THE ALKANOL-AMMONIA-WATER/HEXANE TREATMENT OF CANOLA - AN OVERVIEW

F. Shahidi, Dept. of Biochemistry, Memorial University of Newfoundland, St. John's, NF, A1B 3X9, Canada, and M. Naczk, L.J. Rubin and L.L. Diosady, Dept. of Chemical Engineering, University of Toronto, Toronto, ON, M5S 1A4, Canada.

INTRODUCTION:

Rapeseed is among the world's most important oilseed crops, and in Canada it is second only to wheat in value and in area planted. Rapeseed is used for the production of edible oil and feed-grade meal. Although rapeseed meal has a reasonably well balanced amino acid content, its use as a food-protein source has been thwarted by the presence of undesirable components. These include glucosinolates, phenolics, phytates, and fibre (largely hull). The Canadian plant breeders have developed the low glucosinolate, low erucic acid content varieties referred to as canola. However, the canola meal still contains 1-3 mg glucosinolates per g of meal and it cannot be used as a food products. Over the last three decades, many methods have been developed to upgrade canola meal; however, none of them has reached the commercial stage. Disadvantages such as loss of proteins, poor functional properties, or high processing costs are among the main reasons (Maheshwari et al, 1981).

Recently in our laboratories, a novel method for the removal of glucosinolates from rapeseed and mustard seed was developed (Naczk et al, 1986a; Rubin et al, 1984). In this method, the crushed seed was exposed to a two phase solvent-extraction system. The polar phase was alkanol-ammonia and the second phase was hexane which extracted the oil. The effect of this processing method on the content of glucosinolates, phytates, and phenolics in the meal, its gross composition and functional properties will be discussed in this overview.

THE ALKANOL-AMMONIA/HEXANE PROCESS:

The two-phase solvent extraction process developed in our laboratories for rapeseed and mustard seed (Naczk et al, 1986a; Rubin et al, 1984) is schematically shown in Figure 1.

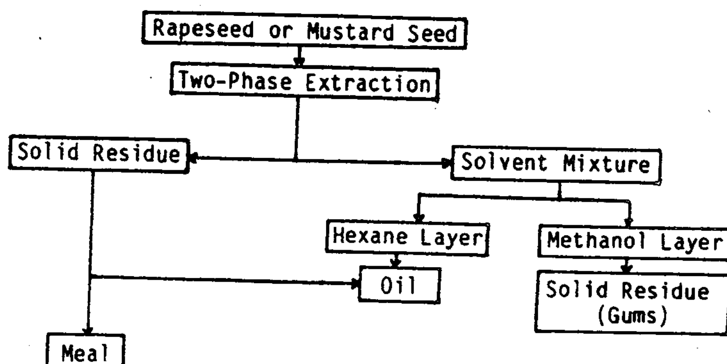

Figure 1. Flowsheet for the two-phase extraction process.

The laboratory procedure includes a two-minute blending period of ground seed with methanol-ammonia-water (solvent-to-seed ratio of 6.7) followed by a quiescent period of 15 minutes. At this point, hexane was added and the mixture was stirred vigorously for a further two-minute period. The meal was then filtered off and the two phases were separated by usual means. This process simultaneously removed the glucosinolates from canola meal to a level below the detection limit of Wetter and Youngs' method (1976). The oil was extracted into the hexane phase which contained about 84% of the total oil present in the seed (Rubin et al, 1986). The resultant oil had a low phosphorus content (unpublished results); and could be considered as a degummed oil. The meal (about 48% yield) still contained some residual oil. The polar phase contained about 9% of the solids present in the original seed (Rubin et al, 1986). The solids included phospholipids, phenolics, carbohydrates, soluble nitrogen compounds, glucosinolates and their breakdown products.

COMPOSITION OF THE MEAL:

The typical chemical composition of canola meals obtained by the two-phase solvent extraction system is shown in Table 1. The glucosinolate-free canola meal contained about 25% more crude protein than the corresponding hexane-extracted meal. The ash content of glucosinolate-free meal was, on average, 18% higher than that of hexane-extracted meal. The increase in crude protein and ash content was the result of dissolution of 8-10% of the seed materials (phospholipids, phenolics, carbohydrates, and glucosinolates) in the polar phase. Furthermore, the non-protein nitrogen content of the treated meals was about half of that of hexane-extracted meal (Table 1).

Table 1: Chemical Composition of Canola Meals.[1]

Meal[2]		Crude Protein % (N x 6.25)	Ash %	Non-protein Nitrogen % of Crude Protein	Glucosinolates[3] mg/g	Phytate %	Phenolics[4] mg/100g
	A	39.0 ± 1.0	6.7±0.1	9.9 ± 0.3	1.72 ± 0.09	3.55±0.10	1543
Tower	B	49.7 ± 1.0	8.0±0.2	4.8 ± 0.3	0.25 ± 0.05	3.78±0.10	472
	C	49.5 ± 2.5	7.8±0.2	4.5 ± 0.3	< 0.2	3.89±0.14	383
	A	39.1 ± 0.2	7.5±0.2	9.0 ± 0.05	0.91 ± 0.05	4.05±0.03	1807
Altex	B	47.1 ± 0.5	9.0±0.1	5.3 ± 0.1	< 0.2	4.70±0.10	521
	C	46.2 ± 3.0	6.6±0.1	4.9 ± 0.3	< 0.2	4.80±0.08	505

[1] From Naczk et al, 1985 and 1986b. All values are on dry basis.
[2] A- extracted with hexane; B- extracted with 10% NH_3 in MeOH and hexane; and C- extracted with 10% NH_3 in MeOH containing 5% H_2O and hexane.
[3] 0.2 mg/g meal is the sensitivity of the analytical procedure (Wetter and Youngs, 1976).
[4] Expressed as mg trans-sinapic acid.

The drop in the non-protein nitrogen content is due to its partial dissolution in methanol layer and this is perhaps in part responsible

for the light colour of the treated meals (Rutkowski, 1970). Removal of phenolic compounds also has a beneficial effect on colour and flavour (vide infra) (Rutkowski and Kozlowska, 1979).

GLUCOSINOLATES:

The glucosinolates of rapeseed and mustard seed all contain a β-thioglucose group, a side chain R and a sulphonated oxime moiety (Figure 2).

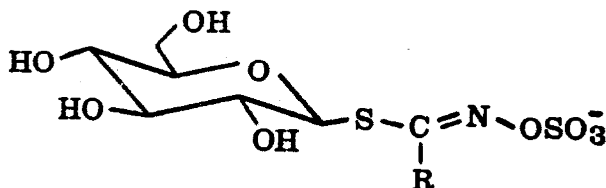

Figure 2. General chemical structure of glucosinolates.

Products of enzymatic hydrolysis of glucosinolates interfere with the function of thyroid glands and adversely affect the growth of experimental animals. Of these 2-hydroxy-3-butenyl glucosinolate and the oxazolidinethione derived from it are of particular concern. Therefore, a number of different techniques have been employed for the removal of glucosinolates from rapeseed or rapeseed meal (Fenwick _et al_, 1986; Maheshwari _et al_, 1981; Rutkowski, 1970). However, none of these methods of glucosinolate removal have as yet been used commercially.

The alkanol-ammonia treatment of rapeseed was developed in our laboratories for simultaneous oil extraction and glucosinolate removal from canola and rapeseed (Diosady _et al_, 1985a; Naczk _et al_, 1986a; Rubin _et al_, 1984 and 1986). The effectiveness of glucosinolate removal depended on the alkanol used, ammonia concentration, solvent-to-seed ratio, and the contact time of ground seed or meal with solvent.

The effectiveness of glucosinolates removal by alkanol and alkanol-ammonia solutions may be ranked as follows:

methanol>> ethanol> isopropanol> t-butanol.

Only methanol and methanol-ammonia solutions, with or without water, were highly effective in removing glucosinolates from canola seed and meal (Table 1). Five percent (v/v) water in methanol in the presence of 10% (w/w) ammonia was sufficient to give excellent glucosinolate removal. It should be noted that for canola seed, the use of more than 5% water in alkanol phase produced a dark coloured and sticky meal, perhaps due to the hydration and precipitation of phospholipids in the meal, which are otherwise dissolved in the alkanol phase.

The concentration of ammonia in methanol was another important variable. The optimum concentration of ammonia in methanol was 10% (w/w) (Diosady _et al_, 1985a; Rubin _et al_, 1984 and 1986).

Removal of glucosinolates from high-glucosinolate rapeseed of the Midas variety and of mustard seed (Naczk _et al_, 1986a) was quite effective when 10% (w/w) ammonia in methanol containing 5% (v/v) water and a 30-minute quiescent period was used. The glucosinolate content was reduced from a level of 17.0 mg/g to 1.6 mg/g of oil-free meal. A two-stage process removed over 98% of the glucosinolates originally present in the meal. The resultant meal contained 0.2 mg glucosinolates per g of meal.

Solvent ($CH_3OH/NH_3/H_2O$)-to-seed ratio (R) was another factor which

affected the efficiency of glucosinolate removal from rapeseed. Increasing R from 3.3 to 6.7 decreased the content of glucosinolates in canola meal by a factor of 2. The residual glucosinolate content was < 0.2 mg/g meal (R = 6.7) which is less than the detection limit of 0.2 mg/g reported by Wetter and Youngs (1976). For Midas rapeseed and mustard seed further dilution from an R of 6.7 to R = 13.3 reduced the glucosinolates content by a factor of 2 (Naczk et al, 1986a), but it had little effect on the values obtained for canola varieties.

After the initial two minutes of blending, 70% of the glucosinolates present in canola meal and Midas were removed. The rapid glucosinolate reduction continued until the quiescent period reached 15 and 30 minutes for canola and Midas, respectively. After these periods, the level of residual glucosinolates in the meal was essentially constant. Based on these results, it was suggested that the glucosinolates were perhaps removed by two different mechanisms from the seeds. Some of the glucosinolates are extracted into the polar phase and these may be converted to other products on standing. The remaining glucosinolates are probably first converted to other products by the influence of ammonia before being extracted into the polar phase. Nearly 85% of the glucosinolates were removed into the polar phase, in the intact form, and some 10% were degraded to other products. These were also found in methanol-ammonia-water phase. The balance of the glucosinolates (about 5%) remained in the meal (unpublished results).

PHENOLIC COMPOUNDS:

The effect of treatment of oilseeds with ammonia (gaseous or in ethanol solution) on the content of phenolic compounds of the resultant meal has been investigated (Goh et al, 1983; Kirk et al, 1966; McGregor et al, 1983). The two-phase solvent extraction of canola reduced the phenolic content of the meals by 72.4 ± 3.5% (Naczk et al, 1986b). An increase in the concentration of ammonia in methanol phase above 4% (w/w) did not alter the extraction efficiency of the phenolic compounds significantly (Diosady et al, 1985b).

PHOSPHORUS COMPOUNDS:

Hexane-extracted canola meals contained between 11.3 and 14.3 mg phosphorus per g meal, on dry basis, depending on the variety of seed used (Naczk et al, 1986b). Uppstrom and Svensson (1980) have reported similar results. However, the two-phase solvent extraction of canola increased the total phosphorus content of the meal by 14.5 ± 5.8% when compared with hexane-extracted meals (Naczk et al, 1986b). This apparent increase in the phosphorus content is due to the dissolution of phospholipids, phenolics, non-protein nitrogen compounds, etc., out of the seed by the methanol phase.

Phytates are the most important class of phosphorus compounds having nutritional implications (Maga, 1982). In canola meal, phytates constitute the major portion of the total phosphorus content, i.e. 78.6 to 88.1% (Naczk et al, 1986b). Uppstrom and Svensson (1980) reported similar results. Methanol-ammonia treatment of Tower seed increased the phytic acid content of the meal from 3.55 to 3.78%. However, the ratio of the content of phytic acid to the total phosphorus did not change when compared to that of hexane-extracted meals. Similar results were obtained for the meals prepared from Altex, Candle, and Regent varieties (Naczk et al, 1986b). It is obvious that our process does nothing to reduce the phosphorus content of rapeseed.

FUNCTIONAL PROPERTIES:

The acceptability of oilseed protein products (meal, isolate, concentrate) as a component of food formulations depends on their sensory quality, nutritive value, and functional properties.

The methanol-ammonia process produces a free-flowing meal with a sandy to light-beige colour, depending on the cultivar used. The product has a bland taste and this is perhaps due to the removal of 68 - 74% of the polyphenols and nearly half of the non-protein nitrogen compounds from the meal. The pH of a 10% dispersion of these meals in CO_2-free distilled water was higher than for hexane-extracted meals by one pH unit (Naczk et al, 1985). The presence of adsorbed ammonia in the meal is perhaps responsible for this increase in the pH. The reduced solubility of the meal protein is perhaps due to the unfolding of protein chains which may in turn form protein aggregates which are insoluble in water.

Water absorption is one measure of the interaction of protein products with water. Both hexane-extracted and methanol-ammonia treated meals have high water absorption (Diosady et al, 1985b; Naczk et al, 1985).

Another important binding property of protein products is their fat absorption. The fat absorption of rapeseed meals is affected strongly by the presence of ammonia in methanol (Naczk et al, 1985). Ammonia at a concentration of $\geq$ 4% increased the fat absorption of canola meals by 50 to 70%, as compared with the hexane-extracted meals (Diosady et al 1985b). This increase may be due to the presence of a large number of hydrophobic groups on the surface of protein molecules which have a great affinity for oil. The high water-and fat-absorption obtained for methanol-ammonia-treated meals results from unfolding of hydrophobic groups of proteins at the oil-water interface as suggested by Morr (1979) for whey protein molecules.

Emulsifying properties of canola meals were not affected by the methanol-ammonia treatment (Naczk et al, 1985). Both hexane-extracted and treated meals had a low water-soluble protein content, their high fat absorption is likely to play an important role in emulsion formation and stabilization (Wolf and Cowan, 1975).

The whippability of methanol-ammonia treated meals, especially in the presence of 5% water, was generally lower than hexane-extracted samples, but was still significantly better than the commercial canola meal. The two-phase solvent-extraction system did not influence the foam-stability. The stability of foam produced from laboratory-prepared hexane-extracted canola meal was much higher than for the commercial canola meal (Naczk et al, 1985).

In conclusion, the two-phase solvent-extraction process of canola produces a meal which is high in protein content (about 50%), light in colour, and is reasonably bland in taste. It is almost free of glucosinolates and is low in phenolics. In addition, it has some interesting functional properties. Its excellent water- and oil-binding and emulsifying properties suggests that it may be used as binder in meat products and as extender for meat proteins. Fibre and phytates were not affected in this process.

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