Further Compositional Analyses of Flax: Mucilage, Trypsin Inhibitors and Hydrocyanic Acid

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Registered Canadian cultivars of flax, and laboratory-prepared and commercially obtained samples of linseed meal (LM), were used to determine extract viscosity and mucilage, trypsin inhibitors and hydrocyanic acid (HCN) concentrations. The mucilage readily leached out from the seed coat (hull) fragments soaked in water, leaving behind pentagon-shaped cells that could be seen clearly in scanning electron micrographs. Extract viscosity significantly varied in the laboratory-prepared (23–48 cS) and commercially obtained (30–68 cS) samples of LM and may be used to obtain an indirect, qualitative estimate of flax mucilage. Mucilage was extracted from whole seed in 5.0–5.3% yields and contained 20–24% protein (about 10% ash and 30% total carbohydrates). Laboratory-prepared LM (raw) contained 42–51 units of trypsin inhibitor activity, commercially obtained samples, 14–37 units, and raw rapeseed and soybean meals, 99 and 1650 units, respectively. Picric acid tests (qualitative) showed only traces of HCN in ten cultivars of freshly ground flax. The acid silver nitrate titration procedure measured HCN quantitatively, but showed its presence only in three of the five cultivars investigated. HCN was conveniently measured by a colorimetric procedure (barbituric acid-pyridine reaction), which may be used to screen flax cultivars. HCN content of flax was significantly influenced by environments (growth location and season) and, to a less extent, by cultivar.

KEY WORDS: Extract viscosity, flax, hydrocyanic acid, linseed meal, mucilage.

The conversion of flax (*Linum usitatissimum* L.) from an industrial to an edible oil crop (low α-linolenic acid) will give added impetus to domestic crushing of flax in Canada, which exports almost all of its flax as seed. Linseed meal (LM), the by-product of the flax crushing industry, has been largely used as a ruminant feed and little, if any, in human foods although flax has been eaten in China and on the Indian subcontinent for centuries. LM has not been investigated in any detail, unlike other oilseed meals, particularly soybean meal (SBM) and rapeseed or canola meal (RSM). LM has potential, as well as limitations, for use in feeds and foods. Its digestible energy (DE) is similar to SBM and greater than RSM for cattle (1). Expeller meal containing 3–4% residual oil is a popular ingredient in calf-feeding formulations in the United Kingdom. The flax seed mucilage is responsible for its high water-hydration capacity (2). Cunnane *et al.* (3) reported that the two components of flax, α-linolenic acid and mucilaginous soluble fiber, have important implications in the management of hyperlipidemia and hyperglycemia in humans. Other studies have reported hypocholesterolemic effects of flax in rats (4,5). However, LM causes problems in swine and poultry (broiler chicks) nutrition. Rats fed more than one-third of the diet protein from flax had lower weight gains and feed intake; in growing pigs (23–57 kg), 16% LM in the diets decreased daily weight gain by 11% and feed intake by 5% (J.M. Bell, personal communication). In broiler chicks (0–14 days old), 20% LM inclusion in the diet decreased body weight gain by 22% (H.L. Classen, personal communication). In poultry, these adverse effects seemed due to beak necrosis and increased gastric viscosity, which led to reduced availability of energy. For swine feeding, LM is deficient in DE, as well as in essential amino acids, lysine and methionine (1). Low-linolenic acid (LINOLA)™ LM was similar in nutritional value to conventional LM for growing pigs, its DE was 9.8 MJ kg^{-1} compared to 9.4 MJ kg^{-1} for conventional LM and 13.0 MJ kg^{-1} for SBM (6). Thus, flax cultivars containing high mucilage (brown-seeded; for use in human foods) or low mucilage (yellow-seeded; for use in swine and poultry diets) may need to be developed. LM contains a vitamin B_6 antagonist and, most likely, inhibitors of mammalian trypsin. Of greater concern for utilization of LM in human foods and animal feeds is the presence of cyanogenic glucosides, which yield hydrocyanic acid (HCN) or prussic acid on enzymatic hydrolysis. Under certain conditions HCN may be converted to the goitrogenic thiocyanate.

This paper reports the mucilage, trypsin inhibitors and HCN contents of flax. The objective was to determine the range and level of these components in samples of flax or LM for their potential utilization in feed and food. The present data complement those published previously (2) on laboratory-prepared and commercially obtained samples of LM.

EXPERIMENTAL PROCEDURES

Samples. Licensed Canadian cultivars of flax (*L. usitatissimum* L.), Andro, Dufferin, Linott, McGregor, NorAlta, NorLin, NorMan and Vimy, each grown at four locations (Morden and Portage la Prairie, Manitoba; Saskatoon and Elrose, Saskatchewan) in 1987 and at two locations (Saskatoon and Elrose) in 1989 were used in the study. The flax samples were from the Flax Cooperative Test and obtained from Dr. E. Kenaschuk (Agriculture Canada Research Station, Morden) and Dr. G. Rowland (Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon). Four samples of commercial LM (cultivar unknown), described previously (2), were obtained from Omega Nutrition (Vancouver, British Columbia; Alberta Linseed, Medicine Hat, Alberta; ADM-Agri Industry, Windsor, Ontario, Canada; and Cargill, Riverside, ND). One sample each of canola, cv. Westar (*Brassica napus* L.) and of soybean (*Glycine max* L.) Merr. were obtained from Dr. F. Sosulski (Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon). Benzoyl-*dl*-arginine-*p*-nitroanilide HCl (lot 68F-0687), porcine pancreas trypsin (lot 44F-0150) and chloramine-T (126F-0624) were Sigma (St. Louis, MO) products. Potassium cyanide (lot 731037) was purchased from Fisher Scientific (Edmonton, Alberta, Canada).

Meal preparation. Samples of flax were defatted and simultaneously crushed to a meal by shaking with petroleum ether (b.p. 35–60°C) in a Swedish ball mill for 2–3 h. After filtration, the meal was air-dried and ground in a micro hammer mill to pass a 0.5-mm screen. The commercial meals contained, on receipt in the laboratory, 1–16% oil (7). They were defatted by stirring with petroleum ether for 30 min, filtered, air-dried and ground as mentioned above.

Residual oil in the meals was determined by extraction with petroleum ether for 5 h in a Goldfisch apparatus. Moisture, ash and protein (N × 6.25) contents were determined by Association of Official Analytical Chemists methods (7), and total carbohydrates by the method of Dubois *et al.* (8), with raffinose as standard.

Viscosity of LM. LM (1 g) was shaken with 15 mL deionized water in a wrist-arm shaker for 45 min. The extract was centrifuged at 1000 × *g* for 20 min and the supernatant was made to volume with water in each case. Viscosity of the extracts was measured at 25°C in an Ubbelohde viscometer (no. A629) (Fisher Scientific) and expressed in centiStokes (cS), calculated by multiplying efflux time in seconds with the calibration constant (0.1024).

Flax mucilage. Mucilage was extracted from the seed by the procedure of Mazza and Biliaderis (9), with minor alterations. Flax seed was extracted with hot water (100°C) for 4 h at a seed-to-water ratio of 1–20 by magnetic stirring. At the end of the extraction period, extract water temperature had reached ambient. The extract was filtered through glass wool, and the filtrate was reduced in volume by rotary evaporation. The mucilage was precipitated from the extract by adding ethanol to a final concentration of 80% (vol/vol). After allowing to stand for 1 h at 5°C, the precipitate was removed by centrifugation (10,000 × *g*; 20 min), homogenized in water and freeze-dried.

Scanning electron microscopy of hulls. Flax was gently cracked, and the seed coat fragments were manually separated from the cotyledons. Some fragments were soaked in water overnight and dried at room temperature. Soaked and unsoaked fragments were placed on aluminum stubs, gold-plated under vacuum and observed under a Phillips SEM (Cambridge Instruments, Cambridge, England) operated at 30 kV.

Trypsin inhibitor activity (TIA). TIA of the meals was determined by the procedure of Kakade *et al.* (10) with minor adjustments. LM, SBM and RSM samples (1 g) were extracted with 50 mL 0.01N NaOH by shaking for 3 h in a wrist-arm shaker, and the extracts were filtered through glass wool and made to volume in each case. The SBM extract was diluted 25-fold with the extract solution. The extract volume added to the assay mixture was adjusted in each case to give about 50% inhibition of the trypsin activity (TA), first established with several samples of freshly diluted trypsin solution. The assay mixture contained an appropriate volume of the extract containing the inhibitor, 1 mL of trypsin solution (20 μg trypsin in 0.001M HCl), 5 mL of the substrate solution (pH 8.2), and the volume was adjusted to 7 mL with water. Assay temperature was 37°C and assay time 15 min. Enzyme activity was stopped by adding 1 mL of 30% acetic acid. Reagent blanks were prepared with each assay. TA was determined by substituting water for the meal extract and was arbitrarily defined as an increase of 0.01 absorbance units under the assay conditions. TIA was defined as TA units inhibited per gram meal and obtained by subtracting TIA from TA.

Determination of hydrocyanic acid. The method used was based on the barbituric acid-pyridine reaction described by Lambert *et al.* (11). Freshly ground flax (4 g) was incubated in a glass-stoppered flask with 100 mL 0.1N sodium citrate buffer, pH 5.5 at 45°C for 1 h. The contents were transferred to a Büchi Kjeldahl nitrogen distillation unit and, after the addition of 20 mL 1% H_2SO_4, distilled into 1 M NaOH. The collected distillate (400 to 450 mL) was then made to 500 mL with water. To a 25-mL aliquot were added dilute HCl to adjust pH to 6.0, 1 mL of chloramine-T (1% solution in water) and 3 mL of the barbituric acid-pyridine reagent, prepared by dissolving 3 g thiobarbituric acid, 30 mL pyridine and 6 mL conc. HCl made to 100 mL with water. After allowing to stand at room temperature for 10 min, absorbance was read at 580 nm. Potassium cyanide (KCN) was used as a reference standard. Concentrations of up to 40 μg KCN gave linear absorbance under the assay conditions. The HCN concentration of the samples was calculated as: (absorbance-blank) × DF × V × 25 × 0.41, where DF was the dilution factor, V the total extract volume, 25 to convert HCN concentration to 100 g meal, and 0.41 (27.0/65.1) to convert KCN to HCN.

Data expression. Data reported are means of at least duplicate determinations, and where LM was used, they are expressed on oil- and moisture-free basis. Analysis of variance and standard error of difference were calculated with a Minitab software program, and least significance differences were obtained from the error mean squares.

RESULTS AND DISCUSSION

Viscosity and mucilage of flax. Flax mucilage, a mixture of neutral and acidic polysaccharides, is present entirely in the seed coat, invariably called true hull or spermoderm, the mucilage layers being on the outermost (epiderm) of the seed coat. The presence of mucilage in the outermost cells of the seed coat was readily apparent from scanning electron micrographs (Fig. 1). The top part of the figure shows the outside of unsoaked seed coat fragment containing the mucilage; the cells were filled with the mucilage and the cellular structure was completely masked. The bottom part of Figure 1 shows scanning electron micrograph of the seed coat after soaking in water. The mucilage was dissolved in water, leaving behind empty-looking cells with clearly defined cell walls; the two micrographs provided a contrasting view of the outer layer of the seed coat. The soaked seed coat cells appeared pentagonal, many with unequal sides. The shape of the mucilage cells in the outermost layer of the seed coat has been described as varying from rectangular to polygonal, and the structural variation is a varietal characteristic (12).

Our previous study (2) reported viscosity of water (pH 6.0) and acid buffer (pH 1.5) extracts of flax hulls. The water extract had three times higher viscosity than the acid-buffer extract. Both extracts were stable at 25°C for 30 min, suggesting that mucilage-degrading enzymes, if present, were not extracted from the seed coat by the two solvents. Therefore, viscosity measurement of water extracts of LM may provide a convenient, indirect procedure for determining mucilage content of flax. To determine optimum extraction time, four samples of Vimy LM were

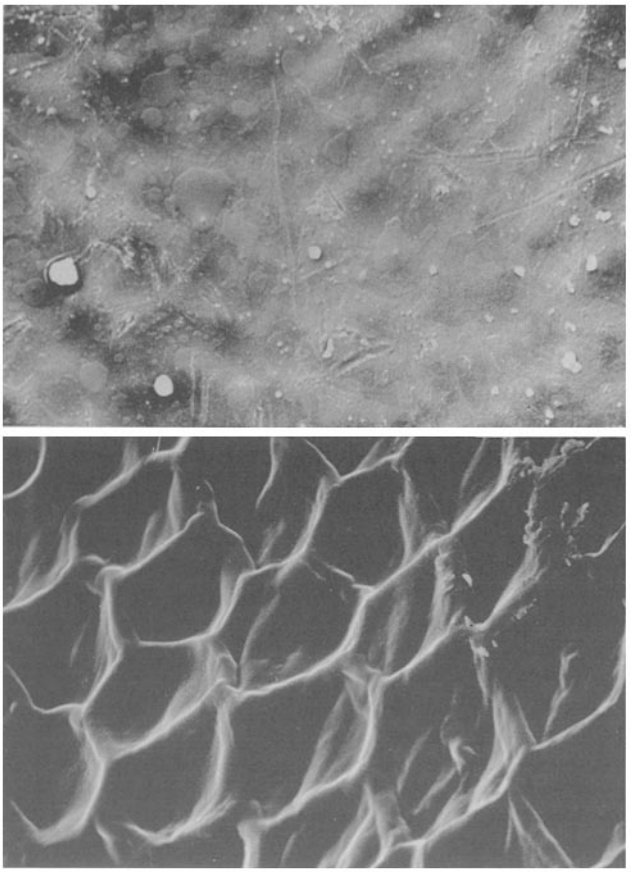


FIG. 1. Scanning electron micrographs of unsoaked and water-soaked fragments of seed coat of Andro flax, showing presence (top) and absence (bottom) of mucilage. Top, magnification 328×; bottom, magnification 630×.

separately extracted for 15, 30, 45 and 60 min at room temperature at a meal-to-solvent ratio of 1:10 by shaking in a wrist-arm shaker. The viscosity values (single determinations) of the extracts, after centrifugation at 1000 × *g* for 20 min, were 22.8, 25.0, 27.3 and 26.1 cS, respectively. In another experiment, one sample of the same LM was sequentially extracted three times for 15 min. The viscosity values obtained were 23.8, 3.0 and 1.4 cS, for a total of 28.2 cS. Thus it made little difference in extract viscosity whether LM was extracted with water directly for 45 min or sequentially for the same total time. Direct extraction is convenient and was used in a subsequent experiment. The water extracts of LM most likely contained, in addition to mucilage polysaccharides, soluble proteins. Their contribution to extract viscosity is not known.

Data in Table 1 show highly significant differences in extract viscosities of nine samples of LM, five laboratory-prepared and four commercially obtained. Analysis of variance of the data showed a highly significant cultivar (sample) effect. McGregor and NorLin LM had twice the extract viscosities of Andro LM. One of the commercially obtained LM samples (Omega Nutrition) had unusually high extract viscosity (68.2 cS) for unknown reasons, possibly due to its contamination at the source. This sample contained high total carbohydrates (2). The viscosities of the other three commercially obtained samples were within the range of the laboratory-prepared samples of LM.

TABLE 1

Viscosity of Water Extracts of Laboratory-Prepared and Commercially Obtained Samples of Linseed Meal

Sample source	Viscosity, cS[a]
Laboratory-prepared[b]	
Andro	23.3 ± 0.8
McGregor	39.7 ± 1.3
NorLin	48.1 ± 1.4
NorMan	29.5 ± 1.2
Vimy	33.8 ± 0.4
Commercially obtained	
Omega Nutrition	68.2 ± 0.4
Alberta Linseed	30.4 ± 1.0
ADM-Industry	33.1 ± 1.7
Cargill	36.9 ± 0.3
Least significant difference ($P < 0.05$)	2.5

[a]Mean ± SD of moisture- and oil-free meals. cS, centiStokes.
[b]Flax cultivars grown at Morden, Manitoba, Canada, in 1989.

Extraction and quantitation of mucilage from flax has been reported for only one cultivar of flax in two separate studies (9,13). The procedures followed in both studies were generally similar: Water extraction followed by precipitation of the dissolved mucilage with acetone or alcohol. This study found whole flax seed preferable to ground seed for extraction of mucilage. In a preliminary experiment, mucilage extracted from ground seed of Vimy flax gave a yield of 15.6%, but the preparation contained 45% protein. Mucilage yield from whole seed of the same cultivar, extracted under identical conditions, was 4%, and the preparation contained only 7% protein. Therefore, it was necessary to use whole flax seed for extraction of low-protein mucilage. The functional properties of flax mucilage have been reported (9,13). Undoubtedly, such properties are affected by protein present in the mucilage. Proteins present in the extracts may be greatly reduced or even eliminated by treatment with a protease, but this step will lengthen the mucilage extraction procedure. Mucilage was then extracted from two cultivars of flax, NorLin and Andro, containing high (48.1 cS) and low (23.3 cS) extract viscosities, respectively (Table 1). The yields and composition of the mucilage obtained are given in Table 2. The mucilage yields (5.0–5.3%) were not significantly different and similar to that (5.0%) reported for one sample of flax by Susheelamma (13). These yields were lower than that obtained from Linott flax (7.6%) by Mazza and Biliaderis (9). However, Linott flax extracted under our conditions also gave a mucilage yield of

TABLE 2

Yield and Composition of Mucilage Extracted from Whole Seeds of Andro and NorLin Cultivars of Flax

Yield/component (%)[a]	Andro[b]	NorLin[b]	SD[c]
Yield	5.0 ± 0.1	5.3 ± 0.1	ns
Protein (N × 6.25)	24.2 ± 0.0	19.7 ± 0.2	**
Ash	9.6 ± 0.2	10.4 ± 0.0	*
Total carbohydrates	31.0 ± 4.7	29.5 ± 0.7	ns

[a]Mean ± SD.
[b]Grown at Morden, Manitoba, Canada, in 1987.
[c]Standard error of difference; ns, not significant; *significant, $P < 0.05$; **highly significant, $P < 0.01$.

5.0 ± 0.5%. The two mucilage samples (Table 2) contained significantly different protein (19.7-24.2%) and ash (9.6-10.4%), but similar total carbohydrates (29.5-31.0%) contents. Although mucilage was extracted only from three cultivars of flax (Andro, Norlin and Linott), their similar yields suggested a limited variation. For this reason, viscosity measurement of water extracts may indicate qualitative differences in flax seed mucilage, viscosity being sensitive to small changes in mucilage content. It will, however, be necessary to first establish a relationship between mucilage content and extract viscosity in a larger number of flax samples.

TIA. TIA of LM has not been reported in the literature. Preliminary experiments were first conducted to determine sodium hydroxide extract volumes that inhibited about 50% of the TA. This volume was 0.4 mL for LM, 0.5 mL (25-fold diluted) for SBM and 0.2 mL for RSM. The relationship between different concentrations of extract volume added to the assay (0 to 0.6 mL) and percent inhibition of trypsin (TIA/TA × 100) obtained was linear for LM. Figure 2 shows the TIA of nine samples of LM as compared to raw samples of SBM and RSM. Laboratory-prepared samples of LM contained 42-51 units of TIA, commercially obtained samples of LM 14-37 units, compared to 99 units of TIA for RSM and 1650 units of TIA for SBM. Analysis of variance of the data, excluding TIA units for RSM and SBM, showed highly significant cultivar differences (LSD = 2.8 units of TIA), largely due to lower TIA values obtained for the three commercially obtained (Alberta Linseed, ADM-Industry and Cargill) samples. These samples were most likely obtained from flax heated or toasted for commercial solvent extraction of oil. The Omega Nutrition sample was a press cake; its TIA (37 units) was closer to the range of the laboratory-prepared (unheated) samples. These data showed that raw LM contained only about one-half the TIA of raw RSM and much less than SBM and, secondly, that TIA of raw LM is reduced to one-half to one-third in commercial samples of LM. The low TIA (17 units) found in the three commercial samples of LM may not be of much significance in livestock and human nutrition.

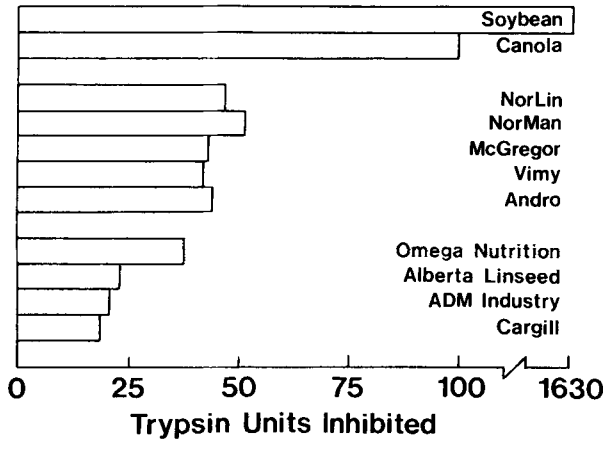


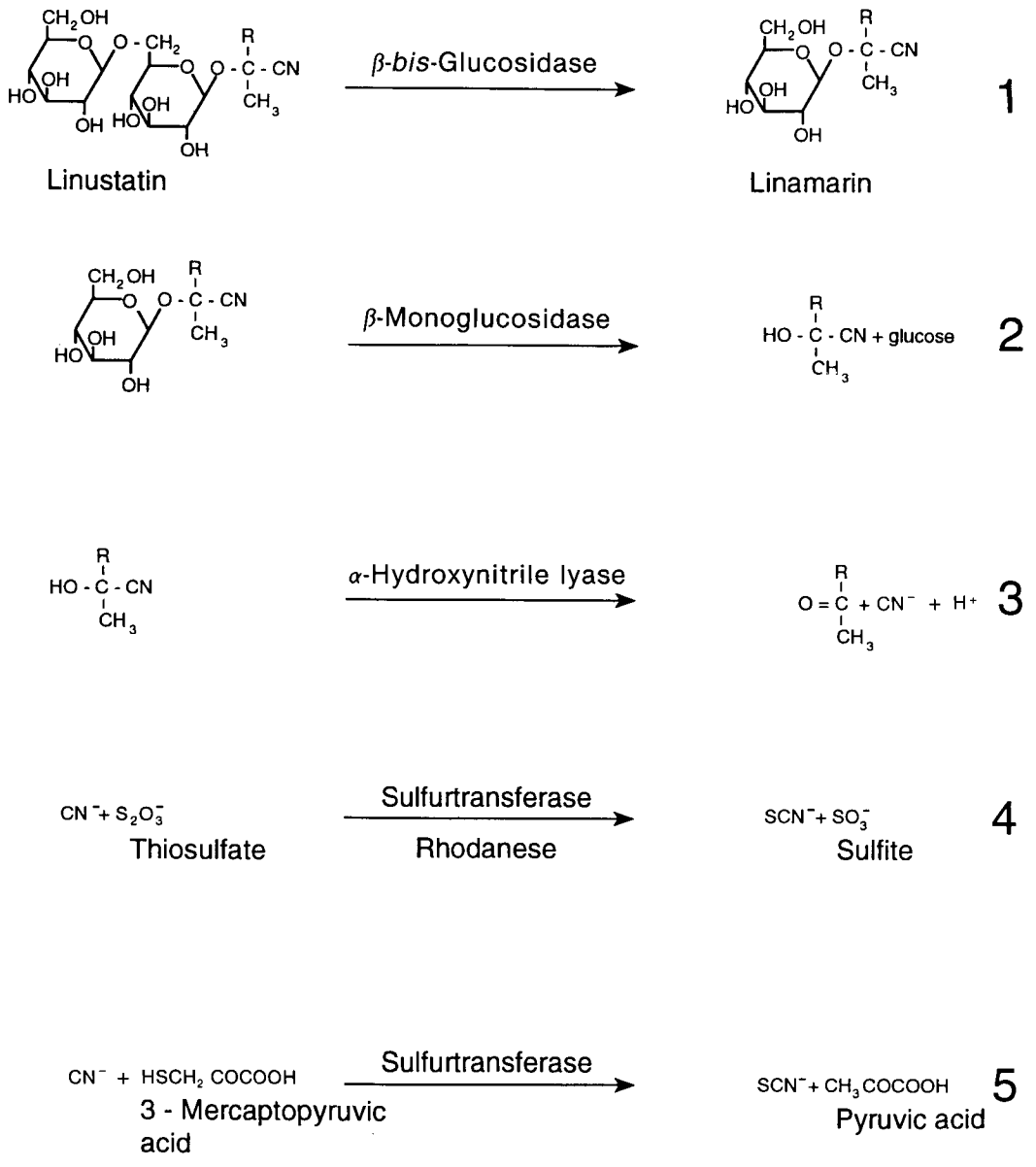
FIG. 2. Trypsin inhibitor activity (TIA) of laboratory-prepared and commercially obtained samples of linseed meal compared to raw soybean and rapeseed meals. The laboratory-prepared samples of linseed meal were from flax cultivars grown at Morden, Manitoba, Canada, in 1987. Least significant difference ($P < 0.05$), 2.8 units of TIA.

HCN. The major cyanogenic glucosides of Canadian cultivars of flax are the diglucosides, linustatin and neolinustatin; linamarin was present only in minor concentrations (14). These findings supported earlier reports on cyanogenic glucosides of flax grown in other countries (15,16). The enzymes involved in the hydrolysis of cyanogenic glucosides for the release of HCN and its conversion to thiocyanate have been reported (15,17). Linustatinase (*β-bis*-glucosidase) hydrolyzes linustatin (R = CH_3) and neolinustatin (R = C_2H_5) to yield the monoglucoside cyanogenic linamarin (Scheme 1, Equation 1), which then is hydrolyzed by β-monoglucosidase to yield the aglycones, acetone cyanohydrin from linustatin and methylethylketone cyanohydrin from neolinustatin (Scheme 1, Equation 2). The liberation of HCN from the aglycones is accomplished by α-hydroxynitrile lyase (Scheme 1, Equation 3). The ingested HCN may be detoxified in the animal body by a sulfurtransferase (rhodanese) in combination with thiosulfate (Scheme 1, Equation 4), or by another sulfurtransferase in combination with 3-mercaptopyruvic acid (Scheme 1, Equation 5) to yield the goitrogenic thiocyanate ion. Goitrogenic effects of LM in sheep have been reported in New Zealand in an earlier study (18).

Unhydrolyzed cyanogenic glucosides of LM may be determined by reverse-phase liquid chromatography (RPLC), where information on individual cyanogens is desired and authentic standards are available (14,16). Where such facilities are not available, HCN, the hydrolytic product, may be conveniently determined by a colorimetric procedure. Colorimetric procedures have been routinely used to determine cyanide contents of soybean products (19), cassava (20) and sorghum leaves (21). In colorimetric determinations, the freed HCN is distilled into an alkaline solution and oxidized, and the product of oxidation is reacted with barbituric acid-pyridine to form a dye complex (pink color), which has a characteristic absorbance at 580 nm.

Preliminary experiments were conducted to determine the presence of HCN in flax by qualitative and titrimetric procedures (7). In the first experiment, ten cultivars of flax were investigated by a picric acid test. Freshly ground flax seed (20 g) was wetted with sufficient water and allowed to autolyze in stoppered test tubes containing sodium picrate paper strips, which turn orange/red on reaction with HCN. This test indicated the presence of only trace quantities of HCN in flax cultivars, judged by color of the paper strips. In the second experiment, ground seed (10 g) of five cultivars of flax (Andro, McGregor, NorMan, NorLin and Vimy) was mixed with 100 mL water in stoppered flasks, and the released HCN was determined titrimetrically (1 mL 0.02N $AgNO_3$ = 0.54 mg HCN). The concentrations of HCN (μg/g meal) obtained were: Andro, 0; McGregor, 24.0 ± 11.3; NorLin, 35.0 ± 4.2; NorMan, 0.4 ± 0.1; and Vimy, 0. The titrimetric procedure lacked sensitivity because subsequent colorimetric determination (Tables 3 and 4) showed Andro, NorMan and Vimy flax to contain considerable quantitites of HCN. HCN values obtained for McGregor and NorLin with the titrimetric procedure were, for unknown reason, substantially higher than those obtained for the same two cultivars with the colorimetric procedure.

Table 3 gives concentrations of HCN, determined by the colorimetric procedure, in cultivars of flax grown at four widely separated locations in 1987, and Table 4 shows



SCHEME 1

TABLE 3

Hydrocyanic Acid (HCN) Content of Freshly Ground Canadian Cultivars of Flax Grown at Four Locations in 1987

Cultivar	(μg HCN per 100 g of as is ground seed)[a]				Cultivar mean
	Morden	Portage la Prairie	Saskatoon	Elrose	
Andro	7.8 ± 0.6	7.6 ± 0.9	8.8 ± 0.2	8.4 ± 0.2	8.2
Dufferin	6.3 ± 0.4	6.6 ± 0.9	8.4 ± 0.1	7.3 ± 0.4	7.2
Linott	6.3 ± 0.2	8.1 ± 0.5	8.2 ± 0.2	6.9 ± 0.2	7.4
McGregor	6.8 ± 0.4	7.6 ± 0.4	8.2 ± 0.6	8.9 ± 0.3	7.9
NorAlta	7.8 ± 0.2	7.5 ± 0.2	7.5 ± 0.7	8.3 ± 0.5	7.8
NorLin	8.0 ± 0.7	7.9 ± 0.6	7.5 ± 0.5	7.8 ± 0.1	7.8
NorMan	5.7 ± 0.3	6.7 ± 0.4	7.5 ± 0.2	7.5 ± 0.2	6.9
Vimy	7.5 ± 0.6	6.5 ± 0.6	8.7 ± 0.4	6.7 ± 0.1	7.4
Least significant difference for cultivar means ($P < 0.05$)					1.0

[a]Mean ± SD.

TABLE 4

Hydrocyanic Acid (HCN) Content of Freshly Ground Canadian Cultivars of Flax Grown at Two Locations in 1987 and 1989

Cultivar[b]	(μg HCN per 100 g of as is ground seed)[a] 1987[c]	1989[c]	Cultivar mean
Andro	8.6 ± 0.3	10.7 ± 0.0	9.7
Linott	7.5 ± 1.0	9.3 ± 0.0	8.4
McGregor	8.6 ± 0.5	9.3 ± 0.6	9.0
NorAlta	7.9 ± 0.2	9.9 ± 1.9	8.9
NorLin	7.6 ± 0.2	11.5 ± 2.2	9.6
NorMan	7.5 ± 0.0	8.4 ± 0.4	8.0
Vimy	7.7 ± 1.4	9.5 ± 1.4	8.6
Total	55.4 ± 3.9	68.6 ± 7.1	
Least significant difference ($P < 0.05$)			1.7

[a]Mean ± SD.
[b]One cultivar (Dufferin) less than in Table 3 due to lack of data.
[c]Data pooled for two locations (Saskatoon, Elrose) in each season.

TABLE 5

Two-Way Analysis of Variance of Data Showing Effects of Growth Location and Season on Hydrocyanic Acid Content of Canadian Cultivars of Flax[a]

Error source	DF	MS	F	$P < 0.05$	$P < 0.01$
Growth location					
Location	3	3.5	19.3	2.9	4.5
Cultivars	7	1.6	8.6	2.3	3.3
Interaction	21	0.8	4.5	1.9	2.6
Season					
Season	1	24.0	22.6	4.6	8.9
Cultivar	6	1.5	1.4	2.9	4.5
Interaction	6	1.0	1.0	2.9	4.5

[a]Abbreviations: DF, degrees of freedom; MS, mean squares; F, F ratio.

concentrations of HCN for the same cultivars grown for two seasons (1987 and 1989). Some of the cultivar means of HCN for the four locations were significantly different (Table 3). The mean HCN concentration was the highest in cultivar Andro and the lowest in cultivar NorMan, the difference between the two means was 19%. This difference was 37% for the same two cultivars grown at Morden but was lower (12–17%) for the cultivars grown at the other three locations. The same two cultivars also contained the highest and lowest concentrations of total cyanogenic glucosides (sum of linamarin, linustatin and neolinustatin) reported by Oomah *et al.* (14), although the difference between the cultivars was larger (50%) due to method of data expression. Furthermore, the HCN content of seven cultivars of flax (Andro, Linott, McGregor, NorAlta, NorLin, NorMan and Vimy) was significantly correlated (r = +0.96) with their total cyanogen content (14). Colorimetric determination of HCN was therefore an acceptable procedure, an alternate to RPLC for quantitation of individual cyanogens. Analysis of variance of the data (Table 5, growth location) showed highly significant effects of location, cultivar and location × cultivar interaction. The location variance (F ratio) was the largest (19.3) and the interaction variance the smallest (4.5). To investigate season effect on the HCN content of the cultivars, data for the two locations (Saskatoon and Elrose) were pooled for each cultivar grown for the two seasons (Table 4). The total HCN content of the seven cultivars for 1989 was 68.6 ± 7.1 μg per 100 g ground seed (mean 9.8 ± 1.0), as compared to 55.4 ± 3.9 μg per 100 g ground seed (mean 7.9 ± 0.6) for 1987 (Table 4). Season effect was highly significant and its variance was the largest (Table 5, season). Although cultivar and cultivar × season effects were significant, their variances were small. The present study showed that HCN content of Canadian-grown cultivars of flax was influenced largely by environments (location and season) and, to a smaller extent, by cultivar. Although flax cultivars may be selected for low HCN content, its critical levels in human and animal nutrition needs to be established first.

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