Simultaneous Extraction of Oil and Antinutritional Compounds from Flaxseed

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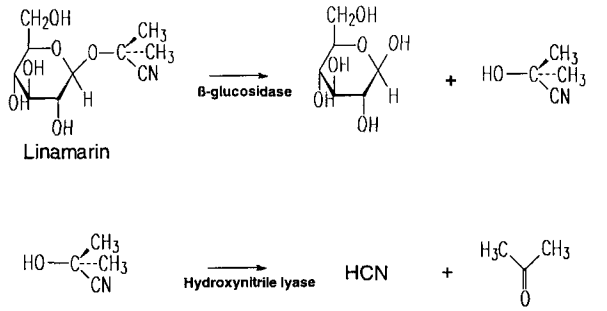
A two-phase solvent extraction process, developed in our laboratory for rapeseed, was used to simultaneously extract oil and toxic, antinutritional components from flaxseed to produce a meal suitable for animal feed. The most effective solvent systems consisted of hexane in combination with a solution of methanol that contained 10% (vol/vol) water and 2.5–5% (w/w) ammonia, or methanol that contained 10% water (vol/vol) and 0.08% (w/w) NaOH. The treatments were carried out both at laboratory and semi-pilot-plant scales. The success of the test with a pilot-scale Karr liquid–liquid extraction column suggested that this process could be readily carried out on an industrial scale. The resulting flax meal had a high protein content (40–47%) and low levels of cyanogenic glycosides (reduced by 90–100% from the starting material). The methanol–ammonia extraction reduced the total polyphenol content by ≈20%. The oil extraction efficiency of the Karr column was high, resulting in meal residual oil contents of ≈1%.

KEY WORDS: Cyanogenic glycosides, flax, Karr column extraction, methanol–ammonia, two-phase extraction.

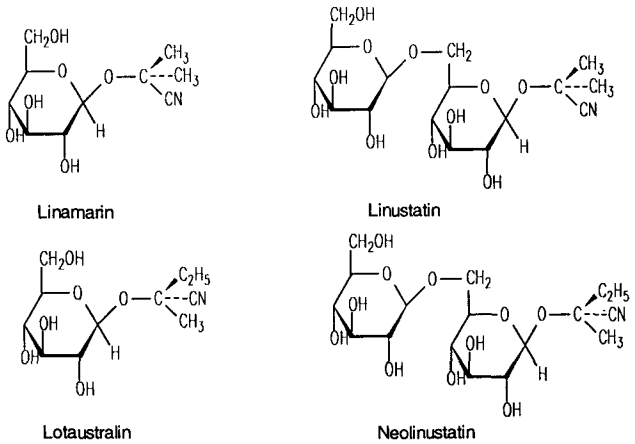
Canada is the world's leading flax producer and exporter with 95% of its annual crop being grown in Manitoba and Saskatchewan (1). Flaxseed is primarily grown for its oil. It is unsuitable for food use but has many important industrial applications, including paints and polymers. Recently, new varieties of flax have been developed which produce oil low in linolenic acid and are suitable for food uses (2). These are now being adapted to the Canadian climate and may generate expansion of flaxseed production in Canada. A recent report by the American Oil Chemists' Society (3) indicates that low-linolenic flaxseed oil will be available for human consumption in Canada in the near future. An increased demand for flax would result in higher crop prices and fewer price fluctuations, benefitting the 10,000 producers as well as crushers, handlers and exporters.

Although the meal contains 35–44% protein (N × 6.25, oil- and moisture-free basis) of reasonable nutritional quality, its use in food and feed is limited primarily to ruminants by the presence of several antinutritional components, including trypsin inhibitors, cyanogenic glycosides and compounds that inhibit the uptake of vitamin-B_6 (vitamin-B_6 antagonists) (4). The cyanogenic compounds release hydrogen cyanide when hydrolyzed in an aqueous acid medium (5) (decomposition of linamarin, Scheme 1). Hence, these compounds are toxic to monogastric animals, and even small doses result in reduced growth, thus limiting the utilization of flaxseed meal as animal feed. They have been identified as linamarin, linustatin, neolinustatin and lotaustralin (6). The chemical structure of the cyanogenic glycosides is illustrated in Scheme 2.

We have developed a process that simultaneously extracts oil and soluble polar components from oilseeds, such as rapeseed, with two immiscible solvents, hexane and a polar solvent consisting of methanol, ammonia and water (7). A similar polar solvent system, where sodium hydroxide replaced ammonia, was also successfully applied to rapeseed (8). Methanol functions by preventing the solubilization of protein while extracting other polar compounds from the seed. These two-phase extraction processes have been tested on a larger semi-pilot scale (8,9), to produce a degummed oil, a high-quality meal and a waste stream that contained the dissolved impurities. Rapeseed was ground as a slurry with the polar solvent in a Szegö mill (Fig. 1) and extracted in a Karr liquid–liquid extraction column (Fig. 2) that was suitably modified for this purpose (10). Such an extraction process seemed to be a logical approach to processing flax with the objective of recovering the oil simultaneously with the reduction of cyanogenic glycoside content in the flax meal.



SCHEME 1



SCHEME 2

MATERIALS AND METHODS

Materials. Two lots of flaxseed were supplied by the Flax Council of Canada (Winnipeg, Manitoba, Canada). Technical-grade methanol and hexane were obtained from BDH Chemicals Ltd. (Toronto, Canada). Technical-grade ammonia was obtained from Canadian Oxygen Ltd. (Toronto, Canada). All reagents used for analytical work were

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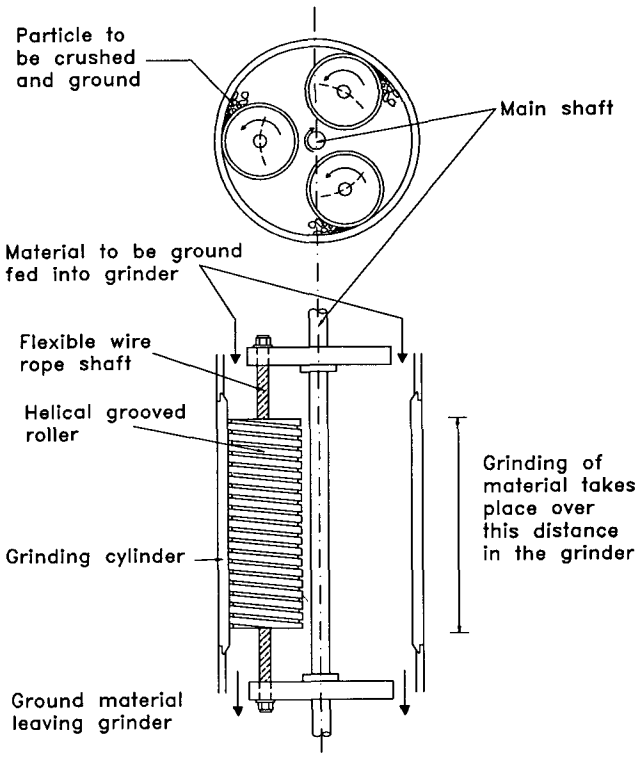

FIG. 1. The Szegö mill.

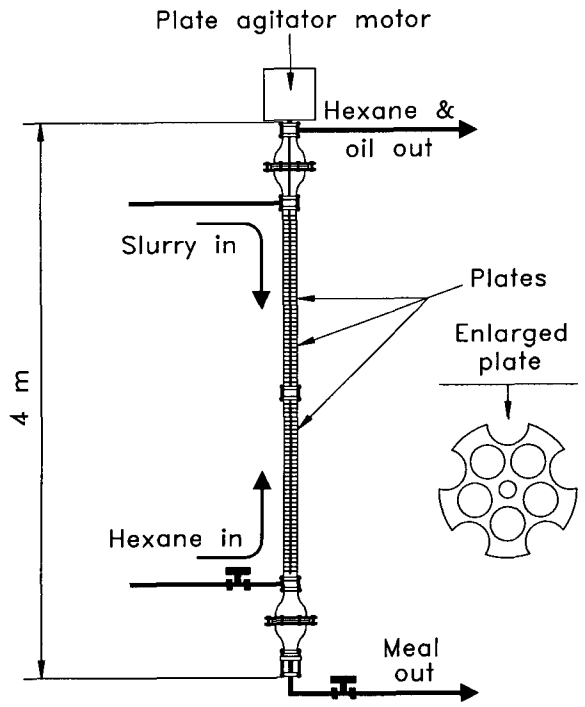

FIG. 2. The Karr column.

reagent-grade and were obtained from major chemical suppliers.

Analytical methods. Moisture was determined by drying samples to constant weight at 110°C in a forced-air oven (11). Fat content was measured by the Soxhlet method (12). Protein was reported as N × 6.25, determined by the Kjeldahl method with a Büchi Model 425 digester and a Büchi Model 320 distillation unit (Büchi Laboratoriums-Technik AG, Flawil, Switzerland). The American Association of Cereal Chemists' (AACC) Method 46-12 (13) was followed.

Cyanogenic glycosides were determined colorimetrically by a technique described by Lambert *et al.* (14) and by Michels and Siegfried (15) and modified as follows. The cyanogenic glycosides of 3 g flax meal were hydrolyzed in a tightly sealed flask in the presence of 1 g freshly ground flaxseed, which provided the active enzyme, linamarase, in 100 mL citrate buffer solution (pH 5.5) at 50°C for 16-24 h. By adding 20 mL 1% H_2SO_4 and a few drops of antifoam, the freed hydrogen cyanide was steam-distilled and trapped in 100 mL 1 M NaOH solution until about 450 mL distillate was collected. The distillate was diluted to 500 mL. A 12.5-mL aliquot of this solution was diluted to about 40 mL; the pH of the sample was adjusted to 6.0 with 0.5% HCl solution; and the solution was then diluted to exactly 100 mL. One mL of 1% chloramine-T and 3 mL of color reagent (3 g barbituric acid in 30 mL pyridine and 6 mL of concentrated HCl, diluted to 100 mL with distilled water) were added to a 20-mL aliquot. The samples were then left for 10 min to allow color development. The absorbance was then measured at 580 nm in a 1-cm cuvette with a Beckman DU-7 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). The results are expressed as mg linamarin equivalent/100 g sample. The cyanogenic glycoside content of the sample was calculated by subtracting the contribution of the added, freshly ground seed. The cyanogenic glycoside content of this untreated flaxseed was determined on the basis of a calibration standard (0.1-0.8 mg/L) KCN solution. To determine the residual cyanogenic glycoside content of the extracted flax meal, another calibration was also carried out with linamarin (Sigma Chemical Co., St. Louis, MO) in the presence of 1 g freshly ground flaxseed, which provided the active enzyme, linamarase.

Polyphenols were isolated from the oil-free seed and meal by the procedure of Krygier *et al.* (16). The free phenolic acids, soluble phenolic acid esters, and insoluble-bound phenolic acids were determined by the gas-chromatographic method of Krygier *et al.* (17). The total phenol content was also determined by the process of Swain and Hillis (18). All phenolic analyses were carried out in triplicate.

Two-phase solvent extraction (laboratory-scale). The two-phase liquid extraction method described by Rubin *et al.* (7) was followed (Fig. 3). The seed was ground in an Osterizer blender and blended with the polar solvent for 2 min. Hexane was added to the slurry after 15 min of contact with gentle stirring. The resulting three-phase system was blended again for 2 min and filtered, and the two liquid layers were separated. The solids were washed with the polar solvent and air-dried, and the residual oil was removed by Soxhlet extraction with hexane. The resulting meal was analyzed. The compositions of the solvent systems used in the laboratory-scale processes will be shown later in Table 2. All laboratory-scale extractions were carried out in triplicate.

Two-phase solvent extraction (semi-pilot-plant scale). The pilot-plant-scale two-phase extraction process

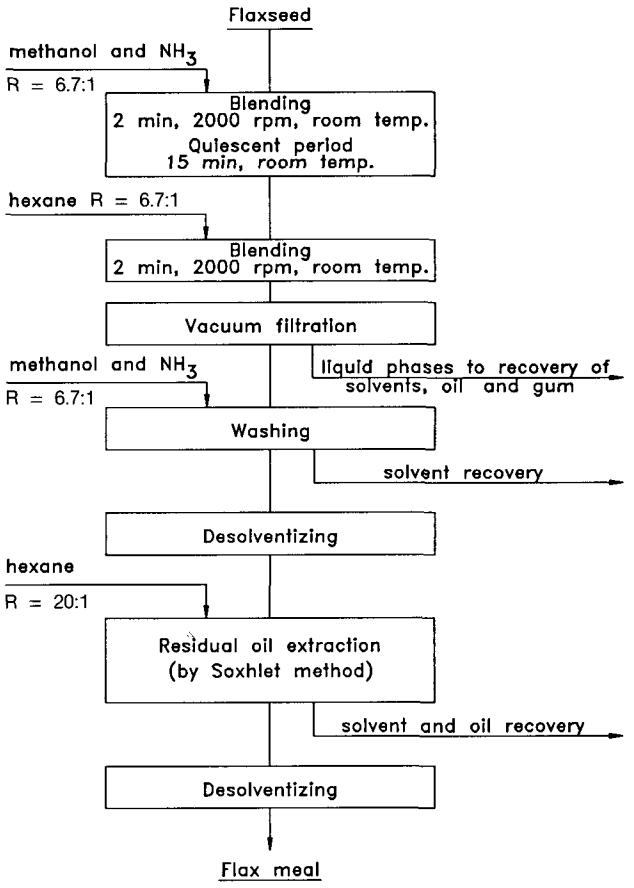

FIG. 3. Flow diagram of laboratory-scale two-phase solvent extraction system. R, solvent to seed ratio (vol/wt).

described by Diosady *et al.* (9) was used to extract oil and antinutritional components from flaxseed. In this process, 10 kg flaxseed was mixed with 20 L of polar-phase solution, and the slurry was then ground with a 100-mm i.d. Szegő mill (General Comminution Inc., Toronto, Canada). The mill was fed from the top with the slurry, which consisted of the methanol–water (10%) and NaOH (0.08%) solution and flaxseed in a 2:1 (vol/wt) ratio. After the initial grinding, the slurry was once again passed through the Szegő mill to thoroughly grind the seed. The slurry was then diluted to a solvent-to-seed ratio of 6.7:1 (vol/wt) with additional polar solvent before the pilot-plant-scale extraction in the Karr column. The entire slurry phase was then poured into a mixing tank and continuously stirred to obtain even distribution of solids throughout the polar phase. Hexane was pumped into the column from the bottom at 0.39–0.40 L/min. When the hexane level reached the middle of the column, the slurry was pumped into the column at 0.80 L/min. The slurry descended by gravity through column plates, which were agitated at a frequency of 65 cycles/min. The extracted slurry phase, known as raffinate, was then removed by adjusting the outlet valve at the bottom of the column. The interface for the two phases was maintained at the top of the column by adjusting the control valve. During the operation of the column, ten samples each of hexane miscella and raffinate were collected over a 10-min interval. The raffinate samples were filtered in a Buchner vacuum filter and washed three times each with methanol. The miscella samples were analyzed for their oil contents. The meal obtained by filtering the raffinate was air-dried and analyzed for oil, protein, moisture and cyanogenic glycoside content. The pilot-plant-scale extraction was carried out in duplicate.

RESULTS AND DISCUSSION

According to the literature, flaxseed contains about 42% oil and 22% protein (moisture-free basis) (19). The cyanogenic glycoside contents of ten flax cultivars were measured by Oomah *et al.* (20), who reported that various Canadian flaxseed varieties contain between 242–338 mg cyanogenic glycosides/100 g seed (moisture- and oil-free basis) expressed as linamarin equivalent. The composition of flaxseed used in our project was representative of the literature values (Table 1).

The effects of the polar solvent composition on protein and linamarin contents of extracted meals are summarized in Table 2. The removal of the cyanogenic glycosides by the laboratory-scale extractions is shown in Figures 4 and 5. Every treatment reduced the cyanogenic glycoside content, indicating that these glycosides are indeed soluble, at least partially, in polar solvents. The presence of ammonia in the polar solvent aided the removal of cyanogenic glycosides (Fig. 4).

For the extraction with 10% water in methanol, even 2.5% ammonia was sufficient to reduce the linamarin content by 90%. However, the 5% ammonia runs yielded higher protein content (Table 2). When the polar solvent contained only 5% water, increased ammonia concentration was necessary to obtain more than 90% cyanogenic glycoside removal.

Up to 50% water, the removal of linamarin was proportional to the water content of the methanol in the absence of ammonia (Fig. 5). Increasing the water content to more than 50% resulted in the formation of a viscous gel-like extract, which was difficult to separate from the solvents. In addition to these practical problems, this increased water content of the solvent, reduced the yield of meal and resulted in loss of protein (Table 2), which would make this

TABLE 1

Composition of Flaxseed

	Moisture[a] (%)	SD	Oil (%)	SD	Protein[c] (%)	SD	Linamarin[c] (mg/100 g)	SD
Lot 1	5.3	±0.7	45.9	±1.2	43.7	±0.9	391.2	±6.3
Lot 2	5.7	±0.3	43.4	±0.9	37.1	±0.8	489.5	±7.0

[a]As is basis.
[b]Moisture-free basis.
[c]Moisture- and oil-free basis.

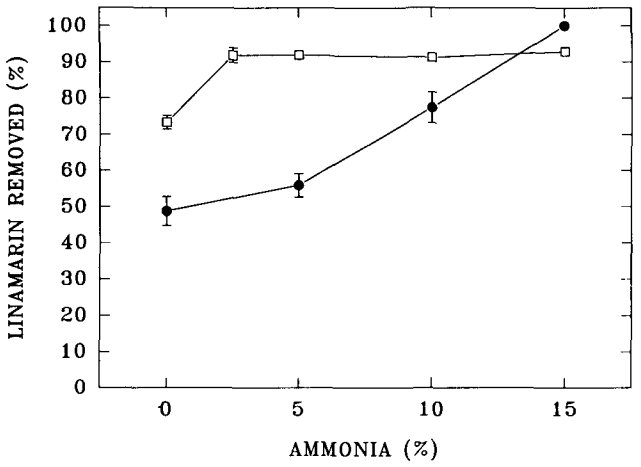

FIG. 4. Effect of ammonia concentration on the removal of linamarin. Methanol/water, 90:10 (□) and 95:5 (●).

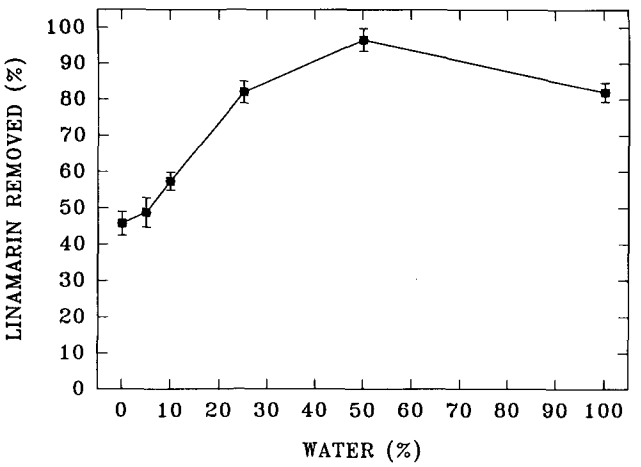

FIG. 5. Effect of water concentration on the removal of linamarin.

approach uneconomical. Because 10% water and 2.5–5% ammonia extraction removed more than 90% of linamarin (Fig. 4), increasing ammonia or water content above these levels offers no practical advantage.

Previous findings that NaOH could replace ammonia during the two-phase extraction treatment of rapeseed suggested that this approach may be suitable for flaxseed as well. Laboratory-scale extractions indicated that the NaOH extraction (0.08% NaOH, 10% water, 90% methanol) removed 83.6 ± 1.2% of the cyanogenic glycosides originally present in the seed. Although ammoniated solvents produced better results, the pilot-plant extractions were conducted with the MeOH-H_2O-NaOH solvent because of safety considerations. As expected, a Szego" mill and a Karr column produced much better results than the laboratory-scale extractions. The meal produced was high in protein (45%), and the residual cyanogenic glycoside content was below the detection limit (8–10 mg/100 g seed) (Table 2). The residual oil in the meal was readily reduced to 1% or less in a typical Karr column operation. Because the Szego mill grinds the meal to a small, uniform particle size with a large aspect ratio, a more effective mass transfer can be expected in this system than in the laboratory apparatus, where the grinding is much less effective.

The results of polyphenol analyses on meals prepared by extraction with 2.5% ammonia, 10% water, 90% methanol are presented in Table 3. Although the methanol-

TABLE 2

Effect of Polar Solvent Composition on Meal Protein and Linamarin Content

Treatment	Protein content[a] (%)	SD	Protein enhancement[b] (%)	Linamarin content[a] (mg/100 g)	SD
Flax Lot 2					
90% methanol, 10% water, 0.08% NaOH[c]	39.2	±0.7	2.1	80.3	±5.9
90% methanol, 10% water, 0.08% NaOH[d]	44.9	±0.2	7.8	ND[e]	
100% methanol, 0% water, 0% ammonia	37.8	±0.2	0.7	265.3	±15.9
95% methanol, 5% water, 0% ammonia	38.3	±0.2	1.2	250.6	±19.6
90% methanol, 10% water, 0% ammonia	39.1	±0.1	2.0	208.5	±12.0
75% methanol, 25% water, 0% ammonia	37.5	±0.2	0.4	88.1	±14.9
50% methanol, 50% water, 0% ammonia	37.1	±0.2	0.0	16.6	±15.6
0% methanol, 100% water, 0% ammonia	34.1	±0.2	−3.0	88.1	±13.0
95% methanol, 5% water, 5% ammonia	41.0	±0.3	3.9	215.9	±15.8
95% methanol, 5% water, 10% ammonia	41.9	±0.8	4.8	110.5	±20.7
95% methanol, 5% water, 15% ammonia	42.7	±0.5	5.6	ND[e]	
Flax Lot 1					
90% methanol, 10% water, 0% ammonia	47.2	±0.4	3.5	103.2	±7.5
90% methanol, 10% water, 2.5% ammonia	45.1	±0.1	1.4	31.9	±8.1
90% methanol, 10% water, 5% ammonia	46.9	±0.1	3.2	31.6	±5.9
90% methanol, 10% water, 10% ammonia	47.4	±0.1	3.7	34.2	±4.2
90% methanol, 10% water, 15% ammonia	47.6	±0.3	3.9	28.0	±3.4

[a]Moisture- and oil-free basis.
[b]Change in protein content compared to the hexane-extracted meal.
[c]Laboratory-scale treatment.
[d]Pilot-plant-scale treatment.
[e]ND, not detectable.

TABLE 3

Polyphenol Content of Hexane-Extracted and Methanol-Ammonia-Extracted Flax Meal

	Hexane-extracted[a] (mg/100 g)	SD	Methanol-ammonia-extracted[a] (mg/100 g)	SD	Reduction of phenolic content (%)
Free phenolic acids	76.3	±15.1	74.9	±4.5	1.8
Soluble phenolic acid esters	238.4	±52.6	189.7	±19.8	20.4
Insoluble-bound phenolic acids	127.2	±25.2	90.8	±9.4	28.6
Total phenolic content	441.8	±31.0	355.3	±11.2	19.6

[a]Moisture- and oil-free basis.

ammonia extraction reduced both soluble phenolic acid esters and insoluble (bound) phenolic acids, it was surprising that free phenolic acids were not significantly reduced. Because the isolation and measurement of soluble phenolic acid esters, insoluble and free phenolic acids gave high standard deviations, the determination of total phenolic content by the method of Swain and Hillis (18) was necessary. This analysis showed a 20% reduction of total phenolic content of the meal, but this decrease was smaller than expected (on the basis of our experience) with canola extraction. As reported earlier, the phenolic content of canola meal was reduced by up to 85% during methanol–ammonia extraction (21).

A preliminary feeding trial, comparing two-phase extracted flax meal to commercially (hexane)-extracted flax, was carried out at the University of Guelph (Guelph, Canada) by Professor S. Leeson. Nutritional tests showed no adverse effects of the two-phase-extracted flax meal on chickens. There was no significant difference between conventional flax meal and the two-phase-extracted meal, although on the basis of the decreased cyanogen content, significant improvement was expected. It is possible that the vitamin B_6 antagonist activity of the meal (22) is retained during the methanol–ammonia–hexane extraction, and its effect on young chicks masks the effect of cyanogen removal.

The semi-pilot-scale tests with the Karr column have demonstrated the technical feasibility of producing high-quality flaxseed oil and flax meal that contained greatly reduced concentrations of cyanogenic glycosides. Based on our experience with canola (10), the process requires similar energy as a conventional hexane extraction, and the equipment is smaller and simpler than the conventional percolating-bed extractors currently in use.

Since the objective for this approach is the reduction of cyanogenic glycosides, the economic feasibility of the process can only be determined by large-scale feeding trials with a number of species, to confirm the cost of meal processing and is justified by the nutritional improvement in the meal.

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