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Effect of Domestic Processing and Crude Extract of α-Galactosidase on Oligosaccharide Content of Red Gram (*Cajanus cajan* L.) Seeds

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Abstract

The aim of the present study was to demonstrate the effect of cooking in 1% mango powder, soaking in mixture of 1% salts and crude extract of α-galactosidase treatment on the raffinose family sugar content of red gram. Cooking of red gram seeds in 1% mango powder for 60 min resulted in a mean reduction of raffinose stachyose and verbascose by 67.26, 63.74 and 51.53% respectively. Pressure cooking of red gram seeds in 1% mango powder for 15 min led to decrease of raffinose by 69.24%, stachyose 65.13% and verbascose 47.42%. Soaking in 1% salt mixture solution led to loss of raffinose by 70.20%, stachyose 67.08% and verbascose 53.43% respectively. The crude α-galactosidase treatment led to a mean hydrolysis of raffinose by 67.18%, stachyose 65.68% and verbascose 56.55%. The domestic processing methods reduced the anti-nutrients content and in turn, improved the nutritional quality of the legume seeds.

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Raffinose;
Stachyose;
Guar seeds;
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Introduction

Legumes play a major role in the diet of humans and provide well balanced diet along with cereals.[1] They are good source of vitamins, minerals and polyunsaturated fatty acids also rich in trace elements such as iron and iodine.[2] Pigeon pea also contains essential amino acids such as cystine, lycine, tyrosine, and arginine.

In India, after chick pea red gram is the most widely grown pulse crop, cultivating in an area of 4.42 mha and production of 2.86 MT and productivity of about 707 kg/ha.[3] Pigeon pea is an important crop in India and contributing around 18 percent and 12 percent to total area and production respectively. In India pigeon pea is used in many food preparations like dal, sambar and green immature seeds are used in vegetable preparation.[4]

Non digestible carbohydrates such as raffinose family sugars of legumes are responsible for the production of flatus, due to lack of α-galactosidase

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enzyme that catalyzes α- (1, 6) glycosidic bonds in human and animals.[5] The consumption of red gram seeds is restricted in human nutrition due to the presence of anti-nutritional components including oligosaccharides. The undigested, raffinose family oligosaccharides are fermented with the microflora of the intestine.[6] As a result of this the byproducts such as carbon dioxide, hydrogen and methane are produced causing flatus and abdominal discomfort.[7]

Therefore, to make pigeon pea more acceptable by several methods of processing is to reduce the flatulence inducing factors. The use of enzymes and soaking or germinating the seeds before cooking can reduce the oligosaccharide content.[8] Traditional processing practices have been followed for many years to convert legumes in to the more acceptable forms. Such processes not only improve the digestibility and palatability of legumes but also help to remove some anti-nutritional factors. Household processing of pulses has been known to improve nutritional quality by increasing protein digestibility and also by reducing anti-nutrients.[9] Ramadoss *et al.,* have reported the oligosaccharides and sucrose content in 32 pigeon pea cultivars.[10] Soaking of pulses in sodium bicarbonate results in significant reduction in raffinose family of oligosaccharides and enhances the protein digestibility and absorption.[11]

Oligosaccharides are known to be low calorie substances and last decade have gained interest in food and pharma industries as functional foods.[12] Oligosaccharides can be found naturally or synthesized from disaccharides or by the hydrolysis of polysaccharides.[13] Oligosaccharide content in soybean of Thailand was proven to be a potential source of prebiotics.[14]

Data regarding the oligosaccharide content in new cultivars of red gram and the effect of different domestic processing methods is not available. Aim of the present study is to screen oligosaccharides and to demonstrate the effect of soaking in a mixture of 1% salt solution, cooking, pressure cooking in 1% mango powder (Vasant Dry Mango Powder) and crude enzymatic hydrolysis for the reduction of oligosaccharide content of red gram seeds. The crude α-galactosidase is a glycoside hydrolase enzyme that removes the terminal α-galactosyl moieties from glycoproteins and glycolipids.[15]

Materials and Methods

Materials

The red gram varieties used in the present study local red and local white were procured from local markets of Hyderabad and Secunderabad, Telangana State. The seeds were cleaned to remove dust and foreign particles and the seeds were stored at 4^0 C for further study.

Cooking in 1% Mango Powder

Mango powder used in the present study was commercially available in the local market (Vasant Dry Mango Powder). 100 gm of red gram seeds were cooked on a hot plate in 1% (w/v) mango powder solution for 60 min at different time intervals. The cooked seeds were rinsed with 500 ml of distilled water and mashed and dried in hot air oven at 50 ^{0}C for 40 h and then milled to flour. The oligosaccharide content was estimated immediately by following the method of Tanaka *et al.,*[16]

Pressure- Cooking in 1% Mango Powder

Hundred grams of red gram seeds were pressure cooked in 1% (w/v) mango powder solution at 15 lbs for 15 min with different time intervals and the seeds were washed with distilled water 1:5 (bean : water ratio). These seeds were mashed and dried in hot air oven for 40 h at 50 ^{0}C. The dried seeds were milled to flour and the oligosaccharide content was analyzed as described in above method.

Soaking in Mixture of 1% Sodium Carbonate, Sodium Bicarbonate and Sodium Chloride ($NaCO_3$ & $NaHCO_3$ & NaCl) Solution

The red gram seeds (100 g) were soaked in a mixture of 1% salt solution of sodium carbonate, sodium bicarbonate and sodium chloride ($NaCO_3$ & $NaHCO_3$ & NaCl) (w/v) (1 liter), for 16 hrs with 4 hrs of time intervals at room temperature (35±2 ^{0}C). The soaked solution was decanted after every 4 h time interval. The soaked seeds were rinsed twice with 500 ml and 1 liter of distilled water and seeds were mashed and dried in hot air oven for 40 h at 50 ^{0}C. The dried seeds were milled to flour and flour was subjected to oligosaccharide analysis.

Crude α-galactosidase Extraction from Guar Seeds

The guar seeds (*Cyamopsis tetragonolobous*) were surface sterilized with 1% mercuric chloride solution for 15 minutes. The seeds were thoroughly washed with distilled water for 4 times and then soaked for 4 hours. The soaked seeds were germinated in a perforated tray on the moist filter paper slightly wetted with distilled water at room temperature for 1-7 days. The de-husked germinated seeds were chilled. The enzyme was extracted from the chilled seedlings by homogenizing with 5 volumes of 50 mM sodium acetate buffer pH 5.5 containing 2mM EDTA, 2 mM - mercaptoethanol, 1mM phenyl methane sulphonyl fluoride (PMSF), 200 mM sodium chloride and 1% insoluble polyvinyl pyrolidone (PVP). Slurry was drained through a muslin cloth and the filtrate was centrifuged at 5000 rpm for a period of 20 min. The supernatant were precipitated with 40% ammonium sulphate saturation with agitating at 4 ^{0}C. The solution was set for sedimentation at 4^{0}C for 12 h and then centrifuged at 12000 xg for 20 min. The pooled supernatant was then saturated to 70% ammonium sulphate, and stored for 4 h at 4^{0}C and then centrifuged at 12,000 rpm and kept at 4^{0}C for 20 min. The precipitate was dissolved in 50 mM acetate buffer (pH 5.5) and dialyzed against 2 liters of 10 mM acetate buffer (pH 5.5) for 12 h with two changes.

α- Galactosidase Treatment of Red Gram Flour

Five grams of red gram flour was treated with 50 ml of crude α- galactosidase (containing 0.45 Units ml^{-1}). In the control experiments 5 grams of red gram flour was treated with 50ml of buffer (0.1 M, pH 6.8) in place of enzyme extract. The reaction was incubated at 37 ^{0}C for 3 h on an orbital rotary shaker (125 rpm). Succeeding treatment red gram samples were filtered through filter paper (Whatman No.1) and the residue was dried in a hot- air oven at 50 ^{0}C for 40 h and oligosaccharide content was analyzed.

Estimation of Total Carbohydrate

The total carbohydrate content of red gram flour was estimated by following the method of Dubois *et al.,*[17]

Estimation of Reducing Sugars

The estimation of reducing sugars of red gram flour was followed by the method of Nelson.[18]

Statistical Analysis

Results were reported as mean ± standard deviation of triplicate determinations and the significance of difference of means at 5% was analyzed by repeated measure of analysis. The variance was denoted with different letters. The analysis of data was performed by using statistical package for social sciences (SPSS) version 19.0.

Results and Discussion

Effect of Cooking in 1% Mango Powder on Oligosaccharide Content

The commercially available mango powder was used for the preparation of legume recipes. Cooking of red gram seeds in 1% mango powder solution resulted in falling off in the raffinose oligosaccharide content. When seeds were cooked in 1% mango powder for 60 min resulted in reduction of raffinose by 67.26%, stachyose 63.74%, verbascose 51.53% respectively (Table 1). From the table it is evident that the mean reduction of raffinose family sugars was correlated with the cooking time. The domestic processing method also resulted in mean decrease of reducing sugars by 70.88%, total soluble sugars 54.44% and sucrose 58.63% respectively.

The α-galactosidase enzyme hydrolyses the galactose from raffinose family oligosaccharides. Several studies reported the effect of domestic processing on oligosaccharide content of pulses and beans. Cooking of mucuna pruriens and soaking in tamarind pulp extract followed by cooking caused a reduction in the raffinose family sugar content.[19] The quality of pulse flours from Algeria has been evaluated and found that inhibition of α-amylase was improved during processing.[20] In a study tender seeds of five been cultivars were assessed for changes in raffinose family oligosaccharides content due to boiling, and sterilization. Boiling immature seeds reduced raffinose family oligosaccharides s by 55%. Sterilization resulted in 65% reduction of both total soluble sugars and raffinose family oligosaccharides.[21] In a similar study raw chick pea was evaluated for changes in functional carbohydrates under cooking, soaking, and dehydration processes. Among these methods dehydration significantly increased raffinose family oligosaccharides by 43%.[22]

Effect of Pressure Cooking in 1% Mango Powder on Oligosaccharide Content

Pressure cooking of red gram seeds in 1% mango powder solution for 15 min resulted in mean reduction of oligosaccharide content. The mean reduction was 69.24%, 65.13% and 47.42% for raffinose, stachyose and Verbascose respectively (Table 2). From the table it is also evident that there was a positive correlation between the pressure-cooking time and percent removal of the raffinose family of sugars. Pressure cooking of red gram seeds in 1% mango powder solution also showed a mean decrease of 65.49%, 60.77% and 69.94% for reducing sugars, total soluble sugars and sucrose respectively. All the commonly consumed cereals and pulses as influenced by domestic food processing[23] and household processing methods like soaking, sprouting, and dehulling in combination with heating cause an increase nutrient digestibility and bioavailability.[24]

Table 1: Effect of cooking in 1% mango powder on raffinose family sugars, reducing, non-reducing and total soluble sugars in red gram (g Kg^{-1} dry basis)[a]

	Cooking in 1% mango powder solution (min)					
Variety	**Raw**	**20**	**30**	**40**	**50**	**60**
			Raffinose			
Local - Red	12.6^a±0.3	5.33^b±0.21	5.40^b±0.4	4.00^c±0.60	4.30^c±0.30	4.90^c±0.60
Local -White	15.8^a ±0.6	5.30^b±0.30	4.90^b±0.7	4.90^b±0.30	4.40^b±0.40	4.13^b±0.70
Mean±SD	14.2^a±0.5	5.31^b±0.25	5.15^b±0.5	4.45^b±0.45	4.35^b ±0.35	4.51^b±0.65
			Stachyose			
Local - Red	17.2^a±0.4	8.7^b±0.5	8.4^b±0.4	7.4^c±0.3	6.00^d±0.40	5.90^d±0.70
Local -White	17.8^a±0.8	7.3^b±0.3	7.0^b±0.2	7.0^b±0.5	7.10^b±0.50	6.80^b±0.40
Mean±SD	17.5^a±0.6	8.0^b ±0.4	7.7^b±0.3	7.2^b±0.4	6.55^c±0.45	6.35^c±0.55
			Verbascose			
Local -Red	48.0^a±4	36^b±3	35^b±3.0	34^b±2	30^b±3	23^c±3
Local -White	51.0^a ±2	48^b±3	45^b±2.0	30^c±2	28^d±3	25^d±1
Mean±SD	49.5^a ±3	42^b±3	40^b±2.5	32^c±2	29^c±3	24^d±2
			Reducing sugar			
Local -Red	8.6^a±0.40	8.0^a±0.40	7.2^b±0.20	6.6^b±0.40	3.53^c±0.31	2.50^d±0.10
Local -White	9.6^a±0.30	8.6^b±0.30	7.4^c±0.30	6.5^d±0.40	3.50^e±0.20	2.80^f±0.20
Mean±SD	9.1^a ±0.35	8.3^b±0.35	7.3^c±0.25	6.5^d±0.40	3.51^e±0.25	2.65^f±0.15
			Sucrose			
Local -Red	24.4^a±0.4	8.20^b±0.20	7.53^c±0.31	7.2^c±0.2	6.20^d±0.20	6.0^d±0.2
Local -White	29.2^a±0.2	10.07^b±0.45	8.50^c±0.30	8.0^c±0.4	7.47^c±0.32	7.2^d±0.2
Mean±SD	26.8^a±0.3	9.13^b±0.32	8.00^c±0.30	7.6^c±0.3	6.83^d±0.26	6.6^d±0.2
			Total soluble sugars			
Local –Red	67.2^a±0.4	40.8^b±0.8	49.6^c±0.2	49.2^c±0.2	40.0^d±1.4	30.0^e±1.00
Local -White	56.4^a±0.3	50.8^b±0.6	50.0^b±0.8	49.2^b±0.4	42.2^c±0.4	23.2^d±0.30
Mean±SD	$61.^{8a}$±0.35	45.8^b±0.7	49.8^c±0.5	49.2^c±0.3	41.1^d±0.9	26.6^e±0.65

[a]Each value is the average of triplicate determinations and expressed as mean ± SD

Means in the same row with different letters are significantly different ($P < 0.05$).

SD, standard deviation

Effect of Soaking on a Mixture of 1% Salt Solution on Oligosaccharide Content

Results of soaking red gram seeds in a mixture of 1% salt solution ($NaCO_3$ + $NaHCO_3$ + NaCl) are summarized in Table 3. The mean decrease in raffinose family sugars was 70.20%, 67.08% and 53.43% for raffinose, stachyose and verbascose respectively. Soaking of red gram seeds also resulted in the loss of reducing sugars by 97.79%, 77.35% for sucrose and 75.93% for total soluble sugars respectively (Fig.1). The effect of soaking on the level of oligosaccharide content in soybean was investigated and upon soaking, the oligosaccharide losses were 0.68% when compared to initial oligosaccharide content of raw soybean[25] In another study effect of cooking, soaking, on raffinose family

Table 2: Effect of pressure cooking in 1% mango powder solution on the raffinose family sugars, reducing, non-reducing and total soluble sugars in red gram (gKg^{-1} dry basis)[a]

		Pressure cooking in 1% mango powder solution (min)		
Variety	**Raw**	**5**	**10**	**15**
		Raffinose		
Local -Red	12.6[a]±0.30	4.80[b]±0.6	4.60[b]±0.60	4.13[b]±0.64
Local - White	15.8[a]±0.60	4.87[b]±0.6	4.50[b]±0.35	4.20[b]±0.20
Mean ± SD	14.2[a]±0.45	4.83[b]±0.6	4.55[b]±0.45	4.17[b]±0.42
		Stachyose		
Local - Red	17.2[a]±0.4	6.90[b]±0.40	6.2[c]±0.17	6.10[c]±0.10
Local -White	17.8[a]±0.8	7.06[b]±0.30	6.8[b]±0.20	6.10[c]±0.10
Mean ± SD	17.5[a]±0.6	7.25[b]±0.35	6.5[c]±0.18	6.10[c]±0.10
		Verbascose		
Local- Red	48.0[a]±40	40.0[b]±1.0	36[b]±4	26[c]±2.0
Local -White	51.0[a]±20	37.0[b]±2.0	36[b]±2	26[c]±1.0
Mean±SD	49.5[a]±2.5	38.5[b]±1.5	36[b]±3	26[c]±1.5
		Reducing sugar		
Local - Red	8.6[a]±0.40	4.70[b]±0.2	3.50[c]±0.3	2.80[d]±0.20
Local - White	9.6[a]±0.30	6.40[b]±0.4	5.40[c]±0.3	3.50[d]±0.30
Mean ± SD	9.1[a]±0.35	5.55[b]±0.3	4.45[c]±0.3	3.15[d]±0.25
		Sucrose		
Local -Red	24.4[a]±0.4	8.9[b]±0.20	8.3[c]±0.30	7.9[d]±0.3
Local - White	29.2[a]±0.2	16.9[b]±0.90	10.1[c]±0.20	8.1[d]±0.1
Mean ± SD	26.8[a]±0.3	12.9[b]±0.55	9.2[c]±0.25	8.0[d]±0.2
		Total soluble sugars		
Local -Red	67.2[a]±0.4	50.8[b]±0.6	40.0[c]±0.5	28.4[d]±0.2
Local -White	56.4[a]±0.3	49.6[b]±0.4	41.6[c]±0.3	20.4[d]±0.4
Mean ± SD	61.8[a]±0.35	50.2[b]±0.5	40.8[c]±0.4	24.4[d]±0.3

[a]Each value is the average of triplicate determinations and expressed as mean ± SD

Means in the same row with different letters are significantly different ($P < 0.05$).

SD- Standard Deviation

oligosaccharide content was studied in lentil and chick pea. The processing resulted in a reduction of soluble carbohydrates of lentil and chick pea by 85% and 57% respectively. Processing of legume flours also exhibited low levels of α-Galactosides.[26] Hithamani and Srinivasan,[27] have studied the effect of soaking on the polyphenol content and bio-accessibility in finger millet and pearl millet. Soaking of mucuna monosperma seed resulted in the low content of oligosaccgarides. Soaking followed by autoclaving of the mucuna pruriens var. utilis in sodium bicarbonate solution was shown to have maximal reduction of oligosaccharides.[28]

Effect of Treatment with α- galactosidase on Red Gram (*Cajanus cajan*, L) flour

When red gram flour treated with crude α- galactosidase enzyme from guar seeds reduced raffinose family oligosaccharides content (Table 4). The enzyme treatment resulted in a reduction of

Table 3: Effect of soaking in mixture of 1% sodium salt ($NaCO_3$ + $NaHCO_3$ + NaCl) solution on the raffinose family sugars, reducing, non-reducing and total soluble sugars in red gram (gKg^{-1}dry basis)[a]

		Soaking in mixture of 1% ($NaCO_3$ + $NaHCO_3$ + NaCl) solution			
Variety	Raw	4	8	12	16
		Raffinose			
Local- Red	12.6^a±0.3	7.6^b±0.5	6.8^b±0.2	4.40^c±0.3	4.0^c±0.4
Local - White	15.8^a±0.6	7.6^b±0.1	6.2^c±0.3	4.83^d±0.31	4.4^c±0.3
Mean ± SD	14.2^a±0.45	7.6^b±0.3	6.5^c±0.25	4.62^d±0.3	4.2^d±0.35
		Stachyose			
Local- Red	17.2^a±0.4	10.4^b±0.4	10.6^b±0.3	9.0^b±0.3	3.2^c±0.4
Local - White	17.8^a±0.8	13.4^b±0.2	11.2^c±0.2	7.4^d±0.5	8.4^d±0.4
Mean ± SD	17.5^a±0.6	10.4^b±0.4	10.6^b±0.3	9.0^c±0.3	3.2^d±0.4
		Verbascose			
Local - Red	48.0^a±4	30^b±2.0	28^b±3.0	26^b±3.0	24^b±1.0
Local -White	51.0^a±2	36^b±3.0	34^b±2.0	30^c±2.0	22^d±2.0
Mean±SD	49.5^a±3	33^b±2.5	31^b±2.5	28^b±2.5	23^c±1.5
		Reducing sugar			
Local - Red	8.6^a±0.40	0.4^b±0.10	0.4^b ±0.10	0.2^c±0.0	0.2^c±0.0
Local - white	9.6^a±0.30	1.0^b±0.20	0.8^b±0.20	0.2^c±0.0	0.2^c±0.0
Mean±SD	9.1^a±0.35	0.7^b±0.15	0.6^b±0.15	0.2^c±0.0	0.2^c±0.0
		Sucrose			
Local - Red	24.4^a±0.4	10.0^b±1.0	8^c±0.20	8.8^d±0.4	5.2^e±0.2
Local -White	29.2^a±0.2	11.2^b±0.4	8.4^c±0.4	10.0^d±0.1	7.0^e±0.4
Mean±SD	26.8^a±0.3	10.6^b±0.7	8.2^c±0.30	9.4^d±0.7	6.1^e±0.3
		Total soluble sugars			
Local - Red	67.2^a±0.40	18.8^b±0.4	20.0^b±2.0	14.0^c±1.0	12.8^c±0.4
Local - White	56.4^a±0.30	21.6^b±0.6	22.4^b±0.4	21.6^b±0.2	16.4^c±0.4
Mean±SD	61.8^a±0.35	20.2^b±0.5	21.2^b±1.2	17.8^c±0.6	14.6^d±0.4

[a]Each value is the average of triplicate determinations and expressed as mean ± SD
Means in the same row with different letters are significantly different ($P < 0.05$).
SD- Standard Deviation

raffinose, stachyose and verbascose by 67.18%, 65.68% and 56.55% respectively. Treatment of red gram flour also demonstrated incomplete hydrolysis of raffinose family oligosaccharides as shown in Fig. 2. Crude α- galactosidase treatment have also resulted in the loss of sucrose by 80.09%, reducing sugars by 69.13%, and total soluble sugars by 75.15%.

In a study oligosaccharides were quantified in eight legume species using mass spectrometry. Among the legumes tested cow pea was observed to be a potent source of oligosaccharides.[29] Effect of soaking followed by cooking and partly purified α-galactosidase from guar seeds on the oligosaccharide content of Jack bean, Sword beans was investigated and proved that such enzymatic treatment will complement the usage of these beans in human nutrition.[30] Zhang W *et al.,*[31] have reported the complete hydrolysis of raffinose and stachyose to galactose within 6 hours time period at 50^{0}C by α-Galactosidase enzyme from Termitomyces eurrhizus. Zhang B *et al.,*[32] have reported the effect of α-Galactosidase treatment on energy metabolism for broilers fed on corn- soybean meal diet.

Conclusion

The present study determined that the processing methods substantially reduce raffinose family oligosaccharides content of pulses. Soaking in 1% salt mixture reduced the flatus inducing oligosaccharides of red gram seeds. Crude enzymes

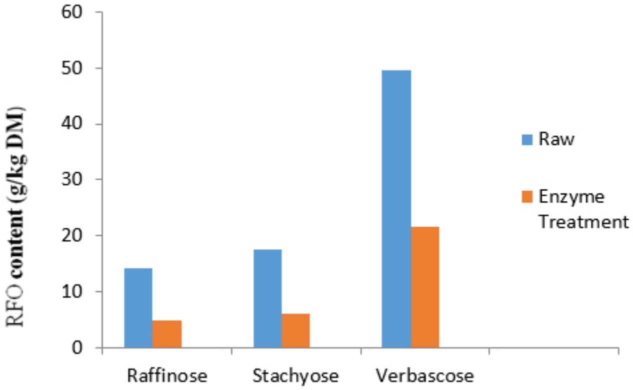

Fig. 1: Effect of crude α-galactosidase treatment on oligosaccharide content of red gram flour for 3 hrs

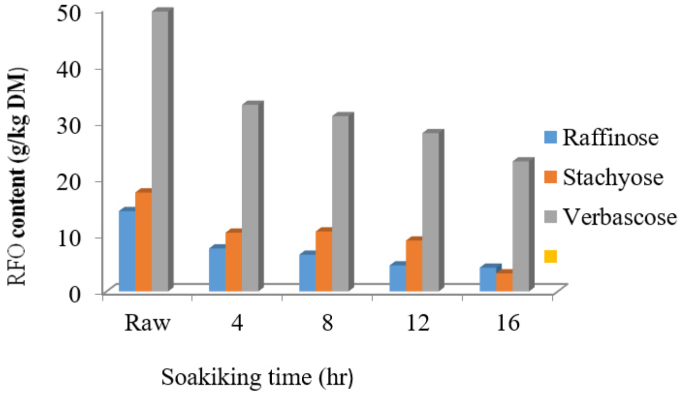

Fig. 1: Effect of soaking in mixture of 1% ($NaCO_3$ & $NaHCO_3$ &NaCl) on Raffinose family oligosaccharide content

preparation from germinating guar seed treatment would seem to be a considerable potential approach to curb the flatus inducing oligosaccharides of red gram and other legume also. Use of all the domestic processing methods which are simple may further complement the usage of red gram as an economical source of micro and macro nutrients for meeting nutritional needs of developing countries.

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Conflict of Interest
The authors declare no conflict of interests.

Table 4: Effect of α-galactosidase enzyme treatment on raffinose family sugars, reducing, non reducing and total soluble sugars in red gram (gKg^{-1} dry basis)[a]

Variety	Raw	Enzyme treatment
	Raffinose	
Local -Red	12.6±0.30	4.80*±0.25
Local - white	15.8±0.60	4.90*±0.30
Mean ± SD	14.2±0.45	4.85*±0.27
	Stachyose	
Local - Red	17.2±0.4	6.2*±0.20
Local -White	17.8±0.80	5.8*±0.50
Mean ± SD	17.5±0.60	6.0*±0.35
	Verbascose	
Local - Red	48.0±4	21.0*±2
Local – White	51.0±2	22.0*±2
Mean ± SD	49.5±3	21.5*±2
	Reducing sugar	
Local -Red	8.6±0.40	2.8*±0.10
Local -White	9.6±0.30	2.8*±0.20
Mean ± SD	9.1±0.35	2.8*±0.15
	Sucrose	
Local - Red	24.4±0.4	5.2*±0.20
Local - White	29.2±0.2	5.4*±0.30
Mean ± SD	26.8±0.3	5.3*±0.25
	Total soluble sugars	
Local 1	67.2±0.40	16.0*±0.6
Local 2	56.4±0.30	14.6*±0.4
Mean ± SD	61.8±0.35	15.3*±0.5

[a]Each value is the average of triplicate determinations and expressed as mean ± SD
Means in the same row with different letters are significantly different ($P < 0.05$).
SD-Standard deviation

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