



Fermentation of Purple Yam (*Dioscorea alata* L.) by *Lactobacillus plantarum* B1765 for Fructooligosaccharide Production

Tri Ayu Sukma Ningsih and Prima Retno Wikandari*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia

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Abstract

Purple yam (*Dioscorea alata* L.) contains inulin that can be hydrolyzed into fructooligosaccharides (FOS) by inulinase from lactic acid bacteria (LAB). FOS are useful prebiotic compounds in functional foods. This study examined the influence of fermentation time on FOS content and its relationship with LAB growth, pH, and total titratable acidity (TTA) during the fermentation of purple yam by *Lactobacillus plantarum* B1765. Purple yam slices were fermented using a 2% (v/v) inoculum of *L. plantarum* B1765 at 37 °C for 0, 6, 12, 18, 24, and 30 hours. LAB counts, pH, and TTA were quantified using the total plate count method, pH meter, and acid-alkalimetric titration, respectively. FOS content in fermented purple yam flour was quantified using high-performance liquid chromatography (HPLC) analysis. The fermentation time significantly influenced LAB growth, pH, TTA, and FOS content. LAB growth peaked at 18 hours (9.21 log CFU ml⁻¹). At the end of the fermentation process, the pH value decreased to 3.90±0.01, and TTA increased to 0.30±0.01%. Fermentation produced 3 commercial FOS, with nystose being the predominant FOS throughout the process, reaching a concentration of 124.773±0.19 mg g⁻¹ at the end of fermentation. These results indicate that fermentation time affects both the levels and composition of FOS in fermented purple yam flour. Therefore, fermented purple yam flour has the potential to serve as a beneficial alternative source of FOS for industrial applications.

Keywords: inulin; inulinase; lactic acid bacteria; prebiotic

INTRODUCTION

Purple yam (*Dioscorea alata* L.) is an underutilized tuber widely cultivated in Indonesia, where it is mostly consumed in the form of traditional dishes such as chips, fried yams, and steamed yams (Rahma et al., 2021; Tamaroh and Sudrajat, 2021). The tuber is largely composed of carbohydrates, the most common type being starch, which constitutes about 70.6 to 83.0% of the dry weight (Lebot et al., 2023). Apart from starch, *D. alata* tubers have also been found to contain reducing sugars that constitute about 0.27 to 1.49% of the dry weight, which can easily be

used as carbon sources by microorganisms during fermentation (Yulifianti et al., 2021). Of great importance, the purple yam has also been viewed as a potential functional food ingredient due to its high inulin content of about 7.54% (Louise, 2020).

Previous research has proven that yam tubers (*Dioscorea* spp.) are a suitable substrate for lactic acid bacteria (LAB) fermentation (Batista et al., 2019). Similarly, fermentation of *Dioscorea hispida* tuber flour with *Lactobacillus plantarum* has been reported to alter its nutritional and

* **Corresponding author:** primaretno@unesa.ac.id

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functional properties, further demonstrating the potential of *Dioscorea* tubers as substrates for LAB fermentation (Kumoro et al., 2020). More recently, fermentation of a plant-based formulation containing *Dioscorea dumetorum* with LAB, including *Lactiplantibacillus plantarum*, was shown to induce changes in carbohydrate metabolism and other nutritional characteristics (Munghang et al., 2025). Fermentation of *gembili* (*Dioscorea esculenta* L.) with *L. plantarum* B1765 has also been shown to affect LAB growth, pH, titratable acidity, and organoleptic properties (Amaliyah and Wikandari, 2025).

Inulin is a fructan-type polysaccharide composed mainly of fructose units connected through β -(2 $\rightarrow$ 1) glycosidic bonds, which can be hydrolyzed into fructooligosaccharides (FOS) by microbial inulinases or acid hydrolysis (Martins et al., 2019; Ningsih and Sanjaya, 2022; Karimi et al., 2025). As a soluble dietary fiber, inulin reaches the colon undigested, where it is fermented by beneficial gut microbiota to produce short-chain fatty acids (SCFAs; Alonso-Allende et al., 2024). The production of SCFAs contributes to digestive health and may help inhibit the growth of pathogenic bacteria in the gastrointestinal tract (Sheng et al., 2023). In addition to these beneficial effects, the hydrolysis of inulin also produces shorter-chain fructans, particularly FOS, which possess prebiotic properties (Nisa et al., 2021).

FOS are a type of short-chain polysaccharide composed of glucose-fructose (GF_n) or fructose (F_m) monomers, where n and m range from 1 to 6 (Cahyaningtyas and Wikandari, 2022). In the food industry, commercially used FOS most commonly have a degree of polymerization (DP) of 3 to 5 and are denoted as GF_n (Fernandes, 2025). Commercially important FOS include kestose (GF₂), nystose (GF₃), and fructosyl-nystose (GF₄), which are widely used as prebiotic ingredients in functional foods (Yuliana et al., 2014). According to Suparwati (2014), commercial production of FOS is more commonly achieved through enzymatic synthesis from sucrose using fructosyltransferase (FTase). FOS are utilized across a broad spectrum of food products because of their ability to (1) positively impact the development of beneficial bacteria in the gastrointestinal system; (2) improve digestive health; and/or (3) offer soluble dietary fiber and/or low-calorie sweeteners as

a carbohydrate source (Rolim, 2015; Setiarto et al., 2017).

As the consumption of FOS increases, Indonesia's reliance on imported FOS (Nisa et al., 2021) indicates a need to use local agricultural products to produce this ingredient. FOS can be produced enzymatically from inulin through the action of microbial endo-inulinases, which randomly hydrolyze long inulin chains into shorter FOS chains (de Araujo Ribeiro et al., 2021; Ibrahim, 2021; Kucuk et al., 2026). Inulinases are produced by diverse microorganisms, including bacteria, yeasts, and fungi (Tufan et al., 2025; Raiciu et al., 2026). Experimental production of short-chain fructooligosaccharides (SCFOS) has also been demonstrated through enzymatic hydrolysis of fructans using endo-inulinase, with optimized conditions yielding high levels of SCFOS (Wongsanittayarak et al., 2024).

Recent experimental work has also demonstrated microbial production of FOS using bacterial systems, such as *Zymomonas mobilis* ZM4, which produced FOS, including 1-kestose and nystose under optimized fermentation conditions (Braga et al., 2025). The ability of *L. plantarum* to utilize carbohydrate substrates is strain-dependent, with substantial variation among strains in their utilization of inulin and other prebiotic carbohydrates (Fuhren et al., 2020). Similarly, previous experimental studies have demonstrated the ability of *L. plantarum* and other *Lactobacillus* strains to grow in media containing inulin and FOS, although utilization capacity varies among strains and carbohydrate conditions (Parhi et al., 2021; Renye et al., 2021).

Lactobacillus plantarum B1765 has been reported to produce inulinase, with optimum enzyme production occurring during the logarithmic growth phase after 18 hours of incubation at 37 °C, yielding an activity of 0.047 U ml⁻¹ (Nabila and Wikandari, 2018). In comparison, *Aspergillus tritici* BGPUP6 produced endo-inulinase with a maximum activity of 25.01 U ml⁻¹ under optimized conditions, and the enzyme was capable of hydrolyzing inulin into FOS (Singh et al., 2016). Furthermore, purified endo-inulinase from *A. tritici* BGPUP6 produced FOS, including GF₂, GF₃, and GF₄ (Singh et al., 2020).

Recent studies have also demonstrated continued interest in optimizing microbial FTase production for FOS biosynthesis, with

fermentation conditions strongly influencing FTase activity and productivity (Wu et al., 2025). Gautam et al. (2026) further reported the production and characterization of microbial FTase from *Bacillus stercoris* S1, demonstrating the continued exploration of bacterial sources for FOS-producing enzymes. Natasya and Wikandari (2022) also demonstrated that *L. plantarum* B1765 produced FOS from *gembili* with a DP of 3.47.

Although these studies demonstrate the potential of microbial inulinases and *L. plantarum* B1765 for FOS production, the synthesis of FOS from purple yam (*D. alata* L.) using this specific strain remains unexplored, particularly regarding the changes occurring around and beyond 18 hours of fermentation. Preliminary observations showed that LAB growth continued to increase up to 18 hours. Therefore, fermentation times of 0, 6, 12, 18, 24, and 30 hours were selected to investigate the effects of fermentation time on LAB growth, pH, titratable acidity, and FOS production from purple yam using *L. plantarum* B1765.

MATERIALS AND METHOD

Research design

Various fermentation times (0, 6, 12, 18, 24, and 30 hours) were evaluated for their effects on LAB growth, pH, and total titratable acidity (TTA) of fermented purple yam, as well as on FOS production in the resulting purple yam flour.

Materials

The materials used in this study included *L. plantarum* B1765 (obtained from a private collection, isolated from fermented fish *bekasam*), MRS broth (GranuCult®, Merck KGaA, Darmstadt, Germany), CaCO₃ (Merck), NaCl (Merck), 0.01 N NaOH (ROFA Lab), phenolphthalein (Merck), agar powder (Swallow Globe Brand), FOS standards (inulobiose, inulotriose, 1-kestose, nystose, and 1,1,1-kestopentaose), distilled water, and purple yam tubers (*D. alata* L.) from Bantul, Yogyakarta, Indonesia. The tubers were 2 to 3 months old, large and irregular, with brown skin and purple flesh. To prepare the starter culture, *L. plantarum* B1765 was cultured in MRS broth, centrifuged, washed with sterile 0.85% NaCl solution, and resuspended (Hidaya and Wikandari, 2020).

Purple yam fermentation

The tubers were washed, peeled, sliced to a thickness of 1.5 mm, and steamed for 5 minutes at 90 °C (Hazizah and Estiasih, 2013). Taxonomic identity was verified by Yayasan Generasi Biologi Indonesia (Mojokerto, East Java, Indonesia). For each treatment, 200 g of tuber slices were placed in a sterilized glass jar, followed by the addition of a 2% (w/v) NaCl solution (1:1) and 2% (v/v) *L. plantarum* B1765 starter culture. Fermentation was conducted at 37 °C for 0, 6, 12, 18, 24, and 30 hours. After fermentation, slices were separated from the brine, dried at 50 °C until a constant weight, milled, and sieved through an 80-mesh sieve (Prihastina and Wikandari, 2025).

LAB, pH, and TTA analysis

For LAB enumeration, 1 ml of fermented sample was serially diluted in sterile 0.85% NaCl solution (10^{-1} to 10^{-8}). A 1 ml aliquot of each dilution was pour-plated with approximately 12 ml of MRS agar containing 1% CaCO₃ and incubated at 37 °C for 48 hours. Colonies were counted, and results were expressed as CFU ml⁻¹ (Insani and Wikandari, 2024). For pH measurement, fermented yam and liquid were homogenized, and the pH of a 20 ml aliquot of this homogenate was measured using a calibrated pH meter (Nugrainglahi and Wikandari, 2025). For TTA determination, homogenized fermented yam and liquid were diluted 50- to 330-fold with distilled water until colorless. A 10 ml aliquot was titrated with 0.01 N NaOH using phenolphthalein, and TTA was expressed as a percentage of lactic acid (Prihastina and Wikandari, 2025).

FOS analysis

FOS were analyzed using a Shimadzu HPLC system equipped with a CBM-20A controller, LC-20AT solvent delivery unit, CTO-10ASVP column oven, and RID-20A refractive index detector. Chromatographic separation was performed using a Shim-pack VP-ODS column (5 µm, 150 mm × 4.6 mm) at 35 °C. The mobile phase consisted of acetonitrile and 0.04% ammonium hydroxide in water (70:30, v/v) and was delivered under isocratic conditions at a flow rate of 1.25 ml minute⁻¹. The injection volume was 10 µl, and the run time was 10 minutes.

Data analysis

LAB and TTA data were analyzed using one-way ANOVA, followed by the least significant difference (LSD) test at a 5% significance level.

pH data were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney U test due to non-normality. FOS data were analyzed descriptively using SPSS version 16.

RESULTS AND DISCUSSION

The impact of fermentation time on LAB, pH, and TTA

LAB growth, pH, and TTA were evaluated to determine the effect of fermentation time on purple yam fermentation. Statistical analysis showed that fermentation duration significantly affected all 3 parameters ($p < 0.05$). LAB growth peaked at 18 hours, then declined at 24 hours, and remained relatively stable until 30 hours. In addition, both pH and TTA differed significantly throughout the fermentation period (0 to 30 hours). LAB growth, pH, and TTA are presented in Figure 1.

LAB growth reached a maximum of $9.21 \log \text{CFU ml}^{-1}$ at 18 hours, with an initial value of $7.98 \log \text{CFU ml}^{-1}$. These results indicate that after 18 hours of fermentation, the bacterial population multiplied rapidly, entering an exponential growth phase. This was verified by the decrease in viable bacterial cells at the end of the 24-hour fermentation period. Because there were fewer viable cells at the 24-hour mark, the bacterial population was in a decline phase; therefore, this research did not demonstrate a stationary growth phase of the microorganisms before the fermentation process was complete.

The rapid growth of LAB in the early stages of fermentation is likely due to the high levels of fermentable carbohydrates present during that period. During fermentation, starch and inulin may be hydrolyzed into simpler sugars through the action of carbohydrate-hydrolyzing enzymes, such as α -amylase and inulinase. These solubilized sugars provide carbon sources to support bacterial growth and organic acid production (Risky et al., 2019; Winarti and Anggreini, 2019; Ramadhan and Wikandari, 2021). In *L. plantarum*, FOS utilization is also regulated by carbon metabolism. Chen et al. (2018) demonstrated that FOS metabolism is subject to carbon catabolite repression, indicating that the availability and utilization of carbohydrate substrates can influence FOS metabolism in this species. This observation is also consistent with the reported carbohydrate utilization capacity of *L. plantarum*, although carbohydrate utilization can vary among strains, including the ability to metabolize inulin and FOS (Cui et al., 2021; Yang et al., 2025).

Previous studies have also shown that several bacterial species, including *L. plantarum*, produce α -amylase (Hattingh et al., 2015; Onilude et al., 2017). The production of inulinase by *L. plantarum* B1765 was 0.047 U ml^{-1} after 18 hours of fermentation (Nabila and Wikandari, 2018), implying that there is a possible capability for the hydrolysis of inulin. *Lactobacillus plantarum* B1765 has also been found to produce other

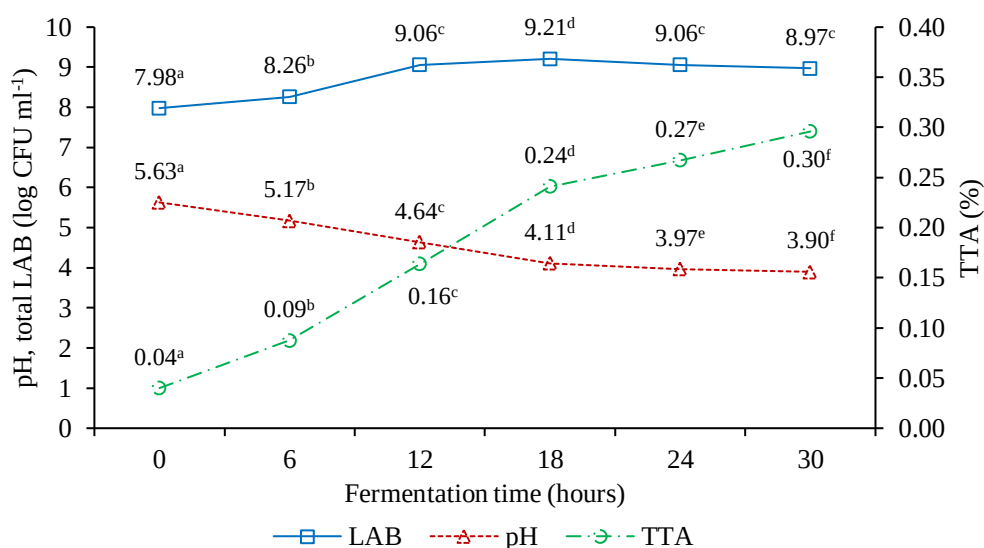


Figure 1. Changes in LAB growth, pH, and TTA during the fermentation of purple yam slices
Note: Values followed by different letters are significantly different ($p < 0.05$)

enzyme activities, such as β -glucosidase and proteolytic activities; however, those activities were examined in other fermentation systems (Huda, 2016; Dhana and Wikandari, 2019). Generally, β -glucosidase activity is known to be important in *L. plantarum* during food fermentation. The presence of other enzymatic and metabolic activities in *L. plantarum* suggests that fermentation changes could arise from various enzymatic and metabolic activities other than inulinase activity (Yilmaz et al., 2022; Paventi et al., 2025). However, since these activities were not directly determined, the exact role of other enzymes remains unknown.

The fermentation of purple yam resulted in a greater increase in the LAB population than that observed during the fermentation of yacon or *gembili* tubers. This is related to the high starch content (approximately 18%) in purple yam. Since LAB can utilize starch as a carbon source, they have more diverse carbon metabolism options than in yacon, which contains mostly fructans as carbohydrate reserves, and *gembili*, which has very little starch and accordingly few fermentable substrates (Maghfiroh et al., 2018; Yan et al., 2019; Permatasari et al., 2025).

The increase in LAB population was accompanied by an increase in organic acid accumulation during fermentation by *L. plantarum* B1765. The initial pH of the medium was 5.63, which decreased to 3.90 after 30 hours of fermentation, while TTA increased from 0.04 to 0.30% over the same period. The highest LAB population was observed at 18 hours, accompanied by a decrease in pH and an increase in TTA, indicating that active LAB metabolism contributed to the accumulation of organic acids during fermentation. As LAB metabolized fermentable carbohydrates, organic acids such as lactic acid were produced, resulting in lower pH and higher TTA. These findings are consistent with those observed during fermentation of yacon and *gembili*, where the LAB population grew progressively and, through its activity, pH decreased (Rafsanjani and Wikandari, 2017; Prihastina and Wikandari, 2025).

The effect of fermentation on FOS production

High-performance liquid chromatography (HPLC) analysis confirmed the presence of several FOS compounds in the fermented purple yam flour. This was achieved by comparing the retention times of the sample peaks with

those of the corresponding FOS reference standards, namely inulobiose (F2), inulotriose (F3), 1-kestose (GF2), nystose (GF3), and 1,1,1-kestopentaose (GF4). A representative FOS chromatogram obtained with the HPLC instrument is shown in Figure 2.

HPLC analysis of the fermented purple yam flour sample at 30 hours showed that its retention times matched those of the FOS standards, namely F2, F3, GF2, GF3, and GF4, confirming that purple yam fermentation produced FOS (Figure 2b). In this research, the fermentation substrate was purple yam, which contains inulin and is known for its prebiotic potential (Birkeland et al., 2020). Louise (2020) reported that purple yam tubers contain 7.54% inulin, which can serve as a substrate for microbial inulinases during fermentation. Microbial inulinases can hydrolyze inulin into fructose and FOS, with endo-inulinases cleaving internal β -(2 $\rightarrow$ 1)-linked fructan chains to generate shorter-chain FOS (Mangunwidjaja et al., 2014; Yuliana et al., 2014; Raiciu et al., 2026). Thus, the detection of Fn-type FOS, including F2 and F3, is consistent with the hydrolysis of inulin by endo-inulinases.

However, the presence of GFn-type FOS, including GF2, GF3, and GF4, cannot be explained solely by endo-inulinase-mediated hydrolysis. GFn-type FOS are commonly associated with FTase-mediated transfructosylation, in which fructosyl residues from sucrose are transferred to sucrose or other acceptor molecules, resulting in the formation and elongation of FOS (Sánchez-Martínez et al., 2020; Khatun et al., 2021; Rawat et al., 2024; Dias et al., 2025). Experimental studies have demonstrated the production of GFn-type FOS through FTase or transglycosylation-mediated reactions (Alvarado-Obando et al., 2022; Wienberg et al., 2022; Wan et al., 2024). Therefore, the coexistence of Fn-type and GFn-type FOS may indicate the contribution of different enzymatic pathways to the observed FOS profile, as microbial FTase can catalyze transfructosylation reactions leading to the formation of FOS with different chain lengths (Karkeszová and Polakovič, 2023).

FOS are recognized as prebiotic carbohydrates that can provide beneficial effects through their utilization by the gut microbiota (Boets et al., 2015; Teferra, 2019; Ojwach et al., 2022). Nevertheless, the activities of endo-inulinase and FTase, as well as the availability of sucrose,

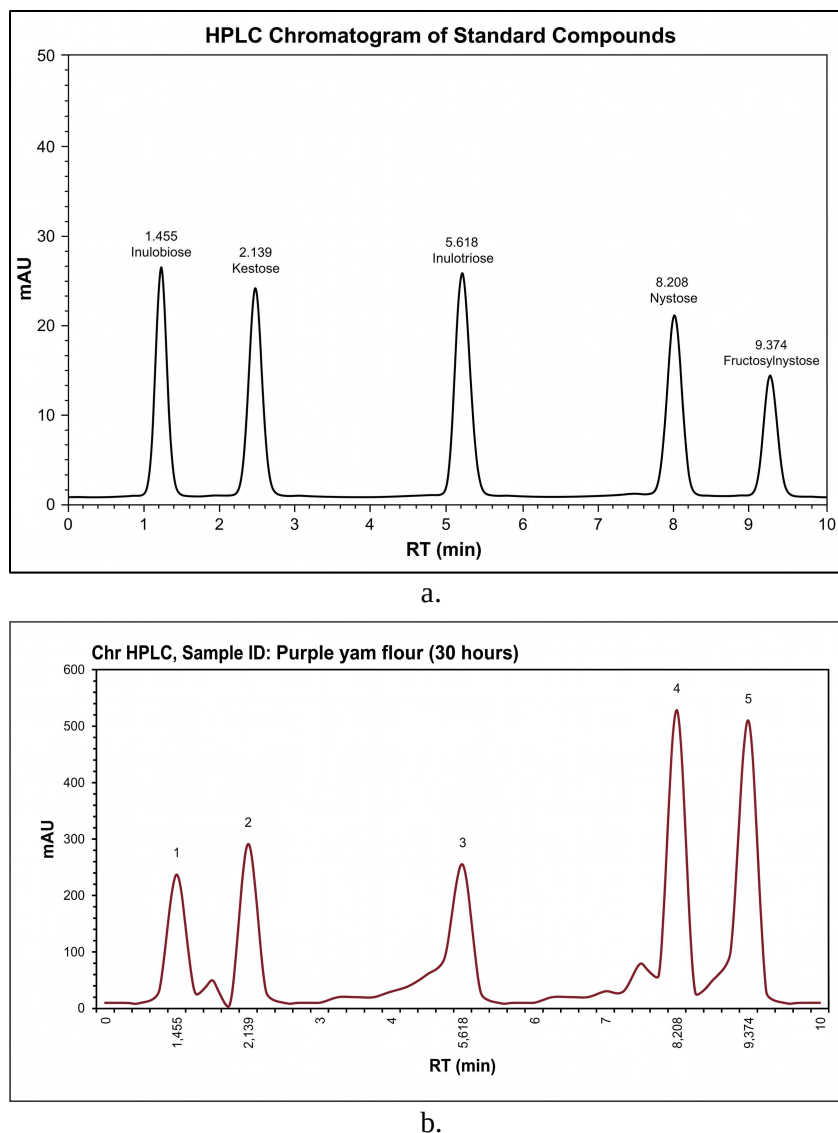


Figure 2. HPLC chromatograms of (a) FOS standards and (b) the fermented purple yam flour sample at 30 hours

Note: RT = Retention time

were not directly determined in the present study. Thus, these pathways are proposed based on the observed FOS profile and established enzymatic mechanisms, rather than being confirmed by direct enzyme activity measurements.

Changes in FOS composition during fermentation are shown in Figure 3. The concentrations of GF_n-type FOS, such as GF₂, GF₃, and GF₄, were relatively high during fermentation, peaking at 24 hours. This trend is consistent with findings from some prior experiments showing that reactions catalyzed by FTase can improve FOS production (Jaswal et al., 2024; Niu et al., 2024). The decline of GF₄ at 30 hours and the increases in GF₂ and GF₃ may

indicate the formation and degradation of GF_n-type FOS during fermentation. Regarding Fn-type FOS, F₃ was highest at 12 hours and declined afterward, while F₂ showed an increasing trend at late times of fermentation. It was apparent that fermentation time affected FOS production in purple yam. However, since the activities of endo-inulinase and FTase were not measured, the mechanisms behind the observed results remain undetermined.

HPLC analysis confirmed the presence of several types of FOS before fermentation (0 hours), including F₂, F₃, GF₂, GF₃, and GF₄. The presence of these compounds at 0 hours indicates that FOS were already present before

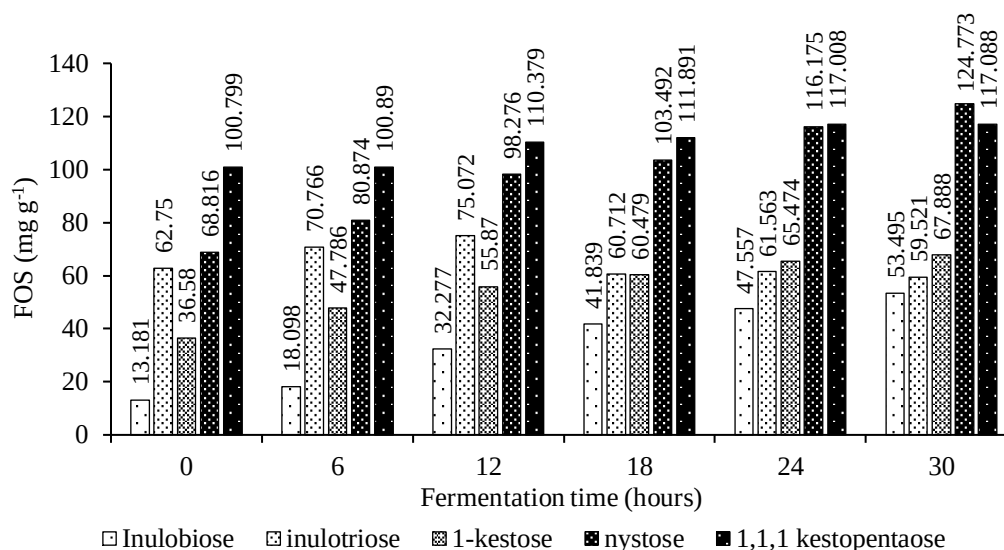


Figure 3. FOS production from fermented purple yam tuber flour

the fermentation period progressed, which may be associated with their natural occurrence in purple yam and/or pre-fermentation hydrolysis of fructan substrates by endogenous enzymes. Therefore, the FOS detected at 0 hours should not be attributed exclusively to the activity of *L. plantarum* B1765.

Among the detected FOS, GF3 showed the highest concentration at the end of fermentation, increasing from 68.816 mg g⁻¹ at 0 hours to 124.773 mg g⁻¹ at 30 hours. This increase indicates that GF3 accumulated during the fermentation process. The accumulation of GF3 may be associated with the formation of GF_n-type FOS through transfructosylation reactions involving FTase activity (Rawat et al., 2024; Dias et al., 2025). A similar FOS profile, including 1-kestose, nystose, and 1,1,1-kestopentaose, was reported in fermented dahlia tuber inulin (Yuliana et al., 2014). Differences in FOS profiles among various studies may result from differences in substrate, such as inulin content, and in the accessibility of inulin to microbial enzymes (Cahyaningtyas and Wikandari, 2022). However, because FTase activity was not directly measured in the current study, the contribution of this pathway to the increase in GF3 remains uncertain.

Total FOS, calculated as the sum of F2, F3, GF2, GF3, and GF4, exhibited an increasing trend during the fermentation process, reaching its highest concentration at 18 hours. The total FOS concentration increased by 96.287 mg g⁻¹ from 0 to 18 hours, whereas only a minimal increase was observed after 18 hours, coinciding with the

decline phase of LAB growth. This pattern was associated with the exponential phase of LAB growth, during which microbial growth was rapid.

Previous research reported that *L. plantarum* B1765 exhibited its highest inulinase activity at 18 hours (Nabila and Wikandari, 2018), while Wijanarka and Sarsa (2019) reported that inulinase production was associated with the exponential growth phase. Therefore, the temporal coincidence between active LAB growth, increased FOS accumulation, and the previously reported inulinase activity of *L. plantarum* B1765 may support a possible contribution of inulin hydrolysis to FOS formation in the present study. However, inulinase activity was not directly measured in the present study. Thus, these findings indicate the potential of purple yam as a raw material for FOS production, particularly for FOS such as GF2, GF3, and GF4.

Study limitations

This study has several limitations. The purple yam used was limited to local tubers obtained from Bantul, Special Region of Yogyakarta, with specific physical characteristics and aged 2 to 3 months. Fermentation was limited to 0, 6, 12, 18, 24, and 30 hours at 37 °C, and LAB growth was determined using the total plate count pour plate method. FOS identification was limited to 5 reference standards with DP 2 to 5, namely F2, F3, GF2, GF3, and GF4; therefore, other FOS compounds were not identified. Furthermore, the exact roles of enzymes such as inulinase and

FTase could not be determined because their specific activities were not directly quantified.

CONCLUSIONS

Fermentation time influenced LAB growth, pH, TTA, and FOS formation in purple yam fermented with *L. plantarum* B1765. LAB growth reached its maximum at 18 hours, accompanied by a decrease in pH and an increase in TTA. Five FOS components were determined using HPLC, including F2, F3, GF2, GF3, and GF4. GF3 was the predominant FOS component at the end of fermentation, whereas total FOS content was highest at 18 hours. These findings highlight the potential of purple yam as a substrate for FOS production using *L. plantarum* B1765.

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DECLARATION OF GENERATIVE AI USE

The authors utilized ChatGPT (OpenAI) to facilitate language editing, improve readability, eliminate redundancy, and summarize parts of the manuscript. Additionally, ChatGPT was utilized to arrange five chromatograms of FOS standards into a single representative figure without changing any scientific information. All scientific information, data, figures, interpretations, and conclusions have been checked by the authors themselves, who are fully responsible for the final manuscript.

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