

Reducing antinutrient content in raw jackfruit bulb flour and seed flour through fermentation by *Saccharomyces cerevisiae*

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Abstract

Koozha type jackfruit in their raw stage was selected. Bulbs and seeds were separated and processed into flour. Considering the fact that flatulence interferes with the consumption of jackfruit, to reduce the level of oligosaccharides, flours were made into batter and subjected to fermentation with *Saccharomyces cerevisiae* @ 5g/kg for 6 hrs and 8 hrs and 12 hrs respectively. Fermentation resulted in proteolytic degradation of proteins into amino acids and amylolytic breakdown of carbohydrates into sugars and organic acids. The oligosaccharide (Raffinose) content in treated and pre treated jackfruit bulb flour treated with *Saccharomyces cerevisiae* at different deviations of fermentation differed in the HPTLC assay. Raffinose content in jackfruit bulb flour was 0.75 $\mu\text{g g}^{-1}$, 0.63 $\mu\text{g g}^{-1}$, 0.58 $\mu\text{g g}^{-1}$ and 0.74 $\mu\text{g g}^{-1}$ in control (F_0), F_1 , F_2 , F_3 respectively. For jackfruit bulb flour and seed flour 8 hour fermentation was found to contain the comparatively less oligosaccharides than other treatments.

Keywords : *Saccharomyces cerevisiae*, oligosaccharides, Fermentation, Proteolytic, Amylolytic Raffinose, Jackfruit bulb flour

Introduction

Anti-nutritional factors are naturally occurring compounds classified as secondary metabolites. These compounds are present in foods consumed by humans and animals and can lead to adverse physiological effects, including gastrointestinal discomfort, impaired reproductive health, and weakened immune response (Zakarial et al., 2011). Jackfruit bulbs and seeds contain oligosaccharides such as raffinose and stachyose, which can cause flatulence and contribute to browning during flour processing. Wichienhot et al. (2010) highlighted oligosaccharide content as a significant challenge in processing jackfruit seeds into raw food materials. Oligosaccharides like raffinose, stachyose, and verbascose are resistant to digestion since mammalian small intestines lack the enzymes necessary for their

breakdown. Upon reaching the large intestine, these compounds undergo fermentation by gut microflora, leading to gas production and flatulence.

Fermentation has been identified as an effective method to reduce oligosaccharide levels in beans and other food products (Gu et al., 2013). Various microorganisms, including lactic acid bacteria (LAB), have been extensively utilized in food fermentation. Strains such as *Lactiplantibacillus plantarum* SMN 25, *L. plantarum pentosus* SMN 01, and *L. plantarum pentosus* FNCC 235, isolated from traditional fermented foods, exhibit the capacity to produce α -galactosidase, an enzyme capable of degrading oligosaccharides (Sumarna, 2008). According to Lee et al. (2011), LAB can ferment carbohydrates, including raffinose and stachyose, which are commonly found in plants, particularly grains. Surono (2004) noted that LAB strains, particularly those from the *L. plantarum* species, can thrive in non-dairy environments. This study explores the characteristics of jackfruit seed flour fermented with *Saccharomyces cerevisiae*, aiming to enhance digestibility and promote increased consumption of jackfruit by mitigating the effects of oligosaccharides.

Materials and methods

Treatment with *Saccharomyces cerevisiae*

Fermentation plays a crucial role in improving nutritional quality by breaking down antinutritional factors, enhancing mineral bioavailability, and increasing protein digestibility. Additionally, it aids in the degradation of oligosaccharides, which are known to cause flatulence. To lower oligosaccharide content, flour is transformed into batter and fermented using *Saccharomyces cerevisiae* at a concentration of 5 g/kg for durations of 6, 8, and 12 hours. The most effective treatments are determined by assessing the extent of oligosaccharide reduction (Krishnaja, 2014).

Statistical Design and treatment details

Experimental design : Completely randomized design (CRD)

Number of treatments : 3x 2

Number of replications : 4

Treatments	Time
F ₁	6 hrs
F ₂	8 hrs
F ₃	12 hrs

Edible Portion

E₁ Bulb

E₂ Seed

HPTLC estimation of Oligosaccharides (Raffinose $\mu\text{g g}^{-1}$)

High Performance Thin Layer Chromatography (HPTLC) is widely utilized for analyzing bioactive oligosaccharides in food, as it is accessible in most laboratories and does not necessitate highly specialized technical expertise. Its popularity stems from the method's simplicity, versatility, and the widespread availability of equipment.

The oligosaccharide content, specifically raffinose, in yeast-treated flours derived from jackfruit bulbs and seeds, was analyzed using HPTLC. A raffinose reference standard with 99% purity was sourced from Sigma–Aldrich Chemie GmbH (Aldrich Division, Steinheim, Germany). High-purity solvents, including isopropanol (99%), methanol (99%), toluene (99%), and ammonia solution (95%), were obtained from Merck, India. All solvents were of HPLC grade, and distilled water used in the process was purified through a Sartorius water purification unit (Arium 61315, USA).

Extraction of samples for HPTLC

Methanolic extracts from treated and pre-treated jackfruit bulb and seed flours were prepared by adding 25 ml of methanol to 10 grams of powdered sample. The mixture was filtered using Whatman No. 41 filter paper, and the filtrate was collected. Extraction was carried out by shaking the solution overnight at room temperature in a rotary evaporator set at 30 rpm.

Mobile Phase

The mobile phase used for detecting oligosaccharide (raffinose) consisted of a mixture of isopropanol, methanol, and toluene in an 8:2:4 ratio, along with a drop of ammonia. The solution was sonicated for 10 minutes to ensure proper mixing.

Stock Solution of Oligosaccharide (Raffinose)

A stock solution of raffinose (1000 $\mu\text{g/ml}$) was prepared by dissolving 10 mg of raffinose reference standard in 5 ml of methanol. The mixture was thoroughly shaken in a 10 ml volumetric flask and brought to volume with methanol.

Working Standard Solution of Oligosaccharide (Raffinose)

Working standard solution (1 to $30\text{ }\mu\text{g ml}^{-1}$) 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 mm apart with a Camag microlitre syringe on pre-coated silica gel aluminium plate 60 F254 having $20 \times 10\text{ cm}$ dimensions.

Linear ascending development was carried out in $20 \times 10\text{ cm}$ 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 treated flour (with *Saccharomyces cerevisiae*) and untreated jackfruit bulb flour at different fermentation times differed in the HPTLC assay and the data is tabulated in Table 1. In HPTLC analysis, the retention factor of standard Raffinose was 0.79 . Retention factor recorded by untreated jackfruit bulb flour was 0.79 , and for bulb flour with 6 hrs fermentation, it was 0.79 , for 8 hrs fermentation it was 0.79 and for 12 hrs it was 0.80 respectively, which were comparable to that of the standard Raffinose. Raffinose content in jackfruit bulb flour was $0.75\text{ }\mu\text{g g}^{-1}$, $0.63\text{ }\mu\text{g g}^{-1}$, $0.58\text{ }\mu\text{g g}^{-1}$ and $0.74\text{ }\mu\text{g g}^{-1}$ in F_1 , F_2 , F_3 treatments respectively. Thus, in this case, 8 hours fermentation was found to contain the least oligosaccharides.

Table 1. Quantification of Oligosaccharide (Raffinose) content in jackfruit bulb flours with different duration of fermentation

Treatments	Retention Factor	Area (AU)	Amount ($\mu\text{g/g}^{-1}$)
Standard	0.79	4974.03	-
F ₀ (control)	0.79	6295.57	0.75
F ₁ 6 hrs	0.79	5222.87	0.63
F ₂ 8 hrs	0.79	6192.57	0.58
F ₃ 12 hrs	0.80	8612.57	0.74

Reduction of oligosaccharide (Raffinose) content in seed flour

The oligosaccharide (Raffinose) content in seed flour treated with *Saccharomyces cerevisiae* and untreated jackfruit seed flour at different durations of fermentation was analysed through HPTLC assay are tabulated in Table 2. In HPTLC analysis, the retention factor of standard Raffinose was 0.57. Retention factor recorded in both untreated and treated jackfruit seed flours were compared to that of the standard Raffinose. Raffinose content in jackfruit seed flour was $1.28 \mu\text{g g}^{-1}$, $0.42 \mu\text{g g}^{-1}$, $0.31 \mu\text{g g}^{-1}$ and $0.62 \mu\text{g g}^{-1}$ in F₁, F₂, F₃ treatments respectively. Raffinose content was found to reduce in these three treatments; F₁ ie; 6 hours fermentation was selected as the best treatment.

Table 2. Quantification of Oligosaccharide (Raffinose) content in jackfruit seed flour with different duration of fermentation

Treatments	Retention Factor	Area (AU)	Amount ($\mu\text{g g}^{-1}$)
Standard	0.57	4999.8	-
F ₀ (control)	0.57	6413.5	1.28
F ₁ 6 hrs	0.57	2107.8	0.42
F ₂ 8 hrs	0.57	1596.2	0.31
F ₃ 12 hrs	0.57	3100.1	0.62

Table 3. One way interaction effect of fermentation time and edible portions of jackfruit on raffinose content

Treatments	Raffinose ($\mu\text{g g}^{-1}$)
Fermentation time	
F ₀	1.02
F ₁	0.52
F ₂	0.45
F ₃	0.68
SEm $\pm$	0.003
CD	0.010
Edible portion	
E ₁	0.68
E ₂	0.66
SEm $\pm$	0.002
CD	0.007

Values are means of triplicates

SEm : standard error of the mean

Table 3 shows the effect of fermentation time and edible portions. Here it was revealed that F₀ (control) contained 1.02 $\mu\text{g g}^{-1}$ and the raffinose content was reduced in treatment F₂ (8 hrs fermentation) to 0.45 $\mu\text{g g}^{-1}$. With respect to edible portions it was observed that E₁ (Bulb flour) contained 0.68 $\mu\text{g g}^{-1}$ and E₂ (Seed flour) contained 0.66 $\mu\text{g g}^{-1}$.

Table 4. Two way interaction effect of fermentation time and edible portions on raffinose content

Treatments	Raffinose ($\mu\text{g g}^{-1}$)
F ₀ E ₁	0.75
F ₀ E ₂	1.28
F ₁ E ₁	0.62
F ₁ E ₂	0.42
F ₂ E ₁	0.58
F ₂ E ₂	0.31
F ₃ E ₁	0.74
F ₃ E ₂	0.62
SEm $\pm$	0.004
CD	0.014

Values are means of triplicates

SEm : standard error of the mean

Table 4 shows the two way interaction effect of varying fermentation time and edible portions. The results revealed that F₀E₁ (Bulb flour control) contained 0.75 µg g⁻¹ and F₀E₂ (Seed flour control) contained 1.28 µg g⁻¹. The lowest raffinose content was observed in F₂E₂ – seed flour fermented for 8 hour (0.31 µg g⁻¹) followed by F₁E₂ – seed flour fermented for 6 hour (0.42 µg g⁻¹), F₂E₁ – bulb flour fermented for 8 hour (0.58 µg g⁻¹). Oluseyi and Temitayo (2015) reported that fermentation resulted in proteolytic degradation of proteins into amino acids and amylolytic breakdown of carbohydrate into sugars and organic acids.

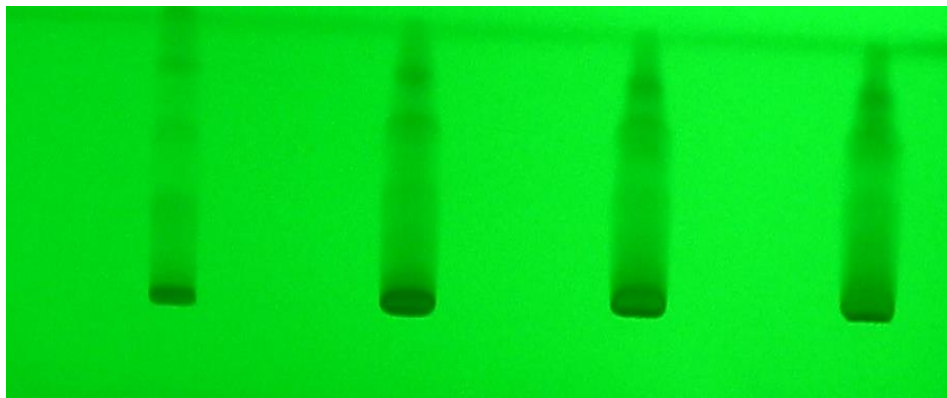


Plate 1. HPTLC plates of reference standard Raffinose and methanolic extracts of yeast treated flour with varying moisture level viewed at 254 nm

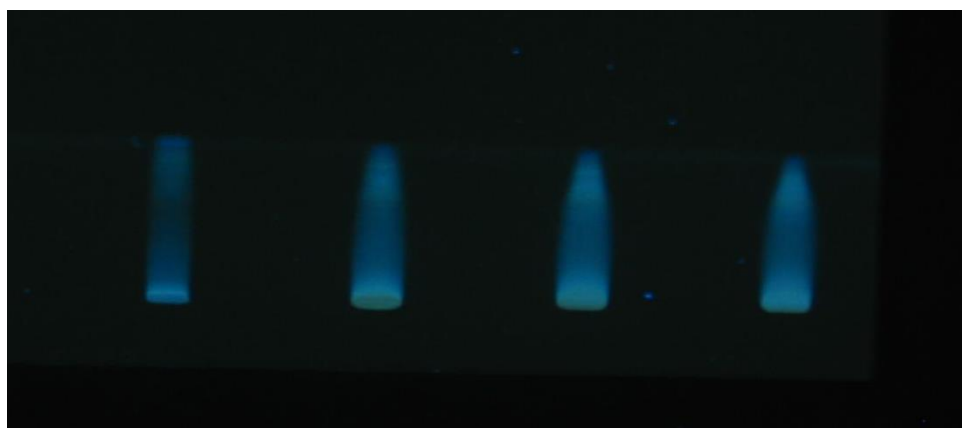


Plate 2. HPTLC plates of reference standard Raffinose and methanolic extract of yeast treated flour of varying moisture level viewed at 366 nm

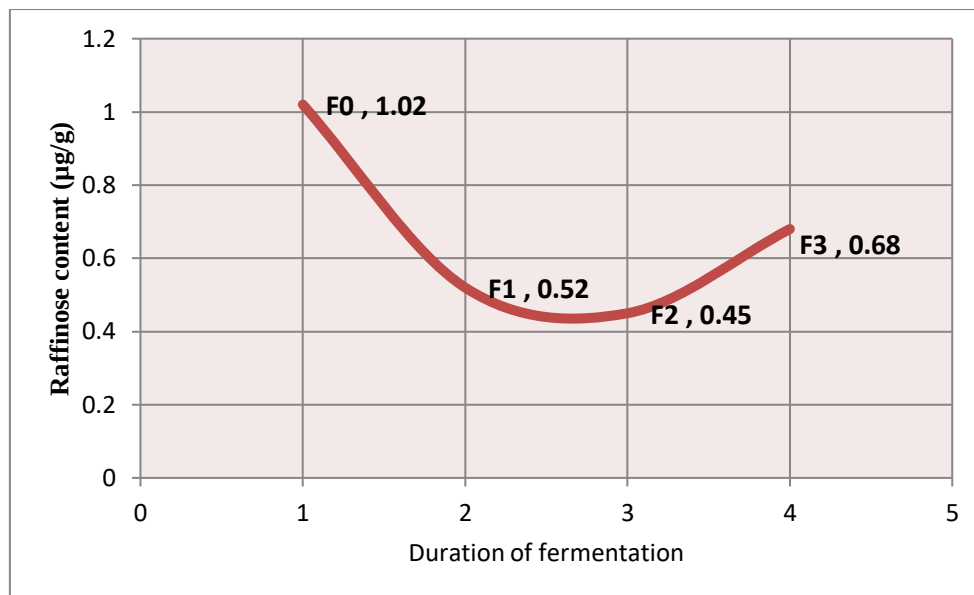


Figure 1. Raffinose content in varying fermentation time

Conclusion

When flours were to be made into batter and subjected to fermentation with *Saccharomyces cerevisiae* @ 5g/kg for 6 hrs, 8 hrs and 12 hrs and analyzed through HPTLC assay; raffinose content in jackfruit bulb flour reduced from 0.75 $\mu\text{g g}^{-1}$ to 0.63 $\mu\text{g g}^{-1}$, 0.58 $\mu\text{g g}^{-1}$ and 0.74 $\mu\text{g g}^{-1}$ after 6, 8 and 12 hours respectively. Raffinose content in jackfruit seed flour reduced from 1.28 $\mu\text{g g}^{-1}$ to 0.42 $\mu\text{g g}^{-1}$, 0.31 $\mu\text{g g}^{-1}$ and 0.62 $\mu\text{g g}^{-1}$ respectively after 6, 8 and 12 hours of fermentation. Considering the reduction of raffinose content and sensory evaluation of the treated flour, eight hour fermentation (F_2) was selected as the best treatment.

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