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Extractability and Evaluation of α-Galactosides of Sucrose in Leguminous Seeds

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ABSTRACT

The levels of sucrose and α-galactosides of sucrose in a range of leguminous seeds extracted by various procedures and analysed by the gel-permeation technique are reported. Cowpeas contained the highest levels of α-galactosides, dry weight basis, the lowest levels being recorded in chickpeas. Levels in lupins are almost comparable with soybeans and intermediate quantities have been measured in adzuki beans. All the legumes examined contained varying amounts of α-galactoside-hydrolysing enzyme (α-galactosidase EC 3.2.1.22) and the defatting process has no effect on its activity. No hydrolysis of these oligomers due to the extraction technique has been observed, except in one procedure. Conditions of cultivation may have been responsible for anomalies in the values of these oligomers observed by various investigators within the same variety and species of a leguminous seed.

INTRODUCTION

The ability of legume seeds to stimulate intestinal gas-formation has been recognised for many years (Calloway & Borough, 1969; Cristofaro *et al.*, 1974; Rackis, 1975). This has often been ascribed to the presence of α-galactosides of sucrose in mature legumes (Murphy, 1973; Wagner *et al.*, 1976; El Faki *et al.*, 1983). These low molecular weight oligomers are of potential importance in the acceptance of legumes as food and are also of industrial significance since their concentration can be greater than 10% of the seed dry weight. In consequence, the extraction and determination of α-galactoside contents of leguminous seeds have been the subject of many

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investigations (Macrae & Zand-Maghaddam, 1978; Kennedy *et al.*, 1985; Saini & Gladstones, 1986; Allen *et al.*, 1986).

A variety of procedures has been employed for the extraction of α-galactosides of sucrose from leguminous seeds. Boiling the seed meals in aqueous ethanol (Saini & Gladstones, 1986), extraction of the previously defatted seed meal under reflux in aqueous solvent (Macrae & Zand-Moghaddam, 1978), heating in aqueous solvents at low temperatures (Allen *et al.*, 1986) or straight water extraction (Kennedy *et al.*, 1985) are some of the most commonly employed procedures. As a result, some anomalies are occasionally noted in the oligosaccharide levels within the same variety and species of a given legume (Saini & Gladstones, 1986; De Almeida *et al.*, 1986; Allen *et al.*, 1986). These anomalies occur for total oligosaccharide contents as well as for individual components. This could be attributed to the inefficient solvent extractability of these compounds, hydrolysis of the individual components due to the presence of endogenous α-galactosidase activity (Kennedy *et al.*, 1985) or the discrepancies occurring due to the methods of analysis and quantification.

This study was undertaken to confirm that the possible use of different methods of oligosaccharide extraction could not be the only source of such anomalies. In this paper, the methods of extraction employed by various investigators have been compared and evaluated for a number of leguminous seeds. The levels of α-galactoside detected are discussed in relation to the amount of endogenous α-galactosidase activity.

EXPERIMENTAL

Leguminous seeds

The seeds used were lupins (*Lupinus angustifolius* cv. Chittick), chickpeas (*Cicer arietinum* cv. Tyson), soybeans (*Glycine max* cv. Forrest), adzuki beans (*Phaseolus angularis* cv. Bloodwood), cowpeas (*Vigna sinensis* cv. Banjo). Whole dry seeds were ground in a Udy cyclone mill and passed through a 100 mesh sieve.

Extraction of α-galactosides

Four extraction methods were compared and evaluated.

Method A (Saini & Gladstones, 1986)
The ground sample (5·0 g) was suspended in 250 ml 70% aqueous ethanol and the mixture boiled for 5 min. After cooling, the solution was filtered

through sintered glass and the residue washed with 50 ml 70% aqueous ethanol. The residue was transferred to a beaker and resuspended in 150 ml 70% ethanol, macerated and filtered. The combined ethanolic filtrates were concentrated to 50 ml on a rotary evaporator below 40°C. The concentrated solution was twice extracted with chloroform in a separating funnel. Final traces of chloroform and denatured protein were removed by centrifugation. The aqueous solution was concentrated under reduced pressure below 40°C to a final volume of 10 ml and used for α-galactoside analysis.

Method B (*Allen* et al., *1986*)
α-Galactosides were extracted from defatted (hexane b.pt. 60–80°C, room temperature) meal (lg) with 70% ethanol/water by sonicating for 40 s, and heating at 65°C for 30 min with frequent shaking. The mixture was then cooled and centrifuged for 10 min at 2500 rpm. The residue was re-extracted and the centrifugates combined. The solvent was removed and aqueous extract concentrated to 2·0 ml for further analysis.

Method C (*Macrae & Zand-Maghaddam, 1978*)
Finely ground defatted (as in method B) meal (2 g) was weighed into a 250 ml round bottom flask and treated with 40 ml of methanol/water mixture (4:6, v/v). The suspension was boiled under reflux in a water bath at 92°C for 2 h. After cooling the suspension was centrifuged at 3000 rpm for 5 min. The residue was re-extracted twice with 40 ml boiling aqueous methanol. The combined extracts and washings were evaporated under vacuum below 50°C to a final volume of 4·0 ml. This extract was used for the analysis of α-galactosides.

Method D (*Kennedy* et al., *1985*)
The extracts of defatted (as in method B), dry meal were prepared by shaking 1 g of meal with 25 ml distilled water for 1 h at 30°C. The suspension was then centrifuged for 5 min at 3000 rpm. The residue was re-extracted and the combined supernatants concentrated under reduced pressure to a final volume of 4·0 ml. The extracts thus obtained were centrifuged (14 000 *g* 10 min, 25°C) prior to analysis.

Estimation of total soluble sugars

Total soluble sugars in various extracts were determined by the anthrone method (Loewus, 1952). Appropriately diluted extracts containing 20–100 μg carbohydrate were aliquoted (0·5 ml) in 2·2 cm × 14 cm test tubes. Water (1·5 ml) was added, followed by 0·5 ml of 2% anthrone (Sigma) freshly prepared in E. Merck grade ethyl acetate. Concentrated sulphuric acid

(5·0 ml) was injected into the tubes and the tubes were immediately stirred on a vortex mixer. Absorbance was read at 620 nm and the weight of carbohydrate was determined from a galactose standard curve. Calculations were made on an anhydrogalactose basis.

Gel-permeation chromatography

Oligosaccharide extracts were chromatographed, separated and quantified as previously described (Saini & Gladstones, 1986).

Measurement of α-galactosidase activity

The ground samples (1 g) were suspended in cold acetate buffer (20 ml pH 5·0, 0·1M) and stored at 4°C for 2 h. The mixture was then homogenised in an Ultra-turrax macerator for 5 min. The suspension was centrifuged (14 000 *g*, 15 min, 5°C) and the clear supernatant was used for enzyme assay. The enzyme extract (0·1 ml) was incubated with *p*-nitrophenyl-α-D-galacto-pyranoside (M/80, 0·1 ml) at 30°C for 15 min. The reaction was stopped with sodium carbonate (2·8 ml, 2%) and the nitrophenyl released measured at 420 mm.

To measure cell-wall bound activities, the residue after extraction of the meal sample was suspended in acetate buffer (0·05M, pH 5·0, 10 ml). An aliquot (0·1 ml) was incubated with nitrophenyl substrate as above, shaken occasionally and filtered before assay. The activity was expressed as mg substrate hydrolysed per min (mg/g sample).

RESULTS AND DISCUSSION

Extractability of α-galactosides

Table 1 shows the results from the extraction of α-galactosides from various legumes using each of the four extraction procedures. The results indicate that treatment of the defatted seed meals with 40% methanol/water mixture (method C) resulted in incomplete extraction of the α-galactoside oligomers. Extraction of the ground material in boiling aqueous ethanol (method A) or heating the defatted meals in aqueous ethanol at 65°C (method B) produced comparable results except for soybeans. Extraction of the previously defatted, dry meals, or the untreated ground samples (method D) produced results close to methods A and B. It is clear that method C is not as satisfactory as methods A, B and D for the extraction of total soluble sugars as well as component α-galactosides (Table 2).

TABLE 1
Comparison of Four Methods for Extracting Total Soluble Sugars from Leguminous Seeds[a]

Leguminous seed	*Extraction method*			
	A	*B*	*C*	*D*
Lupins	12·0	11·2	9·66	10·4
Chickpeas	5·40	5·82	4·77	5·88
Soybeans	15·6	13·0	8·60	14·3
Adzuki beans	7·20	7·70	3·80	7·20
Cowpeas	10·0	10·7	8·20	11·3

[a] Values are g/100 g seed, dry weight.

TABLE 2
Levels of Sucrose and α-Galactosides of Sucrose in Leguminous Seeds Extracted by Four Methods[a]

Sample/ extraction method	*Sucrose*	*Raffinose*	*Stachyose*	*Varbascose*	*Total α-galactoside*[b]
Lupins					
Method A	4·85	1·31	4·10	1·12	6·53
Method B	4·89	1·43	3·97	0·76	6·14
Method C	4·40	1·02	3·42	0·86	5·30
Method D	4·43	0·92	2·89	1·12	4·93
Chickpeas					
Method A	1·98	0·84	3·05	0·42	4·31
Method B	2·30	1·02	3·58	0·29	4·89
Method C	2·87	0·94	2·87	0·33	4·14
Method D	2·15	0·66	3·52	0·00	4·18
Soybeans					
Method A	7·98	0·93	4·56	0·00	5·49
Method B	8·35	1·18	4·83	0·00	6·01
Method C	5·66	0·57	3·63	0·00	4·20
Method D	8·09	1·29	4·85	0·00	6·14
Adzuki beans					
Method A	1·40	0·38	5·04	0·00	5·43
Method B	1·38	0·50	5·35	0·00	5·85
Method C	1·24	0·31	3·45	0·00	3·76
Method D	1·43	0·43	4·82	0·00	5·52
Cowpeas					
Method A	4·32	1·63	6·00	0·00	7·63
Method B	3·72	1·55	5·60	0·00	7·15
Method C	3·29	0·56	4·56	0·00	5·12
Method D	4·89	0·83	6·31	0·00	7·14

[a] Values are g/100 g seed, dry weight.
[b] Total of raffinose + stachyose + verbascose.

Contents of total and component α-galactosides

Figure 1 shows the separation of oligosaccharides of cowpea seed extracts obtained by various extraction procedures. Polyacrylamide mediums resolve carbohydrate oligomers according to molecular weight and the separated oligomers are quantified colorimetrically. Table 2 shows the levels of sucrose and α-galactosides of sucrose in a range of leguminous seeds extracted by various procedures. Of all the legumes examined, cowpeas contained the highest level of total α-galactosides, on a dry weight basis, and the lowest levels were recorded in chickpeas. Levels in lupins were almost comparable with soybeans and intermediate quantities were measured in adzuki beans.

Stachyose was the predominant oligomer present in all the legumes examined with the highest levels recorded for cowpeas. Raffinose represented the second main oligomer and the lowest levels were observed in adzuki beans. Lupins contained a considerable amount of verbascose which represents nearly 17% of the total α-galactosides as compared to 9% present in chickpeas. Soybean, adzuki beans and cowpeas were nearly devoid of verbascose. Soybeans contain a higher level of sucrose (approximately 60% of the total sugars as compared to 20% present in adzuki beans). Sucrose forms almost 40% of the total soluble sugars in lupins and cowpeas.

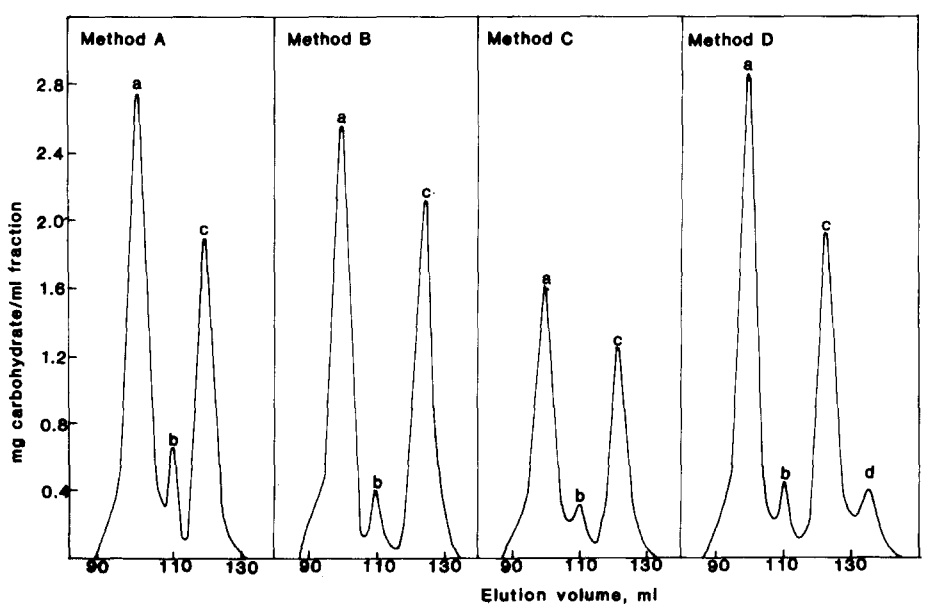

Fig. 1. Gel chromatographic separation of cowpea seed extracts on Biogel P-2 (minus 400 mesh). A, Stachyose; B, raffinose; C, sucrose; D, glucose.

α-Galactosidase activity and hydrolysis of oligosaccharides

To eliminate the possibility of hydrolysis of α-galactosides during the extraction procedure, α-galactosidase activity present in the seeds was monitored (Table 3); α-galactosidase activity has been reported in a number of mature leguminous seeds (Pridham *et al.*, 1969; Harpaz *et al.*, 1977). Cotyledons of lupins and soybeans have been shown to utilize α-galactosides of sucrose on germination, apparently due to the presence of α-galactosidase in the seeds (East *et al.*, 1972; Matheson & Saini, 1977). α-Galactosidase hydrolyses α-galactosides of sucrose to remove galactose residues from stachyose and raffinose with a corresponding release of sucrose. Lupins

TABLE 3
α-Galactosidase Activity in the Cytoplasmic and Cell Wall Fractions of Leguminous Seeds Before and After Defatting at Room Temperature

Leguminous seed	*Before*			*After*		
	Cytoplasmic fraction	*Cell-wall fraction*	*Total activity*	*Cytoplasmic fraction*	*Cell-wall fraction*	*Total activity*
Lupins	0·57	0·36	0·93	0·48	0·30	0·78
Chickpeas	0·22	0·63	0·85	0·20	0·62	0·82
Soybeans	0·29	0·41	0·70	0·37	0·41	0·78
Adzuki beans	0·16	0·23	0·39	0·15	0·23	0·38
Cowpeas	0·23	0·40	0·63	0·23	0·34	0·57

contained the highest levels of α-galactosidase activity and 60% of the activity was present in the cytoplasmic fraction. In all the other legumes a large proportion of the activity was associated with the cell wall fraction. α-Galactosidase activity was also measured after the meals were defatted at room temperature. This process partially removed the α-galactosidase activity from lupin meals but had no effect on the activity in other legumes.

The results indicate that no hydrolysis of the oligomers occurred during the extraction procedure, although a slight hydrolysis was observed in the extracts of method D. This was particularly obvious in the case of lupins which produced low values for raffinose and stachyose as well as the overall total content of α-galactosides. Gel-filtration patterns (Fig. 1) of cowpea extracts obtained by method D also showed a peak due to monosaccharides, apparently galactose removed as a result of α-galactosidase action in the extracts. These extracts contained considerable levels of α-galactosidase activity.

The results suggest that the extraction procedure can contribute to the

incomplete isolation of α-galactoside oligomers. In method C, a low ratio of solvent to solid may have contributed to the poor extractions obtained. Method D should be used with caution to determine component α-galactoside contents of leguminous seeds as the α-galactosidase activity is co-extracted with soluble sugars and will induce hydrolysis of raffinose and stachyose. Methods A and B appear to be quite effective in extracting α-galactosides from leguminous seeds. This suggests that discrepancies reported in the levels of lupin oligosaccharides perhaps occurred due to the difference in methods of analysis and quantification (Saini, 1986; Allen *et al.*, 1986). However, such an occurrence due to conditions of cultivation should not be overlooked.

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