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Comparative study of the polypeptide profiles and functional properties of *Sinapis alba* and *Brassica juncea* seed meals and protein concentrates

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Abstract: Defatted meals and protein concentrates from six accessions of *Sinapis alba* and one accession of *Brassica juncea* mustard seeds were analysed for their polypeptide profile and functional properties. Two types of protein concentrates were prepared using acid-induced and calcium-induced protein precipitations. Meals from the *S alba* seeds had similar polypeptide composition, which was different from that of the *B juncea* meal. Non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that two of the major polypeptides (50 and 55 kDa) in *S alba* seeds were susceptible to acid-induced precipitation but resistant to calcium-induced precipitation. The *B juncea* meal proteins were significantly ($p \leq 0.05$) more susceptible to heat coagulation than the *S alba* meal proteins. Emulsifying activity index was significantly higher ($p \leq 0.05$) in the *B juncea* meal and protein concentrates when compared with similar products from *S alba*. It was concluded that the presence of a high-molecular-weight (135 kDa) disulfide-bonded polypeptide could have contributed to the lower emulsifying power of the *S alba* products when compared with the *B juncea* proteins that do not have this polypeptide.

Keywords: mustard; protein concentrate; defatted meal; polypeptide profile; functional properties

INTRODUCTION

The origin of the word 'mustard' is believed to come from the use of the seeds as condiments in a process which involved mixing the sweet 'must' of old wine with crushed seeds to form a paste, 'hot must' or 'mustum ardens'.[1] Therefore mustard is grown principally as a source of condiment for the spice trade, with two dominant species in the market, ie *Sinapis alba* and *Brassica juncea*. *S alba* is commonly known as 'white' or 'yellow' mustard and contributes a 'hot' principle which results in a sensation of sweetness and warmth.[1] *B juncea*, commonly called 'brown' or 'oriental' mustard, contributes the 'pungent' principle.

In order to meet the anticipated increase in demand for edible vegetable oils in the future, efforts are currently being made to develop the yellow and brown mustard seeds as potential oilseed crops in Canada.[2] These crops can be grown on more than 1.8×10^6 ha in the semi-arid prairies of Canada, with potential for production in other semi-arid regions of the USA, Australia and Asia. The residual meal remaining after oil extraction is rich in proteins (44–48% dry weight basis); therefore mustard seeds could serve as a suitable raw material in the manufacture of protein ingredients for the food and non-food industrial sectors. Previous work in our laboratory has shown that the protein composition and functional properties of defatted flour and protein concentrate prepared from a commercial variety of *S alba* seeds were different from those of similar products prepared from seeds of commercial *B juncea*.[3] Current research efforts at Agriculture and Agri-Food Canada, Saskatoon involve the development of *S alba* and *B juncea* seed varieties that have superior agronomic and seed functional properties when compared with the current commercial varieties. Therefore this work was aimed at determining the physicochemical and functional properties of meals and protein concentrates prepared from six accessions of *S alba* seeds and one variety of *B juncea* seed. The seeds that were used in this work are being developed for release as commercial varieties.

EXPERIMENTAL

Seeds from two species of mustard were used: *Sinapis alba* (M1–M6) and *Brassica juncea* (M7). The seeds were produced in the same year (1998) and at the same location (Saskatoon Research Farm, Saskatoon, SK,

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Canada) in order to minimise environmental variation. Soybean flour (Nutrisoy 7B) and protein isolate (ProFam 781) were obtained from Archer Daniels Midland Company (ADM, Decatur, IL, USA).

Preparation of seed meals

Mustard seeds were ground in small samples in a coffee mill for 2–3 min and the resulting meals were 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 concentrates

Acid-precipitated protein concentrate (APC) and calcium-precipitated protein concentrate (CPC) were prepared according to the procedures previously described by Aluko and McIntosh.[4] Generally, the defatted meal was mixed with 10 volumes 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 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 volumes of distilled water followed by centrifugation, and the precipitate was freeze-dried as the APC or CPC respectively.

Gel electrophoresis

Reduced and non-reduced gel electrophoresis were run separately on 8–25% gradient gels using Phast-System Separation and Control and Development Units according to the manufacturer's instructions (Pharmacia LKB, Montreal, PQ, Canada). Samples were prepared for non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by mixing the seed meal or freeze-dried protein product 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, and an aliquot (1 µl) of the supernatant was loaded onto the gel. Reduced samples were prepared by adding 5% (v/v) 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 AACC[5] method. Protein solubility (PS) was determined according to the method of Aluko and Yada[6] with some modifications. Each sample was mixed with 0.01 M sodium phosphate buffer, pH 7.0 to give a dispersion of approximately 1% (w/v) 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 analysed for protein content according to the method of Markwell *et al*,[7] and this was 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) and centrifuged at 10 000 × *g* for 30 min, and the amount of protein in the supernatant (S2) was determined according to the method of Markwell *et al*.[7] Heat coagulability (HC) was calculated as follows:

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

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 ($p > 0.05$) from that in S2.

Emulsifying activity index (EAI) was determined according to a modification of the spectroturbidimetric method of Pearce and Kinsella.[8] Samples were dispersed in 0.01 M sodium phosphate buffer, pH 7.0 to give 1% (w/v) protein content. An aliquot (5 ml) of the aqueous dispersion was added to 1 ml of pure commercial canola oil. The protein solution and oil phases were homogenised together for 1 min using a Polytron PT 10–35 homogeniser (Kinematica AG, Switzerland) equipped with a 20 mm generator (reduced foam model) with the power control unit (PCU 11) set at #6. Immediately after homogenisation, 10 µl of the emulsion was diluted to 5 ml with 0.1% (w/v) SDS solution and the absorbance at 500 nm was measured using the SDS solution as a blank. EAI ($m^2\,g^{-1}$) was calculated as described by Pearce and Kinsella.[8] The emulsions were allowed to stand at room temperature (23–25 °C) for 30 min and the EAI was determined and expressed as a percentage of the initial EAI to obtain emulsion stability (ES).

Foam expansion (FE) was determined according to the procedure described by Poole *et al*.[9] Sample dispersions containing 1% (w/v) protein were prepared in 0.01 M sodium phosphate buffer, pH 7.0 and homogenised for 30 s using a Polytron PT 10–35 homogeniser 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 analysis of variance and Duncan's multiple-range test were carried out using SAS[10] software.

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. Aoki *et al*[11] found that soybean protein isolates containing high ratios of β-conglycinin/glycinin generally had higher values of emulsifying capacity and emulsion stability than isolates with lower β-conglycinin/glycinin ratios. Therefore the observed differences in the polypeptide composition of the mustard seed meals and protein concentrates may be related to some of the functional properties that were determined in this study. Results of the gel electrophoresis of proteins present in the mustard meals are shown in Figs 1A and 1B for non-reducing and reducing conditions respectively. In the absence of the reducing agent (ME) the *S alba* meals (lanes 1–6) had more protein bands than the *B juncea* meal (lane 7). Four major polypeptides were identified for the *S alba* meals with estimated molecular masses corresponding to 16, 50, 55 and 135 kDa. The 16 kDa protein is equivalent to the 1.7S protein (estimated molecular mass of 15 kDa) that was previously isolated and purified from *S alba* seeds.[12] Absent from the polypeptide profile of the *B juncea* meal but present in the *S alba* meals were the 50 and 135 kDa polypeptide bands. Even though the 55 kDa polypeptide was present in both types of meals, the intensity was higher in the *S alba* meals. A whole range of polypeptides with estimated molecular masses between 29 and 48 kDa which were present in the *S alba* meals were absent in the *B juncea* meal (Fig 1A). The estimated molecular masses of the protein bands of the *B juncea* meal ranged from 12 to 80 kDa, which is similar to the range of 11–70 kDa that was previously reported by Gururaj Rao and Narasinga Rao.[13] In the presence of ME the 16, 50, 55 and 135 kDa polypeptides disappeared, indicating that each of these proteins contain polypeptides held together by disulfide bonds (Fig 1B). The report of Menendez-Arias *et al*[12] also showed that the 16 kDa polypeptide isolated from the seeds of *S alba* dissociated into two lower-molecular-weight polypeptides (5 and 9.5 kDa) upon addition of ME, indicating the presence of disulfide bonds. With the exception of the 63 kDa polypeptide band which is present in the *S alba* meals but absent in the *B juncea* meal, the polypeptide profiles obtained under reducing conditions were similar for both types of meals. Figure 2 also shows the appearance of the 12 and 13 kDa bands which

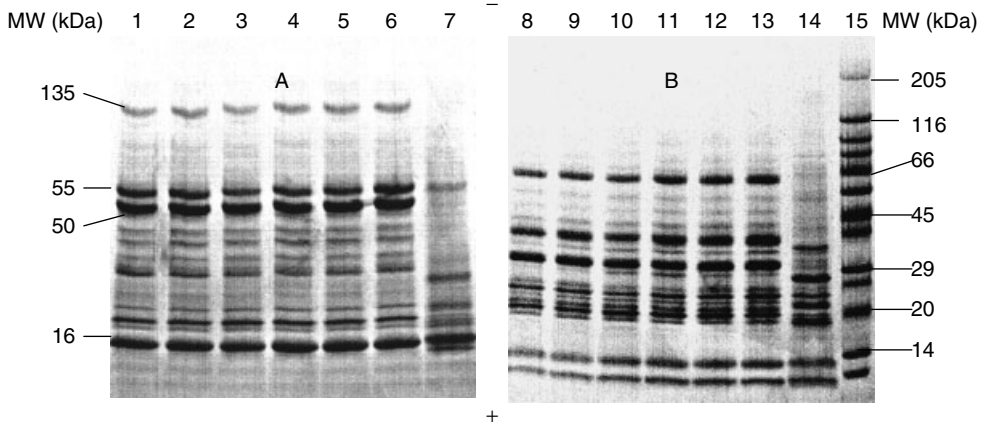

Figure 1. SDS-PAGE patterns of defatted mustard seed meals in (A) absence and (B) presence of 2-mercaptoethanol: lanes 1–6 and 8–13, *Sinapis alba*; lanes 7 and 14, *Brassica juncea*; lane 15, standard proteins.

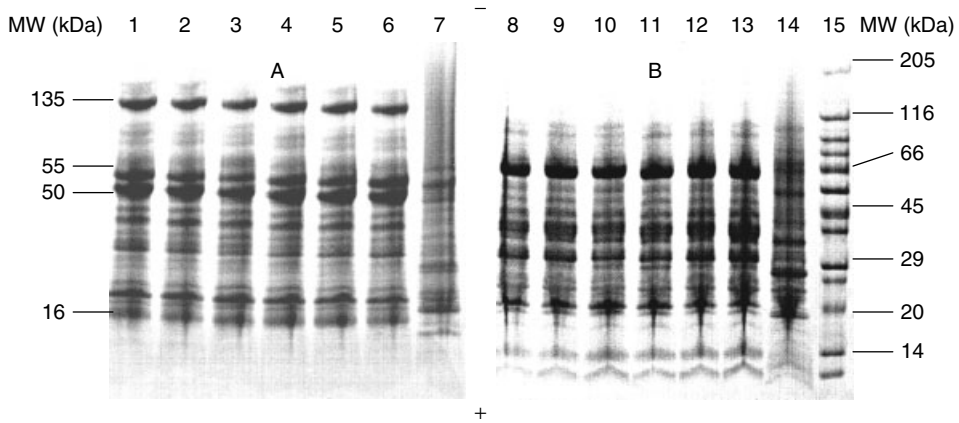

Figure 2. SDS-PAGE patterns of acid precipitated protein concentrates in (A) absence and (B) presence of 2-mercaptoethanol: lanes 1–6 and 8–13, *Sinapis alba*; lanes 7 and 14, *Brassica juncea*; lane 15, standard proteins.

were not present in Fig 1 and is in agreement with the previous work of Venkatesh and Appu Rao[14] who showed that the 12 and 13 kDa polypeptides are subunits (held together by disulfide bonds) of the 2S (or 1.7S) protein of mustard seeds. The polypeptide profile obtained in the presence of ME (Fig 1B) is similar to the results published by Fischer and Schopfer[15] for the seed storage proteins of *S alba*. The results are similar to the polypeptide composition of defatted meals of commercial seed varieties of *S alba* and *B juncea* that we recently reported.[3]

Figures 2A and 2B show results of the gel electrophoresis of protein concentrates obtained by acid precipitation (APCs) under non-reducing and reducing conditions respectively. The gel patterns were similar to those obtained for the meals, except that the 16 kDa polypeptide band was not as prominent in the protein concentrates as in the meals. We have previously shown that APCs from commercial mustard seeds also did not contain high levels of the 16 kDa polypeptide chain.[3] However, there was a significant increase ($p \leq 0.05$) in the intensity of the 135 kDa polypeptide band when compared with the intensity obtained for the meals, which is similar to our previous results.[3] Results of the gel electrophoresis of CPCs are shown in Figs 3A and 3B for non-reducing and reducing conditions respectively. Whereas the 50 and 55 kDa polypeptide bands were very prominent in both the meals (Fig 1A) and APCs (Fig 2A), the intensities were lower in the CPCs (Fig 3A), which is similar to the result obtained for the CPC produced from a commercial variety of *S alba*.[3] The results suggest that the 50 and 55 kDa bands maintained a substantial degree of solubility in 1 M calcium solution at pH 6.0 and could be used to increase incorporation of calcium into liquid foods without causing protein coagulation. Addition of ME completely eliminated the 135 kDa band, which indicates that it contains polypeptides held together by disulfide bonds and may be the same as was detected in the *S alba* meals (Fig 1B) and APCs (Fig 2B).

Protein content and functional properties

With the exception of the M6 meal, protein content was significantly higher ($p \leq 0.05$) in the *B juncea* meal than in the *S alba* meals (Table 1). However, protein contents of the mustard seed meals were significantly lower ($p \leq 0.05$) than that of the soybean flour but were similar to the values obtained for commercial mustard seed meals.[3] Except for the M1 meal, proteins in the *B juncea* meal were significantly ($p \leq 0.05$) more soluble at pH 7.0 when compared with the *S alba* meals; soybean flour proteins were comparatively more soluble than the mustard meals. Our previous report showed very similar PS for *B juncea* meal proteins, but some of the *S alba* accessions in this report (M2–M6)

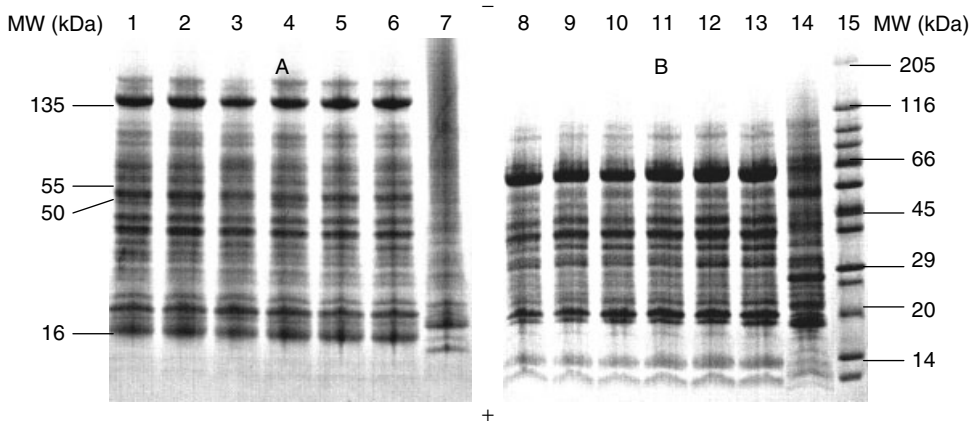

Figure 3. SDS-PAGE patterns of calcium precipitated protein concentrates in (A) absence and (B) presence of 2-mercaptoethanol: lanes 1–6 and 8–13, *Sinapis alba*; lanes 7 and 14, *Brassica juncea*; lane 15, standard proteins.

Table 1. Protein content and functional properties at pH 7.0 of defatted seed meals of *Sinapis alba* (M1–M6), *Brassica juncea* (M7) and soybean (SF)[a]

Sample	P (%)	PS (%)	HC (%)	EAI ($m^2\ g^{-1}$)	ES (%)	FC (%)	FS (%)
M1	45.88cd	55.66b	33.18cd	29.79e	7.64c	226.98cd	50.00c
M2	45.45d	52.59cd	28.95e	36.27cd	30.45b	223.20d	77.97a
M3	40.00f	47.40e	29.75e	32.27de	27.78b	230.76bcd	73.77a
M4	43.52e	51.95d	35.24bc	38.61c	6.79c	242.11ab	45.31c
M5	45.57d	42.30f	30.26de	34.20cde	26.85b	238.33abc	66.67ab
M6	47.16bc	48.88e	37.95b	33.79cde	5.00c	234.55bcd	48.39c
M7	47.52b	55.08bc	43.16a	47.30b	6.12c	249.68a	57.58bc
SF	52.42a	67.70a	2.48f	58.14a	58.59a	196.72e	51.92c

[a] Mean of two determinations. P = protein content; PS = protein solubility; HC = heat coagulability; EAI = emulsifying activity index; ES = emulsion stability; FC = foaming capacity; FS = foaming stability. Within each column, means with different letters are significantly different ($p \leq 0.05$).

had lower PS values than the meal proteins from commercial *S alba*.[3] Proteins in the *B juncea* meal (M7) were significantly ($p \leq 0.05$) more susceptible to heat-induced coagulation than proteins present in the *S alba* meals (M1–M6), which is similar to results obtained for meals from commercial seed varieties.[3] Increased resistance of the *S alba* meal proteins to heat-induced precipitation may be due to the presence of the 135 kDa protein, which probably has a rigid structure as a result of disulfide bonds as indicated by disappearance of the polypeptide in the presence of ME (Fig 1B). On the other hand, the 135 kDa polypeptide is absent in *B juncea* flour (Fig 1A, lane 7). The presence of intramolecular disulfide bonds restricts protein unfolding which is necessary to enable polypeptide chains to associate non-covalently into protein aggregates during heating.[16,17] Similarly, intermolecular disulfide bonds could prevent effective interactions between different polypeptide chains. The present result is similar to a previous report which 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.[18] The low susceptibility of the soybean flour proteins to heat-induced coagulation indicates greater structural stability when compared with the mustard proteins.

The *B juncea* meal had better emulsion-forming ability, since its EAI was significantly higher ($p \leq 0.05$) than those obtained for the *S alba* meals (Table 1). We have previously shown that the EAI of meals from commercial seed varieties of *B juncea* was significantly higher ($p \leq 0.05$) than the value obtained for *S alba* meal.[3] In comparison, the soybean flour had superior EAI when compared with the mustard meals. Increased emulsifying capacity has been associated with proteins that possess lower molecular weights and better interfacial properties at the oil–water interface.[19] Thus the lower EAI of the *S alba* protein products could have been due to the higher levels of high-molecular-mass polypeptides (50, 55 and 135 kDa) when compared with the polypeptides of the *Brassica* proteins. The presence of disulfide bonds in these three polypeptides could also have reduced the overall structural flexibility and interfacial property of the *S alba* proteins; therefore there is a decreased ability to unfold and associate with the oil droplets and hence lower EAI when compared with the *Brassica* proteins which do not contain the 135 kDa polypeptide. The results are comparable to that obtained for soybean proteins, which showed that β-conglycinin with no disulfide bonds had better emulsification properties than glycinin which contained disulfide bonds.[11] Generally, the emulsion made with soybean flour was more stable (higher ES) than those made with the mustard meals, which indicates superior ability of the soybean proteins to form stable interfacial membranes. However, it is possible that non-protein components could have contributed to the observed EAI and ES of the mustard and soybean products; therefore the differences in EAI and ES cannot be explained solely based on the protein composition of the samples. Except for M4 and M5, the FC of *B juncea* meal was superior to those of *S alba* meals and soybean flour (Table 1). In contrast, our previous work showed no significant differences ($p > 0.05$) between the FC of *B juncea* and *S alba* meals.[3] The present results reflect the ability of the proteins in this accession of *B juncea* meal to form interfacial membranes at the air–water interface better than the *S alba* accessions. Even though the EAI of the soybean flour was the highest, its poor FC suggests that the mechanism involved in lowering of surface tension at the oil–water interface may be different from that of the air–water interface. FS of the soybean flour was also poorer than those of some of the mustard samples (M2, M3 and M5).

Table 2 shows the functional properties of the APCs prepared from the various mustard seed meals. With the exception of M1, the *B juncea* APC and soybean protein isolate had significantly higher ($p \leq 0.05$) protein contents when compared with the APCs prepared from *S alba*. Similar to the results obtained for the meals is the fact that the soybean protein isolate and the APC from *B juncea* had significantly higher ($p \leq 0.05$) EAI than those of similar concentrates prepared from *S alba*. We have also shown that APCs prepared from commercial seed varieties of *B juncea* had higher EAI than the APC prepared from a commercial variety of *S alba*.[3] The results may be due to the presence of high-molecular-weight disulfide-bonded proteins in *S alba* which have poorer emulsifying property when compared with the *B juncea* proteins which have lower molecular weights. Stability of the emulsions (ES) was very similar except for M1 and M2. FC was substantially lower for the *B juncea* APC when compared with some of the *S alba* APCs. In contrast to the soybean flour, the soybean protein isolate had significantly higher ($p \leq 0.05$) FC when compared with those obtained for the mustard APCs (Table 2). The results suggest that, unlike the mustard

Table 2. Protein content and functional properties at pH 7.0 of acid-precipitated protein concentrates prepared from defatted meals of *Sinapis alba* (M1–M6) and *Brassica juncea* (M7) seeds[a]

Sample	P (%)	EAI ($m^2\ g^{-1}$)	ES (%)	FC (%)	FS (%)
M1	81.16ab	25.07d	23.93b	200.50bcd	37.68a
M2	75.45c	31.19c	23.94b	192.93cd	35.23b
M3	75.77c	31.17c	37.33a	211.85b	37.50ab
M4	75.46c	35.63c	36.82a	211.85b	37.50ab
M5	78.53bc	33.03c	29.16a	208.07bc	36.38ab
M6	76.67c	31.30c	35.25a	196.72bcd	34.62b
M7	84.32a	42.54b	35.79a	189.15d	37.82ab
SPI[b]	87.72a	64.93a	39.60a	253.46a	41.80a

[a] Mean of two determinations. P = protein content; EAI = emulsifying activity index; ES = emulsion stability; FC = foaming capacity; FS = foaming stability. Within each column, means with different letters are significantly different ($p \leq 0.05$).
[b] Commercial soybean protein isolate.

Table 3. Protein content and functional properties at pH 7.0 of calcium-precipitated protein concentrates prepared from defatted meals of *Sinapis alba* (M1–M6) and *Brassica juncea* (M7) seeds[a]

Sample	P (%)	EAI ($m^2\ g^{-1}$)	ES (%)	FC (%)	FS (%)
M1	69.65d	28.20c	9.85c	196.72c	21.16bcd
M2	68.62d	24.96cd	19.15bc	196.72c	25.00bc
M3	73.13bc	26.06cd	12.24c	189.15d	26.00bc
M4	69.47d	26.41c	10.48c	192.93cd	21.62bcd
M5	75.09bc	20.55d	18.13bc	196.72c	17.31cd
M6	75.45bc	23.86cd	12.07c	204.28b	29.63b
M7	76.82b	35.24b	24.96b	192.93cd	11.77d
SPI[b]	87.72a	64.93a	39.60a	253.46a	41.80a

[a] Mean of two determinations. P = protein content; EAI = emulsifying activity index; ES = emulsion stability; FC = foaming capacity; FS = foaming stability. Within each column, means with different letters are significantly different ($p \leq 0.05$).
[b] Commercial soybean protein isolate.

APCs, the reduction in the level of non-protein components favoured increased foam formation by the soybean proteins. With the exception of M2 and M6, there were no significant differences between the FS values of the mustard and soybean foams.

Results of the functional properties of concentrates from calcium-induced protein precipitation (CPCs) are shown in Table 3. Generally, the CPCs had less amount of protein when compared with the APCs and soybean protein isolate; therefore the proteins in mustard seeds seem to be more resistant to calcium-induced precipitation than acid-induced precipitation. CPCs prepared from commercial mustard seed varieties have also been shown to have lower protein contents than the APCs.[3] In a trend similar to that obtained for the meals and protein concentrates from acid-induced precipitation, the calcium-induced protein concentrate from *B juncea* (M7) and soybean protein isolate had significantly higher ($p \leq 0.05$) EAI value when compared with CPCs from *S alba*. The results are similar to our previous report which showed higher EAI for CPCs from commercial *B juncea* when compared with the CPC from a commercial *S alba*.[3] The results can be explained based on the differences in polypeptide composition as discussed above for the meals and APCs. FC did not differ considerably, except for the M6 concentrate which had a significantly higher ($p \leq 0.05$) value than the other concentrates. However, the FC and FS of the soybean protein isolate were significantly higher ($p \leq 0.05$) than those of the mustard CPCs. Generally, FC values of the APCs and CPCs were very similar but lower than the values obtained for the meals; however, soybean protein isolate produced higher FC than soybean flour. In contrast, the APC foams were more stable than the CPC foams, while the meals produced the most stable foams. It is possible that the higher content of non-protein components in the meals contributed to the high FS of the products, since soybean flour also produced foams that were more stable than that of soybean isolate.

CONCLUSIONS

The poor emulsifying activity index of the *S alba* meals and protein concentrates (M1–M6) was probably due to the presence of high-molecular-weight disulfide-bonded polypeptides which are unable to unfold properly at the oil–water interface. On the other hand, the *B juncea* meals and protein concentrates had relatively better emulsifying activity index as a result of reduced levels of the high-molecular-weight proteins. Generally, foams and emulsions stabilised by APCs were relatively more stable than those stabilised by CPCs. However, it is possible than non-protein components also contributed to the observed functional properties. Calcium was less efficient at precipitating the mustard proteins, and further work is being carried out to determine the potential food or nutraceutical utilisation of the fractions that were soluble in 1 M $CaCl_2$.

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