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RESEARCH NOTE

Effects of processing on the oligosaccharides of oilseed and legume protein meals

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The effects of (alkanol)–hexane, (alkanol/water)–hexane and (alkanol/ammonia/water)–hexane extraction systems on the removal of low-molecular-weight sugars from soybean, cottonseed, field pea and mung bean were studied. The total content of soluble sugars of hexane-extracted meals varied between 4·79 and 12·12%, depending on the type of seed and solvent extraction system used. The predominant sugar of cottonseed was raffinose. Sucrose and stachyose were present in smaller quantities. However, glanded cottonseed contained about four times more stachyose than glandless seeds. Seeds were extracted with methanol, ethanol and isopropanol, with or without 10% (w/w) ammonia, possibly containing 5% (v/v) water. The above extraction systems removed up to 81% of the total flatulence-causing sugars. Methanol was the most effective alkanol used, while isopropanol exhibited the poorest extraction efficiency. (Methanol/ammonia)-treated meals contained from 0·38 to 2·86% raffinose; however, all extraction systems were less effective in removing stachyose and verbascose. Results of this study imply that the removal of flatulence-causing sugars depends on their molecular weight, the seed's structural parameters, interaction within the seed components and polarity of the solvent systems used.

INTRODUCTION

Low-molecular-weight carbohydrates are known to be responsible for the flatulence activity of certain plant protein products. Stachyose and raffinose, galactosides of sucrose, are primary factors causing flatulence (Rackis *et al.*, 1970; Sacks & Olson, 1979; Fleming, 1981). These sugars cannot be absorbed by the human digestive system, as it lacks the α-galactosidase enzyme required for their hydrolysis. Therefore, these oligosaccharides reach the large intestine, where they are anaerobically fermented, thus producing an excessive amount of gas.

Due to the undesirable effects of sucrose-containing oligosaccharides, a number of studies has been carried out in order to remove them by enzymatic degradation (Sherba, 1970; Sugimoto & Van Buren, 1970; Calloway *et al.*, 1971). A significant reduction in the level of oligosaccharides of soybean has been achieved using a combination of soaking, germination and resoaking (Kim *et al.*, 1973). Becker *et al.* (1974) were able to lower the oligosaccharide content of small white beans by autolysis (pH 5·2 at 55°C).

Recently, a processing approach for extraction of

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rapeseed and mustard seed was reviewed (Shahidi *et al.*, 1988). This extraction system, consisting of methanol/ammonia/water and hexane, produced a meal with an enhanced protein content and largely removed phenolic acids and tannins (Naczk & Shahidi, 1989; Shahidi & Naczk, 1989).

The present study was designed to investigate the effect of solvent extraction by ammoniated methanol on soluble sugars of soybean, glanded and glandless cottonseeds, and legumes.

MATERIALS AND METHODS

Commercial soybean meals, glandless and glanded cottonseed, field peas and mung beans were used in this study. Hexane-extracted meals were prepared by grinding the seeds in a Phillips coffee grinder and then extracting them for 12 h using a Soxhlet apparatus. The defatted meal was dried at 40°C in a vacuum oven.

The alkanol/ammonia solutions were prepared by bubbling ammonia into absolute or 95% (v/v) alkanol at 0°C. The concentration of dissolved ammonia was determined by titration with 1·0 M sulphuric acid. The 10% (w/w) content of ammonia in alkanol or 95% (v/v) alkanol was made up by dilution with ammonia-free solvents.

Ground seed (60 g) was blended at approximately 15000 rpm in a Waring blender for 2 min with 400 ml of methanol, 95% methanol, 95% ethanol or 95% isopropanol, or 10% (w/w) ammonia in methanol, 95% methanol, 95% ethanol or 95% isopropanol. After a quiescent period of 15 min, 400 ml of hexane was added to the slurry and the mixture was again blended for 2 min. The meal was separated by vacuum filtration, rinsed three times with a total of 100 ml of alkanol and dried at 40°C in a vacuum oven. Residual oil was extracted with hexane using a Soxhlet apparatus, and the resultant meal was dried again as before.

The soluble sugars were extracted from 5 g of meal, twice, with 40 ml of 75% (v/v) ethanol at 80°C for 1 h with occasional shaking. After centrifugation, the supernatant was separated. The extracts were combined and clarified by adding, twice, 1 ml of 10% (w/v) lead-acetate solution with mild heating. Precipitates were centrifuged and discarded. The deproteinized extract was then concentrated by evaporation to a final volume of 25 ml. Ten millilitres of the concentrated extract were treated with 2 g of ion-exchange resin (Dowex MR-3) for 30 min and then passed through a PrepSep-C_{18} (Fisher Scientific, Fair Lawn, NJ, USA) and a 0·45-μm cellulose acetate membrane filter (Millipore Corp., Bedford, MA, USA). A 20-μl sample was injected on to a high-performance liquid chromatography (HPLC) system (Perkin-Elmer, Norwalk, CT, USA) equipped with a series 410 solvent delivery system, LC 25 differential refractometer, LC 600 sample injector and a Porasil column (10 μm, 30 cm × 5 mm) (Waters Associates, Milford, MA, USA). Prior to analysis, the silica column was modified by passing 1 litre of acetonitrile–water (65:35) containing 0·1% (w/v) amine modifier (3-aminopropyltriethoxysilane) with a flow rate of 2 ml/min (Aitzetmüller, 1978). The column was then stabilized with 1 litre of solvent, consisting of acetonitrile–water (65:35) in which 0·01% (w/w) amine modifier was dissolved. The latter solvent system was used for the elution of carbohydrates at a flow rate of 2 ml/min. A 1% (w/v) solution of glycerol was used as the internal standard. For standardization of sugar quantification, the following sugar concentrations were used: 0·5–1·5% fructose, 0·5–1·5% glucose, 0·5–6·0% sucrose, 0·5–3·0% raffinose, and 0·5–1·5% stachyose. The response factors were 0·42, 0·408, 0·22, 0·15 and 0·116, respectively. The response factor for verbascose was obtained by an extrapolation of the data for its homologues. Total soluble sugars, as raffinose equivalents, were determined colorimetrically by a modified anthrone reagent method (Finley & Fellers, 1973).

RESULTS AND DISCUSSION

The content of individual low-molecular-weight sugars, as determined by HPLC, is shown in Table 1. The total content of monosaccharides and disaccharides in hexane-extracted oilseed meals ranged from 0·77% for glandless cottonseed to 6·97% for 48% commercial soybean meal. This accounted for approximately 9–62% of the total soluble sugars. The α-galactosides of sucrose constituted 41–91% of total soluble sugars. Raffinose was the principle flatulence-causing sugar of cottonseed, while stachyose was the major cause of flatulence in soybean meals. On the other hand, hexane-extracted field-pea and mung-bean meals contained 5·74 and 4·83% total soluble sugars, respectively. Flatulence-causing sugars, predominantly verbascose, accounted for up to 77% of the total soluble sugars. These results are in reasonable agreement with those reported by Bianchi *et al.* (1984), Knudsen (1986) and Liu and Markakis (1987) for soybeans, Cegla and Bell (1976) and Kadan *et al.* (1979) for cottonseed and Sosulski *et al.* (1982) for soybean and other legumes, as well as a recent report by Knudsen and Li (1991) for soybean, cottonseed and field peas.

Methanol alone was quite effective in removing monosaccharides and disaccharides, as well as raffinose, from glandless cottonseed, while stachyose was extracted to a lesser (*ca.* 26%) extent (Table 1). These results are similar to those recently reported (Shahidi *et al.*, 1990) for Triton canola extracted with absolute methanol–hexane. However, methanol was a less effective solvent for extraction of low-molecular-weight carbohydrates from commercial soybean meal.

The addition of 5% (v/v) water to methanol in-

Table 1. Content of individual flatulence-causing sugars (%) in oilseeds and legumes as affected by processing

Seed	Solvent extraction system	Simple sugars[a]	Raffinose	Stachyose	Verbascose
Glandless cottonseed	Hexane	0·77	7·27	0·30	ND[b]
	(Methanol)–hexane	0·28	2·65	0·22	ND
	(Methanol/water)–hexane	0·31	2·15	0·08	ND
	(Ethanol/water)–hexane	0·52	4·50	0·29	ND
	(Isopropanol/water)–hexane	0·64	6·32	0·29	ND
	(Methanol/ammonia)–hexane	0·14	2·52	0·19	ND
	(Methanol/ammonia/water)–hexane	0·14	2·86	0·13	ND
	(Ethanol/ammonia/water)–hexane	0·44	4·15	0·44	ND
	(Isopropanol/ammonia/water)–hexane	0·59	6·01	0·38	ND
Glanded cottonseed	Hexane	1·15	5·73	1·21	ND
	(Methanol/water)–hexane	0·68	1·96	0·52	ND
	(Ethanol/water)–hexane	0·79	3·79	0·95	ND
	(Methanol/ammonia)–hexane	0·32	1·80	0·58	ND
	(Methanol/ammonia/water)–hexane	0·37	2·29	0·58	ND
Soybean meal (48%)	Hexane	6·67	0·64	3·96	ND
	(Methanol)–hexane	6·31	0·63	3·97	ND
	(Methanol/ammonia)–hexane	5·49	0·49	3·72	ND
	(Methanol/water)–hexane	5·76	0·59	3·61	ND
	(Methanol/ammonia/water)–hexane	4·66	0·47	3·20	ND
Soybean meal (44%)	Hexane	6·98	0·79	4·35	ND
	(Methanol/ammonia)–hexane	5·08	0·68	3·68	ND
	(Methanol/ammonia/water)–hexane	4·19	0·56	3·55	ND
Field pea	Hexane	1·31	0·80	1·28	2·35
	(Methanol/ammonia/water)–hexane	0·77	0·51	1·06	2·21
Mung bean	Hexane	1·17	0·40	1·24	2·02
	(Methanol/ammonia/water)–hexane	0·71	0·38	0·56	1·85

[a] Total content of mainly sucrose and small amounts of glucose and fructose.
[b] ND—not detected.

creased the extractability of raffinose up to 23 and 70% and stachyose up to 6 and 74% in soybean and cottonseed meals, respectively (Table 1). In one experiment, the effect of the addition of water to alkanols on the extractability of flatulence-causing sugars from cottonseed was studied. Results presented in Table 1 indicate that ethanol and isopropanol were less effective in removing raffinose and stachyose as compared with 95% (v/v) methanol. In addition to structural organization of seeds and varying molecular interaction within the seed components, polarity of solvent extraction systems is an important factor in removing soluble sugars from oilseeds.

The presence of ammonia in alkanol (ROH) and alkanol–water solvent systems greatly enhanced the extraction of low-molecular-weight sugars. Results in Table 1 indicated that the (methanol/ammonia)–hexane solvent extraction system removed 18–82% of monosaccharides and disaccharides, 5–69% of raffinose, 6–55% of stachyose and up to 8% of verbascose originally present in oilseed and leguminous seed meals. Thus, the removal of flatulence-causing sugars depended on their molecular weight: (raffinose > stachyose > verbascose). Addition of water to methanol/ammonia slightly increased the solubility of stachyose. Treated oilseed meals contained 0·14–5·49% monosaccharides and disaccharides, 0·47–2·86% raffinose and 0·19–3·72% stachyose. On the other hand, (methanol/ammonia/water)-treated mung beans and field peas contained 0·38–0·51% raffinose, 0·56–1·06% stachyose and 1·85–2·21% verbascose.

Table 2 summarizes the content of total flatulence-causing and soluble sugars of oilseed and legume meals. The total content of flatulence-causing sugars, as determined by HPLC, ranged from 3·66 to 7·57% for hexane-extracted meals, from 2·23 to 6·61% for (alkanol)–hexane extracted meals and from 2·71 to 6·60% for (ROH/ammonia/water)-extracted meals. Hexane-extracted glandless cottonseed meal contained about 40 and 52% more flatulence-causing sugars than commercial soybean meals and mung beans, respectively. The (methanol/ammonia/water)–hexane extraction system removed up to 81 and 24% of total flatulence-causing sugars originally present in oilseeds and legumes, respectively (Table 2). Comparison of these results with those reported by Shahidi *et al.* (1990) indicated that methanol/ammonia/water treatment was less effective in removal of flatulence-causing sugars from meals of canola than cottonseeds. However, the methanol/ammonia solvent system was least effective in the removal of flatulence-causing sugars from commercial soybean meals.

Table 2. Effect of processing on the total soluble and flatulence-causing sugars of cottonseed[a]

Seed	Solvent extraction system	Flatulence sugars (%)	Total sugars (%)	
			HPLC	Colorimetric[b]
Glandless cottonseed	Hexane	7·57 ± 0·15	8·34 ± 0·12	8·54 ± 0·13
	(Methanol)–hexane	2·87 ± 0·09	3·15 ± 0·10	3·74 ± 0·10
	(Methanol/water)–hexane	2·23 ± 0·05	2·54 ± 0·05	3·15 ± 0·17
	(Ethanol/water)–hexane	4·79 ± 0·10	5·31 ± 0·10	5·67 ± 0·13
	(Isopropanol/water)–hexane	6·61 ± 0·29	7·25 ± 0·29	8·14 ± 0·06
	(Methanol/ammonia)–hexane	2·71 ± 0·06	2·85 ± 0·08	3·43 ± 0·07
	(Methanol/ammonia/water)–hexane	2·99 ± 0·08	3·13 ± 0·15	3·21 ± 0·21
	(Ethanol/ammonia/water)–hexane	4·59 ± 0·13	5·03 ± 0·15	4·95 ± 0·21
	(Isopropanol/ammonia/water)–hexane	6·60 ± 0·30	6·98 ± 0·33	7·47 ± 0·16
Glanded cottonseed	Hexane	6·94 ± 0·22	8·09 ± 0·21	8·42 ± 0·10
	(Methanol/water)–hexane	2·48 ± 0·11	3·16 ± 0·10	3·83 ± 0·12
	(Ethanol/water)–hexane	4·74 ± 0·20	5·53 ± 0·17	6·91 ± 0·08
	(Methanol/ammonia)–hexane	2·38 ± 0·10	2·72 ± 0·11	2·78 ± 0·08
	(Methanol/ammonia/water)–hexane	2·87 ± 0·10	3·24 ± 0·29	2·83 ± 0·08
Soybean meal (48%)	Hexane	4·60 ± 0·10	11·27 ± 0·04	11·96 ± 0·18
	(Methanol)–hexane	4·60 ± 0·18	10·91 ± 0·20	11·26 ± 0·38
	(Methanol/ammonia)–hexane	4·21 ± 0·09	9·70 ± 0·05	10·33 ± 0·10
	(Methanol/water)–hexane	4·20 ± 0·12	9·96 ± 0·07	11·22 ± 0·36
	(Methanol/ammonia/water)–hexane	3·67 ± 0·11	8·33 ± 0·10	9·17 ± 0·30
Soybean meal (44%)	Hexane	5·14 ± 0·24	12·12 ± 0·35	13·07 ± 0·30
	(Methanol/ammonia)–hexane	4·36 ± 0·16	9·44 ± 0·15	10·12 ± 0·27
	(Methanol/ammonia/water)–hexane	4·11 ± 0·19	8·30 ± 0·21	8·61 ± 0·46
Field pea	Hexane	4·43 ± 0·12	5·74 ± 0·14	5·62 ± 0·20
	(Methanol/ammonia/water)–hexane	3·78 ± 0·15	4·55 ± 0·14	4·63 ± 0·20
Mung bean	Hexane	3·66 ± 0·10	4·83 ± 0·13	4·79 ± 0·15
	(Methanol/ammonia/water)–hexane	2·79 ± 0·12	3·50 ± 0·10	3·82 ± 0·12

[a] All values are given on percentage basis of oil-free and dried meal.
[b] As raffinose equivalents.

In conclusion, the results of this study indicate that the ROH/ammonia processing, originally developed for simultaneous extraction of oil and glucosinolates from canola (Shahidi *et al.*, 1988), greatly reduced the content of low-molecular-weight sugars of oilseeds and legumes. Thus, reducing flatulence activity is one factor that may improve the nutritional quality of the resultant meals.

The potential of the present processing approach warrants further studies with respect to the optimization of variables. The time and temperature of extraction, solvent-to-solid ratio, number of extraction stages and the ratio of different solvent components to one another are among parameters being considered.

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