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Potential Fermentation of Dahlia Tubers (*Dahlia pinnata Cav.*) with *L. plantarum* B1765 in the Production of Fructooligosaccharides

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Abstract: Fructooligosaccharides (FOS) are functional food ingredients used as food additives in the food, pharmaceutical, and health industries. FOS can be produced by hydrolyzing inulin with the enzyme inulinase. One source of inulin is dahlia tubers. *L. plantarum* B1765 is known to possess inulinase activity. This study aimed to investigate the effect of fermentation time on dahlia tubers (*Dahlia pinnata* Cav.) using *L. plantarum* B1765 as a starter culture to produce FOS. An experimental research design was utilized with fermentation conducted for 0, 24, 48, and 72 hours at 37°C using a 3% (v/v) starter culture. Subsequently, LAB growth, pH, total titratable acidity (TTA), and FOS content were measured. Total LAB was determined using the Total Plate Count (TPC) method, pH was measured using a pH meter, and TTA was determined by acid-base titration. FOS content was analyzed using HPLC with a mobile phase consisting of 70% ammonium hydroxide solution and 30% acetonitrile. Fermentation duration influenced increases in LAB population, TTA, and FOS content, as well as decreases in pH. The results showed that optimal bacterial growth occurred after 24 hours of fermentation, with a total LAB count of $2.48 \times 10^8 \pm 0.068$ CFU/mL. FOS production continued to increase throughout the 72-hour fermentation period. The FOS produced after 72 hours of fermentation consisted of inulobiose (32.24 ± 0.16 mg/g), inulotriose (31.49 ± 0.56 mg/g), kestose (49.67 ± 0.24 mg/g), nystose (33.40 ± 0.60 mg/g), and fructosylnystose (23.90 ± 0.43 mg/g). These findings suggest that dahlia tubers fermented with *L. plantarum* B1765 have potential as a substrate for FOS production and may contribute to the development of the FOS industry. Ultimately, these practical implications highlight the potential of this bioprocess to advance functional food development and strengthen the commercial prebiotic industry.

Keywords: Dahlia Tubers; Fermentation; FOS; *L. plantarum* B1765.

Introduction

Fructooligosaccharides (FOS) are composed of fructose units linked by β-(2→1) glycosidic bonds, with a terminal glucose residue attached through an α-(1→2) bond [1]. Because these glycosidic linkages are resistant to hydrolysis by human digestive enzymes, FOS reaches the large intestine intact, where it is selectively fermented by gut microbiota [2]. This characteristic makes FOS an important prebiotic ingredient. During fermentation, intestinal microorganisms metabolize FOS to produce short-chain fatty acids (SCFAs), which contribute to gastrointestinal health by lowering intestinal pH and inhibiting the growth of pathogenic bacteria [3,4]. Furthermore, FOS promotes the growth of beneficial bacteria, particularly *Lactobacillus* and *Bifidobacterium*, thereby enhancing digestive health and supporting immune function [5].

The demand for FOS is increasing due to their widespread use as low-calorie sweeteners, dietary fiber sources, and functional ingredients that improve food texture and stability [6,7]. Compared to sucrose, FOS contains approximately 1.5 - 2,0 kcal/g. Although the global FOS market continues to grow, production in Indonesia remains limited and relies heavily on imports. Dahlia tubers (*Dahlia pinnata* Cav.), which contain 65.7–72.6% inulin on a dry-weight basis, represent a promising local raw material for FOS production via enzymatic hydrolysis [8].

FOS can be produced in two ways, both by utilizing the transfructosylation of sucrose by fructosyl transferase to produce FOS, and hydrolyzing inulin using inulinase enzyme [9]. The type commonly used in industry is referred to as commercial FOS, which consists of kestose (GF_2), nystose (GF_3), and fructosylnystose (GF_4) [10]. Among these three types, kestose is the most widely used in the food industry due to its higher sweetness, good water solubility, and stability under heat and pH changes [10,5].

Previous studies on dahlia tubers have mainly focused on inulin extraction and characterization, while FOS production has received little attention. FOS production from dahlia tuber inulin has been reported using *Kluyveromyces marxianus* DUCC-Y-003 [11]. However, the application of lactic acid bacteria (LAB) remains largely unexplored. LAB offers a promising alternative due to their ability to produce inulinase enzymes and give beneficial metabolites that can enhance the functional value of FOS-containing products. LABs also have potential as probiotics, which supports the development of synbiotic products.

L. plantarum B1765 has been identified as a promising lactic acid bacterium for FOS production. This strain has been reported to exhibit good growth performance during fermentation and to produce the highest inulinase activity during the logarithmic growth phase [12]. Furthermore, *L. plantarum* B1765 can reduce the fermentation pH by producing organic acids and increase the population of lactic acid bacteria during fermentation

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[13,14]. These metabolic characteristics suggest that *L. plantarum* B1765 possesses a strong capability to hydrolyze inulin derived from dahlia tubers into FOS.

Fermentation time is a critical factor influencing the growth of lactic acid bacteria and the production of inulinase. During fermentation, bacterial cells undergo several growth phases, including the lag, logarithmic, stationary, and death phases [15]. The logarithmic phase is characterized by intensified metabolic activity, which promotes both inulinase synthesis and organic acid production. Increased inulinase activity facilitates the hydrolysis of inulin into FOS, whereas the accumulation of organic acids contributes to a reduction in pH and an increase in total titratable acidity (TTA). Therefore, fermentation duration is expected to significantly affect bacterial growth, pH, TTA, and ultimately the FOS content produced during fermentation.

However, despite this background, a clear research gap remains, as previous studies have not explicitly addressed the effect of fermentation duration on the interactive dynamics among LAB growth, acidity changes, and the production profiles of individual FOS fractions using L. plantarum B1765 and a dahlia tuber substrate. Most prior works focus on microbial activity or product yield separately without integrating these parameters, limiting the understanding needed for process optimization. Furthermore, studies employing similar substrates and LAB strains either did not examine *L. plantarum* B1765 specifically or lacked detailed time-course analyses comparing biochemical and microbial growth parameters.

Based on this explanation, this study aims to examine the effect of dahlia tuber fermentation durations of 0, 24, 48, and 72 hours using the *L. plantarum* B1765 starter culture on the total growth of lactic acid bacteria, pH, total titratable acidity (TTA), and the resulting FOS content. This study will provide insights into the potential of dahlia tubers as a source of FOS produced through fermentation and contribute to the development of local functional food products based on synbiotics.

Research Methods

Culture Preparation

The *L. plantarum* B1765 starter culture was prepared in sterilized MRS Broth medium, which had been autoclaved at 121°C for 15 min. A 1 mL aliquot of the stock culture was inoculated into 9 mL of MRS Broth and incubated at 37°C for 20 hours. The culture was subsequently centrifuged at 3500 rpm for 5 min, and the resulting pellet was resuspended in 9 mL of sterile 0.85% NaCl solution and centrifuged again. Finally, the pellet was resuspended in 10 mL of sterile 0.85% NaCl solution to obtain the starter culture [16].

Sample Preparation

The dahlia tubers are peeled, thoroughly cleaned, and grated lengthwise. Approximately 200 grams of pickles were weighed and then blanched for 5 minutes to reduce microbial contamination. Afterwards, 3% NaCl was added at a 1:1 (weight/volume) ratio. The pickles were transferred to sterile containers, each with a capacity of approximately 250 mL. Lastly, *L. plantarum* B1765 was added as a starter culture at a concentration of 3% (v/v). The containers were sealed tightly and fermented in an incubator at 37°C for durations of 0, 24, 48, and 72 hours [17].

Determination of Total Lactic Acid Bacteria

The growth of lactic acid bacteria was determined using the total plate count method. Samples were serially diluted in sterile 0.85% NaCl solution to obtain dilutions ranging from 10^{-1} to 10^{-8}. A 1 mL aliquot from the appropriate dilution was transferred into a sterile Petri dish, followed by the addition of approximately 15 mL of MRS agar supplemented with $CaCO_3$. The plates were incubated at 37°C for 48 hours, and the number of lactic acid bacteria was expressed as colony-forming units per milliliter (CFU/mL). Plates containing 25–250 colonies were considered countable and used for bacterial enumeration. The analysis was performed according to the method described by [18].

Determination of pH

The pH and total titratable acidity (TTA) of the fermented dahlia tubers were determined. Approximately 20 mL of the fermentation sample was transferred into a 50 mL beaker for pH measurement. The pH meter was calibrated using standard buffer solutions (pH 4.0, 7.0, and 9.0) prior to analysis, and the electrode was rinsed with distilled water between measurements [19].

Determination of Total Titratable Acidity (TTA)

For TTA determination, 10 mL of the sample was diluted to 100 mL with distilled water in a volumetric flask. An aliquot of 20 mL of the diluted sample was mixed with 2–3 drops of phenolphthalein indicator and titrated with 0.05 N NaOH until a persistent pale pink endpoint was reached. The TTA values were expressed as the percentage of lactic acid.

Determination of Fructooligosaccharides (FOS)

Fermented dahlia tubers were prepared, and then analyzed by HPLC to determine FOS content. The samples were blended, filtered, and concentrated under vacuum at 30–35°C until dry. The dry concentrate was redissolved in 1 mL of distilled water, filtered through a 0.22 µm Millipore membrane, and analyzed using an Agilent 1100 series High-Performance Liquid Chromatography (HPLC) system, a Shim-pack VP ODS column (5 µm, 4.6 × 150 mm) at room temperature, and an RID detector. A 70/30 (v/v) mixture of 0.04% ammonium hydroxide solution and acetonitrile was used as the mobile phase at a flow rate of 1.25 mL/min at 30°C. Quantitative identification was performed by comparing retention times and peak areas with external standard calibration curves of individual FOS standards (inulobiose, inulotriose, kestose, nystose, and fructosylnystose) across a validated linear concentration range, ensuring analytical quality control and reproducibility [20].

Statistical Analysis: All experimental data on LAB growth, pH, TTA, and FOS content were analyzed using SPSS version 23. Prior to parametric evaluation, data normality and homogeneity of variance were tested using the

Shapiro-Wilk and Levene tests, respectively. Data were then analyzed using One-Way Analysis of Variance (ANOVA) to determine the effect of fermentation duration, with statistical significance set at $p < 0.05$. Significant differences among treatment means were further evaluated using Fisher's Least Significant Difference (LSD) post hoc test.

Results and Discussion

Effect of fermentation on Total Lactic Acid Bacteria (LAB), pH, and Total Titratable Acid (TTA)

The research results indicate a significant difference ($p < 0.05$) in total LAB count between 0 and 24 hours of fermentation, no significant change between 24 and 48 hours, and a significant reduction at 72 hours. Regarding pH and TTA, they showed significant differences across all variables, indicating that pH decreased and TTA increased with increasing fermentation duration. The results of the tests for total LAB, pH and TTA for the fermented dahlia tubers are shown in Fig. 1.

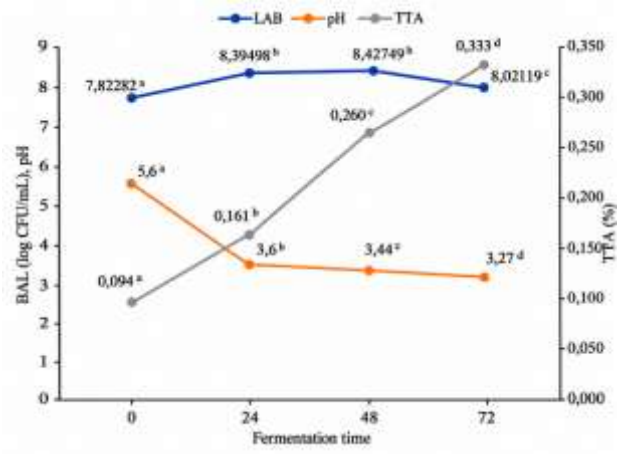


Figure 1. Total LAB, pH, and TTA of Dahlia Tubers during Fermentation Time.

Note: Values followed by different letters indicate a significant difference ($p < 0.05$).

The growth of lactic acid bacteria (LAB) was significantly influenced by fermentation time, with longer fermentation periods generally promoting LAB proliferation [21]. The highest LAB count was observed after 24 hours of fermentation, reaching $2.48 \times 10^8 \pm 0.04$ CFU/mL, indicating that the bacteria had entered the logarithmic growth phase. No significant changes in LAB counts were observed between 24 and 48 hours, suggesting that the bacterial population had reached the stationary phase. After 72 hours of fermentation, the LAB count decreased to $1.05 \times 10^8 \pm 0.08$ CFU/mL, indicating the onset of the death phase.

The increase in the LAB population during fermentation may be attributed to the high inulin content of dahlia tubers (66.76%) [22], which serves as a prebiotic substrate that supports the growth of beneficial microorganisms, including LAB [23,24]. Inulin cannot be digested by human digestive enzymes but can be metabolized by microorganisms as an energy source [25]. During fermentation, native or hydrolyzed inulin serves as the primary carbon source, which is broken down into simpler sugars and fructooligosaccharides (FOS) to sustain bacterial growth and metabolism [26].

The present findings are consistent with previous studies involving other inulin-rich substrates. Fermentation of jicama extract inoculated with 5% *L. plantarum* B1765 increased the LAB population by approximately 2 log cycles after 12 hours, reaching $1.15 \times 10^8 \pm 0.13$ CFU/mL [27]. Similarly, yacon fermentation inoculated with 10% LAB showed the highest bacterial growth after 48 hours, with a total count of 3.25×10^8 CFU/mL [28]. In addition, fermentation of gembili tuber flour (*Dioscorea esculenta*) using *L. plantarum* B1765 resulted in LAB counts of 2.5×10^8 CFU/mL after 72 hours of fermentation [29]. Compared with these substrates, dahlia tubers supported comparable LAB growth despite requiring a lower inoculum concentration and shorter fermentation period. Based on the results, dahlia tuber fermentation yields a relatively high LAB growth rate, which is influenced by factors such as substrate type, the bacteria's ability to produce enzymes involved in metabolism, temperature, pH, and fermentation duration [30].

LAB growth during dahlia tuber fermentation was accompanied by a significant decrease in pH ($p < 0.05$), from 5.60 ± 0.008 to 3.27 ± 0.007, and a significant increase in TTA ($p < 0.05$), from $0.09 \pm 0.005\%$ to $0.33 \pm 0.005\%$. Similar trends have been reported in fermented gembili products, in which pH decreased from 6.43 ± 0.002 to 3.07 ± 0.002 and TTA increased from $0.31 \pm 0.010\%$ to $0.53 \pm 0.008\%$ after 144 h of fermentation [31]. Likewise, fermented sweet potato products showed a reduction in pH from 5.52 ± 0.59 to 3.23 ± 0.20 and an increase in TTA from $0.07 \pm 0.001\%$ to $0.56 \pm 0.02\%$ during 24 hours of fermentation [32].

The decrease in pH and increase in TTA observed in the present study were associated with the metabolic activity of *L. plantarum* B1765, which produces lactic acid during carbohydrate fermentation. The dissociation of lactic acid releases H^+ ions, increasing acidity and lowering pH [33]. Although direct enzymatic assays for inulinase quantification were not performed in this study, the metabolic activity of *L. plantarum* B1765, as evidenced by concurrent pH reduction, biomass accumulation, and progressive release of diverse FOS fractions, strongly suggests active carbohydrate metabolism and enzymatic cleavage of inulin polymers [12]. Therefore, fermentation time governs the interplay among bacterial proliferation, acidogenesis, and FOS accumulation.

The Effect of Fermentation Time on the Production of Fructooligosaccharides

Fructooligosaccharides (FOS) are oligosaccharides consisting of 2 to 10 fructose monomer units linked by β-(2→1) glycosidic bonds, with a single glucose monomer at the end of the chain linked by an α-(2→1) glycosidic bond [34]. This study utilized the inulinase bacterial enzyme of *L. plantarum* B1765 to hydrolyze inulin into FOS. The enzyme inulinase can metabolizze inulin in two ways: both endoinulinase and exoinulinase. Endoinulinase (β-2-1-D-fructan hydrolase) cleaves inulin randomly from within the chain. Meanwhile, exoinulinase (fructan β-fructosidase) hydrolyzes inulin from the terminal fructose end [35]. *L.*

plantarum B1765 is a LAB known to secrete the enzyme inulinase [12].

While direct enzymatic activity rates were not measured in this study, the greatest enhancement in FOS production was observed during the logarithmic phase, indicating that Inulinase activity is highest then. Saraswati et al. [36] stated that inulinase degrades inulin into FOS, reaching its maximum activity during that phase. A similar phenomenon occurs during the stationary phase, where inulinase activity remains high enough to degrade inulin into FOS [37]. In this study, FOS was analyzed using an HPLC instrument with a 0.04% ammonium hydroxide solution and acetonitrile as the eluent in a 70:30 (v/v) ratio. FOS standard chromatogram and an example of a sample chromatogram are shown in Figure 2.

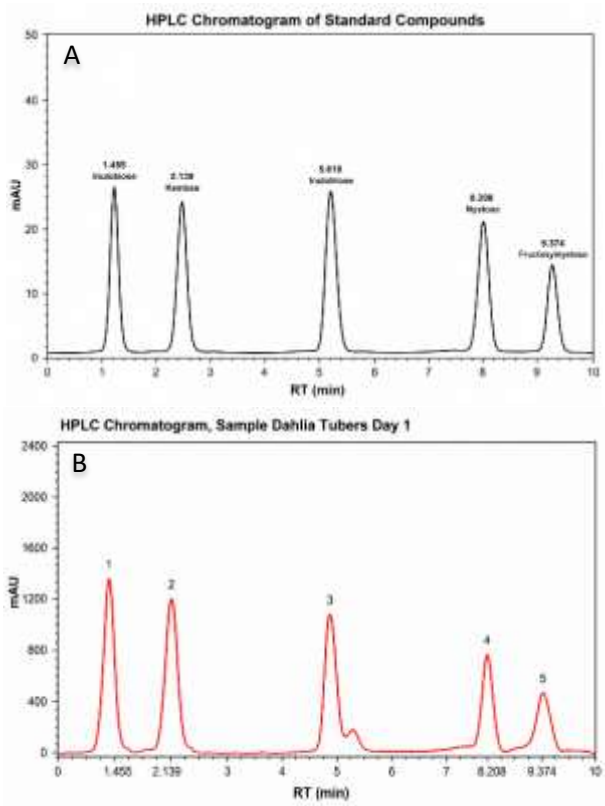


Figure 2. The Fructooligosaccharides Chromatogram: a) Chromatogram of standard compounds, b) Chromatogram of sample

The relationship between fermentation duration and FOS production is presented in Figure 3. HPLC analysis revealed the presence of several FOS, including inulobiose, inulotriose, kestose, nystose, and fructosylnystose. These compounds were detected even in the unfermented substrate (0 hours), indicating that dahlia tubers naturally contain not only long-chain inulin but also short-chain fructans that can be classified as FOS. Previous studies have demonstrated that inulin-producing tubers contain fructans with varying degrees of polymerization [38]. Kriukova *et al*. [39] further reported that dahlia tubers possess a broad fructan chain-length distribution and a relatively high proportion of short-chain fructans, which explains the presence of FOS prior to fermentation.

Dahlia tubers contain a high inulin content (66.76 ± 0.572% dry weight) [24], providing a substantial substrate for microbial hydrolysis during fermentation. To minimize indigenous microbial contamination, the tubers were blanched at 100°C for 5 min and subsequently soaked in 3% NaCl solution before fermentation. The increase in FOS concentration during fermentation therefore primarily reflected the metabolic activity of *L. plantarum* B1765.

Table 1. Fructooligosaccharide (FOS) Production during Dahlia Tuber Fermentation

Parameter	Hours 0	Hours 24	Hours 48	Hours 72
Inulobiose	28.44 ± 0.58	32.41 ± 0.05	31.87 ± 0.19	32.24 ± 0.16
Inulotriose	17.43 ± 0.31	25.12 ± 0.29	31.82 ± 0.71	31.49 ± 0.56
Kestose	19.47 ± 0.39	29.87 ± 0.05	41.74 ± 0.26	49.67 ± 0.24
Nystose	10.30 ± 0.18	17.17 ± 0.20	24.68 ± 0.55	33.40 ± 0.60
Fructosylnystose	3.80 ± 0.06	8.86 ± 0.10	15.49 ± 0.34	23.90 ± 0.43

Based on Table 1, after 24 hours of fermentation (logarithmic phase), all detected FOS fractions increased substantially. This period corresponded to the logarithmic growth phase of LAB, during which bacterial metabolic activity and substrate utilization were maximal. According to Pelczar & Chan [15], Microorganisms exhibit their highest metabolic activity during the logarithmic phase, supporting cell growth and physiological functions. The increased production of inulinase during this phase enhances inulin hydrolysis, thereby increasing FOS formation. From a biochemical perspective, the accelerated breakdown of long-chain inulin polymers into intermediate oligosaccharides is driven by enhanced microbial catalytic efficiency during active growth. Consistent with this observation, Nabila & Wikandari [12] reported that *L. plantarum* B1765 exhibited maximum inulinase activity (0.047 U/mL) at the end of the logarithmic phase (18 hours of fermentation).

At 48 hours of fermentation, LAB growth entered the stationary phase, during which bacterial growth became relatively stable. During this period, the concentrations of kestose, inulotriose, nystose, and fructosylnystose continued to increase, although at a lower rate than that observed during the logarithmic phase. In contrast, inulobiose concentration decreased, suggesting that the shorter-chain oligosaccharides generated during fermentation were either utilized as carbon sources by microorganisms or further hydrolyzed into simpler sugars [40]. Similar observations have been reported for other inulin-rich substrates, in which oligosaccharides formed during hydrolysis subsequently serve as fermentable substrates when the primary carbon source becomes limited [41].

After 72 hours of fermentation, the concentrations of inulobiose and inulotriose showed little further increase, whereas kestose, nystose, and fructosylnystose continued to accumulate. These findings indicate that inulin hydrolysis remained active throughout fermentation and favored the formation of commercially important short-chain FOS. Among the detected compounds, kestose reached the highest concentration (49.67 ± 0.24 mg/g), followed by nystose (33.40 ± 0.60 mg/g), inulobiose (32.24 ± 0.16 mg/g), inulotriose (31.49 ± 0.56 mg/g), and fructosylnystose (23.90 ± 0.43 mg/g).

The biochemical predominance of kestose (a trisaccharide consisting of one glucose and two fructose units) can be explained by the specific cleavage kinetics of inulin by microbial carbohydrate-active enzymes, which preferentially target internal linkages or transfer fructosyl residues to sucrose/short-chain acceptors via fructosyltransferase-like mechanisms. The predominance of kestose is particularly important because it is one of the major commercial FOS components widely used as a prebiotic ingredient in the food industry [10,42]. Kestose possesses high solubility, mild sweetness, and excellent stability during food processing, making it a desirable functional ingredient. Similar results have been reported during the fermentation of gembili tubers using *L. plantarum* B1765, which also produced short-chain commercial FOS, particularly kestose [43]. Therefore, the present findings demonstrate that 72 hours of fermentation provides the optimal conditions for commercial FOS production from dahlia tubers. Overall, dahlia tubers have considerable potential as a locally available substrate for producing high-value FOS and synbiotic functional food ingredients.

Several limitations of this study should be noted to guide future research. First, the experiments were conducted entirely at a laboratory scale, meaning that scaling up the bioprocess to pilot or industrial levels requires further optimization of reactor parameters, mass transfer, and aeration. Second, this investigation utilized a single bacterial strain (*L. plantarum* B1765); evaluating mixed-culture fermentations or other GRAS (Generally Recognized as Safe) probiotic strains could yield higher product titers. Third, inulinase activity was inferred from growth kinetics and product accumulation rather than quantified directly by specific enzymatic assays (e.g., reducing sugar assays with purified inulin substrates). Finally, the study did not evaluate the long-term storage stability, sensory acceptability, or in vivo prebiotic efficacy of the resulting FOS-enriched dahlia products, which represent essential avenues for future applied food science research.

Conclusion

This study showed that fermentation duration affects increases in total LAB count, pH, TAT, and FOS content. The results indicate that optimal growth of total LAB occurs after 24 hours of fermentation, but TTA and FOS continue to increase until the end (72 hours), and the pH decreases to 3.27 by the end of fermentation. FOS was identified as consisting of inulobiose (32.24 ± 0.16 mg/g), inulotriose (31.49 ± 0.56 mg/g), kestose (49.67 ± 0.24 mg/g), nystose (33.40 ± 0.60 mg/g), and fructosylnystose (23.90 ± 0.43 mg/g), with kestose accounting for the highest FOS content. These findings highlight the strong potential of dahlia tubers fermented with *L. plantarum* B1765 as a valuable substrate for producing high-value FOS, thereby contributing significantly to the development of synbiotic and functional food products as well as broader industrial applications. To enhance the practical applicability of these findings, future research should focus on optimizing fermentation parameters, directly evaluating inulinase activity, conducting comprehensive sensory and product storage studies, and exploring pilot-scale production.

Author's Contribution

Y.M.R. Putra: conducted this research, including data collection, analysis, and drafting of the manuscript. P.R. Wikandari: provided supervision, guidance, and evaluation throughout the research process, and reviewed and revised the manuscript.

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