Research Article

Effect of Fermentation (*Saccharomyces cerevisiae*) on Improvement of Jackfruit Based Nutri Flour

Soumya P.S.[1,*], Suma Divakar[2]

[1]Department of Clinical Nutrition and Dietetics, St. Teresa's College, Ernakulam, Kerala, INDIA.
[2]Department of Community Science, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, INDIA.

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Correspondence:
Dr. Soumya P.S
Assistant Professor, Department of Clinical Nutrition and Dietetics, St. Teresa's College, Ernakulam, Kerala, INDIA.

Email: soumyahsc@gmail.com

ABSTRACT

Background: The main deleterious effect of jackfruit consumption is its low digestibility and flatulence factors, caused by the presence of oligosaccharides. Oligosaccharide is a carbohydrate comprised of few saccharides, *i.e.,* about three to ten monosaccharide units. Most types of oligosaccharides are indigestible, so they move through small intestine to large intestine, where bacteria finally break them down and it can result in pathological symptoms such as headaches, dizziness and even mental illness. **Aim:** With the aim of the present study is reducing these antinutritional and anti-inflammatory factors, *Saccharomyces cerevisiae* was utilised to breakdown oligosaccharides to simpler compounds and hence reduce the indigestible factors. **Materials and Methods:** HPLC estimation of Oligosaccharides: The breakdown of Oligosaccharides presents in the jackfruit based nutri flour (untreated and yeast treated) were analysed through High-performance liquid chromatography (HPLC) analysis. The reference sugars were: stachyose (purity 98%), D-(+)-glucose (purity>99.5%), fructose (purity 98%) and D-(+)-galactose (purity>99%), rhamnose (purity 98%) and D-arabinose (purity 99%); which were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division; Steinheim, Federal Republic of Germany). HPLC-grade water. **Results:** In HPLC analysis, the retention time of standard stachyose, untreated nutri flour and treated nutri flour was 6.93. Nutri flour treated with Saccharomyces cerevisiae @ 8 hr was found to be low in oligosaccharides compared to control. **Conclusion:** Nutri flour treated with Saccharomyces cerevisiae had very low sugar content than untreated flour. The fermentation resulted in proteolytic degradation of protein into amino acids and amylolytic break down of carbohydrates into sugars and organic acids.

Keywords: Stachyose, Rhamnose and Arabinose, *Saccharomyces cerevisiae*.

INTRODUCTION

Plant-based foods contain a variety of antinutritional ingredients. Anti-nutritional factors are substances that reduce a plant's or a plant product's ability to consume nutrients and absorb food for human consumption. Anti-nutritional factors can be reduced with proper processing techniques.[1]

Oligosaccharides, despite being classified as prebiotics and often found in dietary fibre, can pose digestive challenges for some individuals. They are malabsorbed by the small intestine and undergo fermentation in the large intestine, leading to gas production and subsequent flatulence. Oligosaccharides of the raffinose series, such as raffinose, verbascose and stachyose, are prevalent in many foods. These oligosaccharides consist of carbohydrate chains composed of 3-10 monosaccharides.[2] While oligosaccharides are considered beneficial as they serve as a food source for beneficial gut bacteria, their consumption can lead to gastrointestinal discomfort in certain individuals. The accumulation of gas in the rectum can result in symptoms like headaches, dizziness and, in severe cases, mental illness. Hence, while oligosaccharides play a role in supporting gut health their effects on digestion vary from person to person.[3]

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Raffinose Family Oligosaccharides (RFOs) are complex sugar molecules comprising galactose chains that remain undigested in the human upper intestine owing to the absence of galactosidase, the enzyme responsible for breaking down these chains. As a result, the RFOs make it to the lower intestine, where they are encountered by bacteria that utilize them as a substrate for fermentation. Methane, carbon dioxide and hydrogen gas are produced, which can cause bloating, stomach pain and excessive flatulence.[4,5] The seeds of the jackfruit contain various oligosaccharides like raffinose and stachyose, which can induce flatulence in humans. Additionally, these compounds contribute to a darker colour during the processing of flour.[6]

Fermented foods, through their microbial action, are crucial for ensuring the necessary stability, safety and sensory characteristics of the product. From a nutritional perspective, fermentation aids in breaking down antinutritional components, enhancing mineral absorption, improving protein digestion and decreasing the presence of oligosaccharides responsible for flatulence. In this study, the level of oligosaccharides and monosaccharides in jackfruit were tried to be reduced by fermentation with *Saccharomyces cerevisiae* was adopted. The level of stachyose, rhamnose and arabinose were ascertained being the prominent member of the oligosaccharide family.

MATERIALS AND METHODS

Selection and collection of materials

Jackfruit cultivars "koozha" and "varikka" were selected for the study. Raw jackfruits were harvested from the trees grown in the Instructional farm, College of Agriculture vellayani. The raw fruits were taken with 12-week maturity, along with the manifestations, such as flattening of spines, colour change from green to pale green, hollow sound and last leaf in the stalk turning yellow.

Preliminary processing

The bulbs, perigones, seeds, rind, core and testa were separated from the jackfruit. All parts were washed under tap water to remove dust and dirt. The raw material was cut in dimensions of 2.5 X 1 cm. All jackfruit parts were subjected to thermal treatment to inactivate antinutritional factors present in it. Then the blanched slices were dried at 650ºC in the electric drier for 6-7 hr. Proper care was given to avoid the cross contamination from other foreign particles. The dried jackfruit parts were milled into fine flours separately. The flours were sieved through a 0.05 mm sieve and packed in metalised laminated and High-Density Polyethylene (HDPE) covers.

Development of nutri flour

A nutri flour developed with jackfruit *cv koozha* and *cv varikka,* using the different parts such as bulbs, perigones, seeds, rind, core and testa. The formulations were made based on the results of glycemic index. The order of glycemic index of jackfruit parts were observed as KJRF> KJTF>VJTF>VJRF≥KJPF≥VJPF>KJCF>VJCF≥KJBF>KJSF≥VJBF>VJSF. The major component (50-60%) of flour was contributed from the fruit parts with lower glycemic index and 40% of the mix was formulated by other components in different proportions. The flours of all jackfruit parts were processed separately after pre-treatments and these processed raw materials were mixed into 10 different formulations. Formulated nutri flour ratio had 20% Koozha *Jack* Rind Flour (KJRF), 10% *Koozha Jack* Testa Flour (KJTF), 9% *Varkka Jack* Testa Flour (VJTF), 8% *Varikka Jack* Rind Flour (VJRF), 8.5% *Koozha Jack* Perigone Flour (KJPF), 2% *Varikka Jack* Perigone Flour (VJPF), 2.5% *Koozha Jack* Core Flour (KJCR), 2% *Varikka Jack* Core Flour (VJCF), 12% *Koozha Jack* Bulb Flour (KJBF), 7% *Koozha Jack* Seed Flour (KJSF), 12% *Varikka Jack* Bulb Flour (VJBF) and 7% *Varikka Jack* Seed Flour (VJSF).

Treatment with *Saccharomyces cerevisiae*

To reduce the level of oligosaccharides, the developed nutri flours were made into batter and subjected to fermentation with *Saccharomyces cerevisiae* @ 5 g/kg for 8 hr.[7,8] The level of oligosaccharides was then compared with that of control (F_1). The treated (F_2) and untreated flour (F_1) was tested for the breakdown of oligosaccharides.

HPLC estimation of Oligosaccharides

The breakdown of Oligosaccharides presents in the jackfruit based nutri flour (untreated and yeast treated) were analysed through High-Performance Liquid Chromatography (HPLC) analysis.

The specified sugars, including stachyose (98% purity), D-(+)-glucose (>99.5% purity), fructose (98% purity), D-(+)-galactose (>99% purity), rhamnose (98% purity) and D-arabinose (99% purity), were obtained from Sigma-Aldrich Chemie GmbH (Aldrich Division; Steinheim, Federal Republic of Germany). High-Performance Liquid Chromatography (HPLC)-grade water was also utilised.

Extraction of sample and HPLC analysis

One gram of both untreated and treated nutrient flour was measured and placed into a container containing

10 mL of Milli Q water. The mixture was vigorously mixed using a vortex for 5 min and then centrifuged at 4ºC at 10,000 rpm for 15 min. The resulting supernatant was filtered through a 0.2 µm nylon membrane. Samples of 10 µl were then injected for analysis using HPLC.

To identify sugars, the retention times of chromatographed samples were compared with those of authentic sugar samples. Calibration curves covering concentrations from 1 to 10 mg/mL were established for each sugar. The quantification of sugars in untreated and treated nutri flour was determined by measuring peak areas and referring to the respective standard curves.

The analysis was conducted using a Prominence UFLC system comprising an LC-20AD system controller, a Rezex RHM-Monosaccharide H+ column (300×7.8 mm), a Column Oven (CTO-20A), an Autosampler injector (SIL-20AC HT) and a RID detector (RID 10A). Milli-Q water served as the mobile phase, with a runtime of 20 min. The injection volume was set at 10 µL and the flow rate was maintained at 0.8 mL/min. The column temperature was held at 80ºC. Sample peaks were identified by comparing retention times with standard peaks. Data acquisition and analysis were performed using LC Lab Solutions software.

RESULTS

The oligosaccharide (Stachyose) and sugar content in untreated and treated (Saccharomyces cerevisiae) nutri flours were analysed by the analytical tool-HPLC and the results are presented in Tables 1 and 2. In HPLC analysis, the retention time of standard stachyose was 6.93. Retention time recorded in the untreated nutri flour and nutri flour with 8 hr fermentation was also 6.93 min. Stachyose percentage in untreated nutri flour was computed as 9.76% and that in treated nutri flour was 1.54%. Nutri flour treated with Saccharomyces cerevisiae was found to be lower in oligosaccharides compared to control.

Table 1 show the monosaccharides such as glucose, fructose and galactose content in untreated and treated flour samples with (Saccharomyces cerevisiae) as observed in HPLC assay. The retention time of standard glucose was 10.13 min, which was similar in both treated and untreated nutri flour (10.13). Glucose percentage in untreated nutri flour was 9.76% and that in treated was 1.54%. The percentage of fructose, in treated and untreated nutri flour was 7.28% and nil, before and after treatment respectively. The retention time of standard galactose, treated and untreated nutri flour was found to be 10.67 min. The level of galactose was compared with treated and untreated nutri flour. The result showed that percentage of galactose was high in untreated nutri flour (6.52%) and was nil in treated flour.

The content of rhamnose and arabinose, treated with Saccharomyces cerevisiae and untreated nutri flour as observed after HPLC assay are tabulated in Table 2. In HPLC analysis the retention time of standard rhamnose was 11.53 min. Retention time recorded in both untreated and treated nutri flours were also same. Percentage of rhamnose was found in very minute quantities in untreated nutri flour (0.00003%) and for treated flour it was totally absent. The retention time of standard arabinose, treated and untreated nutri flour was found to be 11.37min. The result showed that percentage of arabinose was absent in treated nutri flour and in untreated flour arabinose content was found in negligible amount of 0.0001%.

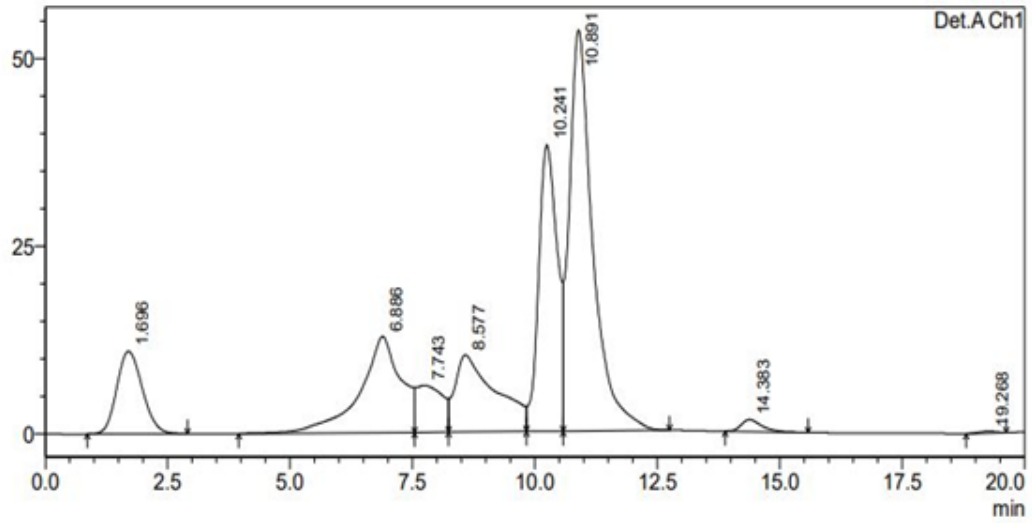
Table 1: Quantification of Oligosaccharide content in untreated and yeast treated nutri flour (@ 8 hr).

Treatments	Retention Time (min)	Area (AU)	Concentration (ppm)	Percentage of stachyose
Standard (Stachyose).	6.93	-	-	-
F1 (control).	6.93	594387	14172.6	9.76
F2 (8 hr) after treatment.	6.93	258785	6182.116	1.54
Standard (Rhamnose).	11.53	-	-	-
F1 (control).	11.53	22935	0.127	0.00003
F2 (8 hr) after treatment.	11.53	Nil	Nil	Nil
Standard (Arabinose).	11.37	-	-	-
F1 (control).	11.37	7908	0.287	0.0001
F2 (8 hr) after treatment.	11.37	Nil	Nil	Nil

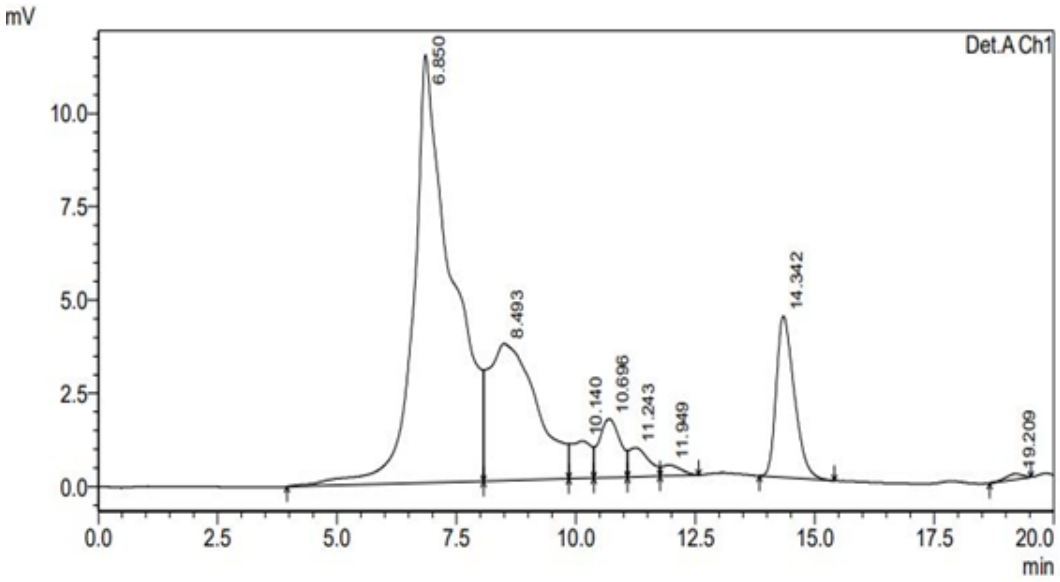
Table 2: Quantification of monosaccharide contents in untreated and yeast treated nutri flour.

Treatments	Retention Time (min)	Area (AU)	Concentration (ppm)	Percentage of monosaccharides
Standard (Glucose).	10.13	-	-	-
F_1 (control).	10.13	1011340	10577	9.76

F_2 (8 hr) after treatment.	10.13	29571	9.118	1.54
Standard (fructose).	10.89	-	-	-
F_1 (control).	10.89	1791793	10658	7.28
F_2 (8 hr) after treatment.	10.89	48726	0.6421	Nil
Standard (Galactose).	10.67	-	-	-
F_1 (control).	10.67	1542684	10725	6.52
F_2 (8 hr) after treatment.	10.67	26418	0.4584	Nil



Chromatogram of untreated nutri flour



Chromatogram of treated nutri flour

DISCUSSION

Reduction of oligosaccharide content in nutri flour

An issue facing in the processing of jackfruit flour into food raw materials is the existence of oligosaccharides.[9] Oligosaccharides such as raffinose, stachyose and verbascose pose a challenge for digestion as mammals lack the enzymes necessary to break them down in the small intestine. Once these oligosaccharides reach the large intestine, they undergo fermentation by the intestinal microflora, resulting in gas production and subsequent flatulence. While flatulence is not toxic, it is deemed a significant issue, as an accumulation of gas in the rectum can lead to pathological symptoms. Hence, it is crucial to remove such oligosaccharides from the jack fruit before utilising them as food. An alternative method to decrease oligosaccharides in the jackfruit flour is through the fermentation process. On the nutritional side, fermentation helps in degradation of antinutritional factors and increase the mineral bioavailability, protein digestibility and reduction of flatulence causing oligosaccharides.[10] Numerous microorganisms, including lactic acid bacteria (LAB) like L. plantarum SMN 25, L. plantarum pentosus SMN 01 and L. plantarum pentosus FNCC 235, obtained from the fermentation of traditional foods, have been extensively employed in food fermentation. These strains exhibit the capability to generate α-galactosidase, an enzyme crucial for the breakdown of oligosaccharides.[11]

In this study, *Saccharomyces cerevisiae* is used in fermenting jackfruit-based nutri flour to reduce the presence of oligosaccharides. A study report that β-Galactosidase production also increased with increased yeast extract concentration. During the entire fermentation, no accumulation of the hydrolysed sugars, glucose and galactose, was observed.[12] Some yeast cell wall enzymes such as glucanase, mannanase, invertase, alkaline phosphatase and lipase is noted to hydrolyze nutrients.[13] The presence of enzymes exhibiting this biochemical property has been reported in several yeast species. A study reported that endo Polygalacturonase (endo-PG) production by yeasts in Saccharomyces fragilis and the properties of these enzymes in comparison to those reported for fungi.[14,15]

The degradation of Polygalacturonic Acid (PGA) by two strains of *S. cerevisiae*.[16] Also, in another study[17] found PG, PE and PL activity in strain SCPP 2180 of this species and characterised the endo-PG from the wild-type strain 1389, as well as the differences between this enzyme and the one synthesised by the genetic strain IM1-8b.[18,19]

A study reported that treated with *Saccharomyces cerevisiae* the common monosaccharides such as D-glucose, D-fructose, D-mannose and D-galactose were completely removed; D-glucuronic acid and D-ribose were partially removed; but D-xylose, D-rhamnose and L-sorbose were not removed and were completely resistant.[20]

The present study result shows that Nutri flour treated with *Saccharomyces cerevisiae* after 8 hr of fermentation was found to be lower in oligosaccharides compared to control. In a similar study[8] it was reported that retention factor of standard Raffinose and pre-treated jackfruit bulb flour was 0.79. Bulb flour with 6 hr fermentation F_1 (0.79), 8 hr fermentation F_2 (0.79) and 12 hr was F_3 (0.80) were analysed through HPLC. Raffinose content in jackfruit flour was 0.75, 0.63, 0.58 and 0.74% in control, F_1, F_2 and F_3 respectively. In the case of jackfruit seed flour, the raffinose contents were 1.28, 0.42, 0.31 and 0.62% in control, F_1, F_2 and F_3 respectively. The raffinose content significantly decreased after 8 hr of fermentation in both jackfruit bulb flour and seed flour treatments.

A study reported that[6] fermenting jackfruit seed flour with *L. plantarum pentosus* for 32 hr led to the breakdown of oligosaccharides such as stachyose and verbascose. In another work reported[8] in order to reduce the oligosaccharide content enriched flours, were treated with *Saccharomyces cerevisiae* @ 5 g/kg for 6 hr, 8 hr and 12 hr respectively. Another study reported that[10,11] yeast fermentation using *Saccharomyces cerevisiae* isolated from Austrian traditional sourdough exhibited the most substantial reduction in total fructan content and demonstrated the highest gas production capability.

Key points

The level of oligosaccharide (Stachyose, Rhamnose and Arabinose) and monosaccharide (glucose, fructose and galactose) content in jackfruit based nutri flour treated with *Saccharomyces cerevisiae* was seen to decrease. Rhamnose and Arabinose content was found in negligible amount even in the untreated nutri flour. During fermentation process, proteins undergo proteolytic degradation into amino acids, while carbohydrates are subject to amylolytic breakdown into sugars and organic acids. The nutri flour treated with *Saccharomyces cerevisiae* @ 8 hr can be considered as cost effective and healthy method for improving the quality of the product.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High-performance liquid chromatography; **UFLC:** Ultra-Fast Liquid Chromatography; **RFOs:** Raffinose Family Oligosaccharides.

SUMMARY

In this study, jackfruit based nutri flour treated with *Saccharomyces cerevisiae* for 8 hr exhibited reduced levels of oligosaccharides. Jackfruit components contain oligosaccharides from the raffinose series, which remain undigested in the upper gut due to the absence of galactosidase. Upon reaching the large intestine, these oligosaccharides undergo fermentation by intestinal microflora, leading to gas production and flatulence. Yeast fermentation enhances the production of galactosidase, the enzyme responsible for breaking the links within galactose chains. This process facilitates the reduction of oligosaccharides and monosaccharides.

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