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Potential Fermentation of Sweet Potato Pickle (*Ipomoea batatas* L.) with *Lactobacillus plantarum* B1765 in the Production of Short Chain Fatty Acids

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Abstract: Short-chain fatty acid (SCFA) is the final metabolic product of inulin that can provide various health benefits. In this study, the effect of fermentation duration of sweet potato pickle using *Lactobacillus plantarum* B1765 starter culture in the production of SCFA was studied, as well as its relationship with total Lactic Acid Bacteria (LAB), pH, and Total Titratable Acid (TTA). Fermentation was carried out with the addition of starter culture as much as 10% (v/v) at 37ºC for 4, 8, 12, 24, and 0 hours which was used as a control. The number of LAB was measured using the Total Plate Count (TPC) method, pH value was measured with a pH meter, TTA was measured using the acid-alkalimetric titration method, and SCFA was measured using an HPLC instrument with 0.1% H_3PO_4 eluent and 25% acetonitrile. Fermentation duration affects LAB growth, TTA, SCFA, and pH. The results showed that total LAB growth was optimal at 8 hours fermentation time at 2.81×10^8 CFU/mL, which increased by 2 log cycles from the initial fermentation time of 0 hours. Still, the pH decreased until it reached 3.23 ± 0.20, and TTA increased to 0,56 ± 0.02% until the end of fermentation at 24 hours. SCFA also increased until the end of fermentation at 24 hours, where acetic acid levels showed the highest levels (6.85 ± 0.30 mg/mL), followed by propionic acid (5.04 ± 0.20 mg/mL) and butyric acid (2.14 ± 0.10 mg/mL). Sweet potato pickles fermented with *L. plantarum* B1765 starter culture have met the SNI 01-3784-1995 standard and have the potential to be developed as a functional food source of SCFA, which has many health benefits.

Keywords: *Lactobacillus plantarum* B1765; Sweet Potato Pickle; SCFA; Total LAB; TTA.

Introduction

Sweet potato (*Ipomoea batatas*) is a dicotyledonous plant from the Convolvulaceae family [1]. Sweet potato production in 2019 was 1,515,739 tons, with a productivity of 180 quintals/hectare, and in 2020, production increased to 1,604,184 tons [2]. The productivity of sweet potatoes is also stated in 2021 at 210.35 quintals/hectare [3]. This high productivity has not been utilized optimally. Sweet potatoes are still limited; most people use them by consuming them directly or processing them, for example, chips [4]. The potential of yellow sweet potato can still be developed as a functional food. Functional food is foods that naturally contain one or more compounds, where these compounds have specific physiological functions that are useful for the health of the human body [5]. Several studies show that sweet potatoes contain a bioactive component in the form of inulin, a dietary fiber known for its benefits as a prebiotic. One of the bioactive compounds that acts as a prebiotic is inulin, a dietary fiber [6]. Sweet potatoes also contain dietary fiber in pectin and inulin; the inulin content in yellow sweet potatoes is known to be 4.6% [7].

Inulin is a polymer containing a fructose group with a β-2-1 fructofuranside bond, where this fiber is soluble in water and cannot be digested by digestive enzymes [8]. Human digestive enzymes cannot digest inulin but can be metabolized by intestinal microorganisms into short-chain fatty acids (SCFA). SCFA is a compound that has many health benefits, so the food processing process that produces SCFA has the potential to be developed as a functional food [9].

SCFA is known to maintain body homeostasis, modulate metabolic processes and the immune system, and directly protect against pathogens [10]. Apart from that, SCFA can preserve the health of epithelial cells in the large intestine, can also act as an anti-tumour and anti-inflammatory, control glucose homeostasis, regulate appetite, and maintain the circulatory system [11]. In the SCFA formation process, inulin will be hydrolyzed into a simple form by the activity of the inulinase enzyme [10]. The inulinase enzyme in inulin from yellow sweet potatoes becomes a mixture of fructose and Glucose [12]. Glucose and fructose will enter the glycolysis process, forming Phosphoenolpyruvate (PEP) and Dihydroxyacetone phosphate (DHAP). Then, PEP will be degraded into pyruvate, divided into several pathways to form acetic acid, butyric acid, and propionic acid [13].

Inulinase can be produced by isolating plants that contain inulin, but inulinase produced by plants has relatively low activity compared to microbes and fungi [14]. Apart from plants, inulinase can also be made by microbes. Several intestinal microorganisms have been confirmed to be capable of producing inulinase enzymes, such as *Pichia guillermondii* strain 1 [15], *Kluveromyces marxianu*s NRRL Y-7571 [16], *Saccharomyces* sp. [17], and *Aspergillus niger* AUMC 93775 [18].

In this research, sweet potatoes as a source of inulin will be used to produce SCFA by fermenting sweet

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potatoes in pickle form using the starter culture *L. plantarum* B1765. Pickel is a solid food from vegetables, fruit, or meat preserved using acid. This acid comes from the fermentation of the fruit or vegetable liquid itself or is obtained from adding vinegar [19]. The use of *L. plantarum* B1765 as a starter culture in this research is based on research results which state that *L. plantarum* B1765 is known to be able to produce inulinase enzyme activity of 0.047 U/mL for 18 hours of incubation time [20]. *L .plantarum* B1765 has been studied to produce SCFA from inulin sources and FOS from yacon tuber sources [21, 22].

Based on the description above, the research aimed to determine the effect of fermentation time for sweet potato pickles on the production of SCFA, which is formed from inulin as a source of prebiotics with *L. plantarum* B1765 activity. As well as to see how LAB, pH, and TTA grow during fermentation.

Research Methods

Tools

The tools in this research include analytical balance (Denver S1-234), autoclave (Hirayama HVE-50), laminar airflow (Thermo Scientific 1300-series A2), micropipette (Eppendorf), incubator (Memmert), centrifugator (Eppendorf), water bath (Labtech LWB 122D), HPLC (Shimadzu CBM 20 A), pH meter, burette, stand, glass tools, 400 mL glass jar, blender, knife, cutting board, spirit burner, lighter, bath, pan, spray bottle, and dropper pipette.

Material

The materials used in this research include sweet potatoes obtained from sellers at Lawang market, Malang, *L. plantarum* B1765 starter culture, MRS Broth (Merck), sterile aqua mineral, 0.85% NaCl, sugar, agar (swallow) flour, $CaCO_3$, acetic acid standard solution (Merck), propionic acid standard solution (Merck), butyric acid standard solution (Merck).

Preparation of *L. plantarum* B1765 Starter Culture

The *L. plantarum* B1765 starter culture is an isolate from fermented bekasam fish developed in deMan Rogosa Sharpe (MRS) Broth medium, which has been sterilized by autoclave at 121ºC and 15 Psi pressure for 3 hours. A total of 1 mL of *L. plantarum* B1765 stock culture was cultured in 9 mL MRS Broth and incubated at 37ºC for 20-24 hours. Next, the *L. plantarum* B1765 starter culture was centrifuged for 5 minutes at 3500 rpm, resuspended in 9 mL of sterile 0.85% NaCl solution, and centrifuged for 5 minutes at 3500 rpm. The pellet was resuspended in 10 mL of sterile 0.85% NaCl solution as a starter culture [23].

Making Sweet Potato Pickle

Making sweet potato pickles follows a modified procedure with *L. plantarum* B1765 bacterial culture inoculum. Sweet potatoes are peeled, washed, cut lengthwise into matchsticks, and blanched for 5 minutes at 85℃. The sweet potato pieces were weighed to ± 100 grams, then put into a 400 mL sterile glass jar, 2% (v/v) salt solution, 2% (v/v) sugar solution, and 100 mL of sterile aquademineral were added. Then added bacteria *L. plantarum* B1765 as much as 10% (v/v), fermented for 0, 4, 8, 12, and 24 hours at 37℃ [21].

Total Lactic Acid Bacteria (LAB)

Total LAB was calculated by counting the total LAB that grew on DeMan Rogosa Sharpe (MRS) culture media. MRS agar media is made from 5,22 grams of MRS Broth dissolved in 100 ml of distilled water, with 1,5% agar and 1% $CaCO_3$ added. Samples of sweet potato pickles that had been crushed with a blender were then diluted with 0,85% NaCl solution to a dilution of 10^1-10^8. Cups were carried out by taking a sample using a micropipette of 1 ml (1000 µl) of the resulting dilution into a petri dish. The dilution media was made in triplicate. Next, ± 10 ml of MRS agar medium is poured into the cup. Immediately after pouring, the petri dish is moved in a figure of eight so that the inoculated bacteria are evenly distributed, then let sit until it solidifies. The media in the petri dish was then incubated upside down at 37ºC for 48 hours. Then, the number of lactic acid bacteria was calculated in CFU (Colony Forming Unit)/mL units. The colonies that grow and can be counted are between 25-250 colonies. All treatments were carried out aseptically [23].

pH and TTA Test

pH and TTA measurements were carried out by crushing the pickel with the pickel liquid. When measuring pH, take 20 mL into a beaker, then measure a pH meter that has been calibrated until you get a stable number on the pH meter [21].

At TTA, 10 mL was diluted in a 100 mL volumetric flask, then pipetted 20 mL to put into an Erlenmeyer flask. After that, three drops of phenolphthalein (PP) indicator were added and titrated with NaOH solution. The titration was stopped if a stable pink color change occurred [24].

Short Chain Fatty Acid Test

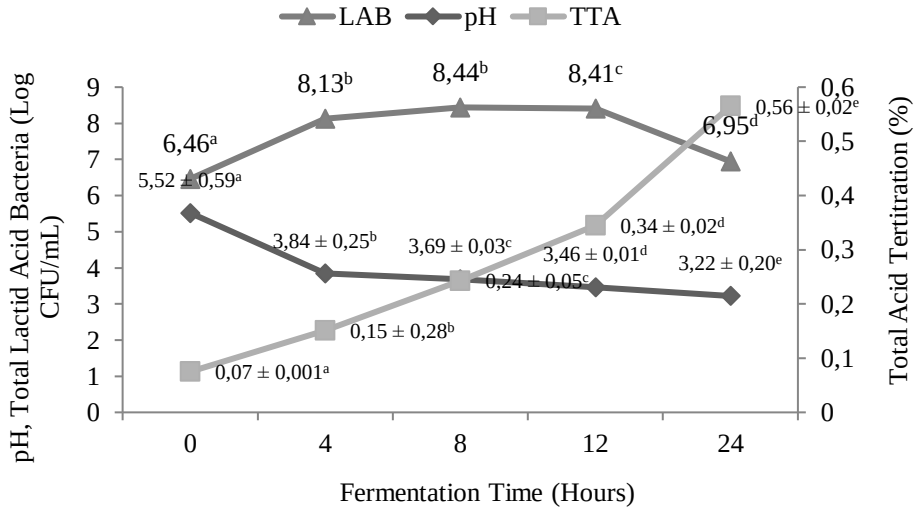
The SCFA standard solution was prepared using graded dilutions with a concentration of 0.001, 0.005, 0.010, 0.025, and 0.050 mg/mL of 0.100 mg/mL standard solution. The standard solutions used are acetic acid, propionic, and butyric acid. Next, sweet potato pickles were crushed with water, a 2 mL sample was taken and then centrifuged within 10 minutes at a speed of 8500 rpm. 1 mL of the supernatant was taken and dissolved with 10 mL of distilled water. Before being injected into HPLC, the standard solution, sample, and mobile phase were filtered using a PTFE membrane with a diameter of 47 mm. Then, the standard solution and sample were injected into the HPLC with a volume of 100 µL with a column temperature of 25ºC. UV-Vis detector, wavelength 280 nm, and eluent consisting of 0.1% H_3PO_4 and 25% acetonitrile using a ratio of 45:55. The SCFA concentration is determined after the retention time of each standard is known and compared with the retention time of the sample [22].

Result and Discussion

LAB growth, pH, and TTA

In this research, LAB, pH, and TTA tests were carried out to determine the growth of LAB, pH, and TTA and the effect of fermentation time on sweet potato pickles. The research results analyzed using One Way ANOVA showed an influence ($p < 0.05$) on total LAB and pH. The TTA data did not meet normality, so data analysis was carried out using the Kruskall Wallis Test and showed the effect of fermentation time ($p < 0.05$) on the TTA value. Further tests with Post Hoc LSD showed that the total LAB was significantly different from the start of fermentation (0 hours) to 8 hours of fermentation. There was no real difference between fermentation from 8 to 12 hours and a significant difference from 12 to 24 hours. However, pH and TTA showed substantial differences from the beginning (0 hours) of fermentation to the end of fermentation (24 hours). The results of the total growth test for LAB, pH, and TTA of sweet potato pickle fermentation can be seen in Figure 1.

In the research that has been carried out, the most significant growth occurred at 8 hours of fermentation, where LAB increased by 2 log cycles from 2.93×10^6 at 0 hours to 2.81×10^8; this indicates that LAB entered the exponential phase. After that, LAB showed no real difference between 8 and 12 hours. This suggests that LAB entered the stationary phase, which showed a decrease at 24 hours of fermentation, which means LAB had entered the death phase.



Figures 1. Total LAB, pH, TTA Test Results of Sweet Potato Pickle Fermentation
Note: Values followed by the same letter indicate that they are not significantly different ($\alpha = 0.05$)

LAB growth was highest at 8 hours of fermentation, where the bacteria entered the exponential phase. In this phase, nutrients in the form of starch or other polysaccharides will be broken down into Glucose by the α-amylase enzyme, which begins to be secreted in this phase. The α-amylase enzyme is known to be produced by several bacteria, including *L. plantarum* bacteria [25]. Glucose then undergoes further metabolism into lactic acid and other organic acids, lowering the product's pH. In this phase, there is also an increase in the inulinase enzyme, which hydrolyzes inulin into Glucose in the media, which will be used as an energy source [26]. *L. plantarum* B1765 is known to produce inulinase enzyme activity of 0.047 U/mL for 18 hours of incubation time [20].

Research by Wikandari et al. [27] showed that the total LAB in fermented yacon pickle as a source of inulin, which was inoculated with 10% *L. plantarum* B1765, reached an optimal of 3.25 x 10^8 CFU/mL at 48 hours of fermentation. Research from Wijayanti & Wikandari [22] showed the highest LAB growth in jicama extract products as a source of inulin with 5% *L. plantarum* B1765 of 1.15×10^8 CFU/mL at 12 hours fermentation time.

LAB growth, when compared with yacon pickle, sweet potato pickle with the same LAB concentration showed faster LAB growth. In sweet potatoes, it reaches 108 at 8 hours, while in yacon pickle, it is 48 hours. This is due to the possibility that sweet potatoes contain higher levels of starch than yacon pickles, thus providing better nutrition for the total growth of LAB.

Meanwhile, when compared with jicama extract, sweet potato pickle has a higher and faster total LAB. This can happen because sweet potato pickles use a higher concentration of starter culture than jicama extract, namely 10%. The effect of increasing total LAB from sweet potato pickles is also thought to occur due to adding 2% sugar (v/v) and 2% salt (v/v) to the sweet potato pickle fermentation product. When this sugar is added later, bacteria will use it as a source of nutrition, which will then be hydrolyzed into a simple form to support LAB growth [28]. Meanwhile, salt can stimulate the growth of lactic acid bacteria, where the salt acts as a selective agent that will inhibit other microorganisms besides lactic acid bacteria [29].

The total growth of LAB during the fermentation process of sweet potato pickles also affects other qualities, namely decreasing pH and increasing TTA. In (Fig. 1), it can be seen that the pH there was a decrease from a pH of 5.52 ± 0.59 at 0 hours to 3.23 ± 0.20 at 24 hours of

fermentation. Meanwhile, TTA increased by 0.07 ± 0.001% at the beginning of fermentation (0 hours) to 0.56 ± 0.02% at the end (24 hours). Research [22] showed that the fermented product of jicama extract also experienced a decrease in pH from 5.79 in fermentation (2 hours) to 3.91 in time (36 hours), and there was an increase in TTA from 2 hours of fermentation by 0.127% to 0.223 % at 36 hours of fermentation time. This decrease in pH and increase in TTA occurs due to a metabolic process by the *L. plantarum* B1765 bacteria, which can produce lactic acid and other organic acids such as acetic acid. Lactic acid is obtained from starch metabolism and added sugar, while inulin metabolism will metabolize into SCFA.

Effect of Fermentation on Short-Chain Fatty Acid Production

This study tested SCFA using an HPLC instrument with 0.1% H_3PO_4 eluent and 25% acetonitrile, where the ratio was 45:55. Example of SCFA chromatogram results with HPLC instruments (Figure 2).

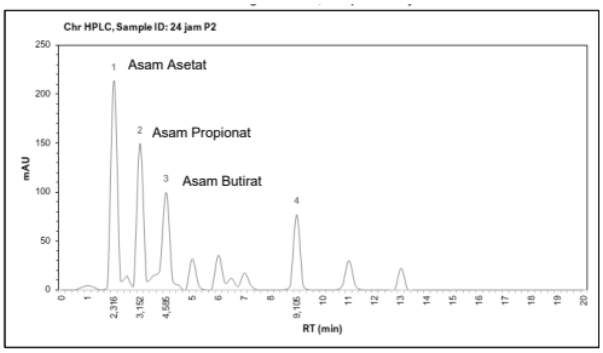


Figure 2. Chromatogram of Short Chain Fatty Acid Solutions

The SCFA production results of sweet potato pickle fermentation are shown in (Fig 3); it can be seen that the longer the fermentation time, the more the SCFA production also increases. SCFA is the final sweet potato pickle product because fermentation uses the starter culture L. plantarum B1765, a facultative heterofermentative bacteria. This type of bacteria can produce lactic acid and types of SCFA such as acetic, propionic and butyric acids [30].

In this study, SCFA, with the highest production, was obtained in acetic acid, which reached 6.85 ± 0.30 mg/mL at 24-hour fermentation from the initial concentration of 0.54 ± 0.06 mg/mL. Acetic acid is the most dominant SCFA product; its highest production is at the end of fermentation (24 hours). This shows acetic acid's metabolic pathway is more active or efficient under sweet potato pickle fermentation conditions. The research results were supported by Ji-Young & Sung-Hee et al. [31], who fermented inulin sources from artichokes with *L. plantarum*, where the most SCFA produced was acetic acid. The acetic acid produced was 25-30 mM after 48 hours of fermentation. Another study by Maryati et al. [32] showed that the highest yield of SCFA was acetic acid using an inulin medium by *L. acidophilus* FNCC 0051, amounting to 60.83 mM.

Acetic acid production is higher because acetic acid can be formed through two pathways, namely the Acetyl Co-A and Acetogenic Cross-feeding pathways. The acetyl Co-A pathway is formed due to changes in Acetyl Co-A from pyruvate accompanied by the release of CO_2 into acetate by the activity of the acetate kinase enzyme [13]. Meanwhile, the Acetogenic Cross-feeding pathway is a direct process from pyruvate to acetate via Acetyl Co-A [13].

Of the two pathways, the first one is suspected to produce more acetic acid because the path is more straightforward and faster, thus allowing quicker and more acetic acid production. Second, other more complex organic acid formation pathways, such as propionate and butyrate, require more steps and energy, resulting in slower production than acetic acid [33].

Apart from acetic acid, there are other SCFA, namely propionic and butyric acids, which also increase during fermentation. However, the production of propionic and butyric acids is not as high as that of acetic acid. Propionic acid is formed from 3 pathways: acrylate, succinate, and propanol [13]. Meanwhile, butyric acid is formed from the butyryl-phosphate pathway and the butyryl Co-A pathway. Although propionic acid and butyric acid have different pathways, these two acids have a more complex process than the more straightforward pathway for forming acetic acid [34].

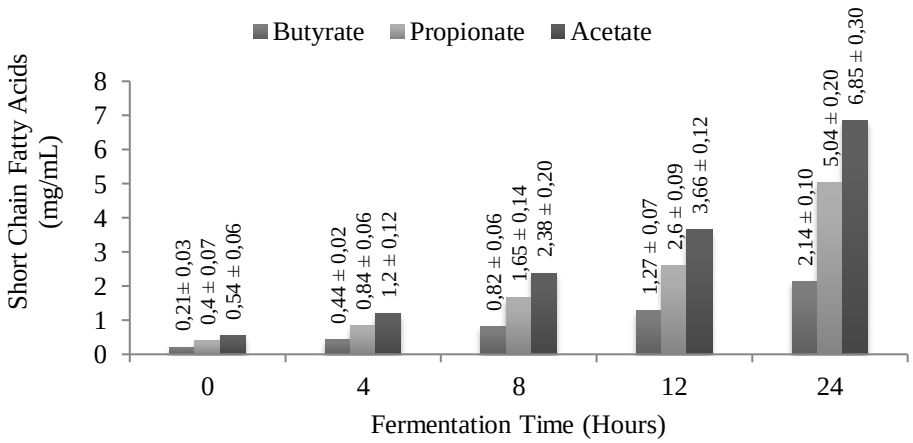


Figure 3. Production of SCFA Fermented Sweet Potato Pickles

Propionic acid production from 0.40 ± 0.07 mg/mL increased to 5.04 ± 0.20 mg/L, and butyric acid production also increased to 2.14 ± 0.10 mg/mL from the initial concentration of 0.21 ± 0.03 mg/mL. Each increase in SCFA production is related to the growth of LAB, TTA, and a decrease in pH. LAB growth directly influences SCFA production during fermentation. When LAB grows and metabolizes substrates, it produces lactic acid and SCFA, such as acetate, propionate, and butyrate. The production of lactic acid and SCFA causes a decrease in pH and an increase in TTA values.

In the process, the formation of SCFA is formed due to the presence of inulin. Inulin is a fibre converted by the inulinase enzyme into a mixture of fructose and Glucose. Inulinase enzyme activity occurs in the exponential phase; in this phase, the inulinase enzyme will form Glucose and fructose and be added to SCFA [35]. It can be seen in Figure 3 that there is an increase in SCFA production from 0 hours to 8 hours, which occurs in the exponential phase.

Furthermore, at 12 hours and 24 hours fermentation time, SCFA production continued to increase. This is thought to occur because the inulinase enzyme, which works in the exponential phase, continues to be active until the death phase. Inulinase continues to carry out the hydrolysis process on substrates, which may be wholly degraded, so the inulinase enzyme will continue to convert inulin into a mixture of fructose and Glucose, which will continue to become SCFA.

Conclusion

This research shows that fermentation time influences the increase in total LAB, TTA, and SCFA, as well as the decrease in pH. The study results showed that the total growth of LAB was optimal at a fermentation time of 8 hours, 2.18×10^8 CFU/mL increased by 2 log cycles from the start of fermentation (0 hours). This is in line with an increase in TTA from 5.52 ± 0.59% to 3.23 ± 0.20%, and pH decreases until the end of fermentation of 3.23. SCFA increased until the end of fermentation, namely at 24 hours, where acetic acid (6.85 ± 0.30 mg/mL), propionic acid (5.04 ± 0.20 mg/mL), and butyric acid (2.14 ± 0.10 mg/mL). SCFA production is most abundant in acetic acid. Therefore, the fermentation of sweet potato pickles with *Lactobacillus plantarum* B1765 has the potential to produce SCFA.

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