Protein Enrichment of Defatted Salicornia Meal by Air Classification

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ABSTRACT: *Salicornia bigelovii* Torr. is a leafless, annual salt-marsh plant. Previous investigators reported that the seed contained 26 to 33% oil, 30 to 33% protein, 5 to 7% fiber, and 5 to 7% ash. Hexane-defatted salicornia meal was ground in a pin mill and separated by air classification into various fractions by particle size. The fine fractions were enriched in protein. The degree of protein enrichment and yield of fine fractions depended on the intensity of grinding. More intense grinding resulted in a higher yield of fine fractions with a smaller increase in protein content compared with less intensive grinding. The amino acid composition and proximate composition of the air-classified fractions are compared with the starting material.

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KEY WORDS: Air classification, amino acid, milling, protein, proximate composition, salicornia.

Salicornia bigelovii Torr. is an annual salt-marsh oilseed plant. Glenn *et al.* (1) reported that the seed averaged 26 to 33% oil, 31% protein, 5 to 7% fiber, and 5 to 7% ash over 6 yr of field trials. Attia *et al*. (2) studied the nutrient composition and feeding value of defatted salicornia meal in broiler diets and found a depression in growth and feed intake proportional to the level of salicornia meal in the diet. However, the inclusion of cholesterol counteracted the depression activity of salicornia meal. Belal and Al-Dosari (3) replaced fish meal with defatted salicornia meal in feeds for Nile tilapia and concluded that salicornia meal can replace up to 40% of the fish meal in tilapia feeds without affecting growth or body composition. This paper studies the enrichment of protein from defatted salicornia meal by fine grinding and air classification.

EXPERIMENTAL PROCEDURES

Materials. The first batch of defatted salicornia meal was prepared by POS Pilot Plant Corp. (Saskatoon, Saskatchewan, Canada). The seed was conditioned at 30 to 40°C for 20 to 30 min prior to flaking and cold pressing. The press cake generated from the expelling process was extracted with hexane at 60 to 67°C for 4 h, and the salicornia meal was desolventized at 22 to 24°C for 3 d in a fume hood. This batch of defatted salicornia meal is designated as POS in subsequent text.

The second batch of defatted salicornia meal was prepared in our laboratory. About 34 kg of salicornia seeds was washed with water and freeze-dried. The dried seeds were pressed with a Gusta Model 10 Lab Press (Winnipeg, Manitoba, Canada). The press was operated at 30 rpm to provide a feed rate of 2 kg/h. The press barrel was maintained at a temperature of 80 to 90°C. Seeds were gravity fed to the press from an overhead hopper. Oil and press cake were collected separately. The press cake was ground at 9000 rpm in an Alpine Model 160Z pin mill (Augsburg, Germany) to break up the lumps prior to hexane defatting at room temperature. The salicornia meal was desolventized at room temperature in fume hoods for 3 d. This batch of defatted salicornia meal is designated as NCAUR in subsequent text. The original seed had 32.0% protein, 24.5% oil, 11.0% crude fiber, and 9.2% ash on a dry basis.

Pin milling and air classification. The salicornia meal was ground in an Alpine Model 160Z pin mill at various speeds and fractionated in a Pillsbury laboratory model air classifier (Minneapolis, MN) according to particle size. The classifier was first set at a 15-μm cutpoint to obtain a fine and a coarse fraction. The coarse fraction was then classified successively with 18, 24, and 30 μm cutpoints to obtain four fine fractions (1 through 4 in increasing size) and a coarse fraction (fraction 4 coarse).

Nitrogen, fat, ash, crude fiber, and moisture were determined by AOAC Official Methods (4). Protein was calculated from N × 6.25. Fat was measured as the ether extract. Ash was determined by heating the sample at 600°C for 2 h. Starch was converted to glucose by amylase digestion, and glucose was measured by a glucose oxidase/peroxidase method (5). Samples were hydrolyzed by 6 N HCl for 4 h at 145°C (6), and the amino acids were determined by cation exchange chromatography in a Beckman 6300 amino acid analyzer (San Ramon, CA). Methionine and cystine were oxidized by performic acid before hydrolysis (7). Tryptophan was measured according to a colorimetric method after enzymatic hydrolysis by pronase (8,9). Duplicate hydrolyses were carried out for each sample, and amino acids were determined for each hydrolysate.

The data were treated by ANOVA. Tukey's Studentized range test was used to determine significant differences from duplicate experiments ($P < 0.05$, Ref. 10).

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RESULTS AND DISCUSSION

Air classification of salicornia meal from POS. Table 1 shows the result of air classification of meal from POS after pin milling three times at 14,000 rpm. Fine fractions 1 and 2 had an increased protein content, whereas the remaining fractions had a lower protein content compared with starting material. Fractions 1 and 2 had low crude fiber, but fractions 4 and 4 coarse had higher fiber. Fractions 3 and 4 had a high ash content. There were statistical differences in oil and starch contents ($P < 0.05$) for the fractions. The high yields of fraction 1 indicated that less intensive grinding of the meal might result in a higher protein content for fine fractions 1 and 2.

Since not enough salicornia meal from POS was left to make four cutpoints at a speed lower than 3 × 14,000 rpm, 1 × 9,000 rpm pin milling followed by a cutpoint of 18 µm was chosen. Table 2 gives the results of pin milling at 9,000 rpm followed by air classification with a cutpoint of 18 µm. Fractions 1 and 2 had protein content of 53.9% and yield of 28.7% compared with the calculated protein content of 46.3% and yield of 62.2% for 3 × 14,000 rpm for the corresponding fractions.

Air classification of NCAUR-defatted salicornia. The NCAUR-defatted salicornia was pin milled at 6,000, 9,000, 12,000, 14,000, and 3 × 14,000 rpm and air classified into five fractions after each treatment. Table 3 shows the results of air classification after pin milling at 12,000 rpm. The first three fractions had a higher protein content (2.9–11%) than the starting material. The first two fractions had low crude fiber, but fractions 4 and 4-coarse had a high crude fiber content. There were statistical differences ($P < 0.05$) for oil, starch, and ash contents of air-classified fractions.

The amino acid content of air-classified NCAUR salicornia fractions is listed in Table 4. Salicornia is rich in glutamic acid (or glutamine) and in arginine. Our values are higher for lysine, histidine, arginine, valine, leucine, phenylalanine, and tyrosine but lower in methionine compared with those reported by Attia *et al.* (2). The amino acid content of POS salicornia meal (not shown) was similar to that in Table 4, although there was a statistical difference ($P < 0.05$) in some cases. In general, there was no large difference in the amino acid content of air-classified fractions and the starting salicornia, although there was a statistical difference ($P < 0.05$) for all amino acids except serine.

Protein shifting. Protein shifting, a calculated value for comparing protein displacement after air classification, equals the sum of the protein shifted into the high-protein fractions and out of the low-protein fractions as a percentage of the total protein present in the starting material (11). Table 5 gives the protein shift of air-classified salicornia meal. The protein shift for air-classified NCAUR salicornia increased from 6.6 for 6,000 rpm pin-milled material to 14.5 for 12,000 rpm pin-milled meal, but dropped to 11.1 for 14,000 rpm pin-milled salicornia. The protein shift for air-classified POS salicornia was considerably higher than for the corresponding pin-milled material from NCAUR salicornia. One obvious difference between the two batches of salicornia is the residual oil content, because a lower oil content will give a better protein shift in general. However, washing the salicornia seeds to reduce ash content for the NCAUR batch and a difference in the temperature of hexane defatting may also have contributed to some of the observed differences between the two batches of salicornia meal.

TABLE 1
Air Classification of Defatted Salicornia Meal from POS, Pin-Milled 3 × 14,000 rpm[a]

Fraction	Size (µm)	% Dry basis					
		Protein	Oil	Starch	Crude fiber	Ash	Yield
1	<15	45.8 A	2.1 A	5.7 A	0.9	9.6 E	44.7
2	15–18	47.5 A	1.7 B	4.4 C	0.6	14.8 C	17.5
3	18–24	28.3 C	1.3 C	3.7 D	6.3	28.6 A	17.2
4	24–30	23.3 D	1.6 B	5.1 B	14.8	26.7 B	6.3
4 Coarse	>30	15.8 E	2.0 A	5.4 B	34.0	11.5 D	14.2
3 × 14,000 rpm		37.4 B	1.8 A,B	3.7 D	6.4	14.3 C	

[a]Means in each column with the same letter are not significantly different ($P > 0.05$). POS, defatted salicornia meal prepared by POS Pilot Plant Corp., Saskatoon, Canada.

TABLE 2
Air Classification of Defatted Salicornia Meal from POS

Pin mill speed (rpm)	Fraction	Size (µm)	% Dry basis		
			Protein	Oil	Yield
9,000	1 and 2	<18	53.9	1.4	28.7
	3, 4, and 4 coarse	>18	31.8	1.7	71.3
3 × 14,000	1 and 2	<18	46.3	2.0	62.2
	3, 4, and 4 coarse	>18	22.7	1.6	37.7

TABLE 3
Air Classification of NCAUR Defatted Salicornia, Pin-Milled at 12,000 rpm[a]

Fraction	Size (µm)	% Dry basis					
		Protein	Oil	Starch	Crude fiber	Ash	Yield
1	<15	49.4 B	3.9 A	4.3 C	0.6 E	9.3 E	17.6
2	15–18	52.4 A	3.5 B	4.3 C	0.8 E	9.7 D	13.2
3	18–24	44.3 C	2.9 C	4.7 B,C	7.0 D	10.6 B	26.7
4	24–30	41.6 D	2.9 C	5.1 A,B	12.6 B	10.2 C	7.1
4 Coarse	>30	33.9 E	1.6 E	5.3 A	17.6 A	11.2 A	35.4
1 × 12,000 rpm		41.4 D	2.6 D	4.8 A,B	8.8 C	10.7 B	

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

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TABLE 4
Amino Acid Content (g/100 g protein) of Air-Classified NCAUR Salicornia Fractions, 1 × 12,000 rpm[a]

		Fraction				
	Salicornia	1	2	3	4	4 Coarse
Aspartic acid	6.78 B,C	6.48 D,E	6.79 B	6.76 B,C	6.77 B,C	7.16 A
Threonine	2.50 B,C	2.36 D,E,F	2.41 C,D,E,F	2.45 C,D,E	2.49 B,C,D	2.60 B
Serine	4.26 A	4.09 A	4.25 A	4.21 A	4.12 A	4.22 A
Glutamic acid	15.98 B,C,D	15.69 C,D,E	16.34 A,B	15.63 D,E	15.29 E,F	16.13 B,C
Proline	2.84 E,F	2.71 G	2.87 D,E	2.81 F	2.91 C,D	3.03 B
Glycine	4.11 D	3.89 E	4.10 D	4.06 D	4.07 D	4.51 B
Alanine	3.04 C	2.87 E,F	3.00 C,D	3.00 C,D,E	3.00 C,D,E	3.33 A
Cystine	1.20 D,E	1.17 E,F	1.22 C,D,E	1.20 C,D,E	1.23 C,D	1.35 A
Valine	3.57 B,C	3.44 B,C	3.62 A,B,C	3.56 B,C	3.48 B,C	3.89 A
Methionine	1.09 B	1.03 C,D	1.06 B,C,D	1.07 B,C,D	1.11 B	1.17 A
Isoleucine	2.97 C,D	2.88 C,D	3.05 B,C	2.94 C,D	2.93 C,D	3.28 A
Leucine	4.87 B,C	4.67 E,F	4.88 B,C	4.78 C,D,E	4.71 D,E	5.13 A
Tyrosine	2.80 E,F	2.68 G	2.82 D,E	2.77 F	2.70 G	2.97 B
Phenylalanine	3.21 B,C	3.08 E	3.23 B,C	3.17 C,D	3.10 D,E	3.42 A
Histidine	2.08 B,C,D,E	2.02 D,E,F	2.10 A,B,C	2.05 C,D,E	2.01 E,F,G	2.17 A
Lysine	3.72 B,C,D	3.51 E,F,G	3.65 C,D,E	3.70 C,D	3.62 D,E,F	4.05 A
Arginine	12.14 C	11.88 DE,	12.36 B	11.99 C,D	11.53 F	12.42 A,B
Tryptophane	1.27 A,B	1.18 C,D	1.22 B,C	1.15 D	1.19 C,D	1.26 A,B

[a]Means in each row with the same letter are not significantly different ($P > 0.05$). Figures are rounded off to two decimal points; figures with three decimal points were used for statistical analysis.

TABLE 5
Protein Shifts of Air-Classified Defatted Salicornia Meal

Pin mill speed (rpm)	Protein shift (%)	
	2.6% Oil, NCAUR	1.8% Oil, POS[a]
6,000	6.6	
9,000	8.6	23.7
12,000	14.5	
14,000	11.1	
3 × 14,000	13.9	29.6

[a]See Table 1 for abbreviation.

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