Food Chemistry **17** (1985) 85–93

Estimation of Sucrose, Raffinose and Stachyose in Soybean Seeds

I. R. Kennedy*, O. D. Mwandemele†‡
& K. S. McWhirter†

* Department of Agricultural Chemistry, † Department of Agricultural Genetics, University of Sydney, New South Wales, 2006, Australia

(Received: 2 January, 1984)

ABSTRACT

Oligosaccharides have been analysed in extracts of soybean seed meal by HPLC using the Waters Dextropak C_{18} reversed-phase column. A procedure employing aqueous extraction of meal, and immediate analysis of extracts without further treatment except for ultrafiltration with a 25 000 molecular weight cut-off filter has been found much more convenient than gas–liquid chromatography of derivatized sugars. This procedure is recommended as the method of choice for such analyses with soybean. Hydrolysis of oligosaccharides in some extracts was observed, a process that may have significance for the use of soybeans as human food.

INTRODUCTION

Soybean seeds are normally valued for their high oil and protein contents. However, they also contain significant amounts of sucrose and the galactosyl oligosaccharides, raffinose and stachyose. These three oligosaccharides are of potential importance in the acceptance of soybeans as food, and are also of industrial significance since their total concentration can be greater than 10% of the seed dry weight.

Such oligosaccharides have been analysed previously in defatted

‡ Present address: Department of Botany, University of Dar es Salaam, PO Box 35060, Dar es Salaam, Tanzania.

Food Chemistry 0308-8146/85/$03·30

soybean seed meal by extraction in 80% ethanol followed by gas–liquid chromatography of silylated derivatives using a temperature programme from 150–330 °C (Hymowitz *et al.*, 1972). However, preparing the derivatives is time-consuming and the gas–liquid chromatography, demanding. Over the past three growing seasons, we have employed high performance liquid chromatography (HPLC), using the Waters Dextropak column, for the assay of the large numbers of samples required for the assessment of possible genetic variation in oligosaccharide content.

The use of HPLC using acetonitrile–water elution solvent for the determination of soybean oligosaccharides was achieved by Black & Bagley (1978). An HPLC procedure for oligosaccharides of lupin seeds using Spherisorb amino columns has also been described (Macrae & Zand-Moghaddam, 1978). The method described here, employing a Waters Dextropak column, is more convenient than either of these procedures. A determination of reducing sugars and sucrose in food by HPLC has recently been described (Wight & van Niekerk, 1983). Their method is complementary to the procedure described here for sucrose and the higher oligosaccharides.

EXPERIMENTAL

The characteristics of the plastic Dextropak columns for the separation of carbohydrate oligomers have been described by Cheetham *et al.* (1981). The Waters Associates (Milford, MA, USA) instruments used in this work included a M6000 pump, a U6K injector, a radial compression module RCM-100 and a R401 refractive index detector. The Dextropak column was stored in methanol to prevent bacterial growth and flushed with methanol:water (50:50) and then used for chromatography with distilled water as solvent (1·0 ml/min). All solvents were filtered (0·45 μm Millipore) and degassed under vacuum before use.

Soybean (*Glycine max* (L.) Merr.) meal was prepared from whole seed by grinding with a Janke and Kunkel water-cooled mill (Type A 10S S2) to pass a 500 micron sieve. Ethanol elutes from the Dextropak column in the same region as raffinose and it was therefore inconvenient to extract soybean meal with the 80% ethanol normally used to inactivate enzymes. We have routinely made extracts of dry meal, previously defatted with a Soxhlet extractor (8–10 h), by shaking 1 g of meal with

25 ml of distilled water for 1 h at 30 °C. Sodium azide (0·2 ml of 1 % (w/v)) was added to inhibit bacterial growth. For some experiments extracts were made using distilled water at 100 °C for 30 min. The suspension was then centrifuged for 5 min at 2000 × *g*, the supernatant decanted and a sample immediately centrifuged at 1000 × *g* through Amicon (Massachusetts, USA) Centriflo Ultrafilters (CF-25). These filters have a membrane retaining macromolecules of 25 000 or greater molecular weight, effectively removing most protein molecules from the filtrate. The clean filtrate was analysed by HPLC at about 1000 psi for oligosaccharide content, without further treatment, using peak heights for estimations. Fifty-microlitre samples contained about 100 μg of oligosaccharides, although the sensitivity of detection with the equipment would allow the measurement of less than one-tenth this amount. Standards in the range 10–100 μg of sucrose (AR), raffinose and stachyose were employed, taking into account the water content of the latter sugars. Filtered samples could be stored, if necessary, at −15 °C, with freezing immediately after preparation.

Colorimetric tests using phenol–sulphuric acid reagent showed that extraction with water was as effective as 80 % ethanol in extracting sugars from soybean meal, and that there was no significant loss of carbohydrate added as sucrose during the period of extraction (97–98 % recovery with water, 96–98 % recovery with 80 % ethanol). The procedure with extraction using water was also attractive in providing conditions similar to those where soybeans are employed for human food.

RESULTS AND DISCUSSION

Chromatographic separation

Figure 1 shows a separation of standard oligosaccharides on a Dextropak column. Single peaks were obtained for each compound. Surprisingly, the peak for stachyose eluted before that for raffinose, despite the higher molecular weight of the former. The Dextropak column normally resolves carbohydrate oligomers according to molecular weight and does not separate the constituent hexoses, glucose, fructose and galactose. With soybean meal samples, the identity of the material eluting before hexoses, and with them, has not been determined, but presumably includes salts and metabolites present in seed. As can be seen from the chromatogram, only trace amounts of other carbohydrates were

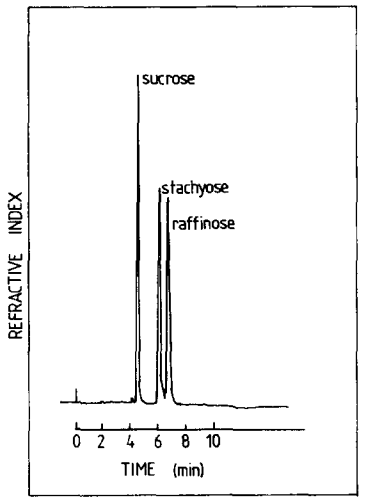

Fig. 1. Separation on a Dextropak column of sucrose and galactosyl oligosaccharides, raffinose and stachyose. Flow rate, 1·0 ml/min; eluent, water; room temperature, 25 °C.

extracted from the soybean meal by the procedure used. The life expectancy of Dextropak columns with the soybean samples was very acceptable; we routinely made several hundred injections before pressure build up above 2000 psi prevented further use of the column.

The chromatographic procedure used has the advantage that oligosaccharides and their immediate breakdown products, hexoses, are quantitatively displayed on the same chromatogram. Unlike gas–liquid chromatography, the HPLC on Dextropak columns does not require derivatization, and slight hydrolysis would be more readily observed.

Hydrolysis of oligosaccharides by endogenous enzymes

For most of the soybean lines examined, minor peaks corresponding to hexoses were observed on the chromatograms, provided extracts were freed of protein immediately following extraction by using Centriflo filters. However, in the case of particular lines, significant hydrolysis of oligosaccharides (more rapid for sucrose but occurring also for raffinose and stachyose) was indicated on the chromatograms. This apparent hydrolysis was first noted when extracts were stored at 4 °C for a period of hours before removal of solution protein with Centriflo filters (see Fig. 2). The pH of extracts was about 7 and hydrolysis did not occur when extracts were made in water at 100 °C for 30 min (Fig. 3), ruling out a

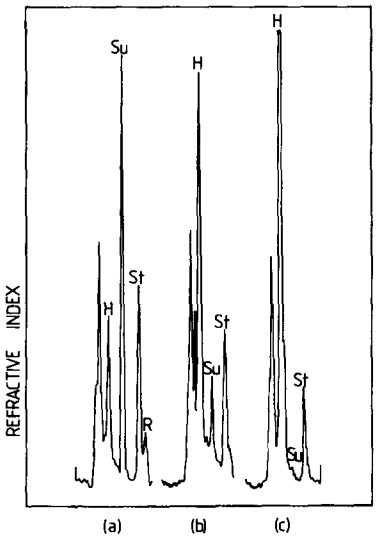

Fig. 2. Chromatograms of hexose and oligosaccharide content of soy meal extract (line 389H.88) after (a) extraction and filtration, 75 min; (b) 24 hours' storage at 4 °C prior to filtration; (c) 48 hours' storage at 4 °C. H = hexose, Su = sucrose, St = stachyose, R = raffinose.

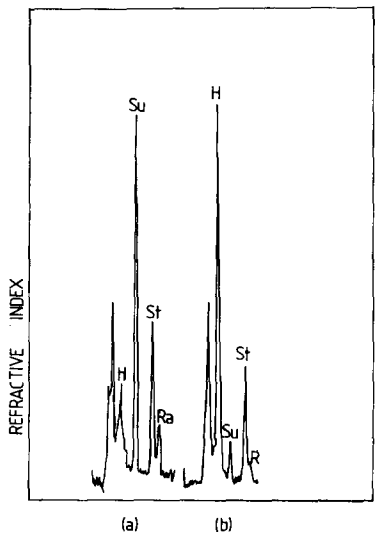

Fig. 3. Chromatograms of hexose and oligosaccharide content of soy meal extract (line 389H.38) after extraction and filtration for 75 min (a) at 100 °C; (b) at 30 °C.

chemical hydrolysis. The rate of breakdown was approximately linear with time and was not affected by the inclusion of sodium azide in the extraction medium. We therefore concluded that the breakdown was not a result of bacterial growth in the extracts, but reflected an endogenous enzyme content of the meal.

Germinating soybean seeds have been shown to utilize oligosaccharides rapidly, apparently employing α-galactosidase to remove galactose residues from stachyose and raffinose (Pazur *et al.*, 1962; East *et al.*, 1972). Pridham *et al.* (1969) showed that α-galactosidase was present in broad bean (*Vicia faba*) seeds, both dormant and germinating, together with other enzymes required to utilize galactose and sucrose (sucrose: UDP glucosyltransferase) in germination. α-galactosidase activity in mature soybean seeds has also been reported (Harpaz *et al.*, 1977; Campillo & Shannon, 1982). We have demonstrated the occurrence of α-galactosidase in several soybean lines by hydrolysis of *p*-nitrophenyl α-D-galactopyranoside at pH 4·5 and 7·0 to assay the activity (Campillo & Shannon, 1982). However, there was no obvious correlation between α-galactosidase activity in a particular line and the occurrence of significant hydrolysis of raffinose and stachyose, since all lines contained similar activities. Possibly, hydrolysis involves a conversion of α-galactosidase to a more active form, as noted by Dey & Pridham (1969) with *Vicia faba* enzyme. The breakdown of sucrose is probably a result of sucrase (invertase) activity. A rapid and complete removal of sucrose would stimulate raffinose and stachyose hydrolysis thermodynamically, so that a high sucrase activity could also stimulate rapid hydrolysis of the galactosyl sugars. It should be noted that the Dextropak column would not resolve mixed reaction products such as melibiose (GAL–GLU) from sucrose.

Reproducibility of estimations

The reproducibility for analyses on standards was high, with about a 1 % coefficient of variation between repeated injections of the same solution. Any significant variation in this reproducibility was found to result from instrument malfunction as when filters or seals in the injector required attention at infrequent intervals.

Some representative analyses on replicated aqueous extractions of the same extracted meals from four soybean cultivars are shown in Table 1. The coefficient of variation (standard deviation as a percentage of the

TABLE 1
Oligosaccharide Content of Soybeans

Cultivar	*Sucrose*	*Stachyose*	*Raffinose*
	(Per cent meal dry weight)		
Williams[a]	5·64 ± 0·23	4·14 ± 0·20	0·94 ± 0·02
Forrest[a]	5·95 ± 0·21	3·93 ± 0·30	0·87 ± 0·16
Big Jule[a]	7·67 ± 0·28	3·86 ± 0·10	0·89 ± 0·08
Beeson[a]	4·00 ± 0·12	2·96 ± 0·13	0·67 ± 0·08
Big Jule[b]	7·73 ± 0·24	4·05 ± 0·26	0·88 ± 0·08

[a] Analyses on replicated extractions from the same defatted meal. Mean values of at least three analyses and standard deviations are shown.
[b] Analyses on replicated samples of meal before Soxhlet extraction.

mean) for replicate analyses was 3·5%, 5·3% and 10·5% for sucrose, stachyose and raffinose, respectively. Similar precision of analysis was obtained for replicated samples of meal, Soxhlet—extracted separately.

Genetic variation of oligosaccharide content

A statistical analysis of the results for three crosses (Big Jule/Bethel; Sen Nari/Dare; Harosoy 63/Hardoo 137) showed that there was genetic variation among lines for total oligosaccharide content, sucrose content, stachyose content and raffinose content (Mwandemele, 1982). Thus, the feasibility for selection of particular lines combining different proportions of sucrose, raffinose and stachyose was indicated.

CONCLUSIONS

The main advantages of this analytical procedure by HPLC are its speed and the simplicity of the sample preparation, thus reducing the possibility of systematic error. The use of water as the chromatograph solvent has advantages of cost and convenience in that the extraction sample can be injected directly, once protein has been removed by ultrafiltration.

The endogenous enzyme activities observed in some samples could possibly be beneficial in reducing the raffinose and stachyose contents,

where soybeans are used for food. Understanding the control of these hydrolyses could lead to a process overcoming the soybean flatus problem in man and monogastric animals, held to be a result of a lack of α-galactosidase in intestinal mucosa, leading to the fermentation of galactosyl oligosaccharides in the lower gut (Rackis, 1975).

ACKNOWLEDGEMENTS

We are grateful to Mr W. Roy Day of Waters Associates for advice, the Rural Credits Development Fund of the Reserve Bank for research grants and Professor J. B. Pridham for suggestions on the manuscript.

REFERENCES

Black, L. T. & Bagley, E. B. (1978). Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. *J. Amer. Oil Chem. Soc.* **55**, 228–32.

Campillo, E. D. & Shannon, L. M. (1982). An α-galactosidase with hemagglutinin properties from soybean seeds. *Plant Physiol.* **69**, 628–31.

Cheetham, N. W. H., Sirimanne, P. & Day, W. R. (1981). High-performance liquid chromatographic separation of carbohydrate oligomers. *J. Chromatog.* **207**, 439–44.

Dey, P. M. & Pridham, J. B. (1969). Substrate specificity and kinetic properties of α-galactosidases from *Vicia faba*. *Biochem. J.* **115**, 47–54.

East, J. W., Nakayama, T. O. M. & Parkman, S. B. (1972). Changes in stachyose, raffinose, sucrose and monosaccharides during germination of soybeans. *Crop Sci.* **12**, 7–9.

Harpaz, N., Flowers, H. & Sharon, N. (1977). α-galactosidase from soybean destroying blood group B antigens. *Eur. J. Biochem.* **77**, 419–26.

Hymowitz, T., Collins, F. I., Panczner, J. & Walker, W. M. (1972). Relationship between the content of oil, protein and sugar in soybean seed, *Agronomy J.* **64**, 613–16.

Macrae, R. & Zand-Moghaddam, A. (1978). The determination of the component oligosaccharides of lupin seeds by high pressure liquid chromatograph. *J. Sci. Fd. Agric.* **29**, 1083–6.

Mwandemele, O. D. (1982). *Thesis*, University of Sydney.

Pazur, J. H., Shadaksharaswamy, M. & Meidell, G. E. (1962). The metabolism of oligosaccharides in germinating soybeans *Glycine max*. *Arch. Biochem. Biophys.* **99**, 78–85.

Pridham, J. B., Walter, M. W. & Worth, H. G. J. (1969). The metabolism of

raffinose and sucrose in germinating broad bean (*Vicia faba*) seeds. *J. exp. Bot.* **20**, 317–24.

Rackis, J. J. (1975). Oligosaccharides of food legumes: Alpha galactosidase activity and the flatus problem. In: *Physiological effects of food carbohydrates* (Jeans, A. & Hodge, J. (Eds)), American Chemical Society, 207–22.

Wight, A. W. & van Niekerk, P. J. (1983). A sensitive and selective method for the determination of reducing sugars and sucrose in food and plant material by high performance liquid chromatography. *Food Chem.* **10**, 211–24.