Chapter III

Enzyme technology for reduction of raffinose family oligosaccharides of black gram

3.1. INTRODUCTION

Legumes serve as the main source of proteins and calories because the animal proteins are too expensive and not readily accepted. Pulse proteins, thus, demand more attention in view of their important function as agents to avert protein malnutrition. Raw legumes are not acceptable as food as because of the antinutritional factors and toxic factors (Reddy et al., 1982).

Most legume seeds store raffinose family of oligosaccharides as reserve in storage organs (endosperm and cotyledons). Typically raffinose oligosaccharides are among the first reserves to be mobilized often before the radicle has emerged from the seed. The key enzyme for the degradation of raffinose family of oligosaccharides is $\alpha$-galactosidase,
which is widespread occurrence in seeds (McCleary, 1988). Certain legumes predominantly contain particular raffinose family of oligosaccharides in higher amounts than others. For instance, verbascose is the major oligosaccharide in black gram, bengal gram, red gram etc. and stachyose is the major oligosaccharides in soybeans, cowpeas, lentils etc. Raffinose is present in moderate to low amounts in most legumes. Ajugose is the other higher molecular weight oligosaccharide of raffinose family of sugars (Reddy et al., 1984).

Legumes are currently interest to the public because they provide high nutrient levels without supplying excessive calories. Flour and concentrates from legumes other than soybeans is also receiving commercial attention. Major constraints in the commercialization of legume products are the presence of flatulence causing raffinose family oligosaccharides.

The raffinose families of oligosaccharides are not digested by human due to the absence of α-galactosidase in the intestinal juice. These oligosaccharides when they reach the lower intestine are digested by bacteria and lead to flatus production (release of CO₂, H₂ and CH₄ gases) (Richard and Steggerda, 1966).

Traditional processing practices have been followed for many years to convert grain legumes into the consumable forms. Such processes not only improve the digestibility and palatability of food legumes but also help to remove deleterious effects of some antinutritional constituents. For human consumption, legumes are processed by various methods like parching, puffing including soaking, sprouting, boiling fermentation etc. depending upon tradition and taste preferences. Domestic processing has been known
to improve nutritional quality of the legumes by increasing the protein digestibility as well as by reducing antinutrients (Reddy et al., 1982).

Unfortunately none of the above methods were able to eliminate sugars of the raffinose family completely (Singh, 1988). α-Galactosidase from microbial sources offers a promising solution in the elimination of such sugars in legume flours (Reddy et al., 1984). In this content, treatment of soymilk and other legume flour with α-galactosidase seems to be appropriate technology for the total elimination of raffinose family of oligosaccharides.

There are few reports available in the literature of the use of α-galactosidase from plant, bacteria and fungal sources for the reduction of raffinose family sugars from legume flour (Thananunkul et al., 1976; Shivanna et al., 1989; Somiari and Balogh, 1993; Mansour and Khalil, 1998).

Information regarding raffinose family oligosaccharides such as raffinose, stachyose, verbascose, ajugose and various processing methods on the levels of oligosaccharides in newly developed cultivars of black gram are not available. In the present study an attempt has been made to reduce raffinose oligosaccharides by various processing methods and the use of α-galactosidase for the hydrolysis of raffinose family oligosaccharides in black gram flour.
3.2. MATERIALS AND METHODS

3.2.1. Seed sample collection

Back gram seeds were collected from Baba Atomic Research Centre, Trombay, Mumbai. Agricultural Research Station, Gulbarga, India, Indian Institute of Pulse Research, Kanpur and local varieties were collected from local market, Gulbarga. The seeds were handsorted to remove foreign material then stored in polythene bags in the refrigerator until used.

3.2.2. Standard calibration curve of RFO

A standard calibration curve of raffinose, stachyose, verbascose and ajugose were constructed according to the method of Tanaka et al., (1975). (Figure. 3.1, 3.2, 3.3, 3.4). Standard solutions were prepared with a concentration of 1 mg/ml of distilled water. Working standard solution (100 μg/ml) were prepared by diluting 10 ml of standard solution to 100 ml. Different volumes of solutions 0.2, 0.4, 0.6, 0.8, and 1 ml taken in a series of test tubes and the volume was made up to 1 ml with distilled water. Then 1 ml of 0.2 M thiobarbituric acid and 1ml of concentrated hydrochloric acid was added and tubes were kept in a boiling water bath exactly for 6 minutes. Soon after the reaction is over, the test tubes were kept in ice-cold water. The optical density of yellow colour formed was measured at 432.5 nm against blank in a spectrophotometer (Elico Ltd India).

3.2.3. Standard calibration curve of sucrose

A standard calibration curve of sucrose was constructed according to the method of Tanaka et al., (1975) (Figure.3.5). Standard sucrose solution was prepared with a concentration of 1 mg/ml of distilled water. Working standard solution (100 μg/ml) were prepared by diluting 10 ml of standard solution to 100 ml. Different volumes of solutions
0.2, 0.4, 0.6, 0.8, and 1 ml taken in a series of test tubes and the volume was made up to 1 ml with distilled water. Then 1 ml of 0.2 M thiobarbituric acid and 1 ml of concentrated hydrochloric acid was added and tubes were kept in a boiling water bath exactly for 6 minutes. Soon after the reaction is over, the test tubes were kept in ice-cold water. The optical density of yellow colour formed was measured at 432.5 nm against blank in a spectrophotometer (Elico India).

3.2.4. Extraction of oligosaccharides from black gram

Seed material was milled to flour and passed through a 400-µm sieve. Five gram of flour was added to an 250-ml Erlenmeyer flask containing 50 ml of 70% ethanol (v/v) and placed on an orbital shaker at 130 rpm for 13 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 below 50°C.

3.2.5. Assay of oligosaccharides

The residue was dissolved in 5 ml of distilled water. 10µl of the above syrup was spotted in triplicate on chromatographic plates (19x19 cm) coated with cellulose G powder (Acme synthetics, Bombay). The plates were kept in a chromatographic chamber containing the solvent system n-propanol:ethylacetate:water (6:1:3). The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugar spots by the method of Albon and Gross (1950). For quantitative estimation, the area (2x3 cm) corresponding to each oligosaccharide was scrapped and soaked in 3 ml of distilled water for 12 h. After 12 h, it was filtered through
Whatman No.1 filter paper and the oligosaccharides in 1ml of filtrate were estimated by the method of Tanaka et al., (1975).

To 1 ml of above filtrate, 1 ml of 0.02M thiobarbituric acid and 1 ml of conc.HCl was added. The reaction mixture was incubated in boiling water bath exactly for 6 min and cooled in ice-cold water. The yellow color produced was measured spectrophotometrically at 432.5 nm. The concentration of sugar/oligosaccharides was calculated from corresponding standard calibration curves.

3.2.6. Separation of oligosaccharides by HPLC

HPLC analysis was performed with Shimadzu (Shimadzu Corporation, Japan) equipment as described in the chapter II, Page No.45.

3.2.7. Processing of RFO of black gram by traditional methods

3.2.7A. Soaking

Hundred grams of black gram seeds were soaked in 1 liter of water for 4, 8, 12 and 16h at room temperature (37±2°C). At 4 h time interval 5 g of sample was removed and the soaking water was decanted, and the soaked seeds washed thoroughly with 500 ml water and treated with 1 liter water. 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 mentioned above.

3.2.7B. Cooking

Hundred grams of different varieties of black gram seeds were cooked in 1 liter of distilled water for 20, 30, 40, 50, 60 min on a hot plate. After cooking, the seeds were rinsed with 500 ml of distilled water, mashed, dried and the oligosaccharide content in the flour was determined as mentioned above.
3.2.8. Use of soluble $\alpha$-galactosidase from microbial source *Aspergillus oryzae*

3.2.8A. Medium and cultural conditions

*Aspergillus oryzae* capable of producing extracellular $\alpha$-galactosidase was isolated by Prashanth and Mulimani (2005). Submerged fermentation was carried out in Erlenmeyer flasks (250 ml) at 37$^\circ$C in basal media with guar gum (1.5%) as carbon source and peptone (6.0 g/l) ammonium sulphate (1.5 g/l), KH$_2$PO$_4$ (6 g/l) was used and pH adjusted to 5.5. After autoclaving, flasks were cooled to room temperature and inoculated with spores (2 x $10^6$) of *Aspergillus oryzae*. The flasks were incubated on an orbital shaker at 120 rpm. On the 5$^{th}$ day mycelium was separated from cultural broth by filtration through Whatman No.1 filter paper and filtrate was used as crude $\alpha$-galactosidase.

3.2.8B. Properties of $\alpha$-galactosidase

The effects of pH and temperature on the relative rates of hydrolysis of p-nitrophenyl-$\alpha$-D-galactopyranoside (PNPG) by $\alpha$-galactosidase was determined at pH and temperature values ranging from 3.5 to 6.5 and 5-65$^\circ$C respectively.

3.2.8C. Processing of black gram flour with $\alpha$-galactosidase from *A. oryzae*

Enzyme Treatment

Five grams flour (fraction which passes 400 $\mu$m sieve) of each cultivar was treated with 15 ml of crude enzyme of $\alpha$-galactosidase containing 0.45 U/ml. For the control, 15 ml of enzyme solution was replaced with 15 ml buffer. The treatment was carried out at 50$^\circ$C for 1, 2 and 3 h of incubation; the contents were removed at different incubation periods, dried at 80$^\circ$C for 24 h and quantified as mentioned above. The flow chart of treatment of black gram with crude $\alpha$-galactosidase is shown below.
Whole black gram
↓
Milling
↓
5 gm sample, which passes 400µm aperture
↓
Crude $\alpha$-galactosidase treatment
↓
Made paste
↓
Kept for incubation at 50°C for 3 hours
↓
Sample removed at 1, 2 and 3 hours
↓
Drying 80°C for 36 h
↓
Fine milling
↓
Enzyme treated black gram flour

3.2.9. Use of $\alpha$-galactosidase from plant source Cassia sericea

3.2.9A. Preparation of $\alpha$-galactosidase

*Cassia sericea* pods were collected from plants grown on the roadside and unused open grounds in Gulbarga city were used as source of $\alpha$-galactosidase. The seeds from the pods were removed and air-dried. The *C. sericea* seeds were surface sterilized by treating with 0.1% mercuric chloride solution for 15 min and the seeds were washed with distilled water, these seeds are placed on moistened filter paper and the seeds are closed with moistened filter paper germination was continued at 27°C for three days. After germination, seeds were homogenized with 0.2 M acetate buffer (pH 5.0) in a
homogenizer for 10 min of full speed. The homogenate was filtered through cloth and kept for few hours to settle down. The supernatant decanted and was centrifuged at 12,000xg at 30 min (Superspin, India). The supernatant was precipitated with ammonium sulfate, precipitate was subjected to centrifugation. Residue was dialyzed against 0.02 M acetate buffer pH 5.0 and was used as a source of enzyme.

3.2.9B. Properties of α-galactosidase from Cassia sericea

The effects of pH and temperature on the relative rates of hydrolysis of p-nitrophenyl-α-D-galactopyranoside (PNPG) by Cassia sericea α-galactosidase was determined at pH and temperature values ranging from 3.5 to 7.0 and 25-70°C respectively.

3.2.9C. Processing of black gram flour with α-galactosidase

5 g flour (fraction which passes 400 µm sieve) of each cultivar was treated with 15 ml of crude enzyme of α-galactosidase containing 0.45 U/ml. For the control, 15 ml of enzyme solution was replaced with 15 ml buffer. The treatment was carried out at 50°C for 1, 2 and 3 h of incubation, the contents were removed at different incubation periods, dried at 80°C for 36 h and quantified as above.

3.2.10. α-Galactosidase assay

α-Galactosidase was assayed by the method of Dey and Pridham (1972). One ml of reaction mixture contains 0.1 ml of suitably diluted enzyme + 0.8 ml of 0.2 M acetate buffer (pH 4.8) + 0.1 ml of 2.0 mM PNPG (p-nitrophenyl-α-D-galactopyranoside) was added and incubated at 50°C for 15 min. Adding 0.2 M Na2CO3 solution arrested the reaction and the absorbance was read at 405 nm in a spectrophotometer (Elico Ltd, India).
One unit of enzyme activity is defined as the amount of enzyme preparation required to liberate one μmol of $p$-nitrophenol from PNPG per minute under the standard assay conditions.

### 3.2.11. Statistical Analysis

Data were statistically analysed using the statistica program. Significant differences between mean values were calculated by analysis of variance (ANOVA) using Duncan’s multiple-range test at $P \leq 0.05$. 
3.3. RESULTS AND DISCUSSIONS

3.3.1. Oligosaccharides content in black gram cultivars

The levels of the raffinose family sugars and sucrose are presented in the table 3.2. From the table it is evident that BDU-2 variety showed highest content of ajugose among all cultivars and IP2K showed less content, whereas cultivars TAU-1 have higher levels of verbascose. BB local has lower concentration of verbascose. The levels of stachyose were highest in T-9 and the cultivar BB.local had the lowest concentration of stachyose. The cultivars TAU-1 had the higher levels of raffinose. The levels of verbascose, stachyose and raffinose observed in the present study are in the same range as reported in black gram by Reddy and Salunke (1980). Mulimani and Devindra (1998) reported oligosaccharides content in 14 cultivars of red gram. Rossi et al., (1984) have studied chemical composition, antinutritional evaluation and oligosaccharides content in 20 cultivars of yellow and black chickpea. The levels of sucrose and stachyose are lower when compared to the earlier report. This could be due to the difference in the cultivars studied and the specific methodology (thiobarbituric acid and conc. HCl) employed for the determination of sucrose, raffinose, stachyose and verbascose. Verbascose is the major oligosaccharide followed by the sucrose, stachyose, ajugose and raffinose. HPLC separation shows that retention times of standards were compared with sample peak. Verbascose was eluted at 20\textsuperscript{th} min and ajugose eluted at 31\textsuperscript{st} min.

3.3.2. Effect of Soaking

Soaking in water (bean to water ratio, 1:10) reduced the levels of raffinose family sugars in the cultivars to various extent (Table 3.3). Soaking led to a mean decrease of 41.66% raffinose, 47.61% stachyose, 28.48% verbascose and 26.82% ajugose in Local I
variety and 43.75% raffinose, 20.58% stachyose, 23.60% verbascose and 15.88% ajugose in Local II variety. It is also evident from table 3.3 that the levels of raffinose family sugars decreased with the increased duration of soaking. Mulimani et al., (1997) reported that soaking of whole soybean seeds for 16 h led to a mean decrease of 80.3% for raffinose and 44.8% stachyose. Mulimani and Devindra (1998) reported that the soaking led to the reduction of 54.6% of raffinose, 55.4% of stachyose, 33.3% verbascose in red gram. 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 also observed that the percent removal of raffinose sugars was higher with soaking in sodium bicarbonate solution than with soaking carried out in distilled water. Khokhar et al., (1996) have studied method of soaking of kesari dhal (Lathyrus sativus) by soaking in tap water, soaking in freshly boiled water and soaking in alkaline medium. Among the above methods, soaking in alkaline medium is effective in reduction of raffinose and stachyose in Lathyrus sativus (Khokhar et al., 1996).

Somiari and Balogh (1993) have shown that soaking of cowpea seeds in distilled water reduced the levels of the raffinose family sugars. Reduction of 26.2% for stachyose and 28% for raffinose were reported after 16 h soaking. Siddhuraju and Becker (2001) reported that a significant reduction of α-galactosides in white and black varieties of mucuna beans observed in soaking in tamarind pulp extract (36.1 and 27%), sodium bicarbonate solution (23.4 and 23.2%) and citric acid solution (32 and 20%). 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 solubility of the individual sugar
and diffusion rate is two factors that could influence the sugar loss during soaking. The diffusion rate in turn depends on the thickness and the permeability of the seed coat (Sidduraju and Becker, 2001).

3.3.3. Effect of Cooking

Cooking brought about a greater reduction in the levels of the raffinose oligosaccharides. Cooking for 60 min resulted in decrease of 100% raffinose, 76.19% stachyose, 36.39% verbascose and 60.97% ajugose in Local I variety and 100% of raffinose, 55.88% stachyose, 48.52% verbascose and 56.07% ajugose. From the table 3.3, it is also observed that the percent removal of the raffinose family sugars positively correlated with the cooking time. Somiari and Balogh (1993) reported that cooking of cowpea for 50 min reduced the raffinose content to 44% and stachyose 28.6%. Oligosaccharide composition of cooked *P. vulgaris* was not intensively affected by cooking time and although about 23% of loss has been observed. Mulimani *et al.*, (1997) reported that decrease in the levels of the raffinose family sugars after cooking of soybean for 60 min. They observed the percent reduction of raffinose for 52.3% and stachyose for 20.7%. Onigbinde and Akinyele (1983) have proposed that decrease 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. 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. Mansour and Khalil (1998) reported that traditional techniques reduced the raffinose content by 13.1-49.2% and stachyose content by 10.1-32.5%.
The variation may be due to the differences in the thickness and permeability of the seed coat between the two varieties. Sidduraju and Becker (2001) reported that loss of oligosaccharides from legumes during cooking is primarily due to leaching into the discarded cooking water.

3.3.4. Use of α-galactosidase from *A. oryzae* for the hydrolysis of raffinose family oligosaccharides

3.3.4A. Properties of *A. oryzae* α-galactosidase

The preparation of α-galactosidase from *A. oryzae* exhibited an optimum pH between 4.5 and 5.5 (Figure 3.6) and optimum temperature at 50 °C (Figure 3.7). Sugimoto and Van Buren (1970) reported that α-Galactosidase from *A. saitoi* showed optimum temperature at 55°C. The optimum temperature for most α-galactosidase is between 37-40°C reported by Ulezlo and Zaprometova (1982). α-Galactosidase from *A. nidulans* and *Humicola sp* showed optimal activity at 50°C and 65°C (Rios et al., 1993; Kotwal et al., 1995). Four major α-galactosidase forms from *A. niger* had optimal activity at 60°C reported by Ademark *et al.* (2001).

Optimum pH value of 4.8 for free α-galactosidase (Figure 3.6) is in agreement with findings of Cruz and Park (1982). α-Galactosidase produced from *A. awamori* and *A. saitoi* were used for the hydrolysis of oligosaccharides in soymilk and had an optimum pH of 5.0 and 5.5 respectively (Sugimoto and Van Buren, 1970; McGhee *et al*., 1978). α-Galactosidase produced by the genus *Aspergillus*, and indeed most fungi have pH optima within the range of 4.5 to 5.5 (Ademark *et al*., 2001; Civas *et al*., 1984; Rios *et al*., 1993; Kotwal *et al*., 1995; Ohtakara *et al*., 1984).
3.3.4B. Enzymatic treatment of black gram flour by \textit{A.oryzae} \(\alpha\)-galactosidase

Enzymatic treatment for 3 hours completely removed the galacto-oligosaccharides, as evidenced by TLC and HPLC (Figure. 3.8, Figure 3.9). The enzyme treatment to black gram flour found to be more effective than soaking and cooking (Table.3.3). There are several reports available in the literature of the use of \(\alpha\)-galactosidase from fungal sources for the removal of the raffinose family sugars from soymilk and legume flours (Somiari and Balogh, 1993; Mulimani and Ramalingam, 1995; Mulimani \textit{et al}., 1997). Somiari and Balogh (1993) have used crude preparations of \(\alpha\)-galactosidase from \textit{A. niger} for the removal of raffinose and stachyose present in cowpea flours. The enzyme treatment to flour was clearly more effective than soaking and cooking in the reduced levels of ajugose, verbascose, stachyose and raffinose of Local-I black gram (Figure 3.10). Enzyme treatment is also effective in Local-II black gram (Figure 3.11). Somiari and Balogh (1993) have shown that the reduction in raffinose of 82.3\% and stachyose of 93.3\% in cowpea flours treated with \(\alpha\)-galactosidase from \textit{A.niger}.

The principle advantage of using \textit{A. oryzae} is the acceptability of the organism for the production of food grade enzyme. It is listed as a ‘GRAS’ (Generally Regarded As Safe) organism by FDA (Food and Drug Administration), USA. (Reichelt, 1983).

3.3.5. Use of \(\alpha\)-galactosidase from \textit{Cassia sericea} for the hydrolysis of raffinose family oligosaccharides

3.3.5A. Properties of \textit{Cassia sericea} \(\alpha\)-galactosidase

The preparation of \(\alpha\)-galactosidase from \textit{Cassia sericea} exhibited an optimum pH 5.0 (Figure.3.12). Shivanna \textit{et al}., (1989) reported that \(\alpha\)-galactosidase from guar seeds showed the optimum pH at 5.0. Mulimani and Devindra (1998) reported that \(\alpha\)-
galactosidase from *Cassia sericea* showed the optimum pH between 5.0 and 5.5. 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*).

The enzyme showed maximum activity at the temperature at 45°C (Figure 3.13). Mulimani and Devindra (1998) reported that α-galactosidase from *C. sericea* showed maximum activity at temperature 45°C. Bhaskar *et al.*, (1990) have reported a temperature optimum of 50°C for two molecular forms of α-galactosidase from *C. sericea*.

**3.3.5B. Enzymatic treatment of black gram flour by Cassia sericea α-galactosidase**

Partially purified α-galactosidase from *C. sericea* seeds was found to be effective in reducing raffinose family sugars. α-Galactosidase treatment for 3 h completely hydrolyzed the galacto-oligosaccharides, as evidenced by HPLC (Figure 3.14). The enzyme treatment to flour could able to reduce raffinose oligosaccharides more effectively than soaking and cooking (Table 3.4). The α-galactosidase treatment from *C. sericea* seeds led to mean decrease of 93% raffinose family oligosaccharides. The enzyme treatment to flour was clearly more effective than soaking and cooking in the reduced levels of ajugose, verbascose, stachyose and raffinose of Local-I black gram (Figure 3.15). Enzyme treatment is also effective in Local-II black gram (Figure 3.16). The oligosaccharides raffinose, stachyose, verbascose and ajugose were almost completely degraded by α-galactosidase treatment.

The partially purified α-galactosidase treatment was clearly more effective than soaking, cooking in reducing ajugose, verbascose, stachyose and raffinose in black gram
flour. *C. sericea* seeds used in the study, a wasteland legume shrub, mainly grown on roadsides and wastelands to eradicate the pernicious weed, *Parthenium hysterophorus* (Shamsundar and Mahadevappa, 1986). Bhaskar *et al.*, (1990) have reported the partial purification and characterization of two molecular forms of $\alpha$-galactosidase from *C. sericea*. Shivanna *et al.*, (1989) have reported the use of $\alpha$-galactosidase from guar seeds for the removal of raffinose and stachyose present in soymilk. $\alpha$-Galactosidase from germinating guar seeds was used as source of enzyme for the decomposition of raffinose and stachyose present in soybean flour (Mulimani *et al.*, 1997). They could able reduce 90% of stachyose and 91% raffinose in local varieties of soybean.

The use of $\alpha$-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. Thus, $\alpha$-galactosidase from *C. sericea* has potential use in the food industry for production of black gram flour free from the flatulence-inducing raffinose family sugars. Plant crude enzyme treatment would seem to have the greatest potential as the technique to control to flatulence-inducing activity of red gram and probably other legume flours.

**IMPORTANCE OF WORK**
• Screening of raffinose family oligosaccharides in newly developed cultivars gives valuable information for growers, processors and consumers.

• α-Galactosidase from A.oryzae and Cassia sericea found to be effective in reducing raffinose family oligosaccharides compared to traditional methods.

• Since the source of α -galactosidase was from a GRAS organism i.e. Aspergillus oryzae, its application poses no problem.

• Cassia sericea grows on the roadside and seeds are the cheap source of α -galactosidase, used for the reduction of oligosaccharides in black gram flour.