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Removal of flaxseed mucilage by chemical and enzymatic treatments

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The amount of flaxseed mucilage extracted into aqueous solutions was determined by monitoring the content of total sugars and pentoses as well as viscosity values. Whole seeds of flax were soaked and then treated with selected enzymes in order to reduce the content of seed coat polysaccharides. Soaking of seeds in water or sodium bicarbonate solutions or treatment with commercially available carbohydrases (Celluclast® 1.5L, Pectinex® Ultra SP and Viscozyme® L) reduced the amount of mucilage remaining in the seeds, as indicated by the low content of pentoses, total sugars and viscosity values of water extracts. Scanning electron micrographs of treated flaxseed coats revealed differences in the cellular structure of the outermost epidermal layer when compared with those of untreated seeds. Nitrogen solubility of defatted meals from low mucilage seeds prepared via sodium bicarbonate soaking (0.10 M, 12 h) and treatment with Viscozyme® L (22.5 mg protein per 100 g, 3 h) was improved. The protein recovery from low mucilage seeds increased since a better isolation of protein from flaxseed was possible due to reduced interference of mucilage. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Flax plant has been cultivated since antiquity, mainly for the use of its oil and fibre. The whole seed and oil of flax have been used for edible purposes in some European and Asian countries. In North America, whole seeds are used in different health foods while the oil is utilized in oil-based industrial products and the meal is used as an animal feed component or fertilizer. There has been a renewed interest to use flaxseed in the human diet in order to improve nutritional and health status. Due to the presence of biologically active phytochemicals in flaxseed, this oilseed has been identified as an item for the 'Designer Foods' project in the USA (Caragay, 1992).

Flaxseed is oval, flat and somewhat beaked, having average dimensions of 2.5 mm×5 mm×1 mm and an average weight of 3–13 mg per seed and a yellow to brown colour (Dybing & Lay, 1981). Its fibrous hulls account for 30–39% of the seed weight; they contain very little protein and oil but are rich in polymeric carbohydrates (Pryde, 1983). The true hull (or spermoderm) is covered on the outside by epiderm containing mucilage (5–8%; BeMiller, 1993) and on the inside by the endosperm (Peterson, 1958). Flax mucilage is a heterogenic polysaccharide and contributes largely to the soluble fibre fraction of flaxseed which is suggested to have a hypoglycaemic effect in humans (Cunnane *et al.*, 1993). Mucilage of flax consists of two polysaccharide components, acidic and neutral, at a ratio of 2:1 (Erskine & Jones, 1957; Hunt & Jones, 1962; Fedeniuk & Biliaderis, 1994). The neutral fraction contains L-arabinose, D-xylose and D-galactose in a mole ratio of 3.5:6.2:1, and is composed of branched arabinoxylan with a significant amount of terminal arabinopyranosyl units. The acidic fraction contains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid in a mole ratio of 2.6:1:1.4:1.7. All D-galactouronosyl units are located in the main chain and all L-fucosyl and about half of its galactosyl units are present as non-reducing end-groups (Muralikrishna *et al.*, 1987). According to Oomah *et al.* (1995), water-soluble polysaccharides of flaxseed consist of glucose, xylose, galactose, rhamnose, arabinose and fucose in a decreasing order of abundance. The functionality of flaxseed mucilage is similar to that of gum arabic (BeMiller, 1993; Mazza & Biliaderis, 1989). Functional properties of the isolated mucilage (Mazza & Biliaderis, 1989; Susheelamma, 1987) and its use as a thickening and stabilizing agent have been reported (Dev & Quensel, 1989; Susheelamma, 1989). The high water absorption capacity of flaxseed meal (Wanasundara & Shahidi, 1994) is mainly attributed to

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the presence of these polysaccharides in the seed coat (Fedeniuk & Biliaderis, 1994).

Development of low linolenic acid flaxseed varieties (LINOLA™ and SOLIN™) in Canada, mainly for their oil for edible purposes, may have an impact on domestic crushing of flax as only 4% of the domestic seed production is currently used for oil extraction for industrial applications. The resultant meal from Canadian varieties contains 31–45% protein (Oomah & Mazza, 1993) and is also rich in seed coat polysaccharides. However, possible utilization of flaxseed meal for non-feed purposes has not been adequately studied. Isolation of proteins from flax meal has previously been achieved mainly by isoelectric precipitation of its aqueous extracts (Smith *et al.*, 1946; Sosulski & Bakal, 1969; Madhusudhan & Singh, 1983). However, the presence of polysaccharides in the seed coat may hinder the separation of proteins due to their swelling in the aqueous medium (Smith *et al.*, 1946; Sosulski & Bakal, 1969). Therefore, use of dehulled or mucilage-free seeds may improve the isolation and the yield of protein. Dehulling of flax is tedious due to the shape of the seed and the nature of seed coats. The liquid cyclone process developed for canola seeds by Sosulski and Zadernowski (1981) may be successfully applied to flaxseed. Mandokhot and Singh (1976) and Madhusudhan and Singh (1985) have reduced the mucilage content of seeds by soaking in water or 1% (v/v) HCl solution for 16–24 h. The present study describes the use of polysaccharide-degrading enzymes and soaking in sodium bicarbonate solutions to remove seed coat polysaccharides of flaxseed in order to improve extraction and recovery of its proteins. Three simple indicators—namely, total sugar and pentose contents as well as viscosity values—were employed to quantitate polysaccharides in flaxseed mucilage extracts.

MATERIALS AND METHODS

Samples of flaxseed (*Linum usitatissimum* Linn., variety 'Somme') were obtained from Omega Nutrition Company (Vancouver, BC). Seeds were stored in air-tight containers at ambient temperatures in the dark. Portions of seeds were processed using a liquid cyclone as described by Sosulski & Zadernowski (1981) to obtain hull-free flour.

Extraction of mucilage

Mucilage present in the seed coat of flaxseed was extracted as described by Bhatty (1993). Whole seeds (3–30 g) were added to 300 ml of boiled distilled water and extracted for 3 h in a wrist-action shaker at room temperature (22°C). Mazza and Biliaderis (1989) have shown that these conditions allow the extraction of over 90% of flaxseed mucilage. In order to obtain extracts containing various amounts of mucilage, different amounts of seeds (1–10%, w/v) were extracted with water. The extract was filtered through a layer of glass wool and the volume was made to 300 ml with distilled water.

Determination of pentose content

The mucilage extract (1 ml) was hydrolysed with 1 ml of 4 N HCl in a tightly capped vial at 100°C for 120 min. After cooling, 0.1–0.2 ml of the hydrolysed sample was diluted to 3 ml with distilled water to which 3 ml of 0.1% (w/v) $FeCl_3$ in concentrated HCl and 0.3 ml of 1.0% (w/v) orcinol in absolute ethanol were added. Reagents were well mixed with the sample and allowed to develop colour at 100°C for 30 min. Absorbance of the reaction mixture was measured at 670 nm (Hashimoto *et al.*, 1987). The pentose content (expressed as milligrams of D-xylose equivalents per millilitre of extract) may then be calculated by multiplying the absorbance reading at 670 nm by a factor of 0.35.

Determination of total sugar content

A 0.05–0.10 ml aliquot of the extract was diluted to 1 ml and 10 ml of ice-cold anthrone reagent (0.05% (w/v) anthrone in 6.5 N H_2SO_4 containing 1% (w/v) thiourea) was added to it followed by mixing. The reaction mixture was held at 100°C for 10 min and the absorbance of the reaction mixture was measured at 620 nm (Carrol *et al.*, 1955). The total sugar content of the mucilage extracts (expressed as milligrams of D-glucose equivalents per millilitre of the sample) was calculated by multiplying the absorbance reading at 620 nm by a factor of 3.06.

Determination of viscosity

Viscosity was determined using a Brookfield Synchro-Lectric viscometer (Model LVT; Brookfield Engineering Inc. Stoughton, MA). Readings were taken at the speed of 60 rpm using spindle No. 1 at 22°C. The viscosity values were calculated in centipoise units by multiplying the dial-reading with corresponding conversion factors provided by the manufacturer.

Preparation of low mucilage flaxseed

Soaking treatment

Whole seeds were soaked in water or in 0.05 M or 0.10 M $NaHCO_3$ (Fisher Scientific, Toronto, ON) solution at a seed-to-solvent ratio of 1:10 (w/v) for 3, 6 or 12 h in glass beakers in a water-bath shaker (200 rpm) at room temperature. After soaking, excess water was drained and seeds were dispersed in 0.10 N HCl (half the volume of liquid used for soaking) and allowed to stand for a few minutes before washing. The resultant slimy suspension of seeds was passed through a wire mesh (mesh size 2 mm) to separate the seeds. Seeds were

rubbed against the mesh to remove more mucilage and then washed five times with water (1:10, w/v) at 30 ± 1°C. After washing and draining, seeds were dried in a forced-air oven at 45°C for approximately 6 h. The dried seeds were stored in 'Nasco' whirl pack plastic bags at room temperature in the dark.

Enzyme treatment

Commercially available carbohydrases used in this study were Celluclast® 1.5L, Pectinex® Ultra SP and Viscozyme® L (kindly provided by Novo Nordisk, Bagsvaerd, Denmark), details of which are given in Table 1. Seeds were added to a 0.01 M acetate buffer of appropriate pH at a seed-to-solvent ratio of 1:5 (w/v) and incubated with different concentrations of each enzyme for 1, 3 or 6 h in a water-bath shaker. The reaction was stopped by adding 50 ml of 0.10 N NaOH to the mixture. Seeds were then treated essentially in the same manner as described for the soaking treatments.

Mucilage remaining in the seed coat after soaking or enzyme treatment was extracted as previously described for untreated seeds. The content of pentoses and total sugars as well as the viscosities of these mucilage extracts were determined as described above.

Nitrogen solubility and protein recovery

Untreated seeds, seeds soaked in 0.10 M $NaHCO_3$ solution for 12 h and seeds treated with Viscozyme® L (22.5 mg protein per 100 g for 3 h) were defatted by blending with hexane (1:5, w/v, five times) at ambient temperatures. Defatted meals were air-dried for 12 h. About 0.3 g of meal was dispersed in distilled water (1:100, w/v) and the pH of the dispersions was adjusted to between 2.00 to 11.00 using 1 N solutions of NaOH or HCl. The dispersions were thoroughly mixed at 200 rpm in an orbital shaker (Model G76; New Brunswick Scientific, New Brunswick, NJ, USA) for 30 min and the pH values were recorded afterwards. The samples were centrifuged at 1500*g* for 10 min and the nitrogen content of supernatants determined by Kjeldahl nitrogen analysis (AOAC, 1990). The content of soluble nitrogen was expressed as the percentage ratio of the content of nitrogen in the supernatant to that in the meal. The pH of the supernatant was then adjusted to 3.55 ± 0.10 followed by centrifugation at 4000*g* for 10 min. The difference in soluble nitrogen content of the supernatants before and after pH adjustment to 3.55 was considered as protein nitrogen content and expressed as the percentage of protein nitrogen recovered from the total nitrogen of the meal. The volume of the aliquots removed for nitrogen determination was considered in calculations. Flour obtained by the liquid cyclone process was also studied for its nitrogen solubility and protein recovery.

Scanning electron microscopy

Seed coats of untreated seeds and of samples treated with water (12 h), $NaHCO_3$ (0.10 M, 12 h) and Viscozyme® L (22.5 mg protein per 100 g, 3 h) were manually dissected and the seed coats were separated from the cotyledons. Seed coat fragments were placed on aluminium stubs covered with double-sided tapes to face outermost side up and then sputter coated with gold under vacuum and observed under a Hitachi S570 scanning electron microscope operated at 20 kV. Photographs were taken using a polaroid type 665 positive/negative film.

Statistical analysis

Mean values of triplicate determinations with standard deviations are reported when appropriate. Analysis of variance was performed and differences in mean values were determined by Tukey's studentized test at $P < 0.05$ using SAS software (SAS Institute Inc., 1990). Regression analysis was also performed with the same software using the GLM procedure.

Table 1. Sources, activities and optimum conditions of enzymes used

Enzyme	Source	Mode of activity	Declared activity[a]	Optimum pH and temperature	Protein content (mg protein ml^{-1})[b]
Celluclast® 1.5L Batch CCN 3041/94-10	*Trichoderma reesei*	Cellulase	1500 NCU g^{-1} (specific gravity 1.2 g ml^{-1})	4.5–6.0 50–60°C	54.0 ± 2.0
Pectinex® Ultra SP Batch T012/94-04	*Aspergillus niger*	Poly-galacturonase, hemicellulase	8800 PSU ml^{-1}	3.5–4.5 30–45°C	76.3 ± 2.5
Viscozyme® L Batch KRN 01108/94-10	*Aspergillus* spp.	Arabanase, cellulase, β-glucanase, hemicellulase, xylanase	100 FBG g^{-1} (specific gravity 1.2 g ml^{-1})	3.3–5.5 40–50°C	90.1 ± 3.0

[a]FBG, fungal β-glucanase; NCU, Novo cellulose units; PSU, pectinose S units.
[b]Determined according to the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Contents of pentose and total sugar and viscosity values of mucilage extracts prepared from untreated flaxseed

Figure 1 shows the content of total sugars and pentoses of the water extract of flaxseed using various amounts of seed. The contents of total sugars and pentoses increased as the amount of seed used to prepare mucilage extracts was increased. Mazza and Biliaderis (1989), Bhatty and Cherdkiatgumchai (1990) and Fedeniuk and Biliaderis (1994) reported that water is the most suitable extraction medium for flaxseed mucilage intended for further studies. Correlation coefficients of the relationship between total sugar and pentose contents in the extracted solutions and the percentage seed in the dispersion were 0.974 and 0.957, respectively (Figs 1(A) and (B)). Glucose (29%) is the major hexose present in the hydrolysed water-soluble polysaccharides of flaxseed; galactose (19%), rhamnose (14%) and fucose (3.5%) are also present. The pentoses present are xylose (23%) and arabinose (11%) (Oomah *et al.*, 1995). Since pentoses constitute only a fraction of the total sugars of hydrolysed water-soluble polysaccharides, their content was lower than those of the total sugars. The polysaccharides solubilized from seed coats are

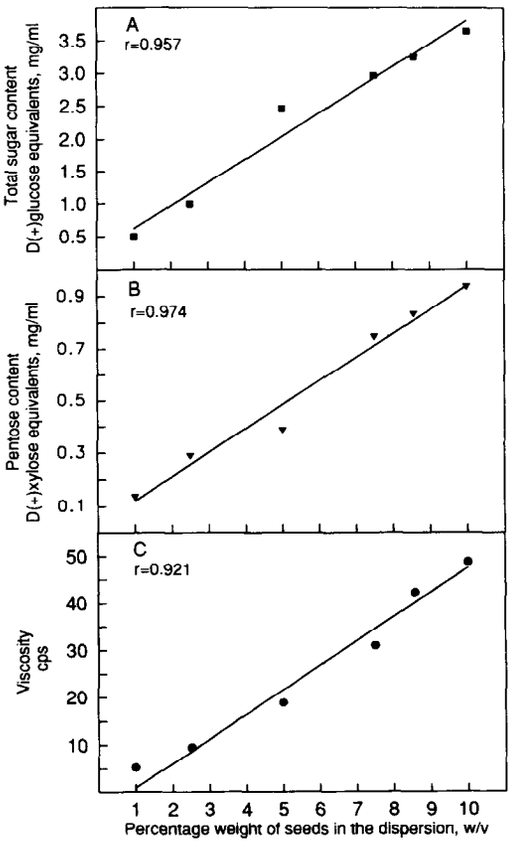

Fig. 1. Relationship between percentage weight of seeds in the dispersion and contents of total sugars (A), pentoses (B) and viscosity values (C) of the mucilage extracts prepared from untreated flaxseed.

responsible for increased contents of total sugars and pentoses as well as the measured viscosity values, the latter exhibiting a good correlation ($r = 0.921$; Fig. 1C) with seed weight in the dispersion. According to Bhatty (1993), the viscosity of mucilage extracts increases as the weight of flaxseed hulls in the dispersion is increased, thus providing a measure of mucilage content in the extract. In this study, contents of total sugars, pentoses and viscosity values provided indirect measurements of mucilage content of the water extracts of flaxseed.

Effect of soaking and enzyme treatments on contents of pentoses, total sugars and viscosity values of flaxseed mucilage extracts

The content of pentoses and total sugars as well as the viscosity values of mucilage extracts prepared from flaxseeds subjected to prior soaking, or treatment with Cellulase® 1.5L, Pectinex™ Ultra SP and Viscozyme® L are given in Tables 2–5, respectively. Mucilage extracts of soaked and enzyme-treated seeds contained a relatively lower amount of pentoses and total sugars and had a reduced viscosity than extracts from untreated seeds. An increase in the concentration of $NaHCO_3$ or soaking time resulted in lowering of the amount of mucilage of seed coats as indicated by reduced amounts of pentoses, total sugars and viscosity values of the extracts. The combination effect of $NaHCO_3$ concentration and soaking time was also significant ($P < 0.05$) for reducing the content of pentoses and total sugars.

Carbohydrase-assisted degradation of mucilage by different enzyme concentrations and reaction times tested lowered the mucilage content in the seed coats. The treated seeds contained a lower amount of pentoses and total sugars and viscosity values when compared with their respective control samples. Treatment of seeds with Celluclast® 1.5L, 45.0 mg protein per 100 g for 6 h and 22.5 mg protein per 100 g for 3 h had a similar effect in reducing the mucilage content of seeds. The effect of Pectinex™ Ultra SP and Viscozyme® L was similar to that of Celluclast® 1.5L. Treating of seeds for 1 h with Viscozyme® 22.5 mg protein per 100 g lowered the contents of pentoses, total sugars and viscosity values of mucilage extracts considerably after 3 or 6 h of treatment.

Polysaccharides have a high affinity for water and are dissolved in it by continuous hydration and changes in the intermolecular binding of the polysaccharides (i.e. polysaccharide–polysaccharide interactions) to polysaccharide–water binding. The amorphous regions of polysaccharides that possess little or no intermolecular H-bonds are particularly available for hydration; as a consequence, the solubility of branched polysaccharides which have no or little possibility of intermolecular association is increased (Whistler, 1973). Therefore, branched polysaccharides of flaxseed coats are solvated during the soaking process and finally get into solution.

As the soaking time increased the solubilization of polysaccharides was also increased. The mild alkali pH of the bicarbonate soaking medium may give rise to depolymerization of polysaccharides (BeMiller, 1986) which increases their solubility. Therefore, the presence of $NaHCO_3$ in the soaking medium improved the removal of mucilage from seeds as compared with that of water alone. Further washing with water resulted in enhanced removal of polysaccharides present from flaxseed coats.

Carbohydrases have been used to degrade cell wall polysaccharides of oilseeds for better oil recovery (Olsen, 1988; Sosulski *et al.*, 1988). The primary modes of activities of the three enzymes tested are summarized in Table 1. Flaxseed mucilage is reported to be devoid of cellulose (Fedeniuk & Biliaderis, 1994); however, Celluclast® 1.5L, which has only cellulolytic activity, was able to liberate mucilage from flaxseed coat which may have cellulosic material. Viscozyme® L, which is a multiactive enzyme (Table 1), was more efficient than the other two enzymes in removing flaxseed mucilage (Table 3–5).

Seed coat microstructure

The scanning electron micrographs of seed coat surfaces of untreated, water (12 h), $NaHCO_3$ (0.10 M, 12 h) soaked and Viscozyme® L (22.5 mg protein per 100 g, 3 h) treated flaxseeds are shown in Fig. 2. The cells of the outermost layer of untreated seed coat (Fig. 2(A)) were not distinctly visual as they were covered with polysaccharides. For treated seeds (Figs 2(B)–(D)), defined cell structures were clearly noted. The hollow cells with visible walls indicated that most cellular contents were removed due to soaking or enzyme treatment. Bhatty (1993) has also observed this contrasting structural difference of flaxseed coat cells after soaking of hull fragments in water. The cells of seeds soaked in water retained some of the cellular contents compared to 0.10 M $NaHCO_3$ soaked and Viscozyme® L treated seeds which were completely empty. According to Freeman (1992), the outermost layer of flaxseed coat varies in shape from rectangular to polygonal and is characteristic of the variety. The seed variety

Table 2. Content of pentoses and total sugars and viscosity values of the mucilage extracts prepared from flaxseed subjected to soaking treatment

Treatment	Viscosity (cps)	Pentose content (mg ml^{-1})[a]	Total sugar content (mg ml^{-1})[b]
	$NaHCO_3$, 0.00 M (pH 6.80)		
3 h	8.25 ± 0.43^{a}	0.80 ± 0.06^{a}	2.60 ± 0.02^{a}
6 h	7.50 ± 0.00^{a}	0.69 ± 0.03^{b}	2.35 ± 0.12^{b}
12 h	5.67 ± 0.29^{bc}	0.55 ± 0.03^{c}	2.05 ± 0.07^{cd}
	$NaHCO_3$, 0.05 M (pH 8.16)		
3 h	6.33 ± 0.29^{b}	0.69 ± 0.03^{b}	2.17 ± 0.05^{c}
6 h	5.17 ± 0.29^{cd}	0.47 ± 0.01^{de}	1.92 ± 0.06^{d}
12 h	3.41 ± 0.14^{e}	0.16 ± 0.01^{f}	1.55 ± 0.06^{ef}
	$NaHCO_3$, 0.10 M (pH 8.35)		
3 h	5.00 ± 0.00^{cd}	0.54 ± 0.03^{cd}	2.10 ± 0.03^{cd}
6 h	4.42 ± 0.38^{d}	0.44 ± 0.02^{e}	1.65 ± 0.05^{e}
12 h	2.83 ± 0.14^{e}	0.13 ± 0.01^{f}	1.45 ± 0.03^{f}

Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another.
[a]As D-xylose equivalents.
[b]As D-glucose equivalents.

Table 3. Content of pentoses and total sugars and viscosity values of mucilage extract prepared from Celluclast® 1.5L treated (1:5 (w/v), 0.01 M acetate buffer, pH 4.0, 50°C) flaxseed

Treatment	Viscosity (cps)	Pentose content (mg ml^{-1})[a]	Total sugar content (mg ml^{-1})[b]
	Celluclast® 1.5L, 0 mg protein per 100 g		
1 h	6.58 ± 0.14^{a}	0.48 ± 0.02^{a}	2.62 ± 0.18^{a}
3 h	6.00 ± 0.00^{b}	0.35 ± 0.01^{b}	2.15 ± 0.15^{b}
6 h	5.50 ± 0.00^{c}	0.32 ± 0.01^{bc}	2.04 ± 0.06^{b}
	Celluclast® 1.5L, 22.5 mg protein per 100 g		
1 h	5.25 ± 0.00^{c}	0.34 ± 0.02^{bc}	2.57 ± 0.05^{b}
3 h	4.50 ± 0.00^{d}	0.27 ± 0.01^{d}	1.65 ± 0.08^{c}
6 h	3.92 ± 0.14^{e}	0.18 ± 0.01^{e}	1.65 ± 0.08^{c}
	Celluclast® 1.5L, 45.0 mg protein per 100 g		
1 h	4.25 ± 0.00^{d}	0.30 ± 0.01^{cd}	2.14 ± 0.07^{b}
3 h	3.67 ± 0.14^{ef}	0.17 ± 0.01^{e}	1.77 ± 0.09^{c}
6 h	3.58 ± 0.14^{f}	0.16 ± 0.01^{e}	1.62 ± 0.02^{c}

Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another.
[a]As D-xylose equivalents.
[b]As D-glucose equivalents.

in this study had mostly pentagonal and hexagonal cells which were clearly visible after soaking or enzyme treatment. It was expected that cell walls may also be solubilized by the Viscozyme treatment; however, the enzyme concentration and reaction time were insufficient to break the complex constituents of flaxseed coat cell walls. Flaxseed (variety, Somme) has a slippery surface with a shiny brown colour coat. However, after soaking or enzyme treatment, seeds lost their sheen as well as their slipperiness. Comparison of the outermost seed coat microstructure before and after soaking or enzyme treatment provided convincing evidence that cellular contents were removed due to the soaking and enzyme treatments, but the cell walls remained intact.

Nitrogen solubility and protein recovery

Figure 3(A) shows changes in the percentage of soluble nitrogen content of untreated, water (12 h), $NaHCO_3$ (0.10 M, 12 h) soaked and Viscozyme® (22.5 mg protein per 100 g, 3 h) treated defatted flaxseed meal at different pH conditions. The solubility pattern indicated that 50% of the total nitrogen content of all four meals was soluble at pH values of above 5.00. A decreased solubility was observed at pH 3.00–5.00. An increase in nitrogen solubility was evident below pH 3.00, but a minimum solubility was observed at pH 3.50–3.60 for all meals. Previous results reported from our laboratory (Wanasundara & Shahidi, 1994) also concur with these observations. Flour obtained from the liquid cyclone process also showed solubility values that were very similar to those obtained for the treated seeds. The flour so obtained is free of hulls and therefore almost devoid of seed coat polysaccharides.

At pH above 7.00, 45–50% of flax proteins were recovered from native seed while $NaHCO_3$ (0.10 M, 12 h) treatment resulted in the recovery of over 70% of proteins present in the seed meals (Fig. 3(B)). Protein recovery of the meals followed a pattern parallel to their nitrogen solubility. However, meal of untreated seeds had a lower protein nitrogen solubility when compared with that of treated seeds or liquid cyclone processed

Table 4. Content of pentoses and total sugars and viscosity values of the mucilage extract prepared from Pectinex® Ultra SP treated (1:5, w/v, 0.01 M acetate buffer, pH 4.0, 30°C) flaxseed

Treatment	Viscosity (cps)	Pentose content (mg ml^{-1})[a]	Total sugar content (mg ml^{-1})[b]
Pectinex® Ultra SP, 0 mg protein per 100 g			
1 h	6.75 ± 0.00^{a}	0.21 ± 0.02^{a}	2.97 ± 0.20^{a}
3 h	6.25 ± 0.25^{b}	0.19 ± 0.01^{a}	2.62 ± 0.11^{a}
6 h	5.83 ± 0.14^{b}	0.15 ± 0.03^{b}	2.42 ± 0.11^{bc}
Pectinex® Ultra SP, 22.5 mg protein per 100 g			
1 h	5.25 ± 0.00^{c}	0.13 ± 0.02^{c}	2.27 ± 0.13^{c}
3 h	3.75 ± 0.25^{d}	0.11 ± 0.01^{de}	1.79 ± 0.08^{de}
6 h	3.75 ± 0.00^{d}	0.08 ± 0.01^{f}	1.65 ± 0.08^{de}
Pectinex® Ultra SP, 45.0 mg protein per 100 g			
1 h	3.83 ± 0.14^{d}	0.12 ± 0.03^{cd}	1.92 ± 0.15^{d}
3 h	3.58 ± 0.14^{d}	0.09 ± 0.02^{ef}	1.65 ± 0.10^{de}
6 h	3.42 ± 0.14^{d}	0.08 ± 0.01^{f}	1.59 ± 0.05^{e}

Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another.
[a]As D-xylose equivalents.
[b]As D-glucose equivalents.

Table 5. Content of pentoses and total sugars and viscosity values of the mucilage extract prepared from Viscozyme® L treated (1:5, w/v, 0.01 M acetate buffer, pH 4.00, 40°C) flaxseed

Treatment	Viscosity (cps)	Pentose content (mg ml^{-1})[a]	Total sugar content (mg ml^{-1})[b]
Viscozyme® L, 0 mg protein per 100 g			
1 h	7.50 ± 0.00^{a}	0.19 ± 0.01^{a}	2.97 ± 0.20^{a}
3 h	5.50 ± 0.00^{b}	0.15 ± 0.04^{b}	2.70 ± 0.08^{ab}
6 h	5.17 ± 0.14^{c}	0.11 ± 0.01^{c}	2.56 ± 0.07^{b}
Viscozyme® L, 22.5 mg protein per 100 g			
1 h	3.75 ± 0.00^{d}	0.10 ± 0.01^{cd}	2.47 ± 0.09^{b}
3 h	3.25 ± 0.00^{e}	0.08 ± 0.00^{de}	2.06 ± 0.16^{c}
6 h	3.25 ± 0.00^{e}	0.07 ± 0.01^{ef}	1.73 ± 0.01^{de}
Viscozyme® L, 45.0 mg protein per 100 g			
1 h	3.75 ± 0.00^{d}	0.10 ± 0.01^{cd}	2.63 ± 0.24^{b}
3 h	3.25 ± 0.00^{e}	0.08 ± 0.01^{de}	2.14 ± 0.04^{c}
6 h	3.17 ± 0.14^{e}	0.05 ± 0.01^{f}	1.71 ± 0.07^{d}

Means followed by different superscripts within a column are significantly ($P < 0.05$) different from one another.
[a]As D-xylose equivalents.
[b]As D-glucose equivalents.

flour. The low mucilage content of the treated seed meals facilitated precipitation of proteins when the pH was lowered to minimum solubility pH. Extracts of untreated seed meal were viscous due to the presence of solubilized seed coat polysaccharides and this prevented precipitation of protein aggregates which were formed at the minimum solubility pH. Thus, untreated seed meals had to be extracted at pH values above 8.00 in order to achieve a maximum of 53% protein recovery. However, in the pH range of 6.00–7.00, 65–75% of protein nitrogens were recovered from the $NaHCO_3$ (0.10 M, 12 h) soaked and Viscozyme® (22.5 mg protein per 100 g, 3 h) treated seed meals which contained low amounts of seed coat polysaccharides. It is important to note that the content of protein nitrogen of liquid cyclone processed flour was always lower than that of $NaHCO_3$ soaked seed meal even at the highest pH solubility.

About 18–20% of solubilized nitrogenous compounds in seed meals was not recovered as protein in the pH range studied. This is comparable to the non-protein nitrogen content of flaxseed (Smith *et al.*, 1946; Sosulski & Bakal, 1969; Wanasundara & Shahidi, 1994) that can not be precipitated by acids. Therefore, consideration of the total nitrogen content of flaxseed extracts results in overestimation of their protein content. Figure 4 shows the relationship between soluble nitrogen and protein nitrogen of seed meals in this study.

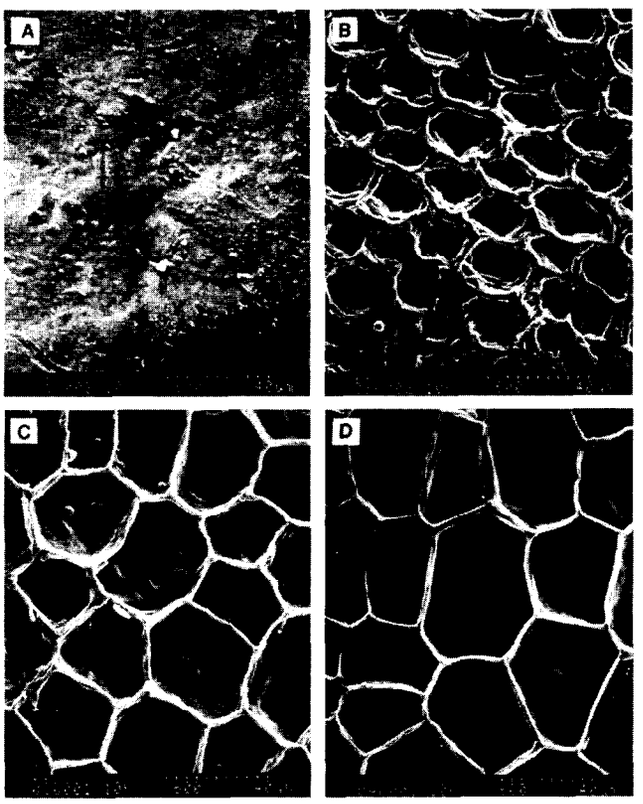

Fig. 2. Scanning electron micrographs of untreated (A), sodium bicarbonate treated (0.10 M, 12 h, B; 0.01 M, 12 h, C) and Viscozyme® L treated (22.5 mg protein per 100 g, 3 h, D) seed coat fragments (magnification ×600).

The regression equations obtained by least square fit for different seed meals were as follows:

Untreated seed meal:

$$\text{Protein nitrogen (\%)} = 0.732 \times \text{soluble nitrogen (\%)} - 19.463$$

$NaHCO_3$ (0.10 M, 12 h) soaked seed meal:

$$\text{Protein nitrogen (\%)} = 0.895 \times \text{soluble nitrogen (\%)} - 14.769$$

Viscozyme® (22.5 mg protein per 100 g, 3 h):

$$\text{Protein nitrogen (\%)} = 0.895 \times \text{soluble nitrogen (\%)} - 19.199$$

Liquid cyclone processed flour:

$$\text{Protein nitrogen (\%)} = 0.890 \times \text{soluble nitrogen (\%)} - 19.199$$

According to the above equations, nearly 16.5–26.6% of soluble nitrogen in different flaxseed meals is not recoverable as protein.

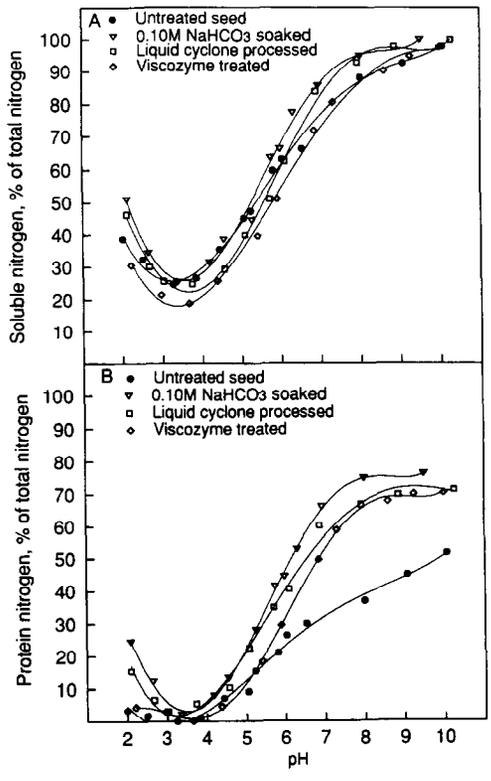

Fig. 3. Percentage of soluble nitrogen (A) and protein nitrogen (B) of untreated, liquid cyclone processed, sodium bicarbonate (0.10 M, 12 h) and Viscozyme® L (22.5 mg protein per 100 g, 3 h) treated flaxseed meal as affected by pH of the extraction medium.

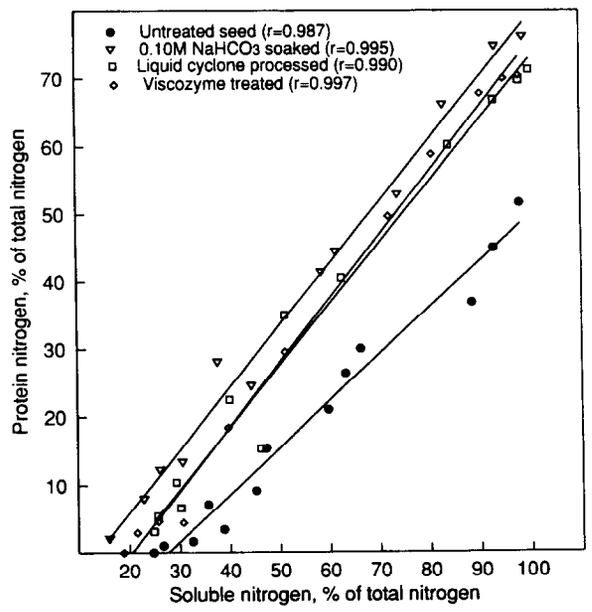

Fig. 4. Relationship between soluble nitrogen and protein nitrogen of untreated, liquid cyclone processed, sodium bicarbonate (0.10 M, 12 h) and Viscozyme® L (22.5 mg protein per 100 g, 3 h) treated flaxseed meal.

Removal of seed coat polysaccharides had a major effect in enhancing protein yield of flaxseed meal via solubilization processes. The pretreatments (soaking or enzyme treatment) did not disintegrate seeds and were low cost when compared with mechanical operations such as the liquid cyclone process. The pretreated seeds may be subjected to conventional solvent extraction and the resultant meal could be used for protein recovery by standard practices such as alkali solubilization and isoelectric precipitation.

CONCLUSIONS

Contents of pentoses and total sugars as well as viscosity values may be used to determine the amount of flaxseed coat polysaccharides dissolved in distilled water. Soaking of whole flaxseed in water or sodium bicarbonate (0.05 and 0.10 M for 6 and 12 h) removed a considerable amount of seed coat mucilage. Polysaccharide-degrading enzymes may also be used to reduce the mucilage content of flaxseed. The decrease in mucilage content of flaxseed was evidenced by reduced viscosity, pentose and total sugar contents of the water extracts of treated seeds. Observation of seed coat microstructure provided convincing evidence for the removal of mucilage from the exposed surfaces of the seed coats. Nitrogen solubility and protein recovery of defatted meals were markedly improved when seeds underwent pretreatments to remove their coat mucilage.

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REFERENCES

AOAC (1990). *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Washington, DC.

BeMiller, J. N. (1986). An introduction to pectins: structure and properties. In *Chemistry and Function of Pectins*, eds M. L. Fishman & J. J. Jen. American Chemical Society, Washington, DC, pp. 2–21.

BeMiller, J. N. (1993). Quinceseed, psyllium seed, flaxseed and okra gums. In *Industrial Gums*, 3rd edn, eds R. L. Whistler & J. N. BeMiller. Academic Press, New York, pp. 232–235.

Bhatty, R. S. (1993). Further compositional analysis of flax: mucilage, trypsin inhibitors and hydrocyanic acid. *J. Am. Oil Chem. Soc.*, **70**, 899–904.

Bhatty, R. S. & Cherdkiatgumchai, P. (1990). Compositional analysis laboratory-prepared and commercial samples of linseed meal and of hull isolated from flax. *J. Am. Oil Chem. Soc.*, **67**, 79–84.

Caragay, A. B. (1992). Cancer preventive foods and ingredients. *Food Technol.*, **46**(4), 65–68.

Carrol, N. V., Longley, R. W. & Roe, J. H. (1955). The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*, **220**, 583–593.

Cunnane, S. C., Ganguli, S., Menard, C., Liede, A. C., Hamadeh, M. J., Chen, Z.-Y., Wolever, T. M. S. & Jenkins, D. J. A. (1993). High α-linolenic acid flaxseed (*Linum ussitatissimum* L.): some nutritional properties in humans. *Br. J. Nutr.*, **69**, 443–453.

Dev, D. K. & Quensel, E. (1989). Functional properties of linseed protein products containing different levels of mucilage in selected food systems. *J. Food Sci.*, **54**, 183–186.

Dybing, C. D. & Lay, C. (1981). Flax. In *Handbook of Biosolar Resources*, Vol. II, ed. O. R. Zoborsky. CRC Press, Boca Raton, pp. 71–85.

Erskine, A. J. & Jones, J. K. N. (1957). The structure of linseed mucilage. Part I. *Can. J. Chem.*, **35**, 1174–1182.

Fedeniuk, R. W. & Biliaderis, C. G. (1994). Composition and physicochemical properties of linseed (*Linum ussitatissimum* L.) mucilage. *J. Agric. Food Chem.*, **42**, 240–247.

Freeman, T. P. (1992). Structural characteristics of the seed coats of two varieties of flax. In *Proceedings of Flax Institute of United States*, 54th Annual Meeting, 30–31 January, Fargo, pp. 93–98.

Hashimoto, S., Shogren, M. D. & Pomeranz, Y. (1987). Cereal pentosans: their estimation and significance 1. Pentosans in wheat and milled wheat products. *Cereal Chem.*, **64**, 30–34.

Hunt, K. & Jones, J. K. N. (1962). The structure of linseed mucilage. Part II. *Can. J. Chem.*, **40**, 1266–1279.

Lowry, O., Rosenborough, N., Farr, A. & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

Madhusudhan, K. T. & Singh, N. (1983). Studies on linseed proteins. *J. Agric. Food Chem.*, **31**, 959–963.

Madhusudhan, K. T. & Singh, N. (1985). Effect of detoxification treatment on the physiochemical properties of linseed proteins. *J. Agric. Food Chem.*, **33**, 1219–1222.

Mandokhot, V. M. & Singh, N. (1976). Studies on linseed as a protein source for poultry. I. Process of demucilaging and dehulling of linseed and evaluation of processed materials by chemical analysis and with rats and chicken. *J. Food Sci. Technol.*, **16**, 25–31.

Mazza, G. & Biliaderis, C. G. (1989). Functional properties of flax mucilage. *J. Food Sci.*, **54**, 1302–1305.

Muralikrishna, G., Salimath, P. V. & Tharanathan, R. N. (1987). Structural features of an arabinoxylan and rhamnogalacturonan derivative from linseed mucilage. *Carbohydr. Res.*, **161**, 265–271.

Olsen, H. S. (1988). *Aqueous Enzymatic Extraction of Oil from Seeds*. A-06041a. Novo Industri, Bagsvaerd, Denmark.

Oomah, B. D. & Mazza, G. (1993). Flaxseed proteins—a review. *Food Chem.*, **48**, 109–114.

Oomah, B. D., Kenaschuk, E. O., Cui, W. & Mazza, G. (1995). Variation in the composition of water-soluble polysaccharides in flaxseed. *J. Agric. Food Chem.*, **43**, 1484–1488.

Peterson, S. W. (1958). Linseed oilmeal. In *Processed Plant Protein Foodstuffs*, ed. A. M. Altschul. Academic Press, New York, p. 539.

Pryde, E. H. (1983). Non-food use of vegetable oils. In *Handbook of Processing and Utilisation of Agricultural Products*, Vol 2, Part 2, ed. I.A. Wolff. CRC Press, Boca Raton, p. 109.

SAS (1990). *SAS User's Guide: Basic Statistics*. Statistical Analysis Systems Institute Inc., Cary, NC.

Smith, A. K., Johnson, V. L. & Beckel, A. C. (1946). Linseed proteins: alkali dispersion and acid precipitation. *Ind. Eng. Chem.*, **38**, 353–356.

Sosulski, F. & Bakal, A. (1969). Isolated proteins from rapeseed, flax and sunflower meals. *Can. Inst. Food Sci. Technol. J.*, **2**, 28–32.

Sosulski, F. W. & Zadernowski, R. (1981). Fractionation of rapeseed meal into flour and hull components. *J. Am. Oil Chem. Soc.*, **58**, 96–98.

Sosulski, K., Sosulski, F. W. & Coxworth, E. (1988). Carbohydrate hydrolysis of canola to enhance oil extraction with hexane. *J. Am. Oil Chem. Soc.*, **65**, 357–361.

Susheelamma, N. S. (1987). Isolation and properties of linseed mucilage. *J. Food Sci. Technol.*, **24**, 103–106.

Susheelamma, N. S. (1989). Functional role of linseed polysaccharide in steamed pudding (idli). *J. Food Sci. Technol.*, **26**, 16–20.

Wanasundara, J. P. D. & Shahidi, F. (1994). Functional properties and amino acid composition of solvent extracted flaxseed meals. *Food Chem.*, **49**, 45–51.

Whistler, R. L. (1973). Solubility of polysaccharides and their behaviour in solution. In *Carbohydrates in Solution*, ed. R. F. Gould. Advances in Chemistry Series 117. American Chemical Society, Washington, DC, pp. 242–255.