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The Effect of Ammonia Concentration on the Properties of Canola Meals Produced by the Ammonia–Methanol/Hexane Extraction System

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ABSTRACT

Rapeseed (Tower variety) was extracted by a two-phase solvent system consisting of methanol containing dissolved anhydrous ammonia, and hexane. The effect of ammonia concentration in the polar phase on the composition and functional properties of the resulting meal was investigated. In the absence of ammonia, the two-phase extraction system removed 76% of the phenolic compounds and 50% of the glucosinolates, resulting in a meal containing 0·8 mg/g of glucosinolates. The glucosinolate content decreased with increasing ammonia concentration until it levelled off at 0·3 mg/g at 10% ammonia. The presence of ammonia did not affect the total phosphorus or phytate levels of the meal. The content of phenolic compounds rose slightly with an increase of the ammonia concentration in the polar solvent.

Extraction with the two-phase solvent system did not affect the water absorption of the meal. However, fat absorption rose sharply with increasing levels of ammonia in the polar phase. The opposite was true for the protein dispersibility index, which fell to 25% of the original level.

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INTRODUCTION

The usefulness of rapeseed as a source of food protein is limited by the presence of undesirable components such as glucosinolates, phenols and phytate.

The glucosinolate content of rapeseed has been significantly reduced, by plant breeding, to some 15% of its original level. Nonetheless, these levels are still too high for inclusion into food products. Reviews of methods for removing glucosinolates from rapeseed meal have been presented by Rutkowski (1970) and by Maheshwari *et al.* (1981). None of these methods is in commercial use due to disadvantages such as loss of protein material, poor functional properties or high processing costs.

The effect of treating rapeseed meal with alkanols containing anhydrous ammonia on the glucosinolate content of the meal has been previously reported (Diosady *et al.*, 1984*a*). As a result of these studies a two-phase solvent extraction system for the removal of glucosinolates from canola seed has been developed (Rubin *et al.*, 1984).

The canola meals produced by the two-phase solvent extraction system have excellent water- and fat-absorption properties, but low protein solubility in water (Diosady *et al.*, 1984*b*).

The objective of the present study was to evaluate the effect of the ammonia concentration in the methanol phase on the properties of Tower meals produced by the methanol–ammonia/hexane extraction system.

MATERIALS AND METHODS

The canola meals were prepared by the two-phase solvent extraction system developed in these laboratories (Rubin *et al.*, 1984). Ground Tower canola seed (60 g) was blended at low speed (approximately 2000 rpm) in a 4-litre Waring blender for 2 min with 400 ml of ammonia–methanol solution. The ammonia concentration in the methanol was varied between 0 and 12% (w/w). After a quiescent period of 15 min, 400 ml of hexane were added, and the mixture was again blended for 2 min. The meal containing residual oil was separated by vacuum filtration using Whatman No. 41 filter paper, rinsed three times with 100 ml of methanol and dried at 40 °C in a vacuum oven. The meal was further defatted with hexane using a Soxhlet apparatus and again dried as before. The properties of these meals were compared with those of meal prepared by extraction of ground Tower seed with hexane for 12 h

using a Soxhlet apparatus. The defatted meal was dried at 40 °C in a vacuum oven.

The crude protein (N × 6·25) and ash content were determined using AACC procedures, (AACC, 1976).

The glucosinolate content was measured by the method of Wetter & Youngs (1976). This measures the isothiocyanate and oxazolidine-2-thione content in the meal, and is useful as a rapid screening technique.

The phenolic compounds were assayed using the Krygier *et al.* (1982*a*) method with the following modification: 2 g of meal were extracted six times in a Polytron (Brinkmann) model A, type PT 10/35 (15 s, approximately 10 000 rpm) at room temperature with a 40 ml mixture of methanol:acetone:water (7:7:6). After centrifugation (15 min, 5000 rpm) the supernatants were combined and evaporated under vacuum at 45 °C to approximately 40 ml. The extract was then hydrolysed with 30 ml of 4N NaOH under nitrogen for 4 h at room temperature. The hydrolysate was acidified to pH 2 using 6N HCl. The resulting cloudy protein precipitate was removed by centrifugation (15 min, 5000 rpm) and discarded. The supernatant was extracted six times with diethyl ether at a supernatant-to-solvent ratio of 1:1. The emulsion formed during extraction was broken by centrifugation. The ether extracts were combined and evaporated to dryness at 30 °C under vacuum. The meal remaining after methanol–acetone–water extractions was treated with 20 ml 4N NaOH at room temperature for 4 h under nitrogen. The mixture was acidified with 6N HCl to pH 2 and centrifuged (15 min, 5000 rpm). The same procedure was used for the extraction of phenolic compounds from the hydrolysate as described above. The two extracts containing the phenolic compounds were dissolved in methanol and combined. The content of phenolic compounds was determined by the Swain & Hill (1959) method. The results were expressed as mg of *trans*-sinapic acid per 100 g of dry meal, since Krygier *et al.* (1982*b*) and Kozlowska *et al.* (1983) have shown that *trans*-sinapic acid is the predominant phenolic acid in Candle and Tower flour (97·8–99·3 % of total phenolic acids). However, sinapine, which is a choline ester of sinapic acid, is the major phenolic acid ester (Fenton *et al.*, 1980). The results for phenolic compounds can be presented on a sinapine basis by multiplying the results reported in Table 1 by a factor of 1·46. Clandinin & Heard (1968) reported the presence of tannins in rapeseed. However, using the procedure developed by Krygier *et al.* (1982*a*), tannins are not determined. The presence of tannins in the meal produced by our process will be investigated at a later date.

The protein dispersibility index (PDI) was measured by the AACC (1976) method.

The pH values were evaluated using a 10% dispersion (w/v) of meal in distilled water free of CO_2.

Fat absorption was determined by the method of Sosulski *et al.* (1976) by dispersing 2 g of sample in 12 ml of soybean oil in a 25 ml centrifuge tube.

Water absorption was determined by a combination of the AACC method (AACC, 1976) with those of Sosulski (1962) and Rutkowski & Kozlowska (1981). A sample of 2 g was dispersed in 16 ml distilled water. The contents were mixed for 30 s every 10 min using a glass rod and, after seven mixings, were centrifuged at 2000 × *g* for 15 min. The supernatant was carefully decanted, the tube was inverted and drained for 15 min, and finally weighed. The water absorption was expressed as the percentage increase of the sample weight.

The total phosphorus content was determined according to Tangkongchitr *et al.* (1981). The phosphorus content in the digest was measured colorimetrically by the AOCS procedure (AOCS, 1983).

The inorganic phosphorus was extracted by the method of Pons & Guthrie (1946). The inorganic phosphorus was measured directly in the extract by the method described by Lindberg & Ernster (1956) as modified by Nahapetian & Bassiri (1975).

Phytate was determined using a procedure adapted from the method given by Wheeler & Ferrel (1971) and Tangkongchitr *et al.* (1981), as previously described (Naczk *et al.*, 1985).

RESULTS AND DISCUSSION

The two-phase solvent extraction of Tower seed by methanol or methanol–ammonia solutions and hexane brings about an increase in the crude protein and ash content of this meal compared to hexane-extracted meal (Table 1). The meals treated with methanol/hexane or methanol–ammonia/hexane contain a similar level of crude protein (N × 6·25) and ash. This suggests that the quantity of ammonia remaining in the meal is very low and should not significantly affect the nitrogen content. The increase of crude protein content is mainly due to the dissolution of polysaccharides, phospholipids and other non-protein substances in the methanol phase.

TABLE 1
The Effect of Ammonia Concentration in Methanol on the Chemical Characteristics of Tower Meal[a]

Concentration of ammonia in MeOH (%)	*Crude protein (N × 6·25) (% of dry meal)*	*Ash (% of dry meal)*	*Glucosinolates (mg/g meal)*	*Phenolic compounds[b] (mg/100 g dry meal)*
Hexane-extracted meal	39·0 ± 1·0	6·70 ± 0·13	1·80 ± 0·10	1 543 ± 30
0	48·5 ± 0·4	8·10 ± 0·30	0·82 ± 0·07	370 ± 10
2	49·0 ± 1·0	8·30 ± 0·24	0·62 ± 0·05	449 ± 19
4	49·1 ± 1·5	8·10 ± 0·15	0·60 ± 0·07	519 ± 25
7·5	50·2 ± 0·6	8·30 ± 0·21	0·45 ± 0·05	503 ± 20
10	49·9 ± 0·8	8·40 ± 0·32	0·30 ± 0·03	507 ± 14
12	48·3 ± 1·5	7·70 ± 0·28	0·32 ± 0·03	555 ± 25

[a] Produced by the two-phase solvent extraction system.
[b] Expressed as mg *trans*-sinapic acid per 100 g dry meal.

In our laboratory we studied the effect of four lower alkanols and different ammonia concentrations on the removal of the glucosinolates from laboratory-prepared Altex meal. We found that 10 % ammonia in methanol, or in methanol containing 5 % of water, was the most effective solvent for the removal of glucosinolates (Diosady *et al.*, 1984*a*). The present study confirmed that the extraction of canola seed using 10 % ammonia in methanol removed over 80 % of the glucosinolate content of the seed, giving a level of 0·3 mg/g of meal (Table 1). In the presence of 5 % water the removal of glucosinolates from seed is further improved, giving levels which are below the sensitivity of Wetter & Youngs' (1976) method (Diosady & Rubin, 1985). The methanol by itself removed only about 50 % of the glucosinolates.

Based on these results we postulate that the removal of glucosinolate from canola seed in the methanol–ammonia/hexane extraction process occurs in two steps. At first some of the glucosinolates are extracted into the methanol–ammonia phase. However, the remaining glucosinolates are probably converted to other products by reaction with ammonia before they are extracted. This step is responsible for the almost complete removal of glucosinolates by this process. The mechanism of glucosinolate removal is presently under investigation. While the Wetter & Youngs (1976) method was suitable during the development of the

process, we fully realize its limitations. At the present stage of development a more accurate and discriminating analytical method will be needed, and such are indeed available (Daun & McGregor, 1981; Maheshwari *et al.*, 1979; Underhill & Kirkland, 1971).

Another advantage of the treatment of oilseed meals with ammonia is the significant drop in the content of phenolic compounds. Goh *et al.* (1983) reported that extraction of Candle and Tower meals with ethanol containing 0·2M ammonia removed 82% and 39%, respectively, of the sinapine initially present in the meal. However, McGregor *et al.* (1983) observed up to 74% sinapine removal from *Brassica juncea* mustard meal by gaseous ammoniation. A higher drop in the sinapine content (90%) was found by Kirk *et al.* (1966) in crambe meal treated with gaseous ammonia. They also found that aqueous ammonia was less effective. The extraction of Tower seed with ammonia-free methanol removed 76% of the phenolic compounds. However, in the presence of ammonia in the methanol phase, the yield of extracted phenolic compounds decreased (Table 1). Concentrations of ammonia above 4% did not significantly affect the extraction of phenolic compounds further, resulting in a reduction of about 67%. The results of our studies are consistent with those reported in the literature.

As expected, the presence of ammonia in methanol increased the pH of a 10% dispersion of the meal in distilled water by about one unit, as compared to methanol- or hexane-extracted meal (Table 2). Kirk *et al.*

TABLE 2
The Effect of Ammonia Concentration in Methanol on Some Functional Properties of Tower Meal[a]

Concentration of ammonia in MeOH (%)	*pH*	*Protein dispersibility index* (%)	*Water absorption* (%)	*Fat absorption* (%)
Hexane-extracted meal	5·97	43·1 ± 2·3	370 ± 5	188 ± 10
0	6·10	18·1 ± 0·4	388 ± 5	213 ± 11
2	7·15	9·9 ± 0·4	334 ± 8	209 ± 11
4	7·15	10·1 ± 0·2	357 ± 16	287 ± 3
7·5	7·12	9·7 ± 0·3	357 ± 10	320 ± 10
10	7·12	9·5 ± 0·3	365 ± 12	309 ± 6
12	7·20	9·4 ± 0·2	369 ± 8	284 ± 9

[a] Produced by the two-phase solvent extraction system.

(1966) reported that in ammonia-treated crambe meal the ammonia was retained as both vacuum-labile and chemically bound ammonia. They found that all labile ammonia was removed from the meal after 20 h of drying under vacuum. It is probable that the vacuum-labile absorbed ammonia is responsible for the observed increase in pH.

The extraction of Tower seed with methanol–hexane resulted in an over 50% drop in the protein dispersibility index (PDI) compared to the PDI of hexane-extracted meal. In the presence of 2% ammonia in the methanol, the PDI decreased to 10%, the level previously reported for the canola meals treated with 10% ammonia in methanol and hexane (Diosady *et al.*, 1984*b*). Concentrations of ammonia in methanol higher than 2% did not decrease the PDI further (Table 2).

Meals treated with methanol containing 0 and 2% ammonia have about 10% lower water absorption than hexane-extracted meal. Increasing the ammonia concentration in the methanol to 4% or more gave an increase in the water absorption to a level similar to that of hexane-extracted meal (Table 2).

We found that fat absorption of the meal was strongly influenced by the ammonia concentration. The meal extracted with methanol or methanol containing 2% ammonia absorbed somewhat more soybean oil than hexane-extracted meal. Higher concentrations of ammonia increased the fat absorption by up to 70% at a concentration of 7·5% ammonia

TABLE 3
The Effect of Ammonia Concentration in Methanol on Total, Phytate and Inorganic Phosphorus Contents of Tower Meal[a]

Concentration of ammonia in MeOH (%)	*Total phosphorus (mg/g dry meal)*	*Phytate as*		*Inorganic phosphorus as*	
		% of dry meal	*% of total phosphorus*	*mg/g dry meal*	*% of total phosphorus*
Hexane-extracted meal	12·6 ± 0·3	3·55 ± 0·10	79·4	0·62 ± 0·01	4·9
0	13·1 ± 0·2	3·76 ± 0·10	80·9	0·65 ± 0·01	5·0
2	13·8 ± 0·4	3·76 ± 0·10	76·8	0·68 ± 0·01	4·9
4	13·4 ± 0·2	3·76 ± 0·10	79·2	0·67 ± 0·02	5·0
7·5	13·7 ± 0·3	3·84 ± 0·10	77·1	0·67 ± 0·01	4·9
10	13·3 ± 0·1	3·84 ± 0·10	81·3	0·67 ± 0·01	5·0
12	14·4 ± 0·3	3·97 ± 0·10	77·7	0·73 ± 0·02	5·1

[a] Produced by the two-phase solvent extraction system.

(Table 2). The fat absorption measurements suggest that the ammonia–methanol treatment makes the rapeseed proteins more capable of reorientating the hydrophobic and hydrophilic groups in relation to the oil phase.

The results given in Table 3 indicate that the ammonia concentration in the methanol phase did not affect the phosphorus content of the treated Tower meals. The small increase of the phosphorus content in these meals compared to hexane-extracted meal was the result of the dissolution of some of the components of the seed in the methanol–ammonia phase. The phytate represents $79 \pm 2\%$ and the inorganic phosphorus $5 \pm 0{\cdot}1\%$ of the total phosphorus. The remaining 16% is present in the meal as organic, non-phytate phosphorus.

CONCLUSIONS

Methanol reduced the glucosinolates content of the meal by approximately 50%. The presence of ammonia in methanol decreased the glucosinolates content in the meal by about 80% to 0·3 mg/g at an ammonia concentration of 10% or more.

Maximum fat absorption was obtained at 7·5% ammonia in methanol. At this concentration it was 70% higher than for hexane-extracted meal. The water absorption rose slowly with increased ammonia concentration, reaching that of hexane-extracted meal at 12% ammonia.

The methanol by itself dropped the PDI of meal by over 50%. In the presence of 2% ammonia the PDI fell to 25% of its original level.

Pure methanol extracted about 76% of the phenolic compounds originally present in the seed. In the presence of ammonia this dropped to about 67%, leaving about one-third of the phenolic compounds in the meal.

The presence of ammonia in methanol did not affect the phytate and inorganic phosphorus contents of the meal.

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