Determination of Oligosaccharides in Protein-Rich Feedstuffs by Gas–Liquid Chromatography and High-Performance Liquid Chromatography

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Gas–Liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) methods were developed for quantitative analysis of oligosaccharides in protein-rich feedstuffs. A total of 18 meal or cake samples derived from soybean, cotton, sunflower, and rapeseed and two samples of field peas were studied. The most suitable extraction media was determined by using water or 50% and 80% (v/v) of alcohol (methanol or ethanol) on meal samples of soybean and cottonseed and a feed mixture (soybean meal/barley, 1:1 w/w). Excellent agreement was found between the HPLC and GLC results for sucrose and raffinose content, while the average value for stachyose was 0.4% (absolute units) lower with GLC compared to HPLC. Sucrose was the main sugar found in the protein-rich feedstuffs (3.00–7.59% of dry weight) but only a minor sugar in cottonseed (0.96–1.14%). Among the raffinose oligosaccharides, stachyose was predominant in soybean (3.94–4.62%) and rapeseed (0.94–1.52%), verbascose in field peas (1.69–2.90%), and raffinose in cottonseed (3.10–4.52%) and sunflower (1.53–2.48%). On the basis of the overall precision and the consistently higher value of stachyose obtained, HPLC should be the method of choice for the type of feed materials described here.

INTRODUCTION

Seeds of many legume, mallow, composite, and mustard species provide an important source of protein for man and animals. Although the byproducts from these plant seeds are used as feed ingredients in animal production, mainly for their high protein and amino acid contents, the carbohydrate fraction also provides an important source of energy. A major concern, however, is the high level of galactosylsucrose oligosaccharides also known as α-galactosides of sucrose: raffinose, stachyose, verbascose, and ajugose. The intestinal mucosa of man and monogastric animals (pig and poultry) (Gitzelmann and Auricchio, 1965: Rackis, 1975) lacks the enzyme α-galactosidase to cleave the α-linked galactose units present in these oligosaccharides. Consequently, raffinose oligosaccharides escape digestion in the upper digestive tract (Saunders and Wiggins, 1981). When present in high concentrations in the small intestine, these sugars increase the osmotic pressure of the luminal content which in turn may cause osmotic diarrhea (Saunders and Wiggins, 1981; Cummings et al., 1986). In the lower gut, the raffinose oligosaccharides are rapidly degraded by the action of bacterial α-galactosidase with subsequent production of carbon dioxide, hydrogen, and methane (Wagner et al., 1976; Rackis, 1975; Nowak and Steinkraues, 1988). Flatulence is one of the physiological effects induced by increased ingestion of legume seeds (Rackis et al., 1970; Rackis, 1975; Wagner et al., 1976; Calloway et al., 1971).

Chromatographic techniques most commonly employed for quantitative determination of oligosaccharides are gas–liquid chromatography (GLC) with packed columns for separation of trimethylsilyl (TMS) ethers or oximes (Delente and Ladenburg, 1972; Janauer and Englmaier, 1978; Åman, 1979; Sosulki et al., 1982; Molnár-Perl et al., 1984) and high-performance liquid chromatography (HPLC) with μ-amino-bonded silica columns (Cegla and Bell, 1977; Black and Bagley, 1978; Dunmire and Otto, 1979; Knudsen, 1986; Shukla, 1987), reverse-phase C_{18} columns (Kennedy et al., 1985), and fixed-ion resin columns (Saravitz et al., 1987; Kuo et al., 1988; Scott and Hatina, 1988). Both chromatographic techniques have advantages and disadvantages (Folkes, 1985). The most notable advantages of the GLC technique are high stability and resolution of the column and sensitivity of flame ionization detector (<0.1 μg). The need for drying and derivatization has frequently been mentioned as a major drawback (Folkes, 1985). In recent years, HPLC has become popular primarily due to its ease of sample preparation; there is no need either to dry the extract or to prepare derivatives. However, sample cleanup for many types of plant materials could be as laborious as sample preparation for GLC determination (Macrae, 1985). Although HPLC columns and detectors have developed rapidly in recent years, separation, elution time, and detector limitations (refractive index detectors, >10 μg) are among the major constraints for quantitation of sugars by HPLC.

Currently there are only few published data available on the composition of raffinose oligosaccharides in protein-rich feedstuffs used in animal production. The objective of the present investigation was therefore to develop a rapid, robust, and reproducible method for quantitative analysis of raffinose oligosaccharides in individual feeds and feed mixtures based on either GLC or HPLC. The parameters studied include (1) the most suitable procedure for extraction of raffinose oligosaccharides, (2) the precision of the chromatographic methods, and (3) a comparison of GLC and HPLC values. Since we have more experience with the GLC technique, the initial study for the most suitable extraction procedures was based entirely on the GLC method. For the GLC determination we used a fused silica cross-linked methyl silicone capillary column and for HPLC a fixed-ion resin column in the sodium form.

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MATERIALS AND METHODS

Samples and Chemicals. Twenty protein-rich feedstuffs commonly present on the Danish feedstuff market were used in the study. These consisted of soybean, cottonseed, sunflower, and rapeseed in meal or cake form and seeds of field peas. In the following text, the samples are referred to simply as soybean, field peas, cotton, sunflower, and rapeseed. The meals or cakes were byproducts from the commercial oil industry. The composition of these samples is also considered representative for feedstuffs found on the world market. Pure standards of D-glucose, maltose, raffinose, stachyose, and phenyl β-D-glucopyranoside were obtained from Sigma (St. Louis, MO), fructose and sucrose were from Fisher (Fair Lawn, NJ), and *myo*-inositol was from Supelco (Bellefonte, PA). Other reagents were of analytical grade.

Extraction. Duplicate samples (500–750 mg) were extracted with 10–15 mL of deionized water or aqueous alcohols: 50% or 80% (v/v) methanol or ethanol. For the HPLC determinations the deionized water contained internal standard (*myo*-inositol; 1 mg/mL). Sample and extracting solvent were mixed continuously at room temperature on a Speci-Mix (Thermolyne, Sybron) and periodically in a sonicator for 45 min. The extracted material was centrifuged at 2000 rpm for 15 min, and aliquots were removed from the supernatant for chromatographic analysis.

Derivatization for GLC. A 0.5-mL aliquot of the above extract was placed in a 1.5-mL vial and dried on a speed vac concentrator (Savant Instrument Inc., Hicksville, NY). Twenty microliters of dimethyl sulfoxide (DMSO) was added and heated for 10 min at 75 °C. After the solution was cooled to room temperature, a pyridine reagent (0.5 mL) containing hydroxylamine hydrochloride (25 mg/mL) and an internal standard (I.S.), phenyl β-D-glucopyranoside (1.0 mg/mL), was added. The solution was mixed vigorously, heated at 75 °C for 30 min, sonicated, heated for an additional 30 min at 75 °C, and then cooled. One milliliter of hexamethyldisilazane and 6 drops of trifluoroacetic acid were added and mixed, and the solution was heated at 75 °C for 20 min. After centrifugation, the trimethylsilyl (Me_3Si) derivatives of sugars were ready for injection.

The individual sugars were identified with their retention times compared to those of known sugars and were quantitated according to the equation

$$\text{sugars, \% of dry matter} = \frac{A_s R_s}{A_{Is} R_{Is}} \frac{W_{Is}}{W_s} \times 100$$

where A_s and A_{Is} are peak areas, W_s and W_{Is} are dry weights of sample and internal standard, respectively, and R_s and R_{Is} are response factors or amount/area for sugars and internal standard in a solution containing a known amount of each component. The actual calculations were done electronically by a microprocessor.

Sample Cleanup for HPLC. Water extracts, containing the internal standard, were submitted to the following procedure. An aliquot of 3–4 mL of supernatant was filtered through a Sep-Pak C_{18} cartridge prewetted with 2 mL of methanol and 5 mL of deionized water; the first 3 mL of eluate was collected and further filtered through a 0.45-μm PTFE filter. From this filtrate an aliquot of 0.5 mL was drawn, and soluble protein and polysaccharides were precipitated by adding an equal volume of absolute ethanol with vortexing. The mixture was allowed to stand for 30 min at −20 °C. After centrifugation at 3000 rpm for 20 min, the clear supernatant was transferred to another tube, taken to dryness at 50 °C under N_2, and finally redissolved in 0.015 M Na_2SO_4.

The individual sugars were identified by their retention times compared to those of standard sugars. The standard sugar solutions were formulated to simulate concentrations in the material under study and were subjected to the same cleanup procedure as used for the samples. The sugars were quantitated as shown above with the exception that height instead of area was used to obtain better repeatability of analyses.

Chromatographic Equipment. *Gas–Liquid Chromatography.* A Hewlett-Packard 5840A was equipped with flame ionization detector, automatic sampler, and capillary injection system. The column was a HP-1 (Hewlett-Packard, Rockville, MD) 10-m wide-bore fused silica cross-linked methyl silicone capillary column with 0.53-mm i.d. and 2.6-μm film thickness. Operation conditions were as follows: injection port 275 °C; detector 340 °C; and column 195–325 or 170–325 °C programmed at 10 or 5 °C/min. Head pressure was 3.5 psi with a 3:1 split ratio and the helium carrier flow rate 6 mL/min. Sample volume was 1 μL.

Gas Chromatography–Mass Spectrometry (GC–MS). A Hewlett-Packard 5890 gas chromatograph was interfaced with HP 5970B mass selective detector via a capillary direct inlet. A fused silica cross-linked methyl silicone capillary column (25-m length, 0.20-mm i.d., and 0.33-μm film thickness) was used for the analyses. Mass detector conditions were as follows: ion source temperature, 220 °C; ionization energy, 70 eV. The interface temperature was 280 °C. Data were acquired with HP 59970 MS ChemStation.

High-Performance Liquid Chromatography. A modular system consisting of a Model 114M solvent delivery pump, Model 156 refractive index detector, Model C-R1A recording integrator with built-in printer/plotter (Beckman Instruments Inc., Irvine, CA), Model 7126 injector valve (Rheodyne Inc., Catati, CA, and an Aminex HPX-87N (300 × 7.8 mm) resin-based column in the sodium form (Bio-Rad, Richmond, CA) was used. The mobile phase was 0.015 M Na_2SO_4 and the flow rate 0.5 mL/min. The column temperature was kept constant at 85 °C by means of a thermostated water bath (Heto, Birkerød, Denmark). Sample volume was 20 μL.

Statistical Analysis. The analytical data were analyzed with a two-way analyses of variance model as outlined by Snedecor and Cochran (1973)

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

where X_{ijk} is a dependent variable (i.e., sucrose, raffinose, etc.), μ an overall mean, α_i the efect of sample, β_j the effect of replication, and ϵ_{ijk} a random variable.

The relationship between the HPLC and GLC results was analyzed with a linear regression model (Snedecor and Cochran, 1973)

$$Y_i = \beta_0 + \beta_1 X_{1i} + \epsilon_i$$

where Y_i is the GLC result, β_0 the intercept, b_1 slope, X_{1i} the HPLC result, and ϵ_i a random variable.

RESULTS AND DISCUSSION

GLC and HPLC Methods for the Determination of Oligosaccharides. The GLC procedure is based essentially on the method of Li et al. (1985). However, because of the chemical nature of raffinose oligosaccharides, it was necessary, as an initial step, to conduct a thorough investigation of the effect of the injection port temperature on the stability of sugar derivatives. In this experiment injection port temperature was varied between 225 and 325 °C (Figure 1). The highest absolute area for I.S. (phenyl β-D-glucopyranoside) was obtained at 275 °C, while the ratio between stachyose and I.S. peaked at 300 °C. At the high temperatures, however, the total area of stachyose was fairly constant, while the area of I.S. decreased. The same was true with glucose and sucrose, indicating thermal degradation of mono- and disaccharide derivatives at temperatures beyond 275 °C. To further verify the GLC conditions, a linearity study with raffinose and stachyose and a spiking experiment with sucrose, raffinose, and stachyose were performed. The detector response was linear for oligosaccharides in the concentration range 0.2–3.0 mg/mL of extract, which corresponds to approximately 0.1–2.0 μg of injected oligosaccharide. Recoveries of the three sugars in spiked samples (20 mg sugar/sample) were 103%, 98%, and 101% (SE = 3.5), respectively.

The fused silica column separated the Me_3Si derivatives in the order of increasing degree of polymerization (Dp) (Figure 2). The column performed baseline separation for all sugars within 20 min. However, the monosaccha-

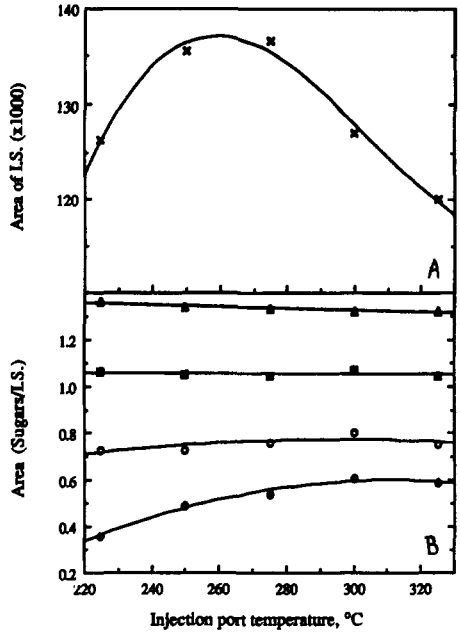

Figure 1. Effect of injection port temperature (°C) on area (integration units × 1000) of internal standard (I.S.) (A) and the ratio between sugars and I.S. (B). Symbols corresponding to the four components in (B) are ▲, glucose; ■, sucrose; ○, raffinose; and ●, stachyose.

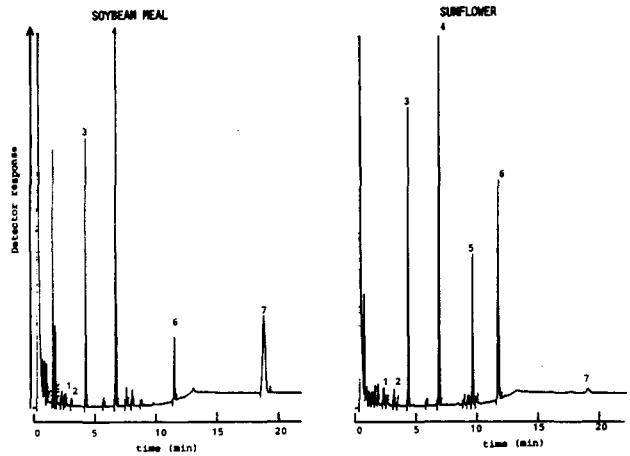

Figure 2. GLC of Me_3Si derivatives of mono- and oligosaccharides in soybean meal and sunflower. 1, Fructose; 2, glucose; 3, internal standard (phenyl β-D-glucopyranoside); 4, sucrose, 5, unknown; 6, raffinose; 7, stachyose.

rides could only be distinguished from each other when the column temperature was programmed at 5 °C/min. Another constraint is that verbascose could not be satisfactorily quantified.

HPLC chromatograms of the same feedstuffs as analyzed by GLC are shown in Figure 3. The sodium-loaded resin column separated individual sugars within 15 min in the order of descending Dp, giving almost baseline separation of raffinose oligosaccharides up to Dp 4. The detector response, based on peak heights, was linear for concentrations of oligosaccharides in the range 0.2–3.0 mg/mL of extract, which corresponds to an injected amount of 4–60 μg. This was the case regardless of the individual concentration of raffinose and stachyose in the analyte. Verbascose (peak B; field peas) could also be quantified; the precision, however, was lower than that for the other oligosaccharides. A recently published HPLC method (Saravitz et al., 1987; Kuo et al., 1988) using a Sugar-Pak I column (Ca^{2+}-loaded resin) promises to give a better separation between stachyose and verbascose. Subse-

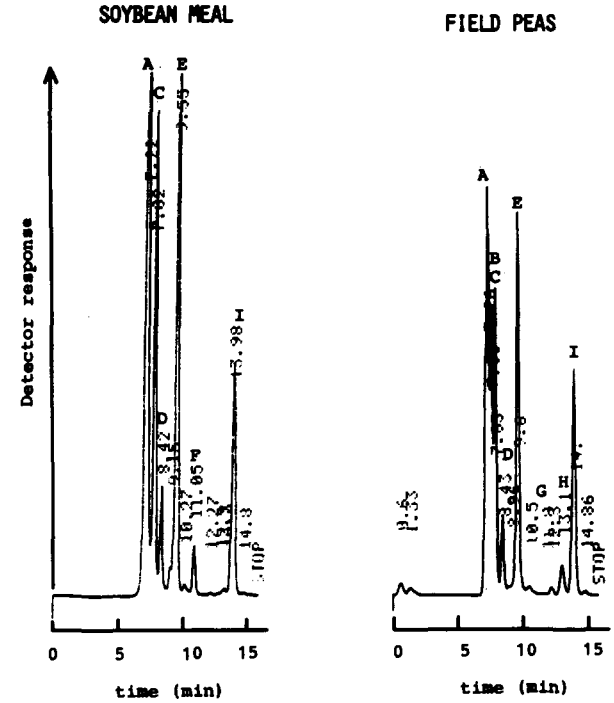

Figure 3. HPLC analysis of mono- and oligosaccharides in soybean meal and field peas. A, Unknown: B, verbascose; C, stachyose; D, raffinose; E, sucrose; F, unknown; G, glucose; H, fructose; I, internal standard (*myo*-inositol).

quently, we tested a calcium-loaded resin column. The calcium column was superior to the sodium column with regard to the separation between stachyose and verbascose but was inferior to the sodium column with regard to the separation between raffinose and stachyose. Compared to other published HPLC methods (Cegla and Bell, 1977; Black and Bagley, 1978; Dunmire and Otto, 1979; Kennedy et al., 1985; Knudsen, 1986; Shukla, 1987; Scott and Hatina, 1988) the sodium-loaded resin column performed a much better separation and is more durable. The life expectancy of the column was also very satisfactory; we made more than 1500 injections before there was any noticeable loss of resolution which prevented further analysis. Recoveries of sucrose, raffinose, and stachyose in spiked samples (20 mg sugar/sample) were 100%, 103%, and 103% (SE = 2.0), respectively.

The chromatographic pattern of oligosaccharides was characteristic for the different protein-rich feedstuffs. Sucrose (peak 4 in Figure 2 and peak E in Figure 3) and stachyose (peaks 7 and C) were predominant in soybean and sunflower; raffinose (peaks 6 and D) was predominant in cottonseed and sucrose (peaks 4 and E) in rapeseed. Significant amounts of verbascose were found in field peas (peak B). Peak 5 found in GLC tracing of sunflower extract was not a carbohydrate. Its mass spectra did not show a m/z fragment of 361, which is characteristic of most di- and trisaccharides we had encountered. Moreover, the component, unlike any sugar, was retained on a Sep-Pak C_{18} filter. Field peas, sunflower, and rapeseed also contain measurable quantities of monosaccharides (peaks 1, 2, G, and H). The peak that elutes as the front peak on HPLC (peak A) is without any doubt a combination of anions and polysaccharides; when the extract was desalted prior to analysis, the peak was greatly reduced.

Extraction Procedure. A variety of procedures have been employed for the extraction of mono- and oligosaccharides from leguminous seeds. These include extraction with water and various concentrations of alcohols at ambient or elevated temperature (Macrae and Zend-

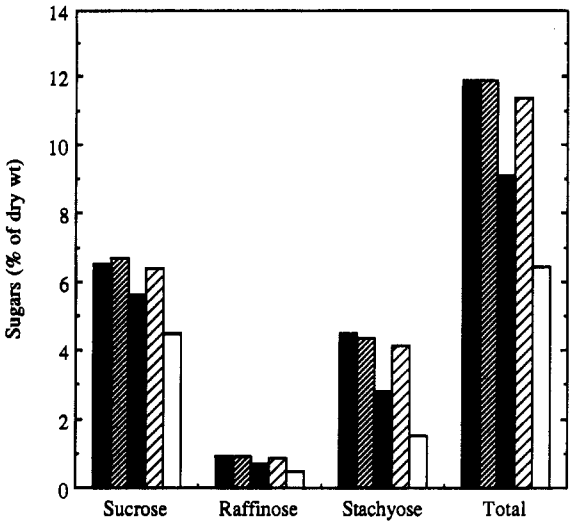

Figure 4. Comparison of five methods for extraction of oligosaccharides from soybean meal. Symbols correspond to, from left to right in each grouping, water, 50% methanol, 80% methanol, 50% ethanol, and 80% ethanol.

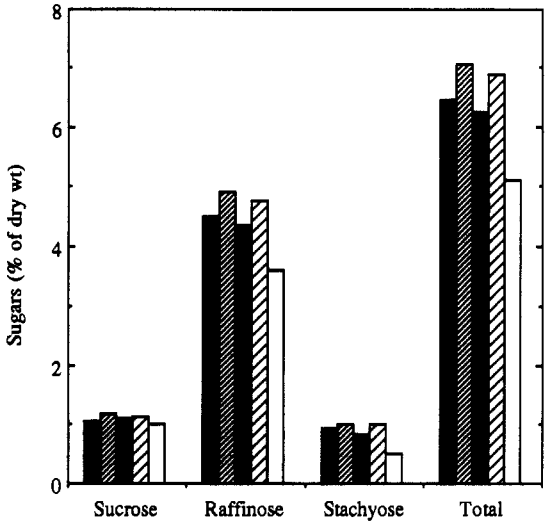

Figure 5. Comparison of five methods for extraction of oligosaccharides from cottonseed meal. Symbols correspond to, from left to right in each grouping, water, 50% methanol, 80% methanol, 50% ethanol, and 80% ethanol.

Table I. Mean Values (Percent of Dry Weight) for Oligosaccharides in Protein-Rich Feedstuffs and Repeatability ($N = 20$) when the GLC or HPLC Method Is Used

	GLC method			HPLC method		
	mean	SE	CV, %	mean	SE	CV, %
sucrose	4.40	0.064	1.5	4.37	0.025	0.6
raffinose	1.32	0.038	2.9	1.39	0.013	0.9
stachyose	1.75	0.088	5.0	2.13	0.027	1.3
total	7.74	0.157	2.0	7.97	0.049	0.6

Moghaddam, 1978; Kennedy et al., 1985; Allen et al., 1986; Knudsen, 1986; Saini and Gladstones, 1986; Shukla, 1987; Saini, 1988; Kuo et al., 1988). In our investigation we found the most efficient extraction was achieved with water at room temperature for 45 min with a ratio of solvent volume (milliliters) to sample weight (grams) of 20:1 (Figures 4 and 5). However, 50% methanol and 50% ethanol gave comparable results, while the more concentrated alcohols gave significantly lower values. This was particularly true for stachyose in the soybean, which only yielded 62% and 33% relative to water when extracted with 80% methanol and ethanol, respectively. This should be expected as, according to the Merck Index (10th ed.), 1 g of raffinose dissolves in 7 mL of water, 10 mL of methanol, and slightly in alcohol (i.e., ethanol). Stachyose is probably even less soluble in ethanol than in methanol. Surprisingly, enough sucrose was also lower when soybean was extracted with the higher strength of aqueous alcohol. This was in contrast to what was found with cotton seed and the study of Li et al. (1985), who found that 80% methanol gave quantitative extraction of sucrose in human diet composites. A spiking experiment with sucrose extracted with 80% methanol gave complete recovery (100%). This might indicate that some of the sucrose in soybean may not be present as free sugar and therefore less extractable using higher than 50% alcohols. Conkerton et al. (1983), working with soybean, have described a stachyose–sucrose complex that could be extracted with 70% ethanol but could not be detected as individual components until after several weeks.

One serious disadvantage of the use of water is that it tends to extract maximum amounts of other polar materials. Those of greatest concern are the enzymes invertase and α-galactosidase. However, the amount of monosaccharides (free glucose and fructose) remained constant for those samples we tested with all extraction procedures mentioned earlier. Furthermore, no free galactose was detected in any of the feed samples. Water extraction is definitely not suited for feed mixtures containing starch (Figure 6). For example, water extract of a mixture consisting of barley and soybean (1:1 w/w) contained higher amounts of free glucose, fructose, and even maltose along with lower levels of sucrose as compared to an aqueous alcohol extract. Since 80% methanol and ethanol gave incomplete extraction of raffinose and stachyose, a 50% strength seems to be most suited for starch-containing samples.

GLC and HPLC Values for Oligosaccharides. Mean values for the determination of the major oligosaccharides by the GLC and HPLC methods are shown in Table I. The regression equations and the standard error of slope (±) for the sugar determinations by HPLC (X) and GLC (Y) are as follows:

sucrose, $Y = 0.094 + 0.985X$; ±0.017 $R^2 = 0.995$

raffinose, $Y = -0.097 + 1.015X$; ±0.025 $R^2 = 0.989$

stachyose, $Y = -0.355 + 1.034X$; ±0.025 $R^2 = 0.990$

total, $Y = -0.262 + 1.006X$; ±0.026 $R^2 = 0.988$

For sucrose and raffinose there was excellent agreement between the two methods, while the value for stachyose obtained with the GLC method was on the average 0.4% (absolute units) lower than obtained with HPLC. This difference is almost within the analytical error of this type of analysis. For the GLC method, the coefficient of variation (CV) ranged from 1.5% to 5.0% and for the HPLC method from 0.6% to 1.3% for sucrose and stachyose, respectively. Compared to literature values the analytical precision is quite acceptable; for a GLC method it was reported as 1.3–3.9% (Sosulki et al., 1982) and for HPLC methods as 3.5–10.5% (Kennedy et al., 1985) and 1.5–24.0% (Kuo et al., 1988). However, the fact that the lower GLC values were obtained for the component of highest molecular weight prompted us to reinvestigate the effect of injection port temperature on sugar quantification. Selected samples together with standard sugar mixtures were subsequently analyzed by using injection

Table II. Content (Percent of Dry Weight) of Oligosaccharides in Protein-Rich Feedstuffs Determined by HPLC

seed	raffinose sugars: verbascose	stachyose	raffinose	total	sucrose	total sugars
			Leguminosae			
soybean meal						
sample 1		4.62	0.75	5.37	7.49	12.86
sample 2		4.67	0.75	5.42	7.59	13.01
sample 3		3.94	0.94	4.88	4.65	9.53
sample 4		4.47	0.84	5.31	6.29	11.60
sample 5		4.50	0.89	5.39	6.41	11.80
sample 6		4.03	0.75	4.78	7.01	11.79
field peas						
sample 1	1.69	4.79	0.39	3.87	2.50	6.37
sample 2	2.90	1.49	0.24	4.63	3.00	7.63
			Malvaceae			
cottonseed						
sample 1		0.83	3.41	4.24	1.14	5.38
sample 2		0.87	3.10	3.97	0.96	4.93
sample 3		1.23	3.49	4.72	1.02	5.74
sample 4		1.52	4.52	6.04	1.02	7.06
			Compositae			
sunflower						
sample 1		0.39	1.77	2.16	3.03	5.19
sample 2		0.33	2.48	2.81	3.77	6.58
sample 3		tr	1.53	1.53	3.06	4.59
			Cruciferae			
rapeseed						
sample 1		1.52	0.38	1.90	5.89	7.79
sample 2		1.14	0.29	1.43	5.84	7.27
sample 3		0.94	0.44	1.38	5.72	7.10
sample 4		0.94	0.43	1.37	5.55	6.92
sample 5		1.38	0.40	1.78	5.38	7.16

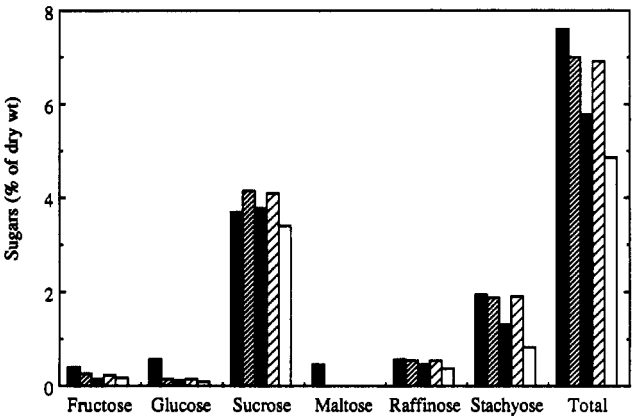

Figure 6. Comparison of five methods for extraction of mono- and oligosaccharides from a feed mixture of soybean meal/barley (1:1 w/w). Symbols correspond to, from left to right in each grouping, water, 50% methanol, 80% methanol, 50% ethanol, and 80% ethanol.

port temperatures of 300 and 325 °C. Even under these conditions, the GLC values were still lower than the HPLC values. An alternative explanation for the higher HPLC value for stachyose could be the overlapping between peaks A, B, and C (Figure 2). At an early stage during our attempt to optimize the HPLC conditions, a guard column was used. Unfortunately, the insertion of a guard column into the system had the disadvantage of making the separation between raffinose and stachyose less efficient than shown in Figure 2, and consequently we felt it was necessary to address the question of whether the overlapping between the two sugars would affect the overall quantification. A linearity study was conducted. In one case, concentration of stachyose was kept constant at 1 mg/mL and the concentration of raffinose varied from 0.2 to 3 mg/mL. In another case, raffinose concentration was kept constant while stachyose concentration was varied. Results indicated that in neither case did the RI response deviate from linearity even when the concentrations of the two sugars were at the farthest range. Thus, it is likely that other constituents present in natural feedstuff may cause interference or it is more difficult to redissolve and prepare derivatives of dried extract from natural feedstuffs as compared to standards. The lower value for stachyose and the fact that the analytical precision of the GLC method was lower (Table I) led us to conclude that HPLC should be the technique of choice for this type of feed material.

The oligosaccharide composition (percent of dry weight) as determined by HPLC of the 20 protein-rich feedstuffs is shown in Table II. Sucrose is the main sugar in all of these protein-rich feedstuffs (3.00–7.59%) except cottonseed (0.96–1.14%). Among the raffinose oligosaccharides, stachyose is predominant in soybean (3.94–4.62%) and rapeseed (0.94–1.52%), verbascose in field peas (1.69–2.90%), and raffinose in cotton (3.10–4.52%) and sunflower (1.53–2.48%). The values found in this study for soybean are comparable to values found by other groups using GLC (Sosulki et al., 1982; Molnár-Perl et al., 1984) and HPLC (Kennedy et al., 1985; Kuo et al., 1988). Reported values for field peas are 1.85% sucrose, 0.60% raffinose, 1.71% stachyose, and 2.30% verbascose (Sosulki et al., 1982).

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LITERATURE CITED

Allen, D. G.; Greirson, B. N.; Wilson, N. L. Chemical analysis of antinutritional factors in sweet lupin seeds. *Proceedings of the 4th International Lupin Conference*, Geraldton, W. Australia; Department of Agriculture: South Perth, Western Australia, 1986; p 291.

Åman, P. Carbohydrates in raw and germinated seeds from mung bean and chick pea. *J. Sci. Food Agric.* **1979**, *30*, 869–875.

Black, L. T.; Bagley, E. B. Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. *J. Am. Oil Chem. Soc.* **1978**, *55*, 228–232.

Calloway, D. H.; Hickey, C. A.; Murphy, E. L. Reduction of intestinal gas-forming properties of legumes by traditional and experimental food processing methods. *J. Food Sci.* **1971**, *36*, 251–255.

Cegla, G. F.; Bell, K. R. High pressure liquid chromatography for the analysis of soluble carbohydrates in defatted oilseed flours. *J. Am. Oil Chem. Soc.* **1977**, *54*, 150–152.

Conkerton, E. J.; Parrish, F. W.; Chapital, D. C.; Ory, R. L. Isolation of a stachyose-sucrose complex from soybeans and peanuts. *J. Food Sci.* **1983**, *48*, 1269–1271.

Cummings, J. H.; Englyst, H. N.; Wiggins, H. S. The role of carbohydrates in lower gut function. In *The nutritional re-emergence of starchy foods*; *Suppl. Naeringsforsk.* **1986**, 30 (23), 17–21.

Delente, J.; Ladenburg, K. Quantitative determination of the oligosaccharides in defatted soybean meal by gas-liquid chromatography. *J. Food Sci.* **1972**, *37*, 372–374.

Dunmire, D. L.; Otto, S. E. High pressure liquid chromatographic determination of sugars in various food products. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 176–185.

Folkes, D. J. Gas-liquid chromatography. In *Analysis of Food Carbohydrate*; Birch, G. G.; Ed.; Elsevier: London, 1985; pp 91–123.

Gitzelmann, P.; Auricchio, S. The handling of soya alpha-galactosides by a normal and a galactosemic child. *Pediatrics* **1965**, *36*, 231–232.

Janauer, G. A.; Englmaier, P. Multi-step time program for the rapid gas-liquid chromatography of carbohydrates. *J. Chromatogr.* **1978**, *153*, 539–542.

Kennedy, I. R.; Mwandemele, O. D.; McWhirter, K. S. Estimation of sucrose, raffinose and stachyose in soybean seeds. *Food Chem.* **1985**, *17*, 85–93.

Knudsen, I. M. High-performance liquid chromatographic determination of oligosaccharides in leguminous seeds. *J. Sci. Food Agric.* **1986**, *37*, 560–566.

Kuo, T. M.; VanMiddleworth, J. F.; Wolf, W. J. Content of oligosaccharides and sucrose in various plant seeds. *J. Agric. Food Chem.* **1988**, *36*, 32–36.

Li, B. W.; Schuhmann, P. J.; Wolf, W. R. Chromatographic determination of sugars and starch in a diet composite reference material. *J. Agric. Food Chem.* **1985**, *33*, 531–536.

Macrae, R. High performance liquid chromatography. In *Analysis of Food Carbohydrate*; Birch, G. G., Ed.; Elsevier: London, 1985; pp 61–89.

Macrae, R.; Zand-Moghaddam, A. The determination of the component oligosaccharides of lupinseeds by high pressure liquid chromatography. *J. Sci. Food. Agric.* **1978**, *29*, 1083–1086.

Molnár-Perl, I.; Pintér-Szakács, M.; Kövagó, A.; Petróczy, J. Gas-liquid chromatographic determination of the raffinose family of oligosaccharides and their metabolites present in soy beans. *J. Chromatogr.* **1984**, *295*, 433–443.

Nowak, J.; Steinkraues, K. H. Effect of tempeh fermentation of peas on their potential flatulence productivity as measured by gas production and growth of Clostridum perfringens. *Nutr. Rep. Int.* **1988**, *38*, 1163–1171.

Rackis, J. J. Oligosaccharides of food legumes: alpha-galactosidase activity and flatus problem. In *Physiological effects of food carbohydrates*; Allen, J., Heilge, J., Eds.; American Chemical Society: Washington, 1975; pp 207–222.

Rackis, J. J.; Sessa, D. J.; Steggerda, F. R.; Shimizu, T.; Anderson, J.; Pearl, S. L. Soybean factors relating to gas production by intestinal bacteria. *J. Food Sci.* **1970**, *35*, 634–639.

Saini, H. S. Extractability and evaluation of α-galactosides of sucrose in leguminous seeds. *Food Chem.* **1988**, *28*, 149–157.

Saini, H. S.; Gladstones, J. S. Variability in the total and component galactosyl-sucrose oligosaccharides of lupinus species. *Aust. J. Agric. Res.* **1986**, *37*, 157–166.

Saravitz, D. M.; Pharr, D. M.; Carter, T. e., Jr. Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes. *Plant Physiol.* **1987**, *83*, 185–189.

Saunders, D. R.; Wiggins, H. S. Conservation of mannitol, lactulose, and raffinose by the human colon. *Am. J. Physiol.* **1981**, *241*, G397–G402.

Scott, F. W.; Hatina, G. HPLC determination of sugars, starch and oligosaccharides in infant formulas using resin-based, fixed-ion columns. *J. Food Sci.* **1988**, *53*, 264–269.

Shukla, K. S. Quantitative determination of oligosaccharides in defatted soybean products by high speed liquid chromatography. *Fat Sci. Technol.* **1987**, *89*, 75–79.

Snedecor, G. W.; Cochran, W. G. *Statistical Methods*; Iowa State University Press: Ames, 1973.

Sosulki, F. W.; Elkowicz, L.; Reichert, R. D. Oligosaccharides in eleven legumes and their air-classified protein and starch fractions. *J. Food Sci.* **1982**, *47*, 498–502.

Wagner, J. R.; Becker, R.; Gumbmann, M. R.; Olsen, A. C. Hydrogen production in the rat following ingestion of raffinose, stachyose and oligosaccharide-free bean residue. *J. Nutr.* **1976**, *106*, 466–470.

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Registry No. Sucrose, 57-50-1; raffinose, 512-69-6; stachyose, 470-55-3; verbascose, 546-62-3.