Electrophoretic and Functional Properties of Mustard Seed Meals and Protein Concentrates

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ABSTRACT: Defatted meals and protein concentrates from five varieties of mustard seeds (four *Brassica* spp. and one *Sinapis alba*) were analyzed for polypeptide composition and functional properties. Nonreducing gel electrophoresis showed that *Brassica* seeds lacked the 135- and 50-kDa polypeptides that were present in the seeds of the *S. alba* variety. On the other hand, the 29-kDa polypeptide found in the *Brassica* seeds was absent from the seed of the *S. alba* variety. Under reducing conditions, the 135 kDa was not detected in the *S. alba* variety and the intensity of the 50-kDa polypeptide was severely reduced; in contrast, the intensity of the 29-kDa polypeptide in the *Brassica* seeds was not affected. Meals from yellow seeds had significantly higher ($P \leq 0.05$) protein contents than meals from the brown seeds. The emulsifying activity indexes (EAI) of meals and protein concentrates from the *Brassica* seeds were significantly higher ($P \leq 0.05$) than those obtained for similar products from *S. alba* seeds. It was concluded that the disulfide-bonded 50- and 135-kDa polypeptides may have contributed to increased rigidity of *S. alba* meal proteins, which resulted in poor EAI when compared to the *Brassica* meals, which do not contain these polypeptides.

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KEY WORDS: *Brassica*, defatted meal, functional properties, mustard, polypeptide composition, protein concentrate, *Sinapis alba.*

The name "mustard" is used to describe seeds from a group of plants that are used in the preparation of condiments in a process that consists of mixing the sweet "must" of old wine with crushed seeds to form a paste, "hot must" or "mustum ardens" (1). Apart from the use of mustard in the preparation of condiments, the seeds have considerable potential as sources of edible oil and protein. In this respect, efforts are currently being made to develop yellow and brown mustard seeds as potential oilseed crops in Canada (2). These crops could be grown on more than 1.8 million ha in the semi-arid prairies of Canada, with potential for production in other semi-arid regions of the United States, Australia, and Asia. Since the residual meal remaining after oil extraction is rich in proteins (30–48%, dry weight basis), mustard seeds could serve as suitable raw materials in the manufacture of protein ingredients for the food and nonfood industrial sectors.

Mackenzie (3) examined the protein profile of 0.5 M NaCl extracts of some varieties of yellow and brown *Brassica juncea* seeds and obtained eight polypeptide bands after PAGE at pH 8.9. Gel permeation chromatography of the NaCl extract yielded three major protein bands, although the M.W. of the bands were not indicated. A low-M.W. protein fraction (1.7 S) was isolated from the seeds of *S. alba* L. and shown to have a molecular mass (MM) of approximately 15 kDa (4). The 1.7 S protein was characterized as an albumin and consisted of two polypeptide chains (MM = 9.5 and 5.0 kDa) linked together by two disulfide bridges. In contrast, Fischer and Schopfer (5) estimated the subunits of the 1.7 S protein to be 10- and 11-kDa polypeptides. Analysis of *S. alba* seed storage proteins revealed the presence of a legumin-like 13 S complex composed of two pairs of disulfide-linked polypeptides (16.5 + 28.5 kDa and 19.5 + 34 kDa, respectively) (5). The *S. alba* seed storage proteins also contained a vicilin-like 9 S complex composed of 64- and 77-kDa glycoproteins (5). However, information on the polypeptide composition and functional properties of seed meals and protein isolates from mustard varieties is scanty.

A detoxified mustard meal containing a zero level of allyl isothiocyanate in addition to low levels of phytic acid (0.52%) and fiber (4.96%) was produced and incorporated into various foods with satisfactory results (6). Therefore, functional use of mustard seed meals and extracted proteins has potential in the food industry. The objective of this work was to determine the functional and electrophoretic properties of various mustard seed protein products, especially varieties that are commercially important. Relationships between polypeptide composition and functional characteristics were also determined.

EXPERIMENTAL PROCEDURES

Materials. The following mustard seed varieties were used: (i) AC Pennant (*S. alba*), a condiment mustard with yellow seed color; (ii) AC Vulcan (*B. juncea* L. Czern.), an oriental-type condiment mustard with yellow seed color; (iii) Commercial Brown (*B. juncea* L. Czern.), a condiment mustard with brown seed color; (iv) S-67 (*B. carinata* Braun), an Ethiopian mustard with brown seed color; and (v) Dodolla (*B. carinata* Braun), an Ethiopian mustard with yellow seed color. The seed varieties were selected based on the differences in seed color and genetic diversity. Seeds were produced in the same year (1998) and at the same location (Saskatoon Research Farm, Saskatoon) to minimize environmental variation.

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Preparation of seed meals. Seeds were ground in small samples in a coffee mill for 2–3 min and the resulting flours defatted in a Soxhlet apparatus using hexane as the solvent. The defatted meals were air dried and ground in the coffee mill to pass through a #40 mesh screen.

Preparation of protein isolates. Acid-precipitated protein concentrate (APC) and calcium-precipitated protein concentrate (CPC) were prepared according to the procedures previously described by Aluko and McIntosh (7). Generally, the defatted meal was mixed with 10 vol of 0.1 M NaOH solution, stirred for 20 min at room temperature, and centrifuged at 10,000 × *g* and 8°C for 30 min. The supernatant was filtered through a Whatman No. 1 filter paper to remove particulate matter. An aliquot of the filtrate was adjusted to pH 4.0 with 0.1 M HCl solution and centrifuged (sample 1). Another aliquot of the filtrate was adjusted to pH 6.0 with 0.1 M HCl solution followed by gradual addition of solid calcium chloride (with continuous mixing) until a concentration of 1 M was reached. The mixture was stirred for an additional 20 min, and the resultant slurry was centrifuged as described above (sample 2). The precipitates from samples 1 and 2 were washed by dispersing each in 200 vol of distilled water followed by centrifugation, and the precipitate was freeze-dried as the APC or CPC, respectively.

Gel electrophoresis. Reduced and nonreduced gel electrophoresis were run separately on 8–25% gradient gels using the PhastSystem Separation and Control and Development Units according to the manufacturer's instructions (Pharmacia LKB, Montréal, Canada). Samples were prepared for nonreduced SDS-PAGE by mixing the defatted mustard meal or freeze-dried protein concentrate with a Tris-HCl buffer solution, pH 8.0, containing 10% SDS and 0.01% bromophenol blue. Samples were placed in boiling water for 5 min, cooled to room temperature, and centrifuged at 16,000 × *g* for 10 min; an aliquot (1 μL) of the supernatant was then loaded onto the gel. Reduced samples were prepared by adding 5% (vol/vol) 2-mercaptoethanol (ME) to an aliquot of the supernatant from 10% SDS extraction, and 1 μL was loaded onto the gel.

Determination of selected functional properties. Total protein content (nitrogen × 6.25) was determined by Kjeldahl digestion according to the American Association of Cereal Chemists' method (8). Protein solubility (PS) was determined according to the method of Aluko and Yada (9) with some modifications. Each sample was mixed with 0.01 M sodium phosphate buffer, pH 7.0, to give a dispersion of approximately 1% (wt/vol) protein content, followed by shaking on a vortex mixer for 5 min and centrifugation at 10,000 × *g* and 10°C for 30 min. The resultant supernatant (S1) was analyzed for protein content according to the modified Lowry method (10) and expressed as a percentage of the initial total protein content of the meal to obtain PS. An aliquot of S1 was heated in boiling water for 15 min, cooled to room temperature (23–25°C), centrifuged at 10,000 × *g* for 30 min and the amount of protein in the supernatant (S2) determined accordingly (10). Heat coagulability (HC) was calculated as follows:

$$\text{HC}(\%) = \frac{(\text{protein content of S1} - \text{protein content of S2})}{\text{protein content of S1}} \times 100 \quad [1]$$

HC was not determined for the protein concentrates because their PS values at pH 7.0 were very low (less than 6%), and the amount of protein in S1 did not differ significantly ($P \geq 0.05$) from S2.

The emulsifying activity index (EAI) was determined according to a modification of the spectroturbidimetric method of Pearce and Kinsella (11). Defatted meals and protein concentrates were dispersed in 0.01 M sodium phosphate buffer, pH 7.0, to give final protein concentrations of 1% (wt/vol). An aliquot of the dispersion (5 mL) was added to 1 mL of pure commercial canola oil followed by homogenization for 1 min using a Polytron PT 10-35 homogenizer equipped with a 20-mm generator (reduced foam model) with the power control unit (PCU 11) set at #6. Immediately after homogenization, 10 μL of the emulsion was diluted to 5 mL with 0.1% (wt/vol) SDS solution, and the absorbance at 500 nm was measured using the SDS solution as a blank. EAI (m^2/g) was calculated as described by Pearce and Kinsella (11). The emulsions were allowed to stand at room temperature (23–25°C) for 30 min and the EAI determined and expressed as a percentage of the initial EAI to obtain emulsion stability (ES). Foam expansion was determined according to the procedure described by Poole *et al.* (12). Sample dispersions containing 1% (wt/vol) protein contents were prepared in 0.01 M sodium phosphate buffer, pH 7.0, and homogenized for 30 s using a Polytron PT 10-35 homogenizer equipped with a 12-mm generator (foam-generating model) with the power control unit (PCU 11) set at #6. The volume of foam obtained was expressed as a percentage of the initial volume of the protein solution. To determine foam stability (FS), the volume of foam that remained after standing at room temperature (23–25°C) for 30 min was expressed as a percentage of the initial foam volume.

Statistical analysis. Each analysis was done in duplicate and ANOVA and Duncan's multiple-range test was carried out using the Statistical Analysis Systems software (13).

RESULTS AND DISCUSSION

Gel electrophoresis. Polypeptide composition has been shown to be an important determinant of the functional properties of various protein ingredients in food systems. For example, soybean protein isolates containing a high level of β-conglycinin and a low level of glycinin generally show higher values of emulsifying capacity and ES when compared to isolates with lower β-conglycinin/glycinin ratios (14). Figure 1 shows the polypeptide profiles of the mustard seed meals under nonreducing (A) and reducing (B) conditions. The *S. alba* meal (lane 1) contained two major polypeptides with M.W. of 50 and 135 kDa, which were not detected in the *Brassica* meals (lanes 2–5). In contrast, the *Brassica* meals contained a 29-kDa polypeptide that was not detected in the *S. alba* meal.

In the presence of ME (Fig. 1B), the 135-kDa band was no longer detected in the *S. alba* meal (lane 6), suggesting a

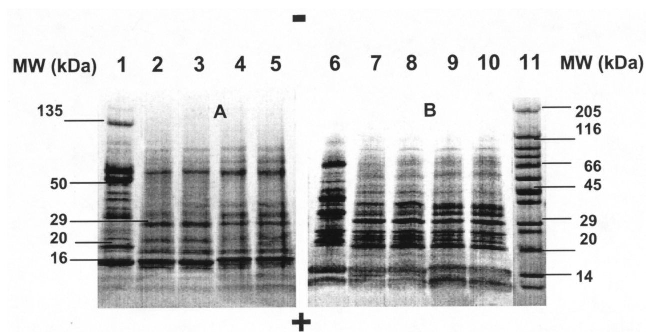

FIG. 1. SDS-PAGE patterns of defatted mustard seed meals in the absence (A: lanes 1–5) and presence (B: lanes 6–10) of 2-mercaptoethanol. Approximately 1 μL of sample (2 mg/mL protein concentration) was applied to each lane. Lanes 1 and 6, AC Pennant (*Sinapis alba*); Lanes 2 and 7, AC Vulcan (*Brassica juncea*); Lanes 3 and 8, Commercial Brown (*B. juncea*); Lanes 4 and 9, S-67 (*B. carinata*); Lanes 5 and 10, Dodolla (*B. carinata*); Lane 11, standard proteins.

polypeptide structure maintained by disulfide bonds. Loss of the 135-kDa band was accompanied by the presence of a polypeptide band with an MM of approximately 66 kDa (Fig. 1B, lane 6), probably a component of the native structure of the 135-kDa polypeptide. A significant ($P \leq 0.05$) reduction in the band intensity of the 50-kDa polypeptide in the presence of ME (Fig. 1B) also suggests that the 135-kDa protein contains disulfide bond(s). On the other hand, Figure 1B shows that the 29-kDa polypeptide found in the *Brassica* meals does not contain disulfide bonds since intensity of the band was not adversely affected by the addition of ME (lanes 7–10). Figure 1B also shows the appearance of the 12- and 13-kDa bands, which were not present in Figure 1A and was in agreement with the work of Venkatesh and Appu Rao (15), who showed that the 12- and 13-kDa polypeptides are subunits of the 2 S (or 1.7 S) protein of mustard seeds. The polypeptide profile obtained in the presence of ME (Fig. 1B, lane 6) is similar to that of Fischer and Schopfer (5) for the seed storage proteins of *S. alba*.

Polypeptide composition of the protein concentrates are shown in Figures 2 and 3, respectively, for APC and CPC. The reducing and nonreducing gel patterns of the protein isolates were similar to the gel patterns obtained for the defatted meals with the following exceptions. Figure 2A shows that the 16- and 20-kDa polypeptides were substantially resistant to acid precipitation as evidenced in the reduced band intensities when compared to the meal profile in Figure 1A. On the other hand, the 16- and 20-kDa polypeptides were very susceptible to calcium precipitation, as shown in the high intensities of the bands (Fig. 3A). A comparison of Figures 2A and 3A showed that the 50- and 55-kDa polypeptides were susceptible to acid precipitation but substantially resistant to calcium-induced precipitation.

Functional properties. The protein content and functional properties of the defatted meals are shown in Table 1. Meals from the yellow seeds (AC Pennant, AC Vulcan, and Dodolla) had significantly higher ($P \leq 0.05$) protein contents than meals from brown seeds (Commercial Brown and S-67). The present results are similar to a previous work that showed that seed meal from Dodolla had significantly higher ($P \leq 0.05$) protein content when compared to the meal from Commercial Brown (16). The *B. carinata* (Ethiopian mustard) seed meals had significantly higher ($P \leq 0.05$) protein solubilities than the meals obtained from either the *B. juncea* or *S. alba* seeds. Surface hydrophobicity and surface hydrophilicity characteristics of a protein have been suggested to be the most important factors that determine solubility properties (17). Therefore, the results suggest that the *B. carinata* proteins have a structure or conformation that exposes more hydrophilic groups, which facilitate increased interaction with the aqueous environment and hence higher PS when compared to the *B. juncea* or *S. alba* proteins.

Proteins in the AC Pennant (*S. alba*) meal were significantly ($P \leq 0.05$) less susceptible to heat-induced coagulation when compared to the *Brassica* proteins. Increased resistance of the *S. alba* meal may be due to the presence of the additional 135-kDa protein, which probably has a rigid structure as a result of the presence of disulfide bonds as deduced from

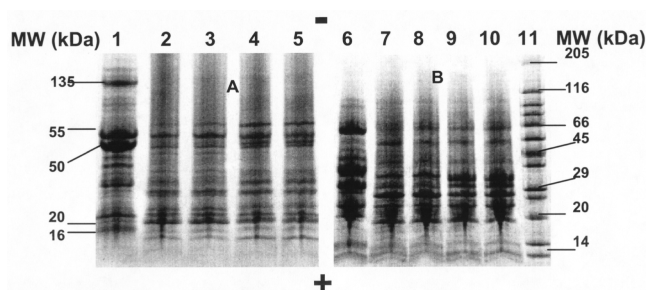

FIG. 2. SDS-PAGE patterns of acid-precipitated protein isolates in the absence (A: lanes 1–5) and presence (B: lanes 6–10) of 2-mercaptoethanol. Approximately 1 μL of sample (2 mg/mL protein concentration) was applied to each lane. Lanes 1 and 6, AC Pennant (*S. alba*); Lanes 2 and 7, AC Vulcan (*B. juncea*); Lanes 3 and 8, Commercial Brown (*B. juncea*); Lanes 4 and 9, S-67 (*B. carinata*); Lanes 5 and 10, Dodolla (*B. carinata*); Lane 11, standard proteins. For abbreviations see Figure 1.

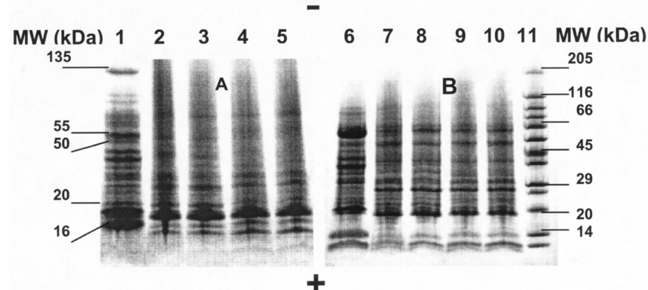

FIG. 3. SDS-PAGE patterns of calcium-precipitated protein isolates in the absence (A: lanes 1–5) and presence (B: lanes 6–10) of 2-mercaptoethanol. Approximately 1 μL of sample (2 mg/mL protein concentration) was applied to each lane. Lanes 1 and 6, AC Pennant (*S. alba*); Lanes 2 and 7, AC Vulcan (*B. juncea*); Lanes 3 and 8, Commercial Brown (*B. juncea*); Lanes 4 and 9, S-67 (*B. carinata*); Lanes 5 and 10, Dodolla (*B. carinata*); Lane 11, standard proteins. For abbreviations see Figure 1.

TABLE 1
Protein Content and Functional Properties at pH 7.0 of Defatted Mustard Seed Meals[a]

Sample	P (%)[b]	PS (%)[b]	HC (%)[b]	EAI (m^2/g)[b]	ES (%)[c]	FC (%)[c]	FS (%)[c]
AC Pennant (*Sinapis alba*)	47.11[b]	53.45[b]	32.68[c]	28.00[b]	7.88	226.98	63.33
AC Vulcan (*Brassica juncea*)	46.71[b]	56.15[b]	43.12[b]	51.58[a]	3.88	245.90	61.54
Comm. Brown (*B. juncea*)	43.23[d]	54.33[b]	48.43[a]	52.34[a]	4.08	230.76	60.66
S-67 (*B. carinata*)	45.32[c]	73.05[a]	48.87[a]	46.27[a]	4.62	242.11	59.38
Dodolla (*B. carinata*)	48.77[a]	71.42[a]	48.88[a]	44.82[a]	5.85	238.33	57.14

[a]P, protein content; PS, protein solubility; HC, heat coagulability; EAI, emulsifying activity index; ES, emulsion stability; FC, foaming capacity; FS, foaming stability.
[b]Within each column, means with different roman superscript letters are significantly different ($P \leq 0.05$).
[c]No significant differences between the values in each column.

TABLE 2
Protein Content and Functional Properties at pH 7.0 of Acid-Precipitated Protein Concentrates Prepared from Defatted Mustard Seed Meals[a]

Sample	P (%)[b]	EAI (m^2/g)[b]	ES (%)[b]	FC (%)[c]	FS(%)[c]
AC Pennant (*S. alba*)	81.85[a,b]	21.44[c]	23.79[b]	200.50	37.68
AC Vulcan (*B. juncea*)	82.04[a]	39.85[a]	38.75[a,b]	200.50	33.91
Comm. Brown (*B. juncea*)	79.28[b]	44.47[a]	47.75[a]	196.72	36.52
S-67 (*B. carinata*)	81.77[a,b]	20.89[c]	45.21[a]	200.50	33.98
Dodolla (*B. carinata*)	83.86[a]	29.17[b]	47.52[a]	181.58	29.05

[a]For abbreviations see Table 1.
[b]Within each column, means with different roman superscript letters are significantly different ($P \leq 0.05$).
[c]No significant differences between the values in each column ($P > 0.05$).

Figure 1. Intramolecular disulfide bonds restrict protein unfolding. Protein unfolding enables polypeptide chains to associate noncovalently into protein aggregates during heating (18). Similarly, intermolecular disulfide bonds could prevent effective interactions between different polypeptide chains. The present result is similar to a previous report that showed that soybean β-conglycinin, which does not have disulfide bonds, was more susceptible to heat-induced coagulation than the glycinin fraction, which contains disulfide bonds (19).

The AC Pennant (*S. alba*) meal and protein concentrates had significantly lower ($P \leq 0.05$) EAI when compared to similar *Brassica* products (Tables 1–3). Increased emulsion formation has been associated with proteins that possess lower M.W. and greater structural flexibilities (20). Thus, the poor EAI of the *S. alba* protein products could have been due to the higher concentration of high MM polypeptide chains (50, 55, and 135 kDa) when compared to the polypeptides of the *Brassica* proteins (Figs. 1–3). The presence of disulfide bonds in these polypeptides could also have reduced the overall structural flexibility of the *S. alba* proteins, leading to decreased ability to unfold and associate with the oil droplets and hence lower EAI when compared to the *Brassica* proteins that do not contain the 135-kDa polypeptide. The results are comparable to those obtained for soybean proteins, which showed that β-conglycinin with no disulfide bonds had better emulsification properties than glycinin with disulfide bonds (14). An important observation is the lower ES values for the seed meals (Table 1), which have lower ratios of protein/nonprotein compounds than the protein isolates (Tables 2 and 3). The results suggest that protein molecules play a more important role in preventing emulsion destabilization than nonprotein molecules. Foaming capacity and FS did not differ significantly ($P > 0.05$) between the seed varieties either as

TABLE 3
Protein Content and Functional Properties at pH 7.0 of Calcium-Precipitated Protein Concentrates Prepared from Defatted Mustard Seed Meals[a]

Sample	P (%)[b]	EAI (m^2/g)[b]	ES (%)[b]	FC (%)[c]	FS (%)[c]
AC Pennant (*S. alba*)	75.17[b,c]	23.72[c]	9.88[b]	189.15	14.00
AC Vulcan (*B. juncea*)	79.83[a]	40.27[a,b]	24.49[a]	170.24	13.33
Comm. Brown (*B. juncea*)	77.04[b]	34.96[b]	23.87[a]	174.02	13.04
S-67 (*B. carinata*)	73.00[c]	43.16[a]	34.19[a]	181.58	12.50
Dodolla (*B. carinata*)	76.15[b]	47.09[a]	30.75[a]	185.37	18.37

[a]For abbreviations see Table 1.
[b]Within each column, means with different roman superscript letters are significantly different ($P \leq 0.05$).
[c]No significant differences between the values in each column ($P > 0.05$).

meals or protein concentrates (Tables 1–3). In comparison to EAI, the foam results indicate that the mechanisms involved in the formation of emulsions stabilized by the meals and protein concentrates are different from that of foam formation.

In conclusion, the results obtained in this work have shown that proteins present in *S. alba* seeds have similar polypeptide compositions as proteins found in *B. carinata* and *B. juncea*, with the exception of three polypeptides. The poor EAI coupled with the low susceptibility to heat-induced coagulation of the meals and protein concentrates from *S. alba* indicates substantial influence of the high-M.W. disulfide-bonded polypeptides (50- and 135-kDa). The 50- and 55-kDa polypeptides that are resistant to calcium-induced precipitation may be used in the food industry to formulate protein beverages that are fortified with calcium. Previous work in our laboratory (Aluko, R.E., and T. McIntosh, unpublished data) showed that the mustard seed protein concentrates contain negligible amounts of allyl isothiocyanate; therefore, the protein concentrates are safe for human consumption.

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