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TECHNICAL NOTE

Application of new methodology to canola protein isolation

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Despite the fact that canola is the second largest cash crop in Canada, the protein is destined for animal feed due to problematic antinutritional factors. A new method, termed the protein micellar mass (PMM) procedure, has been applied to the isolation of protein from canola. The objective of the new methodology was to isolate undenatured canola protein while enhancing the amount of protein extracted and eliminating antinutritional factors. Six different environmental regimes were chosen to range from pH 5·5 to 6·5 and concentrations of 0·01–0·1M NaCl/NaH_2PO_4. Of the six different environments examined, the medium characterized by pH 5·5, 0·1M NaCl/0·1M NaH_2PO_4 was selected to be the most successful at removing antinutritional factors.

INTRODUCTION

Canola is the second largest cash crop in Canada. To date, it holds a very high value for the nation in terms of vegetable oil production and exports. The oil is employed in the food industry while the commercial meal is used as animal feed despite a well rounded amino acid composition (Meith *et al.*, 1983). In addition, canola does not contain the enzyme lipoxygenase that promotes oxidative rancidity in other cereals such as soybean. Despite these positive attributes, the protein present in the defatted commercial meal is destined mainly for animal feed.

The primary reason for the under-utilization of the protein is related to the problematic antinutritional components associated frequently with the canola meal. Although reduced through selective breeding, the antinutritional factors must be reduced further or even removed completely in order for the protein to become suitable for human consumption. These antinutritional factors include glucosinolates, phytic acid and phenolic compounds.

The objective of this study was to extract the main storage globulins from commercially defatted protein meal so as to preserve the native state of the protein while removing the antinutritional factors. The extraction methodology used was based on the protein micellar mass (PMM) process developed by Murray *et al.* (1981). This represents a novel approach to the extraction of protein as the general methodology of acid solubilization/alkali precipitation of proteins is not used. The latter method risks a certain degree of denaturation whereas the PMM process has been shown to preserve protein native structure (Arntfield & Murray, 1981).

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MATERIALS AND METHODS

Protein extraction method

Raw defatted canola meal was obtained from the POS Corporation in Saskatoon, Saskatchewan. The canola protein was subsequently extracted from the meal using methodology derived from the PMM procedure as outlined by Murray *et al.* (1981). Samples of meal weighing 25 g were mixed for 1 h at 22°C in a series of buffers ranging from pH 5·5 to 6·5 with either 0·1M NaCl/0·1M NaH_2PO_4 or 0·01M NaCl/0·01M NaH_2PO_4. The pH values were adjusted with phosphoric acid. These conditions were selected to cover both pH and ionic strength ranges that would largely accomplish the removal of the antinutritional factors while not inflicting any undue damage to the protein.

After 1 h of stirring the mixture was centrifuged using a Sorvall Refrigerated Centrifuge, Model RC-2B (DuPont, Mississauga, Ontario), at 16 300*g* for 30 min in order to remove the seed and hull debris. The supernatant, containing the solubilized protein, was filtered through double-layered pre-moistened cheese cloth to remove any further debris. The supernatant was the concentrated from 200 to 50 ml through a 10^4 molecular weight cut-off PM10 filter in an Amicon ultrafiltration unit under a pressure of 60–70 psi. The solubilized protein concentrate was diluted to 15 times its volume with distilled water at a temperature of 4°C and left overnight (approximately 16 h) in cold storage (4°C). During this time, insoluble protein micelles settled on the bottom of the dilution vessel. The protein was collected by means of centri-fugation of the micellar suspension at 16 300*g* for30 min. The extracted protein was then frozen and freeze-dried.

Protein determination

Analysis of the extract for percent protein was carried out in accordance with the Kjeldahl methodology outlined in the AOAC *Official Methods of Analysis* (1980) in a conventional micro Kjeldahl distillation apparatus.

Antinutritional factor determination

The glucosinolates in the canola protein were analyzed according to the gas chromatographic method described by Slominski and Campbell (1987). Phytic acid analysis was performed according to the method of Latta and Eskin (1980). Phenolic compounds were measured by a variation of the method proposed by Dorrell (1976) with Lowry reagent being substituted for the Folin–Denis reagent as suggested by Schanderl (1970).

Analysis of thermal parameters

An analysis of the degree of denaturation of the extracted protein was performed by examining the thermal properties of the protein using differential scanning calorimetry. A DuPont 9900 computer thermal analyzer with a 910 differential calorimeter cell base was used according to the method of Arntfield and Murray (1981).

RESULTS AND DISCUSSION

Protein extraction

The methodology used to extract protein from the raw canola meal was based on the PMM method devised by Murray *et al.* (1981). The effects of the six extraction conditions on the percentage protein were examined. No significant differences ($P \leq 0.05$) were found among the six extraction conditions and the protein in the isolate, with a mean value being 78·5%.

Removal of the antinutritional factors

Phytic acid

Extraction of the canola protein at pH 5·5, using an 0·1M NaCl/0·1M NaH_2PO_4 buffer, resulted in 75·5% removal of phytic acid (Table 1). Of the six extraction conditions used, this medium was found to be the most effective at removing the phytic acid from the meal protein. This correlates with the findings of others where phytate removal from canola/rapeseed has been best effected at pH values around 5·0 (Carnovale *et al.*, 1988).

The isoelectric point of the main storage globulin is 7·2 (Meith *et al.*, 1983). Subjecting the meal to an extraction environment, such as used in this study, would classify the phytate–protein relationship as electrostatic in nature. The disruption of the electrostatic interactions between phytate and the protein was greatest with 0·1M NaCl/0·1M NaH_2PO_4, pH 5·5.

Phenolic compounds

The extraction buffer that best removed the phenolic compounds from the canola protein was 0·1M NaCl/0·1M NaH_2PO_4, pH 5·5. This buffer was significantly different from the remaining five extraction buffers (Table 1) and was found to reduce the total level of phenolic compounds in the isolate by 85·3%.

Table 1. Effects of the extraction conditions on the phytic acid,[a] phenolic compounds[a] and glucosinolate[a] content in commercial canola meal[b] protein

pH	0·01M NaCl[c]	0·1M NaCl[d]
Phytic acid		
5·5	0·96 ± 0·04[a]	0·49 ± 0·21[c]
6·0	0·92 ± 0·03[a]	0·61 ± 0·02[d]
6·5	1·29 ± 0·23[b]	1·28 ± 0·01[b]
Phenolic compounds		
5·5	0·011 ± 0·001 7[a]	0·005 ± 0·001 3[c]
6·0	0·019 ± 0·001 3[b]	0·016 ± 0·000 8[b]
6·5	0·001 3 ± 0·000[b]	0·015 ± 0·002 7[b]
Glucosinolates		
5·5	0·31 ± 0·05[a]	0·61 ± 0·07[b]
6·0	0·38 ± 0·06[a]	0·62 ± 0·04[b]
6·5	0·40 ± 0·08[a]	0·46 ± 0·02[a]

[a] Phytic acid values expressed as % phytic acid remaining in the protein extract. Phenolic compounds values expressed as mg phenolics/mg sample. Glucosinolate compounds expressed as μmol glucosinolates/g protein.
[b] Canola meal contains 2·00 ± 0·06% phytic acid, 0·034 ± 0·01 mg phenolics/mg meal, and 8·10 ± 0·04 μmol glucosinolates/g protein.
[c] All buffers were prepared using 0·01M NaH_2PO_4.
[d] All buffers were prepared using 0·1M NaH_2PO_4.
Values followed by the same letter are not significantly different ($P \leq 0.05$).

Table 2. Effects of the extraction conditions of thermal parameters, denaturation temperature (T_d)[a] and enthalpy of denaturation (ΔH)[b] for commercial canola meal protein

	pH	0·01M NaCl[c]	0·1M NaCl[d]
T_d			
	5·5	88·31 ± 0·03[a]	87·87 ± 0·99[a]
	6·0	87·92 ± 0·25[a]	88·21 ± 0·65[a]
	6·5	87·18 ± 0·49[a]	89·15 ± 0·50[a]
ΔH			
	5·5	5·30 ± 0·33[a]	5·69 ± 0·13[a]
	6·0	7·64 ± 0·20[a]	6·54 ± 0·41[a]
	6·5	5·75 ± 1·69[a]	8·23 ± 1·28[a]

[a] All T_d values are expressed as °C.
[b] All ΔH values are expressed as J/g of protein
[c] All buffers were prepared using 0·01M NaH_2PO_4.
[d] All buffers were prepared using 0·1M NaH_2PO_4.
Values followed by the same letter are not significantly different at the $P \leq 0.05$ level.

In addition to the tabular values, it was apparent that some phenolic compounds were not removed from the proteins as the freeze-dried extract was a light brown color in appearance. This may suggest that a somewhat stronger form of phenolic–protein compound may also exist in addition to the hydrophobic interactions. Such as association is probably due to strong electrostatic bonding between the acidic phenolic groups and the basic groups on the polypeptide chains. Covalent interactions between highly reactive phenolic groups and the proteins are also a possibility.

Glucosinolates

Maximum glucosinolate removal was achieved in four environments with a mean value of 95·2% elimination (Table 1). Two media were significantly lower in terms of glucosinolate removal with a mean value of 92·4% (Table 1). In this extraction method, dilution of the protein extract results in hydrophobic interactions, which, in turn, accounts for precipitation of the protein. In this environment, the glucosinolates are readily soluble prior to hydrophobic interaction. The glucosinolates are therefore separated from the protein.

Thermal stability of the protein

Thermal denaturation temperature

The relative denaturation of the protein was examined by observing the effects of the six extraction conditions on the thermal properties of the protein. There were no significant differences in denaturation temperature (T_d) among the six conditions (Table 2), with a mean value being 88·12°C.

Enthalphy of denaturation

The ΔH specifically describes the actual heat flow into the protein in the thermal denaturation process. The greater the heat flow, the greater the state of nativity is known to exist in the protein before the heat treatment. There were no significant differences in ΔH among the six conditions (Table 2), with a mean value being 6·19 J/g.

Both the T_d and ΔH values are very similar to the reports of other researchers where proteins from other plant sources, such as soybean and fababean, have been isolated using the PMM process (Arntfield & Murray, 1981). This testifies to the relative mildness of the PMM procedure on the native state of the extracted proteins.

CONCLUSION

From the results described above, it can be seen that the extraction of canola meal using the PMM approach at pH 5·5 with 0·1M NaCl/0·1M NaH_2PO_4 provides a medium for optimal removal of both phytic acid and phenolic compounds.

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REFERENCES

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Arntfield, S. D. & Murray, E. D. (1981). The influence of processing parameters on food protein functionality. 1. Differential scanning calorimetry as an indicator of protein denaturation. *Can. Instit. Food Sci. Technol. J.*, **14**, 289–94.

Carnovale, E., Lugaro, E. & Lombardi-Boccia, G. (1988). Phytic acid in fababean and peas: Effect on protein availability. *Cer. Chem.*, **65**, 114–17.

Dorrell, D. (1976). Chlorogenic acid content of meal from cultivated and wild sunflowers. *Crop Sci.*, **16**, 422–4.

Latta, M. & Eskin, M. (1980). A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.*, **28**, 1313–15.

Meith, G., Schwenke, K., Raab, B. & Bruckner, J. (1983). Rapeseed: Constituents and protein products. Part I. Composition and properties and glucosinolates. *Die Nahrung*, **27**, 675–97.

Murray, E. D., Myers, C. D., Barker, L. D. & Maurice, T. J. (1981). Functional attributes of proteins. A noncovalent approach to processing and utilizing proteins. In *Utilization of Protein Resources.* Food and Nutritional Press, Inc, Westport, CT, p. 158–76.

Schanderl, S. (1970). Tannins and related phenolics. In *Methods of Food Analysis.* Academic Press, New York, pp. 449–87.

Slominski, G. & Campbell, L. (1987). Gas chromatographic determination of indole glucosinolates—A re-examination. *J. Food Sci. Agric.*, **40**, 131–43.