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PII: S0308-8146(97)00142-8

Food Chemistry, Vol. 61, No. 4, pp. 475–479, 1998

0308-8146/98 $19.00+0.00

Effect of soaking, cooking and crude α-galactosidase treatment on the oligosaccharide content of red gram flour

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(Received 22 November 1996; revised version received 12 June 1997; accepted 12 June 1997)

The contents of sucrose, raffinose, stachyose, verbascose, total soluble sugars and reducing sugars in whole seeds of 14 cultivars of red gram (*Cajanus cajan*) grown in Karnataka state were determined. The effects of soaking, cooking and crude α-galactosidase treatment on the levels of the raffinose family of sugars were investigated. The percent losses of raffinose, stachyose and verbascose after soaking the red gram seeds for 16 h were 54.6, 55.4 and 33.3%, respectively. Cooking the red gram seeds for 60 min resulted in a decrease of 80.2% for raffinose, 87.2% for stachyose and 81.6% for verbascose. Thin-layer chromatographic analyses of 4 h enzyme-treated samples revealed complete hydrolysis of galactooligosaccharides. Therefore, α-galactosidase from *Cassia sericea* could have potential use in the food industry. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Legumes and cereals are good, and relatively inexpensive, sources of proteins and energy for third world countries, including India. India is a major pulse-producing country, sharing 36 and 28% of total area and production of these crops, respectively. Red gram seeds are the most important legumes consumed in India in the form of dhal (broken, dehulled) and flour. However, the presence of some undesirable components (antinutritional factors and α-galactosides) present in red gram limits its wider use (Singh, 1988). Most of the antinutritional factors, including phytic acid, tannic acid, amylase and proteinase inhibitors, can easily be removed by traditional cooking and soaking methods (Singh, 1988; Mulimani and Paramjyothi, 1994).

α-Galactosides include raffinose, stachyose and verbascose. These galactooligosaccharides constitute 53% of total soluble sugars. These sugars cannot be hydrolyzed and absorbed, because of lack of α-galactosidase (EC.3.2.1.22) activity in the small intestine. Microorganisms present in the large intestine utilize these sugars and lead to flatus formation (Olson *et al.*, 1981).

There are several reports of the use of α-galactosidase from plants and microorganisms to degrade oligosaccharides present in soymilk and other legume flours (Shivanna *et al.*, 1989; Somiari and Balogh, 1995; Mulimani *et al.*, 1997). To obtain crude α-galactosidase from microbial sources requires a controlled environment for their growth. In the case of plant sources, they have to be cultivated and this adds costs to the enzyme preparation. Information regarding screening of the raffinose family sugar content and processing on the level of oligosaccharides in newly developed cultivars of red gram is not available. Hence, in the present study, an attempt has been made to screen oligosaccharides and to assess the effect of soaking, cooking and enzymatic treatment for eliminating oligosaccharides from red gram flours.

MATERIALS AND METHODS

Source of red gram cultivars

The red gram cultivars used in the present study were obtained from the Agricultural Research Station, Gulbarga and were passed through a screen to remove dust and foreign particles. The varieties local-1 and local-2 were obtained from a local market.

Determination of oligosaccharide content

The red gram cultivars were milled to flour and passed through a 250 μm sieve. Five grams of each flour was

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added to an Erlenmeyer flask (250 ml capacity) containing 50 ml of 70% ethanol (v/v) placed on an orbital shaker at 130 rpm for 12 h. The contents of the flask were filtered through Whatman No. 1 filter paper and the residue was further washed with 25 ml of 70% ethanol. The combined filtrates were evaporated in a rotary vacuum evaporator at 40°C. The concentrated sugar syrup was obtained by dissolving the residue left after vacuum evaporation, in 10 ml of distilled water. Ten microlitres of the above syrup were spotted in triplicate on chromatographic plates (19×19 cm) coated with cellulose powder-G (Acme chemicals, Bombay). The plates were kept in a chromatographic chamber containing *n*-propanol:ethyl acetate:water (6:1:3) as the solvent system (Tanaka *et al.*, 1975). The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugar spots (Albon and Gross, 1952). For quantitative estimation, the area (2×3 cm) corresponding to each oligosaccharide was scraped and soaked in 3 ml of distilled water for 12 h. After 12 h, the mixture was filtered through Whatman No. 1 filter paper and the oligosaccharides in 1 ml of filtrate were estimated by the method of Tanaka *et al.* (1975). Verbascose used in the present study was isolated from red gram (*Cajanus cajan*) and confirmed by the methods of Iyengar and Kulkarni (1975).

Total soluble sugars

Total soluble sugars in the concentrated sugar syrup were estimated by the phenol–sulphuric acid method described by Dubois *et al.* (1956).

Estimation of reducing sugars

The amount of reducing sugars in the sugar syrup was determined by the method of Nelson (1944).

Preparation of α-galactosidase

Cassia sericea pods were collected during December 1995 from plants grown on the roadside and unused open grounds in Gulbarga city. The seeds from the pods were removed and air-dried. Partial purification of α-galactosidase from the seeds was carried out by the method of Bhasker *et al.* (1990). α-Galactosidase activity was determined as described earlier (Mulimani *et al.*, 1996).

Properties of α-galactosidase

The effects of pH and temperature on the relative rates of hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside (PNPG) by α-galactosidase was determined at pH and temperature values ranging from 2.0 to 8.0 and 30–70°C, respectively.

Processing of red gram

Soaking

100 g of whole red grams were soaked in 1 litre of distilled water for 4, 8, 12 and 16 h at room temperature (32 ± 1°C). At 4 h time intervals, the soaking water was decanted, and the soaked seeds washed thoroughly with 500 ml distilled water and treated with 1 litre distilled water. A fraction of soaking water was retained to check the presence of oligosaccharides in it. The soaked seeds were mashed in a porcelain mortar with pestle, dried at 55°C for 36 h and milled to obtain flour. The oligosaccharide content in the flour was determined as above.

Cooking

100 g of whole red grams were cooked in 1 litre of distilled water for 20, 30, 40, 50 and 60 min on a hot plate. After cooking, the beans were rinsed with 500 ml of distilled water, mashed, dried and the oligosaccharides were estimated as above.

Enzyme treatment

5 g of flour (fraction which passes 200 μm) of each cultivar was treated with 50 ml of partially purified preparation of α-galactosidase containing 0.45 units ml^{-1}. For the control, 50 ml of enzyme solution was replaced with 50 ml of buffer. The treatment was carried out on orbital shaker (120 rpm) at 45°C for 3 h. After 3 h, the contents were filtered through a Whatman No.1 filter paper, dried and the oligosaccharides were quantified as above.

RESULTS AND DISCUSSION

The levels of the raffinose family sugars, sucrose, total soluble sugars and reducing sugars are presented in Table 1. From the table it is evident that the cultivars Maruti, Sujatha 1-2 and selection-23 had the higher levels of verbascose. PT-22 1 had the lowest concentration of verbascose. The level of stachyose was highest in Sujatha 1-2 and the cultivar selection-23 had the lowest concentration of stachyose. The concentration of raffinose was lowest in the cultivars selection-23 and selection-27. The cultivars GS-1 and local-2 had the highest levels of raffinose. The levels of verbascose and raffinose observed in the present study were in the same range as reported earlier (Reddy *et al.*, 1984). However, the levels of stachyose in the present study are lower when compared to the values reported earlier (Reddy *et al.*, 1984). This could be (a) the difference in the cultivars studied, (b) specific methodology (thiobarbituric acid and conc. HCl) employed for the determination of sucrose, raffinose, stachyose and verbascose in the present study.

The cultivars Sujatha 1-2 and local-2 had the highest levels of sucrose. The lowest concentration of sucrose was observed in selection-23. The values obtained for the levels of sucrose are well within the values reported

Table 1. Total soluble sugars, reducing sugars, sucrose and galactooligosaccharides in 14 cultivars of redgram flour (g per 100 g dry basis)[a]

Variety	Verbascose	Stachyose	Raffinose	Sucrose	Total soluble sugars	Reducing sugars
TS-1	4.0±0.10	1.06±0.07	0.62±0.05	1.16±0.10	6.64±0.20	1.20±0.07
Maruti	6.0±0.20	0.74±0.06	0.62±0.08	1.32±0.10	7.12±0.21	1.44±0.10
Sujatha 1-2	6.0±0.20	1.20±0.10	0.78±0.04	2.06±0.20	7.36±0.25	1.02±0.05
PT-221	3.6±0.09	1.04±0.07	0.66±0.09	1.80±0.10	6.80±0.30	1.02±0.06
ICPL-270	4.6±0.10	1.06±0.08	0.54±0.06	1.48±0.08	7.12±0.40	1.56±0.10
Selection-23	6.0±0.20	0.72±0.04	0.52±0.03	0.92±0.05	6.64±0.30	1.98±0.20
GS-1	4.4±0.10	1.00±0.06	0.92±0.05	1.78±0.10	6.80±0.28	1.20±0.09
Selection-27	4.8±0.30	0.94±0.05	0.52±0.07	1.78±0.08	7.12±0.30	1.08±0.05
Selection-23	4.0±0.10	0.80±0.04	0.80±0.04	1.78±0.10	7.12±0.36	2.64±0.20
GPS-36	4.0±0.16	1.00±0.10	0.56±0.03	1.70±0.17	7.12±0.45	1.74±0.10
GC-11-39	4.0±0.10	1.04±0.06	0.68±0.03	1.72±0.07	7.16±0.25	1.92±0.08
ICPL-87	5.7±0.20	1.16±0.10	0.76±0.06	1.72±0.10	7.16±0.48	1.92±0.05
Local-1	4.8±0.10	1.04±0.08	0.80±0.04	1.78±0.20	3.92±0.09	0.36±0.02
Local-2	6.0±0.20	1.16±0.10	0.92±0.05	2.06±0.20	4.00±0.18	0.44±0.03
Mean±SD	4.8±0.15	0.92±0.05	0.69±0.03	1.52±0.06	6.57±0.19	1.36±0.01

[a]Each value is the average of triplicate determinations.
±, One SD.

in the literature (Reddy *et al.*, 1984). The concentration of total soluble sugars was highest in the cultivar selection 1-2 and local-2 had the lowest levels among the cultivars studied. The values reported for total soluble sugars in red gram cultivar ranged between 3.5 and 10.2 (Reddy *et al.*, 1984). The values obtained for total soluble sugars in the present study fall within the values stipulated earlier (Reddy *et al.*, 1984). The cultivar ICPL-87 had the highest amounts of reducing sugars and Local-1 had the lowest concentration of reducing sugars. Trugo *et al.* (1990) have reported oligosaccharide composition and trypsin inhibitor activity of *Phaseolus vulgaris* and the effect of germination on the α-galactoside composition.

Properties of α-galactosidase

The partially purified preparation of α-galactosidase from *C. sericea* exhibited an optimum pH between 5.0 and 5.5 (Fig. 1). Bhasker *et al.* (1990) have reported a pH optimum of 5.0 for two molecular forms of purified α-galactosidases from germinating seeds of *C. sericea*. Shivanna *et al.* (1989) have reported a pH optimum of 5.0 for the crude preparation of α-galactosidase from germinating guar (*Cyamopsis tetragonolobus*). From Fig. 1, it is also evident that partially purified enzyme showed a temperature optimum of 45°C. Bhasker *et al.* (1990) have reported a temperature optimum of 50°C for two molecular forms of α-galactosidases from *C. sericea*.

Effect of soaking

Soaking in distilled water (bean to water ratio, 1:10) reduced the levels of the raffinose family of sugars in the cultivars to various extents (Table 1). Soaking led to a mean decrease of 54.6% for raffinose, 55.4% for stachyose and 33.3% for verbascose (Fig. 2). It is also evident from Fig. 2 that the levels of the raffinose family of sugars decreased with the increased duration of soaking. Mulimani *et al.* (1996) have reported that soaking of whole soybean seeds for 16 h led to a mean decrease of 80.3% for raffinose and 44.8% for stachyose. Vijayakumari *et al.* (1996) have reported that soaking the seeds of tribal pulse *Mucuna monosperma* in distilled water and in sodium bicarbonate solution decreased the levels of the raffinose family of sugars. They have observed that the percent removal of raffinose sugars was higher with soaking in sodium bicarbonate solution than with soaking carried out in distilled water.

Somiari and Balogh (1993) have reported that soaking of cow pea seeds in distilled water reduced the levels of the raffinose family of sugars. Price *et al.* (1988) have proposed that leaching could be one of the mechanisms for their removal. Upadhyay and Garcia (1988) have demonstrated that the removal from cow pea during soaking could be attributed to (a) the differential solubility of individual sugars and (b) their diffusion rates.

Effect of cooking

Cooking brought about a greater reduction in the levels of the raffinose oligomers. Cooking of red gram seeds for 60 min resulted in a mean decrease of 80.2% for raffinose, 87.2% for stachyose and 81.6% for verbascose (Table 2). From the table it is also observed that the percent removal of the raffinose family of sugars positively correlated with the cooking time. Somiari and Balogh (1993) reported that cooking of cow pea for 50 min reduced the raffinose content to 44% and stachyose to 28.6%. Rao and Belavady (1978) have reported that the levels of the raffinose family of sugars increased during cooking. Oligosaccharide composition of cooked *P. vulgaris* was not intensively affected by cooking time and, although about 23% of loss has been observed in total oligosaccharides after 90 min cooking, this was mainly due to some sucrose degradation (Trugo *et al.*, 1990).

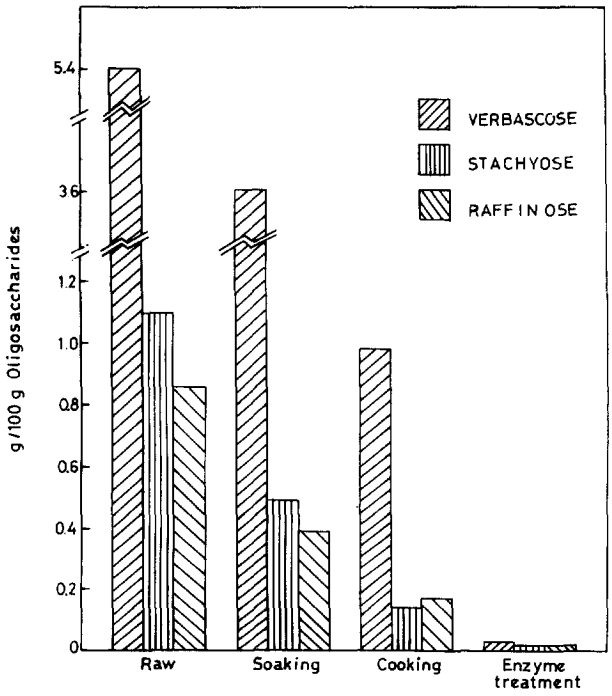

Fig. 1. Effect of pH and temperature on the hydrolysis of PNPG by α-galactosidase from germinating *C. sericea* seeds.

Mulimani *et al.* (1996) reported a decrease in the levels of the raffinose family of sugars after cooking of soybean for 60 min. Onigbinde and Akinyele (1983) have proposed that decreases in the levels of raffinose, stachyose and verbascose during cooking might be attributed to heat hydrolysis to disaccharides and monosaccharides or to the formation of other compounds. Rao and Belavady (1978) have reported that cooking led to an increase in the levels of the raffinose family of sugars. Price *et al.* (1988) have reported that cooking alone is not sufficient to bring about any significant reduction in the flatulence-inducing activity of cowpeas. Trugo *et al.* (1990) have reported a decrease of 15% in the levels of α-galactosides during cooking of *P. vulgaris* for 60 min.

Enzyme treatment

Partially purified α-galactosidase from *C. sericea* was effective in reducing the levels of the raffinose family of sugars in all the cultivars studied (Table 2). Enzymatic treatment for 3 h completely removed the galactooligosaccharides, as evidenced by TLC analysis (Table 1). There are several reports available in the literature of the use of α-galactosidase from plant and fungal sources for the removal of the raffinose family of sugars from soymilk and legume flours (Somiari and Balogh, 1993; Mulimani and Ramalingam, 1995; Mulimani *et al.*, 1997). Somiari and Balogh (1993) have used crude preparations of α-galactosidase from *Aspergillus niger* for the removal of raffinose and stachyose present in cow pea flours. Mulimani *et al.* (1996) reported the use of α-galactosidase from germinating guar (*Cyamopsis tetragonolobus*) for the hydrolysis of galactooligosaccharides present in soybean flours. Thananunkul, *et al.* (1976) have tested the ability of *Mortierella vinacea* mycelium in three different forms (undisrupted, mycelial homogenate and entrapped) for the hydrolysis of raffinose and stachyase present in soymilk.

The crude enzyme treatment was clearly more effective than soaking or cooking in reducing the level of verbascose, stachyose and raffinose in red gram flours (Fig. 2). Treatments such as soaking and cooking can change the physiochemical properties of legumes (Price *et al.*, 1988). *Cassia sericea*, a wasteland legume shrub, is mainly grown on roadsides and waste lands to eradicate the pernicious weed, *Parthenium hysterophorus* (Syamasundar and Mahadevappa, 1986). Plant crude enzyme treatment would seem to have the greatest

Table 2. Raffinose family sugars content in raw, soaked, cooked and enzyme-treated red gram flour (g per 100 g dry basis)[a]

Variety	Raw	Soaked (h)				Cooked (min)					Enzyme treated
		4	8	12	16	20	30	40	50	60	
						Raffinose					
Local-1	0.80	0.56	0.52	0.51	0.46	0.40	0.24	0.24	0.22	0.21	0
Local-2	0.92	0.92	0.63	0.54	0.31	0.57	0.54	0.52	0.46	0.13	0
Mean ± SD	0.86	0.74	0.57	0.52	0.39	0.48	0.39	0.38	0.34	0.17	0
	+0.08	+0.25	+0.08	+0.02	+0.11	+0.12	+0.21	+0.19	+0.17	+0.06	
						Stachyose					
Local-1	1.04	0.68	0.64	0.61	0.58	0.69	0.42	0.25	0.24	0.13	0
Local-2	1.16	0.79	0.66	0.44	0.41	0.84	0.81	0.79	0.73	0.15	0
Mean + SD	1.10	0.73	0.65	0.52	0.49	0.76	0.61	0.52	0.48	0.14	0
	+0.08	+0.08	+0.01	+0.12	+0.12	+0.11	+0.27	+0.38	+0.35	+0.01	
						Verbascose					
Local-1	4.80	3.00	3.40	3.40	3.30	4.00	3.60	1.50	1.20	1.20	0
Local-2	6.00	4.40	3.00	2.80	3.90	3.75	2.70	3.60	3.00	0.75	0
Mean ± SD	5.40	3.70	3.20	3.60	3.60	3.87	3.15	2.60	2.10	0.98	0
	+0.85	+0.99	+0.28	+1.13	+0.42	+0.18	+0.64	+1.48	+1.27	+0.31	

[a]Each value is the average of triplicate determinations.
±, One SD.

potential as the technique to control the flatulence-inducing activity of red gram and probably other legume flours.

This is the first report on the use of α-galactosidase from *C. sericea* for the hydrolysis of raffinose family sugars present in red gram flour. Bhasker *et al.* (1990) partially purified and characterized two molecular forms of α-galactosidase from germinating seeds of *C. sericea*. The use of α-galactosidase from *C. sericea* in the present study is explored mainly for two reasons: (a) seeds are available easily and practically at no cost and (b) they are a rich source of enzyme. α-Galactosidase from cultivated plants and fungal origin adds cost of production to the enzyme-treated flour. Thus, crude preparations of α-galactosidase from *C. sericea* have potential use in the food industry for production of red gram flour free from the flatulence-inducing raffinose family sugars. Before scale-up studies on the enzymatic treatment of red gram flour are undertaken, palatability, acceptability and other nutritional aspects of enzyme-treated red gram flour should be thoroughly studied.

ACKNOWLEDGEMENTS

The authors wish to thank S. Thippeswamy, Research Scholar, for his excellent technical assistance.

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