Subsequent outside dialysis solutions from the first sodium chloride extract and other extracts were discarded.

Analyses. Nitrogen was determined in duplicate by AOAC Official Methods (6). Samples were hydrolyzed by 6 N HCl for 4 h at 145°C (7), and the amino acids were determined by cation exchange chromatography in a Beckman 6300 amino acid analyzer (Beckman Instruments, San Ramon, CA). Cystine and methionine were oxidized by performic acid before hydrolysis (8). Tryptophan was measured by a colorimetric

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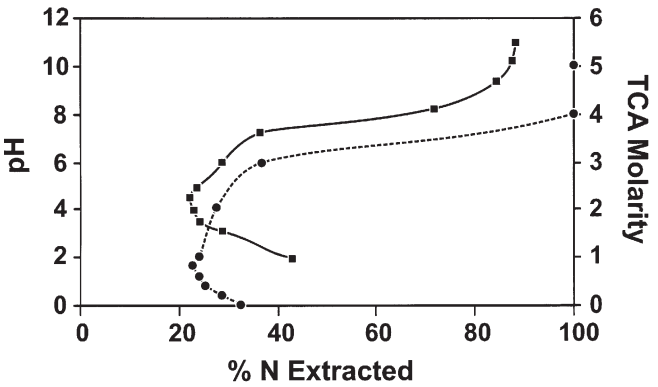

FIG. 1. Nitrogen solubility of defatted salicornia meal at various pH values: (–■–) left scale; and as a function of TCA concentration: (····●····) right scale.

method after enzymatic hydrolysis by pronase (9,10). Duplicate hydrolyses were carried out for each sample, and amino acids were determined for each hydrolysate.

SDS-PAGE. The SDS-PAGE experiment was performed by the Laemmli (11) procedure as modified by Fling and Gregerson (12). For reduced SDS-PAGE, the solvent contained mercaptoethanol, SDS, urea, glycerol, and Tris-HCl, and the samples were boiled. For native SDS-PAGE, no mercaptoethanol was part of the solvent. Details of the procedure were described previously (13).

The data were treated by ANOVA (14), 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 salicornia meal as a function of pH. The starting pH was 6.23 and nitrogen solubility was 32.7%. The minimum nitrogen solubility was around 22% at a pH near 4.5. The nitrogen solubility was 43% at pH 2 and was above 84% at pH 9.3 to 11.0.

Nonprotein nitrogen. At zero TCA concentration, 32.7% of the nitrogen of defatted salicornia meal was soluble (Fig. 1). As the concentration of TCA increased, the percent nitrogen extracted decreased to a minimum around 23, near 0.8 M TCA. Since protein is expected to be precipitated at this TCA concentration, the percent nitrogen extracted will correspond to the nonprotein nitrogen of defatted salicornia meal. As the concentration of TCA increased above 0.8 M, protein became soluble again and all nitrogen was soluble at 4 to 5 M TCA. Extractability of nitrogenous constituents of defatted salicornia meal as a function of TCA concentration is similar to that of undecorticated jojoba meal (5) but higher than defatted soybean, sunflower, and rapeseed meals.

Protein extraction. Preliminary sequential extraction of defatted salicornia meal with 0.5 M NaCl, water, 70% ethanol, and 0.1 N NaOH indicated that the highest percentage of nitrogen was in the NaCl extract, followed by the NaOH extract and residue (not shown). Fifty-seven percent of the total defatted salicornia meal nitrogen was in the first 0.5 M NaCl extract. Table 1 shows that globulin accounted for the highest amount of meal nitrogen, followed by albumin and nonprotein nitrogen from the first NaCl extract. Apparently, some nonprotein nitrogen and possibly some low M.W. protein were lost from dialysis, because only the first outside dialysis solution from the first NaCl extract was recovered. The second NaCl extract accounted for 8.5% of total meal nitrogen, and globulin was again responsible for most of the total. The globulin fractions were essentially pure protein, as the nitrogen contents were 16.9 and 18.7%. The albumin fractions had nitrogen contents of 8.7 and 8.9%. The purpose of the water extraction after the second sodium chloride extraction was to reduce the NaCl concentration, because prolamin from wheat is known to be less soluble as NaCl concentration increases (15). There was 1% or less of total meal nitrogen in 70% ethanol extract, and the freeze-dried solids after dialysis against water and centrifugation had only a 2.1% nitrogen content. The first NaOH extract accounted for about 16% of total meal nitrogen, and the freeze-dried solids (glutelin) contained 12.3% nitrogen. The second NaOH extract had about 1.6% of total meal nitrogen, and the freeze-dried solids had a considerably lower nitrogen content than the first glutelin fraction. The residue after sequential extraction accounted for about 10% of total meal nitrogen with a nitrogen content of 2%. Total nitrogen recovered from all extractions and residue was 94% of meal nitrogen before dialysis.

Amino acid composition. Table 2 shows the amino acid composition of defatted salicornia meal and the extracted protein fractions. The amino acid composition was expressed in g/16 g

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

	% of Meal N		N content, %		% of Meal N	N content, %
	Albumin	Globulin	Albumin	Globulin	Glutelin	Glutelin
First NaCl extract	12.0	26.1	8.7	18.7		
Second NaCl extract	2.1	4.9	8.9	16.9		
Total NaCl extracts	14.1	31.0				
First NaOH extract					16.1	12.3
Second NaOH extract					1.6	7.7
Total NaOH extracts					17.7	

[a]Nonprotein nitrogen recovered from the first NaCl extract was 9.9% of total meal nitrogen, and the residue after sequential extraction had 10.3% of total meal nitrogen and a nitrogen content of 2%.

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

Amino acid	Meal	Albumin	Globulin	Glutelin	Residue
Aspartic acid	6.72 C	7.81 B	7.05 C	8.55 A	7.85 B
Threonine	2.47 D	3.47 B	2.11 D	3.07 C	4.20 A
Serine	4.10 A,B	3.42 B	5.03 A	4.86 A	4.43 A,B
Glutamic acid	14.94 B	18.41 A	18.03 A	16.19 B	11.77 C
Proline	2.64 C	2.88 B,C	2.91 B	3.56 A	3.73 A
Glycine	4.11 C	5.01 B	4.11C	4.75 B	6.63 A
Alanine	3.09 C	4.06 B	2.62 D	3.78 B	4.71 A
Cysteine	1.46 B	1.99 A	1.32 B,C	0.77 D	1.18 C
Valine	3.64 D	4.22 C	3.59 D	5.10 B	5.41 A
Methionine	1.05 D	1.74 B	1.06 D	1.52 C	2.00 A
Isoleucine	3.14 D	2.83 E	3.43 C	4.50 A	4.24 B
Leucine	4.90 C,D	4.57 D	5.12 C	6.71 B	7.41 A
Tyrosine	2.83 D	2.66 D	3.30 C	3.59 B	3.84 A
Phenylalanine	3.22 D	2.95 E	3.63 C	4.51 A	4.24 B
Histidine	2.09 C,D	2.06 D	2.28 C	2.78 A	2.47 B
Lysine	3.68 D	6.35 A	2.92 E	4.29 C	4.79 B
Arginine	12.05 B	11.49 B	13.61 A	11.70 B	7.49 C
Tryptophan	1.28 B	1.29 B	1.15 C	1.42 A	1.02 D

[a]Means in each row with the same letter are not significantly different ($P > 0.05$).

N in Table 2 because of the unknown conversion factors for each fraction from nitrogen to protein. If the customary conversion factor, or 6.25, is used, then g/16 g N is equivalent to g/100 g protein. Although the amino acid composition could be presented as a percentage, the difference in nitrogen (protein) content of the fractions cannot give a meaningful comparison of the individual amino acids among the fractions. All fractions had a high glutamic acid content, and all fractions except the residue had a high arginine content. Albumin had the highest lysine and sulfur amino acid contents, globulin had the lowest lysine content, and glutelin had the lowest sulfur amino acid content. The 70% ethanol extract (1% or less of meal nitrogen) had only 6.0 g total amino acids per 16 g nitrogen (not shown in Table 2), and indicated salicornia meal was essentially devoid of prolamin. Globulin was the closest in amino acid composition, and the residue was the most different from that of meal.

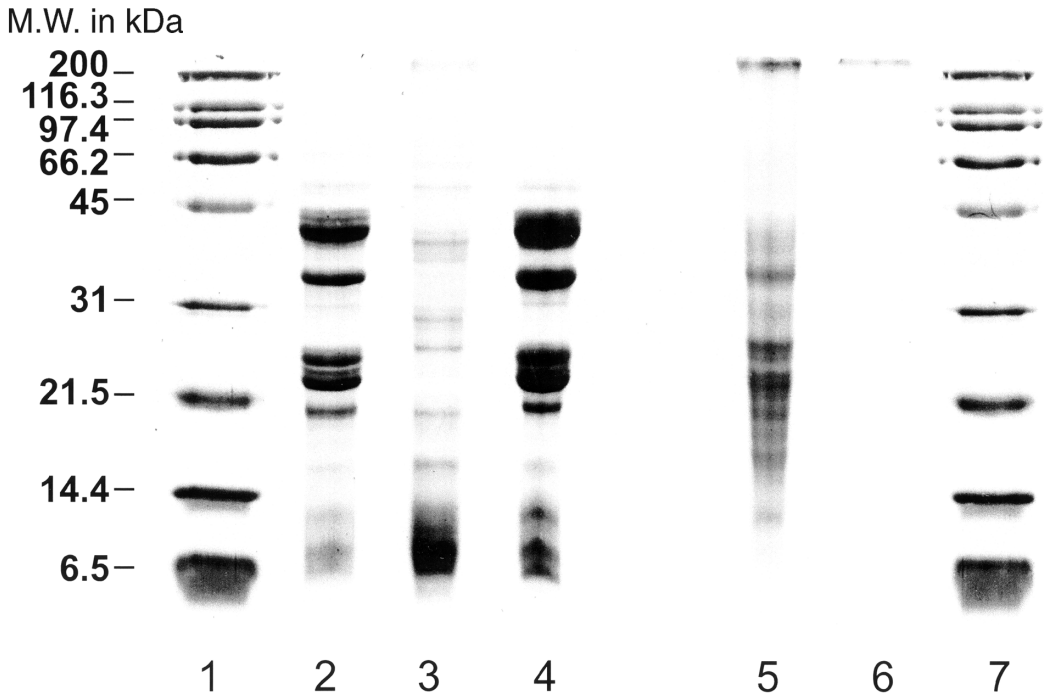

FIG. 2. SDS-PAGE of reduced defatted salicornia meal and protein fractions. Lanes 1 and 7: M.W. standards consisting of myosin, β-galactosidase, phosphorylase b, BSA, ovalbumin, carbonic anhydrase, Kunitz soybean trypsin inhibitor, lysozyme, and aprotinin; lane 2: reduced meal; lane 3: reduced albumin; lane 4: reduced globulin; lane 5: reduced glutelin; lane 6: reduced residue.

SDS-PAGE. The SDS-PAGE pattern of reduced salicornia meal is shown in Figure 2. The estimated M.W. in kDa for reduced albumin bands are >200, 85, 70, 52, 42, 37, 30, 27.5, 24, 21, 18.5, and 7.5; for reduced globulin bands: >200, 79, 56, 46, 40, 34, 32, 27, 24, 21.5, 19, 14, and 7; for reduced glutelin bands: >200, 70, 47, 42, 38, 29, 24, 21.5, 20, 19.5, 14.4, 13, and 7.5; and for reduced residue bands: >200, 80, 55, 42, 29.9, 25, 21, and 19.5. The SDS-PAGE pattern of reduced defatted meal was similar to that of globulin, because globulin accounted for the most nitrogen in the meal. There was more smearing for reduced glutelin patterns compared with those of meal, albumin, and globulin. The native protein without reducing agent dissolved only partially in the SDS-PAGE solvent and showed heavy smearing for albumin and glutelin, medium smearing for salicornia meal, and no movement for globulin and residue (not shown).

The results of this study show that defatted salicornia meal contained a large proportion of relatively soluble protein (albumin and globulin) as well as some glutelin. It appears that an alkaline extraction of salicornia meal can recover a large amount of meal nitrogen, and it may be feasible to obtain a protein concentrate from salicornia meal. The potential practical uses or applications of the proteins will require further studies.

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REFERENCES

1. Glenn, E.P., J.W. O'Leary, M.C. Watson, T.L. Thompson, and R.O. Kuehl, *Salicornia bigelovii* Torr.: An Oilseed Halophyte for Seawater Irrigation, *Science 251*:1065–1067 (1991).
2. Attia, F.M., A.A. Alsobayel, M.S. Driadees, M.Y. Al-Saiady, and M.S. Bayoumi, Nutrient Composition and Feeding Value of *Salicorinia bigelovii* Torr. Meal in Broiler Diets, *Anim. Feed Sci. Technol. 65*:257–263 (1977).
3. Belal, I.E.H., and M. Al-Dosari, Replacement of Fish Meal with Salicornia Meal in Feeds for Nile Tilapia *Oreochromis niloticus, J. World Aquacult. Soc. 30*:285–289 (1999).
4. Wu, Y.V., and T.P. Abbott, Protein Enrichment of Defatted Salicornia Meal by Air Classification, *J. Am. Oil Chem. Soc. 80*: 167–169 (2003).
5. Wolf, W.J., M.L.Schaer, and T.P. Abbott, Nonprotein Nitrogen Content of Defatted Jojoba Meals, *J. Sci. Food Agric. 65*:277–288 (1994).
6. Association of Official Analytical Chemists*, Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th edn., edited by K. Helrich, AOAC, Arlington, VA, 1990.
7. Gehrke, C.W., P.R. Rexroad, R.M. Schisla, J.S. Absheer, and R.W. Zumwalt, Quantitative Analysis of Cystine, Methionine, Lysine, and Nine Other Amino Acids by a Single Oxidation–4 h Hydrolysis Method, *J. Assoc. Off. Anal. Chem. 70*:171–174 (1987).
8. Moor, S., On the Determination of Cystine as Cysteic Acid, *J. Biol. Chem. 238*:235–237 (1963).
9. Spies, J.R., and D.C. Chambers, Chemical Determination of Tryptophan in Proteins, *Anal. Chem. 21*:1249–1266 (1949).
10. Holz, F., Automatic Determination of Tryptophan in Proteins and Protein-Containing Plant Products with Dimethylaminocinnamaldehyde, *Landwirsch. Forsch. Sonderh. 27*:96–109 (1972).
11. Laemmli, U.K., Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4, *Nature 227*:680–685 (1970).
12. Fling, S.P., and D.S. Gregerson, Peptide and Protein Molecular Weight Determination by Electrophoresis Using a High Molarity Tris Buffer System Without Ureas, *Anal. Biochem. 155*:83–88 (1986).
13. Sessa, D.J., and W.J. Wolf, Bowman-Birk Inhibitors in Soybean Seed Coats, *Ind. Crops Prod. 14*:73–83 (2001).
14. SAS Institute, Inc., *SAS/STAT Guide for Personal Computers*, version 6, edited by J.C. Parker, SAS Institute, Cary, NC, 1987.
15. Meredith, P., On the Solubility of Gliadinlike Proteins II. Solubility in Aqueous Acid Media, *Cereal Chem. 42*:64–71 (1965).

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