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EFFECT OF PROCESSING METHODS ON REDUCTION OF α–GALACTOSIDE CONTENT OF RED GRAM (*CAJANUS CAJAN* L.) FLOUR

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SUMMARY

The effect of processing methods such as defatting following by dry heating and cold soaking on the levels of α-galactosides in two locally grown cultivars of red gram seeds has been investigated. The levels of raffinose, stachyose and verbascose were found to decrease as a result of defatting followed by dry heating and cold soaking, microwave cooking and cooking in 1% turmeric solution. The defatting followed by dry heating was effective as compared to cold soaking, microwave cooking and cooking in 1% turmeric solution. The per cent losses of raffinose, stachyose and verbascose in red gram seeds after defatting followed by dry heating for 1 h at 100°C were 63.21%, 64.65% and 48.91%, respectively. Cold soaking of red gram seeds resulted in a decrease of 48.80% for raffinose, 56.63% for stachyose and 51.08% for verbascose. Microwave cooking the red gram seeds for 15 min resulted in a decrease of 46.73%, 24.81% and 31.12% for raffinose, stachyose and verbascose, respectively. Cooking in 1% turmeric solution for 60 min led to a decrease of 62.27% for raffinose, 61.77% for stachyose and 53.67% for verbascose, respectively.

Key words : **Cold soaking, defatting, dry heating, flatulence, oligosaccharides, red gram.**

INTRODUCTION

Red gram (*Cajanus cajan* L.) is an important legume grown and consumed in the tropics and semiarid parts of the world. India is a major pulse producing country, sharing 36% and 28% of the total areas and production of these crops, respectively. Red gram is the most important legume consumed in the form of dhal (broken, dehulled) in India. Although legumes constitute one of the richest and least expensive sources of protein in human diet, their utilization is somewhat limited owing to the presence of antinutritional factors such as protease inhibitors, polyphenols, amylase inhibitors, phytic acid, non-protein amino acids and oligosaccharides (Singh 1988). Among the soluble sugars, oligosaccharides of the raffinose family constitute 31-76% (Reddy and Salunke 1980). Flatulence is the most common symptom associated with pulse consumption (Olson *et al.* 1981). The production of flatus in monogastric animals is due to colonic fermentation of the sugars of raffinose, family, that escape digestion and absorption in the stomach and small intestine. Raffinose, stachyose and verbascose are common in red gram seeds, and are thought to be the major cause of flatulence on the consumption of red gram based foods. These sugars contain one, two or three galactose units joined to sucrose by α-1-6 galactosidic linkages. Owing to the absence of a α-galactosidase enzyme capable of hydrolyzing the α-1-6 galactosidic linkage, these oligosaccharides accumulate in the lower intestine and undergo anaerobic fermentation by bacteria, which may result in the production of flatus gases (H_2, CO_2 and small amount of CH_4) (Liener 1994, Flaming 1981).

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In India, legume seeds are processed and consumed in a variety of forms. The most common methods include soaking, cooking and dehulling. Therefore, to utilize legumes as more acceptable sources of proteins, it is necessary to reduce the flatus inducing agents. However, information on the oligosaccharides and the effect of processing of red gram seeds appears to be lacking. Hence, the present attempt has been made to examine the effect of processing methods like defatting followed by dry heating and cold soaking, etc. on the reduction of oligosaccharides in red gram seeds.

MATERIALS AND METHODS

The seeds of red gram cultivars ICPL 87 and GS-1 used in the present study were procured from the local market at Gulbarga, Karnataka, and were passed through a screen to remove dust, other materials and any broken or immature seeds. The seeds were stored in a plastic container at room temperature (30°C) until processing.

Cold soaking was carried out by keeping 100g seeds in distilled water (1:10, seeds to water ratio) overnight at 20±1°C. Following this the water was decanted and the seeds were dried in a hot-air oven (Toshniwal Brothers Pvt. Ltd. India), at 50°C for 36 h and the dried seeds were then milled in a flourmill (D-P International, India, size 6, 250 mm sieve). The flour was immediately analyzed for its oligosaccharide content.

The red gram sample (100 g) was separately soaked overnight in double distilled water (0 to 5°C) and the soaked seeds were pressed between the folds of filter paper to remove any adhering water. The seeds were then defatted by homogenizing with acetone. The homogenate was filtered under suction, and the residue was repeatedly washed with acetone and finally placed on a filter paper and rapidly air-dried. The defatted meal was heated in a hot-air oven as above sequentially at 60°C, 80°C or 100°C for 1 h each, followed by the determination of oligosaccharide content.

Seeds (100g) were cooked in a microwave oven (Operational frequency 2480 MHZ) for 5, 10 or 15 min. The cooked seeds were dried in a hot-air oven at 50°C for 36 h, and then milled. The flour was immediately analyzed for its oligosaccharide content. Dry seeds (100g) were also cooked in 1% (w/v) turmeric powder solution for 20, 30, 40, 50 or 60 min. The cooked seeds were dried in a hot-air oven at 50°C for 36 h, and then milled before the determination of oligosaccharide content.

The oligosaccharides in the flour were extracted, isolated and determined by the method of Tanaka *et al.* (1975). Five grams flour following various treatments was added to an Erlenmeyer flask (250 ml capacity) containing 50 ml of 70% ethanol (v/v) kept on an orbital shaker (Steelmet Industries, India; 120 rpm, 12 h). The contents of the flasks were filtered (Whatman No. 1 filter paper) and the residue was further washed with 25 ml of 70% ethanol. The combined filtrates were evaporated *in vacuo* (Heidolph, Laborota 4000, Germany) at 40°C. The concentrated sugar syrup was obtained by dissolving the residue in 5 ml distilled water. Five microliters of this syrup was spotted (in triplicate) on chromatographic plates (Cellulose powder-G, 19 x19 cm). The plates were developed in n-propanol: ehtyl acetate: water (6:1:3, v/v). The plates were sprayed with 1% ∝-naphthol in ethyl alcohol containing 10% orthophosphoric acid (Albon and Grass 1952). For quantitative estimation, the area corresponding to each oligosaccharide was scrapped and soaked in 2 ml of distilled water for 12 h. Afterwards the mixture was passed through Whatman No. 1 filter paper, and the individual oligosaccharides in 1 ml of filtrate were estimated by the method of Tanaka *et al.* (1975). Total soluble sugars in the concentrated sugar syrup were estimated by the phenol sulphuric acid method of Dubois *et al.* (1956), and the amount of reducing sugars was determined by the method of Nelson (1944).

RESULTS AND DISCUSSION

Cold soaking of seeds decreased the levels of raffinose, stachyose and verbascose (Table 1). The per cent removal of verbascose (52.1%) and raffinose (48.8%) was higher than that of stachyose (56.5%). Cold soaking also reduced the levels of total soluble sugars (40.2%), reducing sugars (92.3%) and sucrose (61.75%). The cold soaking of red gram seeds for 16 hrs led to a mean decrease of 54.6% for raffinose, 55.4% for stachyose and 33.3% for verbascose. The reduction of sugars of the raffinose family after cold soaking of cowpeas in distilled water for 12 h at 22°C have been reported in cowpea (Ogun *et al.* 1989). Ku *et al.* (1976) and Silva and Braga (1982) reported greater losses of oligosaccharides when beans were soaked in 1:10 bean

Table1. Effect of cold soaking on the levels of α-galactosides, reducing, non reducing and total soluble sugars in red gram (g /100 g dw).

Variety	Raw	Cold soaking
	Total soluble sugars	
ICPL 87	6.72±0.61	3.36±0.32
GS-1	5.64±0.71	3.92±0.31
Mean±SD	6.18±0.66	3.64±0.31
	Reducing sugars	
ICPL 87	0.86±0.11	0.06±0.01
GS-1	0.96±0.09	0.08±0.02
Mean±SD	0.91±0.10	0.07±0.01
	Sucrose	
ICPL 87	2.44±0.21	0.88±0.70
GS-1	2.92±0.33	1.18±0.28
Mean±SD	2.68±0.27	1.03±0.49
	Raffinose	
ICPL 87	1.26±0.18	0.70±0.03
GS-1	1.58±0.20	0.74±0.05
Mean±SD	1.48±0.19	0.72±0.04
	Stachyose	
ICPL 87	1.72±0.12	0.78±0.03
Local 2	1.78±0.15	0.74±0.02
Mean±SD	1.75±0.13	0.76±0.02
	Verbascose	
ICPL 87	4.80±0.91	2.20±0.06
GS-1	5.10±0.83	2.55±0.08
Mean±SD	4.95±0.87	2.37±0.07

to water ratio than in the lower ratio. This is probably due to the increased solubility and leaching of sugars in the medium at higher bean to water ratio. Mulimani and Devindra (2000) have reported the reduction in oligosaccharide content when red gram seeds were soaked in hot distilled water (1:10 bean to water ratio) for 5 min. Upadhyay and Garcia (1988) demonstrated that leaching of sugars of the raffinose family sugars from cowpea seeds during soaking could be attributed to the differential solubility of individual sugars and their diffusion rates.

The effect of defatting followed by dry heating at 100°C led to a decrease in the levels of raffinose, stachyose and verbascose (Table 2). The per cent removal of verbascose (49.2%) and raffinose (63.2%) was higher than that of stachyose (64.6%). Soaking followed by dry heating significantly reduced the levels of total soluble sugars (47.5%), reducing sugars (92.9%) and sucrose (70.5%). Delente and Ladenburg (1972) have reported a similar reduction of sugars of the raffinose family in soymeal by defatting. Kawamura (1967) analyzed the defatted cotyledons, the hull and the defatted hypocotyls of six American varieties and three Japanese varieties of soybeans and gave the average composition of six American varieties, as sucrose 6.2%, raffinose 1.4% and stachyose 5.2%.

The results of the present investigation clearly show that defatting followed by dry heating was more effective than cold soaking for removal of flatulence inducing sugars such as raffinose, stachyose and verbascose thus increasing the nutritional quality of red gram flour.

Results of microwave cooking on raffinose sugars are given in Table 3. Microwave cooking of seeds also led to the reduction in the levels of oligosaccharides. Microwave cooking of red gram seeds for 15 min resulted in a mean decrease of 46.73% for raffinose, 24.81% for stachyose and 31.12% for verbascose. The reduction in the levels of sugars of the raffinose family positively correlated with the cooking time. Microwave cooking also reduced the levels of total soluble sugars, reducing sugars and sucrose. The mean decrease of 45.39% for total soluble sugars, 76.78% for reducing sugars and 62.72% for sucrose was recorded.

Among the spices, turmeric is extensively used in cooking for its colour, taste and flavour. It is also added to the food as a preservative. Cooking in 1% turmeric powder solution for 60 min resulted in a mean reduction of 62.27% for raffinose, 61.77% for stachyose and 53.67% for verbascose (Table 4). This also led to a mean decrease of 52.41% for total soluble sugars, 57.01% for reducing sugars and 61.09% for sucrose. Vijaya Kumari *et al.* (1996) have reported that cooking for 3 h significantly decreased raffinose, stachyose and verbascose in two varieties of *Mucuna monosperma.* Gas formation was totally inhibited by raw as well as autoclaved cinnaman and garlic, whereas ginger pepper and turmeric caused only partial inhibition. Studies carried with curcumin isolated from turmeric indicated that *in vitro* effect was observed at 0.05% level of curcumin and *in vivo* effect at 0.05-0.5% levels (Bhavanishanker and Sreenivasmurthy 1985). Further studies employed *Escherichia coli* and intestinal microflora on the mechanism of inhibition by

Table 2. Effect of defatting followed by dry heating on the levels of α-galactosides, reducing, non reducing and total soluble sugars in red gram (g /100g dw).

Variety	Raw	Defatting followed by dry heating (h)		
		60°C	80°C	100°C
		Total soluble sugars		
ICPL 87	6.72±0.61	3.26±0.31	3.30±0.21	3.00±0.28
GS-1	5.46±0.71	3.80±0.33	3.60±0.23	3.40±0.23
Mean±SD	6.18±0.66	3.53±0.32	3.45±0.22	3.20±0.25
		Reducing sugars		
ICPL 87	0.86±0.11	0.08±0.00	0.07±0.01	0.06±0.01
GS-1	0.96±0.09	0.08±0.01	0.06±0.02	0.07±0.00
Mean±SD	0.91±0.10	0.08±0.00	0.06±0.01	0.06±0.00
		Sucrose		
ICPL 87	2.44±0.21	1.18±0.09	0.86±0.07	0.72±0.05
GS-1	2.92±0.33	1.04±0.07	0.96±0.09	0.86±0.09
Mean±SD	2.68±0.27	1.11±0.08	0.91±0.08	0.79±0.07
		Raffinose		
ICPL 87	1.26±0.18	0.56±0.15	0.66±0.01	0.56±0.07
GS-1	1.58±0.20	0.78±0.19	0.68±0.05	0.46±0.05
Mean±SD	1.42±0.19	0.67±0.17	0.67±0.03	0.51±0.06
		Stachyose		
ICPL 87	1.72±0.12	1.20±0.22	0.88±0.01	0.52±0.14
GS-1	1.78±0.15	0.88±0.19	0.88±0.02	0.72±0.12
Mean±SD	1.75±0.13	1.04±0.20	0.88±0.01	0.62±0.13
		Verbascose		
ICPL 87	4.80±0.91	4.00±0.42	3.60±0.14	2.90±0.56
GS-1	5.10±0.83	4.60±0.45	3.80±0.19	2.10±0.43
Mean±SD	4.95±0.87	4.30±0.43	3.70±0.16	2.50±0.49

curcumin showed that the latter effectively chelates Fe^{+3} ions present in diet which inturn led to the reduced activity of formic hydrolyzate which catalyses the conversion of formic acid in to carbon dioxide and hydrogen (Bhavanishankar and Sreenivasamurthy 1986).

Table 3. Effect of microwave cooking on the levels of α-galactosides, reducing, non reducing and total soluble sugars in red gram (g /100g dw).

Variety	Raw	Microwave cooking (min)		
		5	10	15
		Total soluble sugars		
ICPL 87	6.72±0.61	3.24±0.00	3.44±0.14	3.24±0.14
GS-1	5.64±0.71	3.24±0.12	3.24±0.12	3.44±0.12
Mean±SD	6.18±0.66	3.24±0.06	3.34±0.13	3.34±0.13
		Reducing sugars		
ICPL 87	0.86±0.11	0.20±0.25	0.22±0.08	0.22±0.01
GS-1	0.96±0.09	0.22±0.21	0.12±0.09	0.20±0.02
Mean±SD	0.91±0.10	0.21±0.23	0.17±0.08	0.21±0.01
		Sucrose		
ICPL 87	2.44±0.21	1.50±0.25	1.12±0.09	1.00±0.01
GS-1	2.92±0.33	1.86±0.23	1.00±0.05	0.98±0.00
Mean±SD	2.02±0.27	1.68±0.24	1.06±0.07	0.99±0.00
		Raffinose		
ICPL 87	1.26±0.18	1.60±0.31	1.18±0.02	0.80±0.08
GS-1	1.58±0.20	1.16±0.23	1.14±0.13	0.68±0.05
Mean±SD	1.42±0.19	1.38±0.27	1.16±0.07	0.74±0.06
		Stachyose		
ICPL 87	1.72±0.12	1.70±0.15	1.62±0.12	1.28±0.03
GS-1	1.78±0.15	1.48±0.12	1.44±0.14	1.20±0.05
Mean±SD	1.75±0.13	1.59±0.33	1.53±0.13	1.24±0.04
		Verbascose		
ICPL 87	4.80±0.91	4.20±0.03	4.00±0.14	3.60±0.28
GS-1	5.10±0.83	4.20±0.05	3.80±0.19	3.20±0.25
Mean±SD	4.95±0.87	4.20±0.04	3.90±0.16	3.40±0.26

Table 4. Effect of cooking in 1% turmeric powder on the levels of α-galactosides, reducing, non reducing and total soluble sugars in red gram (g /100g dw).

Variety	Raw	Cooking in 1% turmeric powder (min)				
		20	30	40	50	60
			Total soluble sugars			
ICPL 87	6.72±0.61	3.40±0.13	3.32±0.06	3.20±0.12	3.00±0.05	0.82±0.19
GS-1	5.64±0.71	3.82±0.18	3.73±0.09	3.60±0.09	3.20±0.07	3.00±0.17
Mean±SD	6.18±0.66	3.76±0.15	3.60±0.07	3.40±0.15	3.10±0.15	2.91±0.18
			Reducing sugars			
ICPL 87	0.86±0.11	0.10±0.00	0.09±0.03	0.08±0.04	0.10±0.21	0.39±0.21
GS-1	0.96±0.09	0.12±0.03	0.10±0.05	0.10±0.02	0.11±0.18	0.39±0.33
Mean±SD	0.91±0.10	0.11±0.01	0.10±0.04	0.09±0.03	0.10±0.19	0.39±0.27
			Sucrose			
ICPL 87	2.44±0.21	1.74±0.14	1.34±0.19	1.16±0.09	1.06±0.70	1.00±0.32
GS-1	2.92±0.33	1.34±0.21	1.48±0.20	1.10±0.10	1.12±0.91	1.06±0.29
Mean±SD	2.68±0.27	1.54±0.17	1.41±0.19	1.13±0.09	1.09±0.80	1.03±0.30
			Raffinose			
ICPL 87	1.26±0.18	0.82±0.38	0.60±0.19	0.66±0.05	0.64±0.02	0.52±0.09
GS-1	1.58±0.20	0.58±0.32	0.66±0.15	0.66±0.07	0.60±0.07	0.54±0.07
Mean±SD	1.48±0.19	0.70±0.35	0.63±0.17	0.66±0.06	0.62±0.04	0.53±0.08
			Stachyose			
Local 1	1.72±0.12	0.60±0.79	1.06±0.12	0.64±0.04	0.66±0.09	0.64±0.23
GS-1	1.78±0.15	0.60±0.68	0.88±0.18	0.82±0.08	0.66±0.19	0.62±0.32
Mean±SD	1.75±0.13	0.60±0.73	0.97±0.15	0.73±0.06	0.66±0.14	0.63±0.27
			Verbascose			
ICPL 87	4.80±0.32	4.00±0.23	3.60±0.08	2.80±0.07	3.20±0.17	2.00±0.12
GS-1	5.10±0.39	4.60±0.19	4.20±0.09	4.00±0.05	3.00±0.20	2.60±0.15
Mean±SD	4.95±0.87	4.30±0.21	3.80±0.08	3.48±0.06	3.10±0.18	0.30±0.13

The active principle in turmeric is curcumin has strong antioxidant and anti-inflammatory potency (Krishnaswamy 2001). From our data, it is proposed that the active principle curcumin may be responsible for reduction of oligosaccharide in red gram. Hence, traditional methods like dry heating, cold soaking, microwave cooking and cooking in 1% turmeric solution can be applied to reduce α-galactoside in red gram.

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