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Removal of cyanogenic glycosides of flaxseed meal

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Flaxseed meals were prepared by a two-phase solvent extraction system consisting of hexanes and an alkanol (methanol, ethanol or isopropanol) phase with or without added water and/or ammonia. The effect of the extraction process on the contents of protein and cyanogenic glycosides in the meals was studied. The crude protein content of the extracted meals varied from 43·5 to 48·6, compared with a value of 41·2% for hexane-extracted meals. Of the 4·42 mg/g linustatin and 1·90 mg/g neolinustatin originally present in the meals, over 90% of each cyanogenic glycoside was removed under optimum conditions using methanolic solutions.

INTRODUCTION

Flaxseed (*Linum usitatissimum* L.) is an important oilseed crop of the world. It ranks third in Canada and this contributes 28% to the total global production (Anon., 1989). Flaxseed is one of the richest sources of α-linolenic acid, and is used mainly for production of industrial linseed oil. However, development of low linolenic acid flaxseed varieties may allow the production of food-grade linseed oil (Dorrel, 1972; Prentice, 1992). In North America, flaxseed has been used at low levels in some cereals, specialty breads and in various bakery products (Dybing & Lay, 1981; Ratnayake *et al.*, 1992).

Linseed oil is produced either by cold pressing or by hexane extraction of seeds. The resultant meal, after oil extraction, contains some anti-nutritional factors, particularly cyanogenic glycosides (Butler, 1965; Smith *et al.*, 1980; Conn, 1981) and an anti-pyridoxin (anti-vitamin B6) component (Klosterman *et al.*, 1967). Toxicity of cyanogenic glycosides (Figure 1) is due to the release of hydrogen cyanide by the action of a β-glycosidase, which acts as a potent respiratory inhibitor by complexing with metalloporphyrin-containing enzymes (Poulton, 1989).

The deleterious effects of the anti-pyridoxin component of flaxseed are caused by linatine (1-[(*N*-γ-L-glutamyl)-amino]-D-proline), and this has limited the use of flaxseed meal in poultry feed formulations. However, the adverse effects of linatine could be circumvented by pyridoxin supplementation of the feed. Therefore, attempts have been made to remove the cyanogenic compounds of flaxseed meal by boiling in water, dry and wet autoclaving, and acid treatment followed by autoclaving (Madhusudhan & Singh, 1985*a,b*). These processes generally require a high-temperature treatment and/or a leaching procedure; both of which either adversely affect the quality of the resultant meal or afford a low protein-recovery yield (Madhusudhan & Singh, 1985*a*).

The objectives of the present study were to prepare a high-protein flaxseed meal devoid of cyanogenic glycosides. The use of a two-phase solvent extraction system consisting of alkanol-ammonia and hexane, originally developed for single-cell proteins and canola, was tested.

MATERIALS AND METHODS

Sample preparation

Flaxseed and a commercial flaxmeal were obtained from Omega Nutrition, Vancouver, British Columbia. Laboratory preparation of meals involved grinding seeds in a Moulinex coffee grinder followed by extraction of oil. Hexane-extracted seeds/meals were prepared by blending 75 g of sample with 500 ml of hexanes in a 4 litre Waring blender for 2 min at low speed (approximately 2000 rpm).

Treated samples were prepared by first blending the ground seed/meal (75 g) with 500 ml of 95% or absolute alkanol (methanol, ethanol or isopropanol) with or without 10% (w/w) ammonia for 2 min at 2000 rpm. After a 15-min quiescent period, 500 ml of hexanes was added to the mixture and the slurry was again blended

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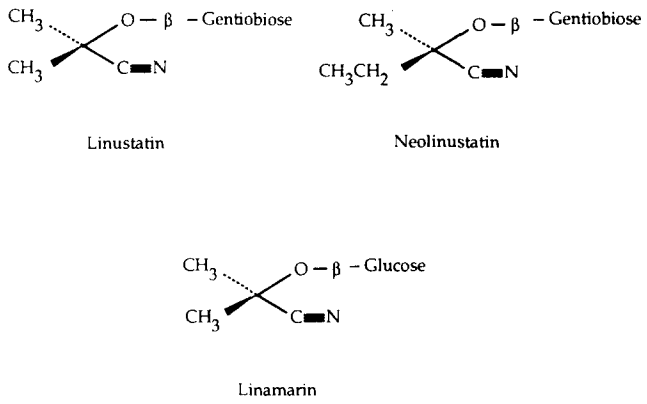

Fig. 1. Possible cyanogenic glycosides of flaxseed.

for 2 min at 2000 rpm. Meal was then recovered on Whatman No. 41 filter paper by vacuum filtration. Samples were rinsed three times with a total of 125 ml of alkanol and then dried overnight at 40°C in vacuum oven. The meal was further defatted with hexanes using a Soxhlet apparatus and dried as described above. The hexane and alkanol phases were separated and the polar fraction was re-extracted three times with hexanes to recover any residual oil. The hexane and alkanol phases were evaporated to recover the oil and the solid residues (gums), respectively.

In another set of experiments, the amount of water in methanol was varied between 5 and 15% to examine the influence of additional water on the detoxification efficiency. A third experiment was also carried out in which recovered meals were treated with the polar phase for a second or third time. Experimental conditions were similar to those reported earlier. In some cases, a longer quiescent period (up to 30 min) or a larger ratio of polar phase-to-seed (up to 13·3, v, w) was allowed.

Analyses

The moisture content of meals was calculated gravimetrically after drying of the samples for 12 h in a forced-air convection oven at 104 ± 1°C (Association of Official Analytical Chemists (AOAC), 1990). The crude protein contents were determined using the Kjeldahl nitrogen data (%N × 6·25) (AOAC, 1990). Ash content of samples was calculated gravimetrically after combusting (AOAC, 1990).

Determination of cyanogenic glyosides

Each meal (100 g) was extracted with 80% ethanol 1:10, w/v) at 7°C for 1 h. Ethanol extracts were dried and residues were dissolved in 10 ml of methanol followed by the addition of 20 ml chloroform; precipitates were recovered by centrifugation. The supernatant was evaporated to dryness using a rotavapor. The dried residue was redissolved in 4 ml of 15% (v/v) high-performance liquid chromatography (HPLC) grade methanol in water. The solution was then filtered through a 0·45 μm nylon filter (Cameo II, MSI, Westboro, MA) into the HPLC sample vial and was then injected onto the machine.

The Shimadzu (Kyoto, Japan) HPLC system used consisted of two LC-6A pumps with a mixing chamber, a SIL-6B autoinjector, a SCL-6B system controller; a CR501 Chromatopac data processor and a differential refractometric detector (Waters Associates, Milford, MA) were also used. A reversed-phase analytical column, CSL-Spherisorb-ODS2 (4·5 mm × 50 mm), obtained from Chromatographic Specialities Inc. (Brockville, Ont.) and a guard column (4·5 mm × 59 mm) was used for chromatographic analysis. The mobile phase consisted of 15% (v/v) methanol in water and was used at a flow rate of 0·8 ml/min. In each run, 20 μl of sample was injected and the chromatogram was run for 14 min.

Linamarin was obtained from Sigma Chemicals Co. (St Louis, MO), and linustatin and neolinustatin (separated by column and thin-layer chromatography (TLC) were used as standards. Identification of the latter compounds was confirmed by mass spectrometry.

RESULTS AND DISCUSSION

Table 1 summarizes mass balance data for solvent extraction processing of flaxseed meals. A loss of 0·80–4·6% in the recovery of processed components, was noticed as transfer of material from one unit operation to the next, on a laboratory scale, was not quantitative. Flaxseed meals so obtained constituted 46·4–50·0% of the total weight of the original seeds, on a dry basis. The oil content of the seeds ranged between 45·9 and 49·2%. This process removed up to

Table 1. Mass balance of flaxseed due to different solvent extractions

Solvent	Yield, on a dry weight basis (%)			Loss (%)
	Meal	Oil	Solids	
Hexanes only	48·9 ± 1·0	49·2 ± 0·6	0·0	1·9
Methanol–hexanes	46·7 ± 1·0	45·9 ± 1·9	5·2 ± 0·1	2·2
Methanol–water/hexanes	46·7 ± 2·0	48·0 ± 0·5	4·8 ± 0·1	0·5
Methanol–ammonia/hexanes	47·6 ± 2·0	46·6 ± 0·8	4·8 ± 0·7	1·0
Methanol–ammonia–water/hexanes	46·4 ± 2·0	47·1 ± 0·8	5·7 ± 0·1	0·8
Ethanol–ammonia–water/hexanes	48·1 ± 1·0	46·8 ± 1·8	4·2 ± 0·1	0·9
Isopropanol–ammonia–water/hexanes	50·0 ± 3·0	48·8 ± 0·5	No phase separation	1·2
Commercial meal: hexanes only	82·5 ± 5·0	15·1 ± 3·0	0·0	2·4
Methanol–ammonia–water/hexanes	74·2 ± 2·0	14·3 ± 1·0	6·9 ± 0·2	4·6

Table 2. The effect of solvent extraction on the content of crude protein (N% × 6·25) of flaxseed meals, on a dry–defatted basis[a]

Alkanol	Solvent			
	Alkanol/hexanes	Alkanol–ammonia/hexanes	Alkanol–water/hexanes	Alkanol–ammonia–water/hexanes
Methanol	43·5 ± 0·7	46·0 ± 0·1	46·8 ± 0·3	48·6 ± 0·3 (47·6 ± 0·2)[b]
Ethanol	—	—	—	46·3 ± 0·2
Isopropanol	—	—	—	46·0 ± 0·4

[a] Protein content of hexanes-extracted was 42·9 ± 0·3. Commercial meal contained 41·2 ± 0·5% crude protein.
[b] Prepared from commercial meal.

5·2% of the polar solid matter (gums) from the seeds and into the alkanol phase.

The crude protein content of solvent-extracted meals is summarized in Table 2. Results indicate an enhancement of 5·3–13·5% in protein content of meals treated with the two-phase solvent extraction system. Whereas the crude protein content of hexane-extracted meal was 41·2%, the corresponding values for meals treated with alkanols varied from 43·5 to 48·6%. Alkanols alone had little influence on the concentration of flaxseed proteins as exemplified for methanol; however, addition of water or ammonia to the solvent system had a pronounced effect on their content. Addition of both water and ammonia to the methanol phase gave the best protein enrichment to treated meal. Furthermore, results in Table 2 indicate the following trend for the efficiency of the alkanol–ammonia–water system: methanol–ammonia–water>>ethanol–ammonia–water >isopropanol– ammonia–water. In comparison with canola and rapeseed (Shahidi *et al.*, 1988) protein enrichment values for flaxseed meals were somewhat less pronounced. Removal of polar components such as phenolic acids, tannins, soluble sugars, glucosinolates, non-protein nitrogen compounds and phospholipids was reported to be responsible for protein enhancement of canola (Shahidi *et al.*, 1988). Further work is required to determine the chemical nature of the polar components of flaxseed and their possible removal by the two-phase solvent extraction system.

Table 3 summarizes the content of cyanogenic glycosides of flaxseed (Fig. 1) as affected by processing. No linamarin was detected in the seeds examined in this study. Oomah *et al.* (1992) reported the absence of linamarin in some Canadian flaxseed cultivars, and Frehner *et al.* (1990) indicated that mature seeds predominantly contain linustatin and neolinustatin, which are disaccharide cyanogenic glycosides. Linustatin was the dominant cyanogenic glycoside present in flaxseed, and neolinustatin accounted for approximately 30% of the total. Extraction of cyanogenic glycosides was most effective when the polar phase was 95% (v/v) methanol containing 10% (w/v) ammonia, which in that case removed over 50% of both the linustatin and neolinustatin present in the meals. Further addition of water (10 or 15%, v/v) in the methanol phase enhanced the removal of cyanogenic glycosides (Fig. 1). However, increasing the water content in methanol gave sticky meals, perhaps as a result of the deposition of phospholipids.

Removal of cyanogenic glycosides as affected by the length of quiescent period during the extraction and the amount of solvent (solvent to seed ratio, *R*) used was also investigated. Figure 2 indicates that doubling of both the volume of the extraction solvent and duration of the extraction period resulted in the removal of over 80% of both linustatin and neolinustatin.

Finally, a multistage process involving extraction of meals, up to three times, with methanol–ammonia–water/hexanes was employed. Figure 3 indicates that extraction of the meals for a second and a third time with this solvent enhanced the removal of cyanogenic glycosides from approximately 56% to 80% and to over 90%, respectively. A similar trend for the removal of glucosinolates from rapeseed meals and canola was

Table 3. The effect of solvent extraction on contents of cyanogenic glycosides (mg/g) of flaxseed meal, on a dry–defatted basis[a]

Solvent	Linustatin	Neolinustatin	Total cyanogenic glycosides as HCN equivalents
Hexanes only	4·42 ± 0·08	1·90 ± 0·03	0·41 ± 0·01
Methanol/hexanes	4·26 ± 0·09	1·81 ± 0·23	0·40 ± 0·03
Methanol–water/hexanes	2·69 ± 0·17	1·19 ± 0·09	0·25 ± 0·02
Methanol–ammonia/hexanes	3·26 ± 0·38	1·39 ± 0·17	0·31 ± 0·03
Methanol–ammonia–water/hexanes	1·92 ± 0·17	0·81 ± 0·04	0·18 ± 0·01
Ethanol–ammonia–water/hexanes	3·99 ± 0·17	1·79 ± 0·09	0·38 ± 0·02
Isopropanol–ammonia–water/hexanes	3·36 ± 0·15	1·31 ± 0·06	0·31 ± 0·02
Commercial meal: hexanes only	4·41 ± 0·32	1·97 ± 0·14	0·42 ± 0·03
Methanol–ammonia–water/hexanes	1·72 ± 0·22	0·69 ± 0·05	0·16 ± 0·02

[a] Mean values of four HPLC determinations ± SD.

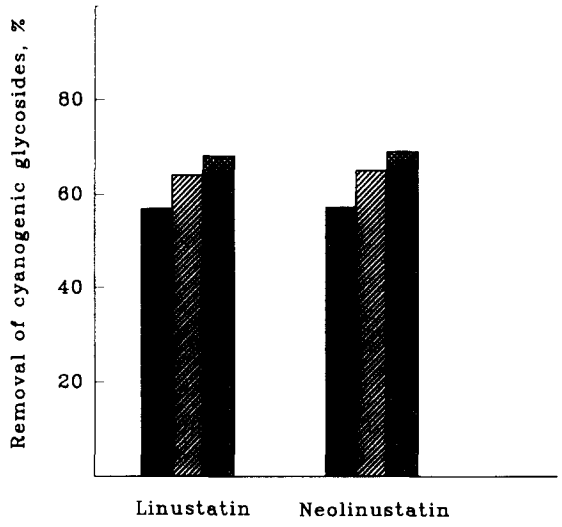

Fig. 2. Removal of cyanogenic glycosides of flaxseed meal as affected by the content of water in the polar phase of methanol–ammonia–water/hexanes extraction: ■, 5%; ▨, 10%; ▩, 15%.

noted when a multistage process was employed Shahidi *et al.*, 1988).

In conclusion, extraction of flaxseed with alkanol–ammonia–water/hexane resulted in simultaneous enhancement of crude protein content of the meals and removal of cyanogenic glycosides. Methanol was the most effective alkanol used. Increasing solvent volumes, duration of extraction period and number of extraction stages enhanced the removal of cyanogenic glycosides. Hence, choice of solvent and extraction variables would allow removal of cyanogenic glycosides to low levels, which may be desirable for their utilization in feed as well as food formulations.

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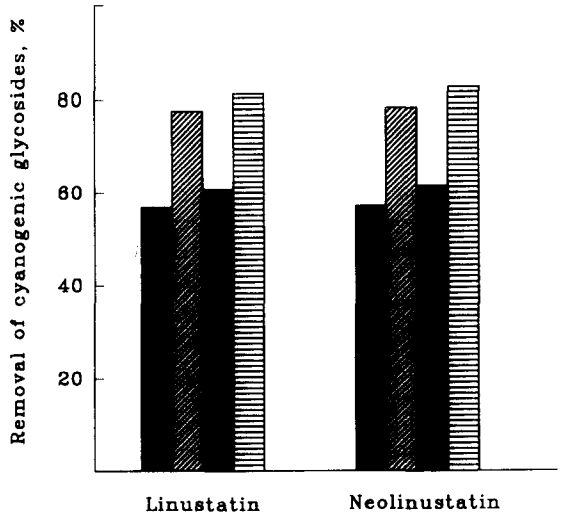

Fig. 3. Removal of cyanogenic glycosides of flaxseed meal as affected by the quiescent period and polar-phase volume to seed ratio (R, v/w): ■, 15 min, $R = 6{\cdot}7$; ▨, 15 min, $R = 13{\cdot}3$; ▩, 30 min, $R = 6{\cdot}7$; ▤, 30 min, $R = 13{\cdot}3$.

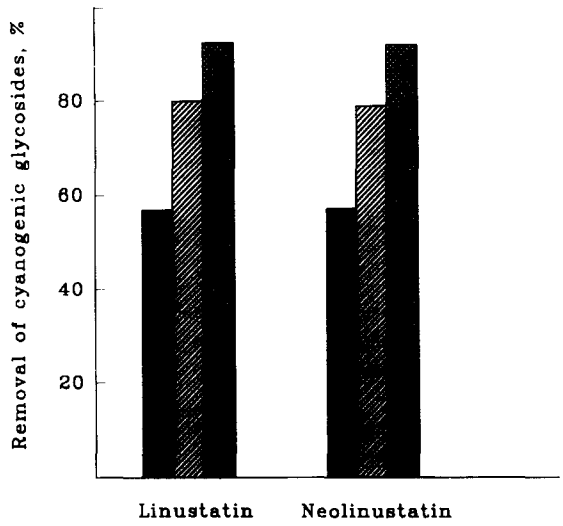

Fig. 4. Removal of cyanogenic glycosides of flaxseed meal as affected by the number of extraction stages: ■, one extraction; ▨, two extractions; ▩, three extractions.

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