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Enzymatic degradation of oligosaccharides in soybean flours

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The oligosaccharide content was determined in the flour of six soybean cultivars grown in India. The effect of cooking and soaking on the removal of oligosaccharides from the whole dry seeds was also studied. Crude α-galactosidase treatment on soybean flour reduced the raffinose and stachyose contents by 90·4% and 91·9%, respectively. This information could stimulate interest in the large-scale production of oligosaccharide-free soybean flour, by modulating its flatulence-causing properties. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Soybeans (*Glycine max*) differ from grain legumes in that they contain high amounts (up to 50%) of proteins. Soybean has a well-balanced amino acid pattern (Smith & Circle, 1972). Soybean-based foods are not widely accepted mainly because of their flavour, cookability and digestibility (Mital & Steinkraus, 1975). A variety of food products completely lacking in flavour can be made from soybeans (Nelson *et al.*, 1971). Soymilk is suitable for children who are allergic to cow's milk.

Soybean also contains a variety of antinutritional factors, including oligosaccharides of the raffinose family. These oligosaccharides, which are not digested by normal human carbohydrases but are fermented by intestinal microflora, lead to flatus formation (Steggerda *et al.*, 1966).

The purpose of the present investigation was to reduce sugars of the raffinose family in soybean varieties. In addition, an attempt has been made to use a crude preparation of α-galactosidase from guar (*Cyamopsis tetragonolobus*) seeds to degrade oligosaccharides present in soybean flours. This information could be useful for the commercial-scale production of soybean flours containing low levels of oligosaccharides, thereby enhancing increased and versatile utilization of soybean as a food and protein supplement.

MATERIALS AND METHODS

Sample preparation

Six cultivars of soybean (*Glycine max*) were collected from the Agricultural Research Station, Gulbarga, Karnataka, India, and were used in the present study. The whole mature raw beans in the form of flour were analysed. All the samples were soaked and cooked from the dry state. Distilled water was used for soaking and cooking experiments (bean:water ratio, 1:10). The beans were soaked in water for 4, 8, 12 and 16 h and the soaking water was decanted at 4 h intervals. The beans were also cooked in water for 20, 30, 40, 50 and 60 min and the cooking water was decanted and discarded. Both the soaked and cooked beans were mashed and dried in a hot-air oven at 45°C for 36 h. One hundred grams of whole, soaked or cooked product of each soybean variety were milled into flour and 5 g samples of the bean flour were used for the extraction of oligosaccharide.

Extraction of oligosaccharide was followed by the method of Somiari and Balogh (1993). The oligosaccharide content in the syrup was separated and estimated by the method described by Tanaka *et al.* (1975). The total soluble sugars were determined using the phenol–sulphuric acid method of Dubois *et al.* (1956).

Preparation and properties of guar α-galactosidase

Preparation of α-galactosidase from locally available guar seeds was followed by the method of Shivanna *et al.* (1989). The activity of α-galactosidase was determined as described by Mulimani *et al.* (1996).

Enzymatic treatment

Five grams of flour (which passes through a 650 μm sieve) of each cultivar was treated with 40 ml of crude α-galactosidase (1·6 U ml^{-1}) at 50°C for 4 h with occasional shaking. For a control, the volume of enzyme

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was replaced with 50 mM phosphate buffer (pH 6·5). After 4 h incubation, the contents were filtered through Whatman No. 1 filter paper. The residue was dried at 60°C for 24 h. The dried samples were milled to obtain flour; oligosaccharides in the flour were determined as above.

RESULTS AND DISCUSSION

The oligosaccharide content of the whole raw beans is presented in Table 1. The data from the raw samples showed that among oligosaccharides, concentration of stachyose is highest in all soybean cultivars. The data obtained from the whole raw samples showed that the local variety showed highest concentration of stachyose and MACS-58 had the least concentration of stachyose. The raffinose concentration of the local variety was the highest and the JS-335 variety had the least amount of sucrose. It is evident from Table 1 that the local cultivar had the highest concentrations of stachyose, raffinose and sucrose, compared to the other five cultivars analysed. The relative levels of raffinose and stachyose found in our study were comparable with those presented by other workers (Hymowitz *et al.*, 1972; Tanusi *et al.*, 1972) while the levels of sucrose were lower, compared to the values reported for sucrose in soybean by other investigators (Tanaka *et al.*, 1975; Kawamura, 1954; Hymowitz *et al.*, 1972). The lower level of sucrose could be attributed to (1) difference in the cultivars and (2) specific methodology used for the estimation of oligosaccharides separated from the concentrated sugar syrup.

Table 1. Oligosaccharide content of soybean from six cultivars (g per 100 g dry basis)

Cultivar	Total soluble sugars	Sucrose	Raffinose	Stachyose
Local	9·6 ± 0·3	0·94 ± 0·04	1·80 ± 0·1	1·82 ± 0·03
Monetta	4·1 ± 0·2	0·82 ± 0·02	0·84 ± 0·04	1·82 ± 0·02
KHSB-2	4·4 ± 0·1	0·50 ± 0·05	0·93 ± 0·03	1·54 ± 0·04
JS-335	4·1 ± 0·2	0·86 ± 0·04	0·73 ± 0·05	1·53 ± 0·03
PK-472	6·4 ± 0·3	0·90 ± 0·05	0·89 ± 0·02	1·59 ± 0·08
MACS-58	3·2 ± 0·2	0·76 ± 0·06	0·81 ± 0·02	1·42 ± 0·02

Data are the average ± 1 SD of triplicate determinations.

Total soluble sugars

The concentration of total soluble sugars in six cultivars also varied considerably. The MACS-58 had the least concentration of total soluble sugars and the local variety had the highest concentration of total soluble sugars. The levels of total soluble sugars observed in our studies are comparable to those of earlier works as reviewed by Reddy *et al.* (1984).

Effect of cooking

Cooking of dry whole soybeans resulted in a mean decrease in the concentration of sucrose, raffinose and stachyose. The percent removal of raffinose (52·3%) and stachyose (20·7%) is higher than the decrease in the sucrose content (16·6%) (Table 2). The decrease in the stachyose and raffinose content is greatest in the local cultivar and least in the Monetta variety. Ku *et al.* (1976) employed a cooking technique for the removal of oligosaccharides from whole dry soybean seeds. They observed that a lower bean:water ratio (1:10) resulted in maximum decrease in the oligosaccharide content (33%).

The mechanism of removal of the raffinose family sugars from beans during cooking is exactly not known, but Price *et al.* (1988) and Reddy and Salunkhe (1980) proposed that the removal of oligosaccharides during the cooking could be due to leaching. Onigbinde and Akinyele (1983) reported that the sucrose concentration almost doubled during cooking of cowpeas. They attributed this increase mainly to the breakdown of storage polysaccharides to sucrose or any other storage oligosaccharide having a similar chromatographic mobility. However, the formation of sucrose from raffinose or stachyose by partial hydrolysis during cooking

Table 2. Stachyose and raffinose content (g kg^{-1} DM) of raw, soaked, cooked and enzyme-treated soybean flour

Variety	Raw	Soaked (h)					Cooked (min)				Enzyme-treated
		4	8	12	16	20	30	40	50	60	
						Stachyose					
Local	18·3	14·2	11·1	14·7	12·4	17·5	17·1	13·6	13·9	13·5	0·1
Monetta	18·2	15·5	14·0	9·9	7·8	16·0	15·9	15·2	15·1	15·4	0·2
Mean[a]	18·3	14·9	12·6	12·3	10·1	16·8	16·5	14·4	14·5	14·5	0·2
± 1 SD	± 0·1	± 0·9	± 2·1	± 3·4	± 3·3	± 1·1	± 0·8	± 1·1	± 0·8	± 1·3	± 0·1
						Raffinose					
Local	13·0	12·0	8·1	6·9	2·1	8·8	7·8	7·6	6·1	5·2	0·1
Monetta	8·4	4·0	3·0	2·5	2·1	6·6	6·1	6·2	6·1	5·0	0·2
Mean[a]	10·7	8·0	5·6	4·7	2·1	7·7	7·0	6·9	6·1	5·1	0·2
± 1 SD	± 3·3	± 5·7	± 3·6	± 3·1	± 0·0	± 1·6	± 1·2	± 1·0	± 0·0	± 0·1	± 0·1

[a]Average of triplicate determinations.

of soybeans or, in fact, any legumes in distilled water (pH 7·0) is not possible.

Effect of soaking

Soaking also brought a decrease in the concentrations of sucrose, raffinose and stachyose in the two cultivars studied. Soaking of soybean seeds up to 16 h resulted in a mean decrease of 44·8% for stachyose and 80·3% for raffinose. Soaking of blackeye bean and pink beans decreased the oligosaccharide contents to 90·9% and 88·1%, respectively (Silva & Luh, 1979). The percent removal of oligosaccharides is proportional to duration of soaking of soybeans (Table 2).

The data from our results clearly suggest that reduction in the raffinose content is higher during soaking of soybeans than the reduction in stachyose content. The reason for this difference in removal is not known. The percent removal of oligosaccharides during soaking is high, compared with the values obtained after cooking of soybeans (Fig. 1). This could be explained from our observation that, during cooking, the absorption of water by the seeds was low, because of exposure of seeds to water for only a short duration.

Enzyme treatment

The crude enzyme from guar seeds reduced the stachyose content by 90·4% and 91·9%. In a control experiment the percent removal of stachyose was 8·5% and of raffinose was 6·2%. The level of sucrose in enzyme-treated sample was lower, compared to control experiments (data not shown). The mean reduction in the oligosaccharide content by α-galactosidase treatment is higher when compared with soaking and cooking treatments (Fig. 1). To our knowledge, this is the first report of the use of guar α-galactosidase for the removal of oligosaccharides present in the soybean flour. Several food scientists used microbial α-galactosidase to degrade the raffinose family sugars in soymilk (Sugimoto & van Buren, 1970; Thananunkul *et al.*, 1976; Mulimani & Ramalingam, 1995). Mital and Steinkraus (1975) used lactic acid bacteria for the utilization of oligosaccharides present in soymilk.

Thananunkul *et al.* (1976) used α-galactosidase in three different forms (undisrupted, disrupted and entrapped in polyacrylamide gel) for the hydrolysis of oligosaccharides and they found that disrupted mycelium of *Mortierella vinacea* gave the highest hydrolysis ratio. Shivanna *et al.* (1989) successfully used a crude α-galactosidase preparation from germinating guar seeds and observed 80% hydrolysis of stachyose and complete hydrolysis of raffinose in 30 min.

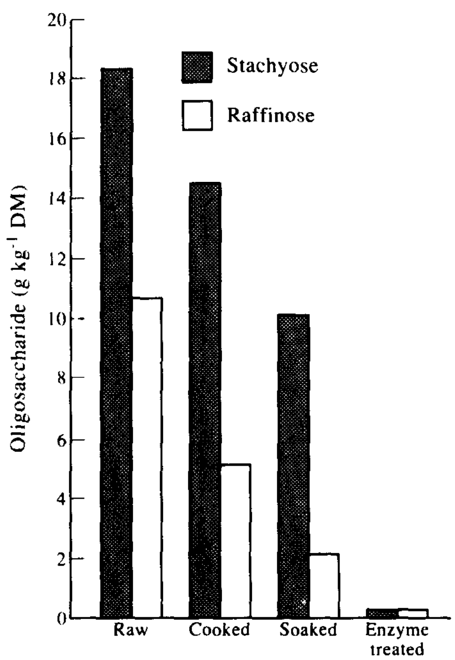

Fig. 1. Mean level of raffinose and stachyose in soybean before and after soaking (for 16 h), cooking (for 60 min) and enzymatic treatment (for 4 h at 50°C).

CONCLUSIONS

In the present work, we have used α-galactosidase from inexpensive guar seeds for the hydrolysis of oligosaccharides present in soyflour. The data from our studies showed that promising results are obtained in the removal of oligosaccharides in soyflour. α-Galactosidase from guar seed may be a better choice than α-galactosidases produced by surface or submerged or solid state fermentation from microbial origin.

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