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# Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude α-galactosidase from *Streptomyces griseoloalbus*

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**Abstract**

The effectiveness of using crude extracellular α-galactosidase from *Streptomyces griseoloalbus* for the treatment of horse gram and green gram flours was investigated by comparing with traditional treatments such as soaking and cooking. The enzymatic treatment was most effective and the raffinose content in horse gram flour was reduced by 97.5% and stachyose content by 93.2%. The reduction in the raffinose content of green gram flour was 96.3% and that for stachyose was 91.8%. The information obtained from the present investigation is advantageous for the large-scale production of horse gram flour and green gram flour free of flatulence-causing oligosaccharides.


*Keywords:* Horse gram flour; Green gram flour; Flatulence; α-Galactosidase

## 1. Introduction

Legumes are widely grown throughout the world and their dietary and economic importance is globally appreciated and recognized. Legumes not only add variety to human diet, but also serve as an economical source of proteins, dietary fibres and a variety of micronutrients and phytochemicals, for a large human population in developing countries, e.g., India, where most of the population is vegetarian. Recently research efforts are being directed towards identifying and evaluating under-exploited legume food sources such as protein crops for the future, so as to meet the nutritional demands of an increasing population.

Horse gram (*Dolichos biflorus*) and green gram or mung bean (*Vigna radiata* L.) are among the most important food legumes grown and consumed in India. In addition to proteins, horse gram is a rich source of iron and molybdenum. Green gram is an excellent source of carbohydrates, proteins and minerals and its protein quality is similar to or better than other legumes, such as chickpea, black gram, peas, pigeonpea (Jood, Mehta, & Singh, 1986). Horse gram and green gram are consumed as whole seeds or sprouts by a large population in rural areas of southern India. Like other legumes, the utilization of horse gram and green gram for human nutrition is constrained by the presence of raffinose family oligosaccharides (RFO) which have a tendency to induce flatulence. The production of flatulence is regarded as being due to the lack of ability of the human intestinal tract to synthesize the enzyme α-galactosidase, which is necessary to hydrolyze oligosaccharides containing α-galactosidic linkages. The predominant RFO, raffinose and stachyose, are relatively large molecules and are hence not resorbed by the intestinal wall. The intact oligosaccharides therefore enter the lower intestine where they are metabolized by the microflora into carbon dioxide, hydrogen and, to a lesser extent, methane. It is the production of these gases which leads to the

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characteristic features of flatulence, namely nausea, cramps, diarrhea, abdominal rumbling, and the social discomfort associated with the ejection of rectal gas (Cristofaro, Mattu, & Wuhrmann, 1974). The problem area in the manufacture of protein foods is therefore the breakdown of RFO which are present in the leguminous seeds.

Many methods are being practised for the processing of legume seeds, such as soaking, cooking and sprouting (Mulimani & Devendra, 1998; Viana et al., 2005), which may bring about changes in the levels of RFO. The newly released high-yielding cultivars may not only have different grain quality characteristics, but also may behave differently from existing cultivars after processing and cooking. Of all the techniques proposed, the enzymatic processing by α-galactosidase has proved most effective (Mansour & Khalil, 1998; Viana et al., 2005). α-Galactosidase or melibiase (α-D-galactoside galactohydrolase, EC 3.2.1.22) is an exo-glycosidase that cleaves, the terminal non-reducing α-D-galactose residues from α-D-galactosides, including galactose oligosaccharides, such as melibiose, raffinose and stachyose, and branched polysaccharides, such as galactomannans and galacto (gluco) mannans (Naumoff, 2004). Currently, there is a lot of interest in the scientific community around the world, in exploiting novel microorganisms for the production of industrially important enzymes and actinomycetes which have immense potential as source of exo-enzymes are yet to be harnessed as source of α-galactosidase for commercial application. We have previously identified the filamentous actinobacterium, *Streptomyces griseoloalbus* as a novel source of α-galactosidase (Anisha & Prema, 2006) and the potential of this enzyme in soymilk hydrolysis has also been demonstrated (Anisha & Prema, 2007). The present study was aimed at evaluating the suitability of α-galactosidase from this novel source, for the reduction of RFO in horse gram and green gram flours. The effect of enzymatic treatment was compared with traditional techniques, such as soaking and cooking.

## 2. Materials and methods

### 2.1. Microorganism

*Streptomyces griseoloalbus* MTCC 7447 used in the study was isolated in our laboratory from a soil sample collected from mangrove regions along the West Coast of India. The organism was maintained at 4 °C on starch casein agar (SCA) slants and was sub-cultured fortnightly.

### 2.2. Production and extraction of crude α-galactosidase

Solid-state fermentation was carried out for the production of α-galactosidase from *S. griseoloalbus*. Inoculum was prepared by transferring a loopful of culture from fresh SCA slants into sterile medium (100 ml in 250 ml Erlenmeyer flask) composed of (g/l): locust bean gum, 10; yeast extract, 3; $(NH_4)_2HPO_4$, 3.03; $KH_2PO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.49; and 1 ml of trace elements solution. The trace elements solution was composed of (g/l): $FeSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.1 and $ZnSO_4 \cdot 7H_2O$, 0.1. The flasks were incubated at 30 °C on a rotary shaker at 175 rpm. A 48 h old culture containing $3 \times 10^6$ CFU/ml was used as the inoculum. For solid-state fermentation, 10 g of soybean flour, taken in a 250 ml Erlenmeyer flask, was moistened with mineral salt solution (g/l: $KH_2PO_4$, 1; $MgSO_4$, 0.4; pH 7.0), thoroughly mixed and autoclaved at 121 °C for 30 min. The cooled medium was inoculated with $2.25 \times 10^7$ CFU of inoculum and incubated at 30 °C for 5 days. The final moisture content of the medium was 40%.

Enzyme extraction was carried out by mixing the fermented matter with distilled water (1:5, w/v) on a rotary shaker at 200 rpm for 1 h. The thoroughly agitated fermented matter was then filtered through muslin cloth and the filtrate obtained was centrifuged at 10000 rpm and 4 °C for 20 min. The resultant supernatant was used as the enzyme preparation.

### 2.3. Enzyme assay

The activity of α-galactosidase was routinely determined according to the method of Dey and Pridham (1969) using *p*-nitrophenyl α-D-galactopyranoside (*p*NPG), with minor modifications. The *p*NPG hydrolyzing activity was estimated by incubating 100 μl of enzyme with 50 μl of 2 mM *p*NPG and 850 μl of 0.1 M McIlvaine buffer (Citrate–$Na_2HPO_4$, pH 7.0) at 55 °C for 10 min. The reaction was terminated by the addition of 2 ml of 1 M sodium carbonate. The *p*-nitrophenol released was estimated spectrophotometrically by absorbance at 400 nm. One unit (U) of enzyme activity was expressed as the amount of enzyme that liberated 1 μmol of *p*-nitrophenol/min under the assay conditions.

### 2.4. Processing of legume seeds

#### 2.4.1. Soaking

Dry whole seeds of horse gram and green gram, purchased from the local market, were soaked in distilled water (1:10, w/v) for 12 h at room temperature. After 12 h, the water was drained off and the soaked seeds were washed three times with distilled water.

#### 2.4.2. Cooking

Whole horse gram and green gram seeds were cooked in distilled water (1:10, w/v) on a hot plate for 60 min. After cooking, the seeds were rinsed three times with distilled water.

#### 2.4.3. α-Galactosidase treatment

Five grammes of horse gram and green gram seed flour, which passes through a 500 μm sieve, were treated with 1 ml of α-galactosidase (40 U) diluted to 50 ml with 0.1 M McIlvaine buffer (pH 7.0), in a rotary shaker at 120 rpm and 55 °C for 2 h. After incubation, the treated seed flour samples were filtered through a Whatman No.

1 filter paper, dried and the oligosaccharide content was determined. For control, the volume of enzyme was replaced with an equal volume of buffer.

#### 2.4.4. Determination of oligosaccharide content

The raffinose oligosaccharides were extracted by treating 5 g of seed flour sample with 50 ml of 70% ethanol (v/v) in a rotary shaker at 120 rpm for 12 h. The alcoholic extract obtained after filtration through Whatman No. 1 filter paper was concentrated under vacuum at 40 °C in a rotary evaporator. The concentrated sugar syrup was made up to 10 ml with distilled water. Ten microlitres each of the sugar extract were applied to silica gel G plates (20 × 20 cm) and developed by ascending chromatography, using *n*-propanol:ethyl acetate:water (6:1:3, v/v) as the solvent system (Tanaka, Thananunkul, Lee, & Chichester, 1975). The sugar spots were located by keeping the plates in an oven at 140 °C for 5 min after spraying with 1% α-naphthol in absolute ethanol containing 10% of *ortho*-phosphoric acid (Albon & Gross, 1950). For quantitative determination, the area (2 × 2 cm) corresponding to each oligosaccharide spot was scraped from unsprayed duplicate plates and eluted with 3 ml distilled water for 12 h. The mixture was centrifuged to remove silica gel and 1 ml of the supernatant was used for the estimation of oligosaccharides by the method of Tanaka et al. (1975).

### 2.5. Analytical procedures

Total soluble sugars in the concentrated sugar syrup were estimated by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The reducing sugars were estimated by the method of Nelson (1944).

### 2.6. Statistical analysis

All experiments were carried out in triplicate to check the reproducibility of results. The data presented here are the averages of triplicate determinations and the standard deviations for all the values were $<\pm 5\%$.

## 3. Results and discussion

The levels of RFO in raw horse gram and green gram flour samples are presented in Table 1. The results showed that green gram contained more RFO than did horse gram and the concentration of stachyose was highest in both horse gram and green gram. The relative levels of raffinose and stachyose obtained in our study were in accordance with those presented by other workers (Adsule, Kadam, & Salunkhe, 1986; Rathbone, 1980).

Table 1
Oligosaccharide content of raw horse gram and green gram

| Seed flour sample | Total soluble sugars (g/kg DM[a]) | Raffinose (g/kg DM[a]) | Stachyose (g/kg DM[a]) |
|---|---|---|---|
| Horse gram | 28.9 ± 0.2 | 6.8 ± 0.15 | 19.4 ± 0.17 |
| Green gram | 59.2 ± 0.25 | 16.5 ± 0.11 | 27.5 ± 0.26 |

The data are means and standard errors of three independent samples with triplicate determinations.
[a] Dry matter.

### 3.1. Effect of soaking

The reduction of RFO in dry whole seeds of horse gram and green gram by various treatments is given in Figs. 1 and 2, respectively. Soaking of dry whole seeds of horse gram in distilled water for 12 h resulted in mean reductions of raffinose content by 23.8% and stachyose content by 12.3%. For green gram flour samples, the reduction of raffinose content was 19% and that of stachyose was 10%. The reduction of raffinose content was higher than that of stachyose content in both the cases. The sucrose content of both the seed flour samples decreased after soaking (Table 2). Mulimani, Thippeswamy, and Ramalingam (1997) have reported that soaking of whole soybean seeds led to a mean decrease of 80.3% for raffinose and 44.8% for stachyose. Reduction in raffinose and stachyose contents of red gram flour by 54.6% and 55.4%, respectively, was reported by Mulimani and Devendra (1998). Reduction of RFO in cow pea seeds by soaking was reported by Somiari and Balogh (1993). Leaching could be one of the reasons for the reduction of the raffinose family of

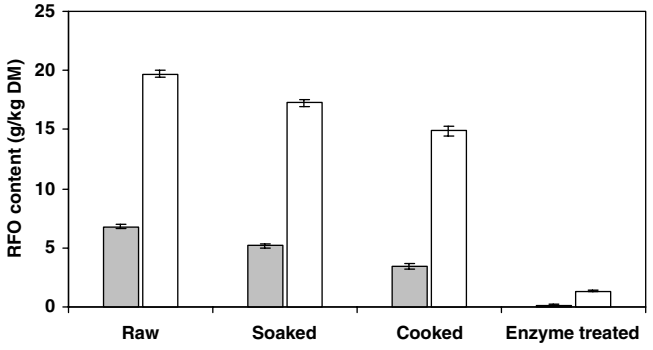


Fig. 1. Raffinose ▩ and stachyose □ contents of raw, soaked, cooked and enzyme-treated horse gram flour.

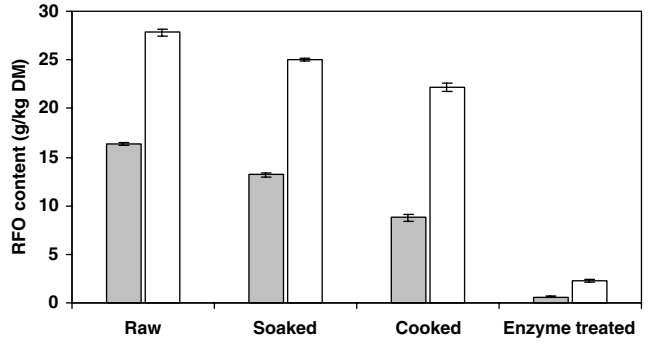


Fig. 2. Raffinose ▩ and stachyose □ contents of raw, soaked, cooked and enzyme-treated green gram flour.

Table 2
Sucrose content of horse gram and green gram before and after various treatments

| Treatments | Sucrose (g/kg DM[a]) | |
|---|---|---|
| | Horse gram | Green gram |
| Raw | 2.3 ± 0.03 | 6.5 ± 0.04 |
| Soaked | 0.7 ± 0.02 | 2.0 ± 0.03 |
| Cooked | 5.1 ± 0.08 | 11.2 ± 0.17 |
| α-Galactosidase-treated | 8.9 ± 0.06 | 22.4 ± 0.1 |

The data are means and standard errors of three independent samples with triplicate determinations.
[a] Dry matter.

sugars during soaking (Price, Lewis, Wyatt, & Fenwick, 1988). Upadhyay and Garcia (1988) have demonstrated that the differential solubilities of individual sugars and their diffusion rates are the two factors that influence the sugar losses during soaking. The extent of reduction in level of oligosaccharides can be enhanced by increasing the soaking time and employing different soaking media (Pugalenthi, Siddhuraju, & Vadivel, 2006). However, the presence of off-odour in flours obtained from the legume seeds after soaking would affect the acceptability of such products (Somiari & Balogh, 1993).

### 3.2. Effect of cooking

Cooking brought about a greater reduction in the raffinose family sugars than did soaking (Figs. 1 and 2). Cooking of horse gram and green gram seeds for 60 min resulted in mean decreases of 49.6% and 46.3%, respectively, for raffinose and 24.3% and 20.1%, respectively, for stachyose. Mulimani et al. (1997) reported 52.3% removal of raffinose and 20.7% removal of stachyose from soybean seeds after cooking. Somiari and Balogh (1993) reported that cooking of cow pea for 50 min reduced the raffinose content by 44% and the stachyose by 28.6%. Onigbinde and Akinyele (1983) have proposed that decrease in the levels of raffinose and stachyose during cooking might be attributed to heat hydrolysis to disaccharides and monosaccharides or the formation of other compounds. In contrast, Rao and Belavady (1978) reported an increase in the level of oligosaccharides after cooking of pulses. The sucrose content of both horse gram and green gram increased after cooking (Table 2). This might be due to the breakdown of storage polysaccharides to sucrose, as reported by Onigbinde and Akinyele (1983).

Though cooking resulted in a decrease in the level of RFO, it affected the colour, texture and aroma of the seed flours. It is also reported that legumes, such as horse gram, require prolonged cooking to obtain products of acceptable nature (Kadam & Salunkhe, 1985). Price et al. (1988) have reported that treatments such as soaking and cooking could change the physicochemical properties of legumes. Moreover, soaking and cooking alone will not be sufficient to bring about any significant reduction in the flatulence-inducing activity of legumes (Price et al., 1988).

### 3.3. Effect of crude α-galactosidase treatment

Horse gram flour, when treated with α-galactosidase, resulted in a reduction of raffinose content by 97.5% and stachyose content by 93.2% (Figs. 1 and 2). The enzyme treatment of green gram samples resulted in 96.3% and 91.8% reductions of raffinose and stachyose, respectively. On the other hand, no reduction of RFO was observed in the control. The sucrose content of the enzyme-treated seed flour samples was higher than that of the soaked and cooked samples (Table 2). The reduction in RFO by crude α-galactosidase treatment was due to the conversion of oligosaccharides into di and monosaccharides by the hydrolysis of α-galactosidic linkages between the sugar molecules. The increase in sucrose content could be due to the formation of sucrose through raffinose hydrolysis. The crude α-galactosidase extracts from *S. griseoloalbus* markedly reduced the levels of raffinose and stachyose in horse gram and green gram flours.

There are several reports available in the literature of the use of α-galactosidase from fungal and plant sources for the removal of the RFO from soymilk and legume flours. Somiari and Balogh (1993) have used crude preparations of α-galactosidase from *Aspergillus niger* for the removal of raffinose and stachyose present in cowpea flours. Mansour and Khalil (1998) have reported 100% reduction of raffinose oligosaccharide content in chickpea flours by crude fungal α-galactosidase treatment. Mulimani et al. (1997) have used crude α-galactosidase from germinating guar seeds for the hydrolysis of galactooligosaccharides in soybean flour and have reported 90.4% reduction of raffinose and 91.9% reduction of stachyose.

## 4. Conclusions

The crude α-galactosidase extracts from *S. griseoloalbus* were clearly more effective for reducing the levels of raffinose and stachyose in legume seed flours than were the conventional methods, soaking and cooking. Crude enzyme treatments would seem to have the greatest potential as the technique for controlling flatulence-inducing activity of horse gram, green gram and other legume seed flours. This is the first report documenting the suitability of using *Streptomycete*-α-galactosidase for the treatment of legume flours. Although the results suggest that α-galactosidase from *S. griseoloalbus* has a great potential in the treatment of legume flours, safety, palatability, functionality and storage properties of enzyme-treated flours have to be determined before they can be commercialized. Adoption of effective processing methods may further enhance the utilization of horse gram and green gram as potential sources of proteins, especially among the economically weaker section of people in developing countries.

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