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Research Article

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Enzymatic Treatment (α galactosidase) to Reduce Antinutrient Content in Raw Jackfruit Bulb Flour and Seed Flour

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ABSTRACT

Oligosaccharides are reported to be the cause of flatulence in jackfruit. With the aim of reducing these antinutritional factors, 'α galactosidase' enzyme was utilised to breakdown oligosaccharides to simpler compounds and hence reduce the indigestible factors. Koozha type jackfruit in their raw stage was selected. Bulbs and seeds were separated and processed into flour. Flours were made from dough to batter by adding water and subjected to enzymatic treatment. The enzyme 'α galactosidase' was premixed with the flour of jackfruit seed and jackfruit bulb separately in the ratio 1:100 and the moisture level was varied from 25 – 200% (dough to batter stage). The hydrolysis was carried out for 90 minutes in both jackfruit bulb flour and seed flour. The products were evaluated for the breakdown of oligosaccharides (Raffinose) using HPTLC method. The results showed that the level of Raffinose after treatment with bulb flour and seed flour was seen to decrease with increase in moisture content (25-100%). Thereafter the content slightly staggered and then reduced (125%, 150%, 175%, 200%). Overall the level of oligosaccharide decreased in comparison to levels in control (0.97 µg g^{-1}).

Key words: *Enzymatic treatment, α galactosidase, Oligosaccharides, Raffinose, Jackfruit bulb flour, Jackfruit seed flour, Moisture level*

INTRODUCTION

Anti-nutrients may occur naturally in plants as secondary metabolites, protecting plants from viral and fungal attack analogous to the immune system of animals. Anti-nutrients may also be produced in large amounts, as a direct result of some adverse environmental condition[7].

Robin and Ross[17] highlight that antinutrients are not something to be alarmed, since most foods typically have one or more antinutrients and that the issue is their concentration and type and whether that specific antinutrient profile will adversely affect health. All plants have some anti-nutrient properties, soybean plant is especially rich in these chemicals. If they are not removed by extensive preparation such as fermentation or soaking, soybeans can become one of the worst foods a person can eat[16].

Oligosaccharides of the raffinose-series (namely raffinose, verbascose and stachyose) are major components in many foods.

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Raffinose-series of oligosaccharides are not hydrolysed in the upper gut due to the absence of α-galactosidase. In the lower intestine they are metabolised by bacterial action, producing methane, hydrogen and carbon dioxide, which lead to flatulence and diarrhoea. Raffinose-series oligosaccharides are thus a major factor limiting the use of grain legumes in monogastric diets. Flatulence is a complaint even among healthy individuals and is one of the common causes of abdominal discomfort. It is also associated with dyspepsia, constipation and diarrhoea[14].

Raffinose family oligosaccharides (RFOs) are complex sugars containing chains of 'α galactose' which are unable to be digested in the human upper intestine, due to the absence of α galactosidase, the enzyme required to break the links in the α galactose chains. The RFOs therefore survive through to the lower intestine, where they encounter bacteria which use them as the substrate for fermentation. This produces methane, carbon dioxide and hydrogen gas, which can cause bloating, abdominal discomfort and excessive flatulence[2,3,10,18].

MATERIAL AND METHODS

Treatment with enzyme α galactosidase

The enzyme α-Galactosidase catalyzes the hydrolysis of α-1,6-linked galactoside residues in simple oligosaccharides, such as melibiose, raffinose, and stachyose and the polysaccharides of galactomannans and guar gum. It also acts on glycoconjugates, glycoproteins and glycosphingolipids and is known to catalyze transglycosylation reactions, especially with higher substrate concentrations[19]. Thus, interest in this enzyme stems from its potential biotechnological and medicinal applications. Currently, the most important industrial applications of α-galactosidase are related to the beet sugar industry, paper pulp industry, soya food processing and animal feed processing[15].

The enzyme α galactosidase was premixed with the dry flour of jackfruit seed and jackfruit bulb separately in the ratio 1:100[4]. The moisture level of the flour was varied from 25 – 200% (dough to batter stage). The hydrolysis was carried out for 90 minutes in both jackfruit bulb flour and seed flour. The products were evaluated for the breakdown of oligosaccharides. The details of variations in moisture content are explained below.

Statistical Design and treatment details

Experimental design : Completely randomized design (CRD)

Number of treatments : 8 x 2

Number of replications : 2

Treatments	Moisture level (%)
M_1	25
M_2	50
M_3	75
M_4	100
M_5	125
M_6	150
M_7	175
M_8	200

Edible Portion

E_1 Bulb (flour)

E_2 Seed (flour)

HPTLC estimation of Oligosaccharides (Raffinose µg g^{-1})

High Performance Thin Layer Chromatography (HPTLC) is a commonly used method for the analysis of food bioactive oligosaccharides because it is available in most of laboratories and does not require much technical support. This method is popular due to its simplicity and adaptability and the availability of the equipment.

The concentration of Oligosaccharides (Raffinose) were analysed for enzyme treated flours of jackfruit bulbs and seeds, through High Performance Thin Layer Chromatography (HPTLC) analysis.

Raffinose (reference standard of 99 per cent purity) was procured from Sigma – Aldrich Chemie GmbH (Aldrich Division; Steinheim, Federal Republic of Germany). Isopropanol (purity 99 per cent), methanol (purity 99 per cent), toluene(purity 99 per cent), and ammonia solution (95 per cent) were procured from Merck, India. All solvents were of HPLC grade and distilled water used was purified with Sartorius water purification unit (Arium 61315, made in USA).

Extraction of samples for HPTLC

Methanolic extract of the treated and pre treated jackfruit bulb flour and seed flour was obtained by collecting filtrate (Whatmann No.41 filter paper) of ten gram powder added with 25 ml methanol, shaken in a rotary evaporator (30 rpm) over night at room temperature.

Mobile Phase

Mobile phase selected for detection of oligosaccharide (Raffinose) was a mixture of isopropanol, methanol and toluene in the ratio of 8: 2: 4 and ammonia (1 drop), sonicated for 10 minutes.

Stock Solution of Oligosaccharide (Raffinose)

Stock solution of Raffinose (1000 µg ml^{-1}) was prepared by mixing 10 mg Raffinose reference standard and five millilitre methanol by thoroughly shaking it in a volumetric flask (10 ml) and making up to the mark using methanol.

Working Standard Solution of Oligosaccharide (Raffinose)

Working standard solution (1 to 30 µg ml^{-1}) was prepared by obtaining the aliquots (0.01 – 0.30 ml) from the stock solution of Raffinose and each of its volume made up to 10 ml with methanol.

Operating System Conditions

In HPTLC analysis, the samples were spotted in the form of bands of width six millimetre, which were 14 millimeter apart with a Camag microlitre syringe on pre- coated silica gel aluminium plate (60 F254 having 20x 10 cm).

Linear ascending development was carried out in 20x10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 20 minutes at room temperature. The length of chromatogram run was 80 mm. Densitometric scanning was performed on Camag TLC scanner III in the reflectance fluorescence mode at 254 nm, as well as 366 nm and was operated by CATS software (V3. Camag).

RESULTS AND DISCUSSION

Reduction of oligosaccharide (Raffinose) content in bulb flour

The oligosaccharide (Raffinose) content in pre treated and treated jackfruit bulb flour (JFBF) at different moisture levels differed customarily in the HPTLC assays. In HPTLC analysis, the retention factor of standared Raffinose was 0.58 minutes. The distance that each component of a mixture travels can be quantified using retention factors (Rf). The retention factor of a particular material is the ratio of the distance the spot moved above the origin to the distance the solvent front moved above the origin. Retention factor recorded by jackfruit bulb flour (control) and bulb flour with enzyme treated at varying moisture levels were 0.58, 0.58, 0.58, 0.58, 0.58, 0.57, 0.57, 0.57, 0.57 at 25%, 50%, 75%, 100%, 125%, 150%, 175%, 200% respectively (Table 1).

These values were compared to that of the standard Raffinose. Raffinose content in jackfruit bulb flour was 0.97in control (without treatment); while the amount in treated samples were in 0.89 in M_1, 0.85 in M_2, 0.80 in M_3, 0.71 in M_4, 0.74 in M_5, 0.83 in M_6, 0.75 in M_7, 0.82µg g^{-1} in M_8 respectively. The level of Raffinose after treatment was seen to decrease with increase in moisture content (25-100%). Thereafter the variation was not uniform (as 0.74 in M_5, 0.83 in M_6, 0.75 in M_7 and 0.82 in M_8) but the level of oligosaccharides decreased in comparison to control (0.97 µg g^{-1}), which indicates that the enzyme was effective in breakdown of oligosaccharides. The enzyme α galactosidase is reported to have the ability to hydrolyse stachyose and raffinose[1,5,9,8,12].

Reduction of oligosaccharide (Raffinose) content in seed flour

The oligosaccharide (Raffinose) content in pre treated and enzyme treated jackfruit seed flour (JFSF) at different moisture levels differed in HPTLC assay and the data is tabulated in Table 2. In the HPTLC analysis, the retention factor of standard Raffinose was 0.61 minutes. Retention factor recorded by pretreated (0.61 minutes) and treated jackfruit seed flour was compared to that of the standard Raffinose. Raffinose content in plain jackfruit bulb flour was 1.16 and 0.83, 0.68, 0.63, 0.56, 0.50, 0.58, 0.74 and 1.08 µg g^{-1} at 25%, 50%, 75%, 100%, 125%, 150%, 175%, 200% dilutions respectively. The level of Raffinose content in seed flour after treatment was seen to decrease with increase in moisture content (25-100%). Thereafter the variation was not uniform (125%, 150%, 175%, 200%). However it may be noted that the level of oligosaccharides decreased in comparison to control (1.16 µg g^{-1}), which indicates the asymptotic effect of enzyme on breakdown of oligosaccharides.

Guimaraes *et al.*[11], found that incubation of soymilk with α galactosidase for 8 hours at 30^0C resulted in a 73.3 per cent reduction in raffinose and a 40.6 per cent reduction in stachyose. In another study conducted by Matella *et al.*[13], it was found that incubation of black, red and navy beans with α galactosidase for 1 hour at 23^0C was more effective (30%-50% reduction) in the reduction of RFOs than soaking of beans for 5 hours at 23^0C (1%-35% reduction). Song and Chang[20] found that two hours of incubation with α galactosidase at 55-60^0C removed 100 per cent of RFOs in pinto beans. This is a significantly greater reduction, than was achieved by soaking followed by boiling for 90 minutes (54.2%).

Table 1: Quantification of Oligosaccharide (Raffinose) in jackfruit bulb flour at different moisture levels and enzyme treatment

Treatments (%)	Retention Factor	Area (AU)	Amount (µg/g^{-1})
Standard	0.58	6319.7	-
M_0 (control)	0.58	6178.0	0.97
M_1 25	0.58	5677.3	0.89
M_2 50	0.58	5394.8	0.85
M_3 75	0.58	5117.0	0.80
M_4 100	0.58	4500.9	0.71
M_5 125	0.57	4734.9	0.74
M_6 150	0.57	5307.7	0.83
M_7 175	0.57	4795.3	0.75
M_8 200	0.57	5185.9	0.82

Table 2: Quantification of Oligosaccharide (Raffinose) in jackfruit seed flour at different moisture levels and enzyme treatment

Treatments (%)	Retention Factor	Area (AU)	Amount (µg g^{-1})
Standard	0.61	5102.6	-
M_0 (control)	0.61	5963.0	1.16
M_1 25	0.61	4265.2	0.83
M_2 50	0.61	3510.5	0.68
M_3 75	0.61	3253.0	0.63
M_4 100	0.61	2866.2	0.56
M_5 125	0.61	2595.2	0.50
M_6 150	0.61	2986.6	0.58
M_7 175	0.61	3824.3	0.74
M_8 200	0.61	5557.7	1.08

Table 3: One way interaction effect of moisture levels and edible portion on raffinose content of jackfruit flours

Treatments	**Raffinose (μg g^{-1})**
Moisture level	
M_0	1.07
M_1	0.86
M_2	0.76
M_3	0.71
M_4	0.63
M_5	0.62
M_6	0.70
M_7	0.74
M_8	0.95
SEm $\pm$	0.001
CD	0.004
Edible portion	
E_1	0.82
E_2	0.75
SEm $\pm$	0.001
CD	0.002

Table 3. shows the effect of varying moisture levels and edible portion on raffinose content (2 way interaction). From this table it is observed that the raffinose content was higher in control samples of jackfruit bulb flour and seed flour (1.07 μg/g^{-1}). The lowest raffinose content was observed in treatment M_5 (125% moisture level). With respect to edible portion, E_1 (Bulb flour) contained 0.82 μg $g^{-1}$and E_2 (Seed flour) contained 0.75 μg g^{-1}. Dey and Pridham[6] observed that generally α-galactosidase may hydrolyze a variety of simple α-d-galactosides as well as more complex molecules, such as oligosaccharides and polysaccharides. α-Galactosidase (α-galactoside galactohydrolase EC 3.2.1.22) is widely distributed in microorganisms, plants and animals.

Table 4: Two way interaction effect of moisture level and edible portion on raffinose content

Treatments	**Raffinose (μg g^{-1})**
M_0E_1	0.97
M_0E_2	1.16
M_1E_1	0.89
M_1E_2	0.83
M_2E_1	0.85
M_2E_2	0.68
M_3E_1	0.80
M_3E_2	0.63
M_4E_1	0.71
M_4E_2	0.56
M_5E_1	0.74
M_5E_2	0.50
M_6E_1	0.83
M_6E_2	0.58
M_7E_1	0.75
M_7E_2	0.74
M_8E_1	0.82
M_8E_2	1.08
SEm $\pm$	0.002
CD	0.005

Table 4 shows the two way interaction effect of varying moisture level and edible portion on raffinose content. Here it is observed that M_0E_2 (Control seed flour) contained highest level of raffinose content (1.16 µg g^{-1}), the lowest raffinose content was observed in M_5E_2 (125% moisture level of seed flour), which contained 0.50 µg g^{-1}. Brain[4] reported the dependence of enzyme α galactosidase activity on the moisture content of the system with a steady increase in the proportion of both stachyose and raffinose hydrolysed with increasing moisture content. The results also showed a steady decrease in the concentration of sucrose and a steady increase in the concentration of galactose as moisture content increased. Sucrose and galactose are the hydrolysis products of stachyose and raffinose.

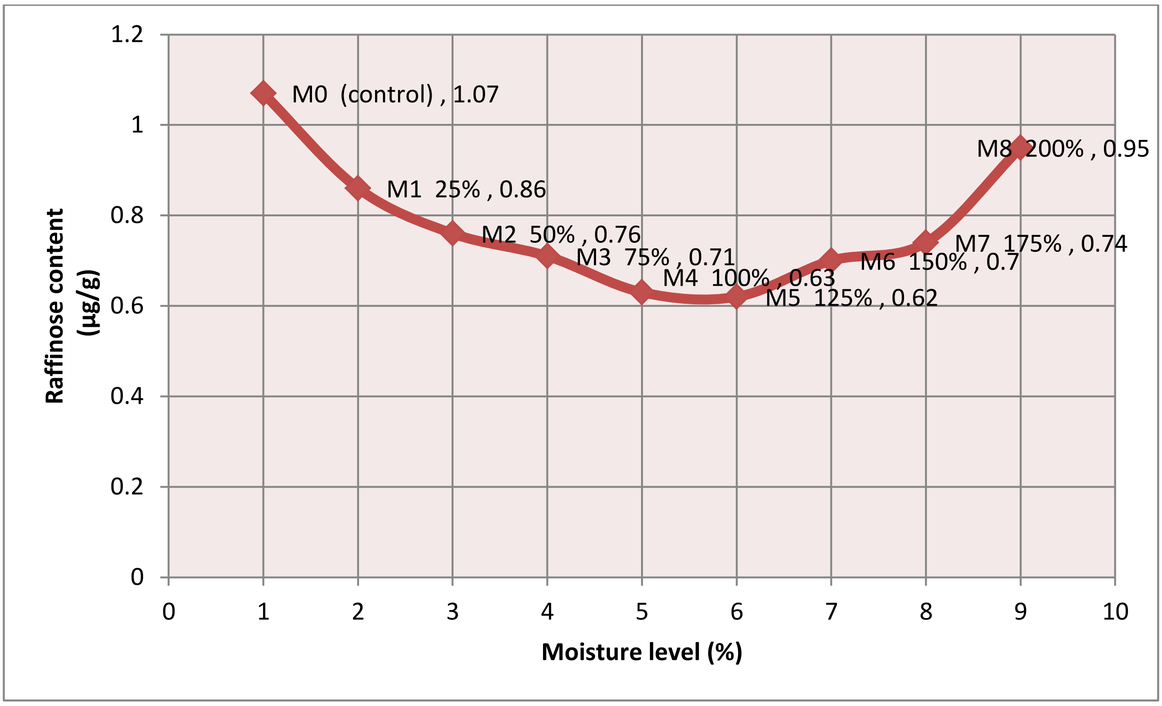

Fig. 1: Raffinose content with varying moisture levels

CONCLUSION

The level of Raffinose content in seed flour after treatment was seen to decrease with increase in moisture content (25-100%). Here the raffinose content was seen to reduce up to 125%. Thereafter the variation was not uniform. The level of oligosaccharide however decreased in comparison to control (1.16 µg g^{-1}), which indicates the asymptotic effect of enzyme on breakdown of oligosaccharides.

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