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Oligosaccharide distribution in Brazilian soya bean cultivars

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The composition of digestible sugars (ds) and non-digestible sugars (nds) was determined in 20 soya bean cultivars grown in Brazil. In addition, a comparison of three different extraction procedures for sugar analysis by HPLC is presented. Total α-galactosides in the samples were in the range 3·9–5·3 g% while fructose + sucrose varied from 4·0 to 6·1 g% dry basis. Based on the analysis of protein and oil data together with total galactosides, stachyose and ds/nds ratios, one variety (IAS-4) appears to be more appropriate for selection.

INTRODUCTION

The commercial and nutritional importance of soya beans for human and animal feeding is well known. However, together with high levels of protein and oil, some undesirable components are also found in the seeds. Anti-nutritional factors such as trypsin inhibitors and phytic acid and indigestible oligosaccharides are present in legume seeds including soya beans (Mohamed *et al.*, 1991). Consequently, it is important to determine the composition of different cultivars in order to select those with high levels of protein and oil and low contents of undesirable factors.

The soya bean crop in Brazil is now well established and represents an important item for the food industry and for export. Much work has been carried out for chemical characterisation of Brazilian soya bean cultivars; however, specific, data on α-galactoside composition are scarce. In the present work we report the oligosaccharide distribution in 20 soya bean cultivars largely used in Brazil. In addition, a comparison between three different extraction methods for oligosaccharide analysis by HPLC is presented.

MATERIALS AND METHODS

Samples

Soya bean cultivars were supplied by EMBRAPA (Brazil) and were harvested in different Brazilian regions. The sample used for method comparison was obtained from the local market. All samples were milled to pass a 0·75 mm sieve and defatted using a Soxhlet extractor with light petroleum prior to sugar analysis.

Extraction

Defatted samples (2·0 g) were extracted with shaking and 15 ml of methanol:water (4:6, v/v) in a water bath at 80°C for 30 min. The suspension was then centrifuged at 3000 *g* for 10 min and the supernatant transferred to a 50 ml volumetric flask. This extraction was repeated twice plus one more time using pure distilled water. All supernatants were transferred to the same flask and the volume made up with distilled water. The extract was filtered, activated charcoal was added (10 mg/ml) and the mixture vigorously shaken and centrifuged for 1 min in an Eppendorff centrifuge. The supernatant was then diluted with pure acetonitrile in the proportion 1:1 (v/v), and the mixture centrifuged if necessary. This solution was then used directly for chromatography. This procedure was compared with the methods described by Macrae and Zand-Moghaddam (1978) and Quemener (1988).

Chromatography

Chromatography was carried out using an HPLC pump (Isco-USA) with a Rheodyne injection valve (20 μl loop) and a refractive index detector (Waters 410,

Table 1. Comparison of methods of extraction for the analysis of free sugars by HPLC[a]

	Method								
	1			2			3		
	Sucrose	Raffinose	Stachyose	Sucrose	Raffinose	Stachyose	Sucrose	Raffinose	Stachyose
Average	5·9	0·7	4·2	5·8	0·5	3·9	6·0	0·7	4·1
SD	0·30	0·04	0·08	0·15	0·02	0·07	0·02	0·02	0·09
CV(%)	5·1	5·9	1·9	2·7	6·3	1·9	3·8	3·4	2·2

[a]Results are averages of six replicates on g% dry basis.

USA). The column was a Lichrospher-5-NH2 (250 × 4 mm i.d. — Merck, Germany) and the mobile phase was acetonitrile:water (72:28, v/v) at 1·0 ml/min. Quantification was achieved by peak height comparison with standards of fructose, sucrose, raffinose (Merck) and stachyose (Sigma, USA). Precision was assessed by determination of the coefficients of variation using six replicate extractions of the same sample. Recoveries were checked by the method of standard addition to the samples.

Protein and oil

Protein and oil were determined by standard Kjeldahl and Soxhlet procedures, respectively (Pearson, 1976).

RESULTS AND DISCUSSION

Extractions using one sample were carried out by three different methods and the results were compared (Table 1). Method 1 is based on the extraction under reflux with methanol:water followed by clearing with Carrez solutions (Macrae & Zand-Moghaddam, 1978). Although this is a very time-consuming method it is widely used for oligosaccharide analysis in seeds. Method 2 is a more recently reported procedure which uses only water for extraction also followed by Carrez clearing (Quemener, 1988). Method 3 is an alternative procedure proposed in the present work which is based on a more rapid and simple methanol:water extraction followed by clearing with activated charcoal and final dilution of the extract with acetonitrile. Method 2 is more rapid but in the present study it showed lower raffinose extraction and gave significantly different results from Method 1 ($P < 0·01$) for raffinose and stachyose. Methods 3 and 1 showed no significant differences ($P < 0·01$) and the use of methanol for extraction in both methods produced a very clear extract. In addition, the use of acetonitrile to make the final extract gives a more similar solution to the chromatographic mobile phase avoiding any artifact formation during chromatography (Tsimidou & Macrae, 1984). Besides, it provides a further control for possible formation of insoluble material in the acetonitrile:water solution which otherwise would precipitate into the column. Recoveries were checked showing values of 95% for sucrose and 97% for raffinose.

Table 2. Free sugar composition of different soya bean cultivars[a]

Cultivar	Fructose	Sucrose	Raffinose	Stachyose	α-Galactosides	Total	ds/nds[b]
Bossier	0·5	4·6	1·0	3·3	4·3	9·4	1·2
BR-4	0·6	5·3	0·7	3·2	3·9	9·8	1·5
BR-5	0·7	4·0	0·8	3·2	4·0	8·7	1·2
BR-9	0·4	5·3	0·7	4·6	5·3	11·0	1·1
BR-15	0·4	5·0	0·7	4·1	4·8	10·2	1·1
BR-27	0·7	4·4	0·8	3·8	4·6	9·7	1·1
Bragg	0·7	4·1	0·8	3·4	4·2	9·0	1·1
Buriti	0·4	4·1	0·6	3·7	4·3	8·8	1·1
Cobb	0·5	5·3	0·7	4·1	4·9	10·7	1·2
Cristalina	0·4	4·3	0·7	4·2	4·9	9·6	1·0
Davis	0·3	4·7	0·6	3·7	4·3	9·3	1·2
Doko	0·3	4·0	0·7	4·1	4·8	9·1	0·9
Dourados	0·3	4·9	1·1	3·8	4·9	10·1	1·1
FT-2	0·6	4·2	0·7	3·9	4·6	9·4	1·0
IAC-12	0·4	4·3	0·7	4·1	4·8	9·5	1·0
IAS-4	0·3	4·8	0·7	3·2	3·9	9·0	1·3
IAS-5	0·3	4·7	0·7	3·8	4·5	9·5	1·1
Ocepar-4	0·3	4·9	1·0	3·8	4·8	10·1	1·1
Parana	0·5	5·6	1·4	3·4	4·8	10·9	1·3
Santa Rosa	0·3	3·7	0·4	4·1	4·5	8·5	0·9

[a] Results are averages of duplicate determinations on g%, dry basis.
[b] ds/nds, digestible sugars/non-digestible sugars.

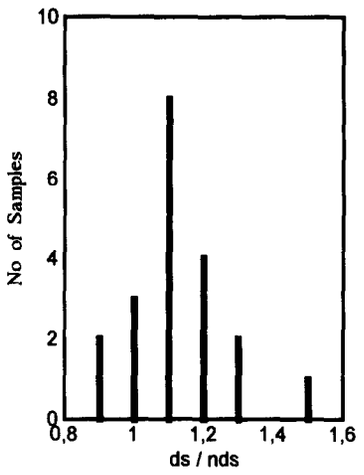

Fig. 1. Distribution of ds/nds ratios in soya bean cultivars. ds = digestible sugars; nds = non-digestible sugars.

Method 3 was then applied to the analysis of free sugars including the α-galactosides of 20 soya bean cultivars adapted to different regions of Brazil (Table 2). The results of total galactosides in the samples were in the range 3·9–5·3 g% on the basis of the dry soya beans (DB) with stachyose being the main component in all samples. Digestible sugars were mainly fructose and sucrose with the latter being predominant in all cases (3·7–5·3 g%, DB). These values are in perfect agreement with data from the literature (Eldridge *et al.*, 1979). It has been pointed out that stachyose is positively correlated with protein content and that this may be a problem for selection of cultivars with both low galactoside and high protein (Turatti *et al.*, 1984). We also observed a positive correlation between protein and stachyose, (r = 0·4834, $P < 0·05$). However, cultivars BR-5, BR-4 and IAS-4, showed high protein and oil contents (41·8 and 21·4; 38·5 and 21·2; 40·3 and 21·2 g%, DB, respectively) and also a lower level of stachyose (3·2 g%). To further assess the sugar bioavailability of the seeds the ratio between digestible sugars (ds) and non-digestible sugars (nds) may be a useful complementary parameter. The greater the ratio the better is the sugar bioavailability. The ds/nds ratios of the samples studied in relation to the frequencies of the samples are shown in Fig. 1. Based on these considerations we may conclude that cultivar IAS-4 should be the choice if a low galactoside sample is desired because it has an excellent ds/nds ratio, low α-galactoside content and also high levels of protein and oil.

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REFERENCES

Eldridge, A. C., Black, L. T. & Wolf, W. J. (1979). Carbohydrate composition of soya bean flours, protein concentrates and isolates. *J. Agric. Food Chem.*, **27**(4), 799–804.

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

Mohamed, A. I., Mebrahtu, T. & Rangappa, M. (1991). Nutrient composition and anti-nutritional factors in selected vegetable soya bean. *Plant Foods Human Nutr.*, **41**, 89–100.

Pearson, D. (1976). *The Chemical Analysis of Foods.*, Churchill Livingstone, Edinburgh, UK, p.206.

Quemener, B. (1988). Improvements in the high-pressure liquid chromatography determination of the amino sugars and α-galactosides in faba bean, lupine and pea. *J. Agric. Food Chem.*, **36**, 754–9.

Tsimidou, M. & Macrae, R. (1984). Influence of injection solvent on RP chromatography of triglycerides. *J. Chromatogr.*, **285**, 178–81.

Turatti, J. M., Tango, J. S., Silva, M. T. C., Shirose, I. & Yotsuyanagi, K. (1984). Caracterizacao dos graos de cultivares de soja cultivadas em algumas regioes do Brasil. *Bol. Ital.*, **21**(1). 73–99.