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POTENCY OF FERMENTED JICAMA EXTRACT CULTURED WITH *Lactobacillus plantarum* B1765 FOR PRODUCING SHORT CHAIN FATTY ACID

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Abstract: Short-chain fatty acid (SCFA) is the end product of inulin metabolism, which has various health benefits, such as affecting lipid metabolism and as an antidiabetic agent. This research studied the effect of the fermentation time of jicama extract with *Lactobacillus plantarum*B1765 as a starter culture on producing SCFA. Fermentation was conducted for 2, 12, 24, and 36 hours at a temperature of 37°C with 5% (v/v) of the starter culture, then determined the growth of LAB, pH, Total Titratable Acid (TTA), fructose, and Short Chain Fatty Acid content. The total LAB count was measured using the Total Plate Count; fructose was measured using the Nelson-Somogyi method, while SCFA was measured using HPLC with eluents H_3PO_4 0.1% and acetonitrile 25%. The length of fermentation affected the increase in total Lactic Acid Bacteria (LAB), Total titratable acidity (TTA), fructose, SCFA, and the decrease in pH. The results showed that the optimal growth of Total LAB at 24 hours of fermentation correlated with fructose content, but increasing TTA and SCFA until 36 hours of fermentation and pH decreased to 3,92 at the end of fermentation and decreasing pH due to lactic acid and SCFA producing. SCFA was identified as consisting of acetic acid (1.31±0.006 mg/mL), propionic acid (1.58±0.0055mg/mL), and butyric acid (1.05±0.0038mg/mL), with the highest SCFA was propionic acid. Therefore, fermented jicama extract with *Lactobacillus plantarum* B1765 has the potential to produce SCFA, which could be used for various health benefits.

Keywords: *Jicama Extract, Fermentation, Lactobacillus plantarum B1765, SCFA*

INTRODUCTION

Inulin is a prebiotic dietary fiber that has many benefits for the body, such as affecting lipid metabolism by lowering triglycerides and LDL so that it can have a positive influence on cholesterol sufferers, forming short-chain fatty acids (SCFA), which can help increase insulin formation for people with diabetes mellitus, improve the immune system, and improve digestive system health [1]. Digestive enzymes cannot digest inulin, so it requires the role of intestinal microorganisms to degrade it into monosaccharide [2].

Jicama (*Pachyrhizus erosus*) is a tuber cultivated in Indonesia that originated from the Americas. Jicama tuber is characterized by a flat shape, white tuber flesh, thin skin that is pale yellow and tastes like a pear or apple but a texture like a radish [3]. Jicama is a source of inulin. The inulin content in fresh jicama was 6.51%, and the inulin content in jicama extract was 4.41% [4]. The inulin content in jicama extract was 12.322% [5]. Inulin from jicama has an inulin polymerization degree of 36% [6].

Jicama can be utilized as a functional food by utilizing inulin as a source of prebiotics that can be used by intestinal microflora. The potential of jicama as an available food can be enhanced by developing synbiotic available food. Synbiotics are a combination of probiotics and prebiotics that work together to provide health benefits to the body [7]. Synbiotic functional food of jicama was developed by utilizing jicama inulin as a prebiotic source that will be fermented using a probiotic starter culture.

Fermented jicama as a synbiotic product provides an advantage over its new form, namely the formation of SCFA in the product. SCFA has many benefits; it can maintain the health of epithelial cells in the colon, be anti-tumor and anti-inflammatory, strengthen immune cells and antibodies against antigens, prevent obesity, control glucose homeostasis, regulate appetite, and maintain the circulatory system [8]**.** SCFA can also act as an antidiabetic by improving glucose homeostasis and activating *Free Fat Acid Receptor 2 (*FFAR2) to stimulate pancreatic β cells to increase insulin with the help of the GLP-1 hormone [9]. Consuming products that have formed SCFA will make it easier for the body to utilize it immediately. Another benefit is that probiotic microorganisms in symbiotics will improve gut microflora in producing other beneficial secondary metabolites for health. Consumption of dietary fiber or other carbohydrates that digestive enzymes cannot digest through bacterial cross-feeding will help create SCFA in vivo [10].

SCFA is produced from inulin metabolism. Monosaccharides formed due to inulin degradation include glucose monomers, and the majority are fructose monomers. Both glucose and fructose will be further metabolized to form pyruvate. Pyruvate will then split through several pathways to produce SCFA, which consists of

three main components, namely acetic acid, propionic acid, and butyric acid [11]. Degrading inulin into monosaccharides requires the activity of inulinase secreted by microbes. Inulinase is divided into two, namely exoinulinase and endoinulinase. Both can run simultaneously. Exoinulinase cuts inulin sequentially and produces fructose monomers, while endo inulinase cuts inulin randomly and produces an oligosaccharide form, namely fructooligosaccharide (FOS) [12,13].

Several gut microorganisms have been confirmed to be capable of forming inulinase enzymes, such as *Bacillus sp., Streptomyces sp.,* Xanthomonas *sp.,* and *Clostridium* sp. [14,15]. Few studies have been conducted on inulinase production from lactic acid bacteria. *L. casei* AP is known to secrete inulinase with an activity of 20.53 U/mL in inulin media [16]. Lactobacillus *plantarum* B1765 was shown to be able to form an inulinase enzyme with an activity of 0.047 U/ml for 18 hours of incubation time [17]. It is known that *L. plantarum* is one of the microorganisms present in the intestine [18]. *L. plantarum* B1765 can also act as a probiotic characterized by its ability to withstand the pH of the gastrointestinal tract and is antagonistic to pathogenic bacteria [19]. In addition, *L. plantarum* B1765 has been known to form SCFA using inulin from yacon [20] and inulin from single garlic [21].

Our study aimed to study the effect of fermentation time on the SCFA profile formed from the prebiotic inulin source of jicama extract by the activity of *L. plantarum* B1765 as a probiotic. The growth of total LAB, pH, TAT, and fructose during fermentation was also observed.

RESEARCH METHODS

Culture Preparation

A starter culture of *L. plantarum* B1765 in MRS Broth medium was sterilized by autoclave at 121o C for 2 hours. 1 mL of *L. plantarum*B1765 stock culture was cultured in 9 mL MRS Broth and incubated at 37°C for 20 hours. Next, the starter culture of *L. plantarum* B1765 was centrifuged for 5 minutes at 3500 rpm, resuspended in 9 mL of 0.85% sterile NaCl solution, and centrifuged again. Pellets were resuspended in 10 mL of 0.85% clean NaCl solution for starter culture [22].

Sample Preparation

Jicama was peeled, washed, and cut into small pieces. Jicama was weighed as much as ± 500 grams, then blanched for 5 minutes to minimize microbes that could cause contamination. Then, it was blended with the addition of water in a ratio of 1: 2 (w/v) until it became a slurry. The slurry was shaken at 150 rpm for 60 minutes, then filtered. The filtrate of jicama extract was put into each sterile container labeled as much as 250 ml [23]. Added 12.5% (v/v) sugar, then pasteurized for 15 minutes at 70ºC. The last stage was adding *L. plantarum* B1765 bacteria as a starter culture of as much as 5% (v/v). Tightly closed and fermented for 0, 12, 24, and 36 hours in an incubator at 37ºC.

Determination of Total Lactic Acid Bacteria

To determine the growth of lactic acid bacteria, samples were diluted using 0.85% NaCl solution to a dilution of 10^{-1}-10^{-8}. Dilution was carried out by taking samples using a micropipette as much as 1 ml (1000µl) of the dilution results into a petri dish. Furthermore, MRS agar medium (MRS Agar and $CaCO_3$) was poured into the cup as much as ± 15 ml. The media in the petri dish was then incubated at 37º C for 48 hours. Then, the number of lactic acid bacteria was counted with CFU *(colony forming unit)*/mL. Colonies that grew and could be counted amounted to between 25-250 colonies (CFU = Colony Forming Unit). This method was conducted by [24].

Determination of pH and TTA

To determine pH and TTA, Jicama extract was taken about ± 20 mL and placed in a 50 ml beaker glass. Before use, the pH meter is calibrated using pH 4 and 9 buffers and then cleaned with distilled water, and then the sample pH is measured. Every time you want to measure the pH of another sample, the pH meter is previously cleaned with distilled water [25].

A sample of 10 ml was diluted to 100 ml in a volumetric flask, then 20 ml was taken, and 2-3 drops of phenolphthalein indicator were added. Then, the sample was titrated with 0.1 N NaOH solution until the color changed to pink. Total Titrated Acid (TAT) is expressed in percent lactic acid [25].

Determination of Fructose

To determine fructose content, fructose standard solution was prepared by taking 1 mL of fructose average solution concentrations 0.050, 0.100, 0.150, 0.200, 0.250 mg/mL. Added Nelson Somogyi reagent as much as 1 mL, heated in a bath for 10 minutes, and cooled for 10 minutes. Then added, with 1 mL of arsenomolybdate reagent and 5 mL of distilled water, the solution was shaken until homogeneous, and the absorbance was calculated at a wavelength of 754.50 nm so that the regression equation for the fructose standard is obtained. Furthermore, the fermentation results were evaporated with a rotary evaporator to be used as samples. Samples were taken as much as 1 mL of sample jicama extract solution with a concentration of 3 mg/mL. Added Nelson Somogyi reagent as much as 1 mL, heated in a bath for 10 minutes, and cooled for 10 minutes. Then, added with 1 mL of arsenomolybdate solution and 5 mL of distilled water, the answer was shaken until homogeneous, and the absorbance was calculated at a wavelength of 754.50 nm with a

UV-Vis Spectrophotometer. Fructose content was calculated using the regression equation that has been obtained.

Determination of Short Chain Fatty Acid

The determination of SCFA followed the procedure from Rawat [26]. Preparation of ALRP standard solution by performing multilevel dilutions with concentrations of 0,001, 0,005, 0,010, 0,025, and 0,050 mg/mL from 0,100 mg/mL common solution. The standard solutions used are the acetic acid standard, propionic acid standard, butyric acid standard, and lactic acid standard. Furthermore, the fermentation results were evaporated with a rotary evaporator to be used as a solid sample. The sample was taken 0.1 grams and dissolved with distilled water in a 10 ml volumetric flask. Then, the sample was homogenized using a vortex for 10 minutes and allowed to stand for 30 minutes. Then, the solution was filtered using Whatman filter paper no. 42, take the filtrate. The filtrate that has been obtained is then centrifuged at 8000 rpm for 20 minutes, and the supernatant. Take 1 ml of supernatant solution, then dissolve with 10 ml of aqua bikes. Before being injected into HPLC, both standard solution, sample solution, and a mobile phase (eluent) were filtered with PTFE membrane (PTFE Membrane Filter Diameter 47 mm Pore 0.45 µm) and degassing (Waters HPLC Degasser AF degassing unit LC). Then, the standard solution and sample were injected into HPLC with a volume of 100 µL with column conditions at 25°C, UV Vis detector, wavelength 280 nm, and eluent consisting of H_3PO_4 0.1% and acetonitrile 25% in a ratio of 45:55. The concentration of ALRP was determined after the retention time of each standard was known and compared with the retention time of the sample.

RESULTS AND DISCUSSION

The Growth of LAB, Acidity, and TTA Profile

The research results indicate a significant difference ($p < 0.05$) in total LAB between 2 hours and 12 hours of fermentation, but there was no significant change between 12 hours to 36 hours of fermentation. Regarding TTA, there was a substantial difference between 2 hours to 24 hours of fermentation, but no significant change was observed between 24 and 36 hours of fermentation. The data pH analysis showed a significant difference at 0, 12, and 36 hours of fermentation, but no significant difference occurred between 12 hours and 24 hours of fermentation. The results of the total LAB, pH, and TTA tests for jicama extract fermentation can be seen in (Figure 1).

Enumeration of total LAB showed a significant increase ($p < 0.05$) with the highest count of $1.15x10^8 \pm 0.13$ CFU/mL until 12 hours of fermentation, after which the total LAB count didn't show significant changes. The increase in the entire LAB is influenced by the fermentation time; the longer the fermentation time, the higher the resulting total LAB count [27]. In this study, the maximum growth of BAL occurred at 12 hours of fermentation, where there was a rapid increase in the bacterial count by 2 log cycles, indicating the exponential phase of fermentation. After 12 hours of fermentation, the growth of LAB did not show significant results between 12 hours to 36 hours, indicating that the LAB had entered the stationary phase. The highest growth of BAL in yacon pickle as a source of inulin with 10% inoculation of *L. plantarum* B1765 was achieved at 48 hours, with a count of 3.25×10^8 CFU/ml [20]. Fermentation of inulin from *Dioscorea esculenta* tuber flour with 5% *L. plantarum* B1765 increased the total LAB count by 2 log cycles over 72 hours [28]. Fermented jicama extract beverage promoted faster BAL growth than yacon-pickle and fermented inulin from *Dioscorea esculenta* tuber flour. It is suspected that the addition of 12.5% (v/v) sugar in the fermented jicama beverage influenced the growth of BAL. Bacteria can utilize sugar as a nutrient source, further hydrolyzed into monosaccharides to support BAL growth [29].

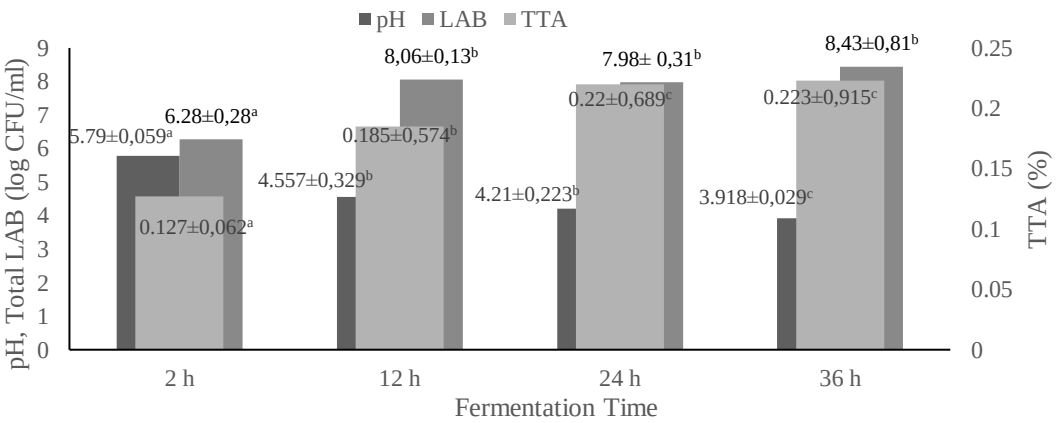

Figure 1. Total LAB, Acidity, and TTA of Fermentation of Jicama Extract During Time Fermentation

LAB growth during jicama extract fermentation also affects the decrease in pH and the increase in TTA. The pH decreased from 5.79±0.059 to 3.92±0.029, while the TTA showed a significant increase ($p < 0.05$) from 0.127±0.062% to 0.22±0.689% during the 2-hour to 24-hour fermentation period. However, there was no significant increase in TTA during the 24-hour to 36-hour fermentation period. The amount of lactic acid produced during fermentation affects the decrease in pH, as lactic acid dissociates into H^+ ions and $CH_3CHOHCOO^-$ ions. An increase in H^+ ions can decrease the pH of the sample [30]. Besides that, the formation of SCFA also causes a reduction in pH in the model. This is due to the increased number of lactic acid bacteria that will hydrolyze inulin into SCFA [20].

The Effect of Fermentation Time on the Production of Fructose

Jicama is a tuber rich in inulin. Inulin is a prebiotic dietary fiber that cannot be digested by digestive enzymes but can be utilized by gut microbes as a source of energy, making it a suitable medium for the growth of gut microbes [2]. Inulin can be further hydrolyzed into monosaccharides and oligosaccharides. The formed monosaccharides primarily consist of glucose and fructose.

The research results showed a significant difference ($p < 0.05$) during the 2-hour to 36-hour fermentation period. The highest fructose content was found at 24 hours of fermentation, measuring 163.81±2.89 mg/mL, and then decreased to 129.69±2.17 mg/mL at 36 hours of fermentation (Fig 2). Natasya & Wikandari [28] stated that the total fructose content increases with the longer fermentation time, as more inulin chain bonds are broken down into fructose due to prolonged fermentation. Their research findings also support this statement during the 24-hour to 72-hour fermentation period, where the fructose content increased from 163.44 mg/mL to 324.67 mg/mL. Jicama inulin produces a lower amount of fructose compared to *Dioscorea esculenta* tubers. This is likely due to the lower inulin content in jicama, which is around 6.51% [4], while *Dioscorea esculent* tubers contain 10.10% - 21.13% inulin [31]. The fructose content resulting from inulin hydrolysis in *Dahlia pinata* tubers is around 75-98%, depending on environmental conditions and storage duration [32].

The formation of monosaccharides results from the sequential cleavage of β-2,1 bonds in inulin by the activity of exoinulinase. Fructooligosaccharides (FOS) are the end products of random division by the action of endo-inulinase enzymes [33]. To degrade inulin into simpler forms, the assistance of inulinase enzymes is present in microorganisms and inulin-containing plants. Nabila & Wikandari [17] demonstrated in their research that *L. plantarum* B1765 produced inulinase with an activity of 0.047 U/ml within 18 hours of incubation. The decrease in fructose content is caused by further fructose metabolism into short-chain fatty acids (SCFA).

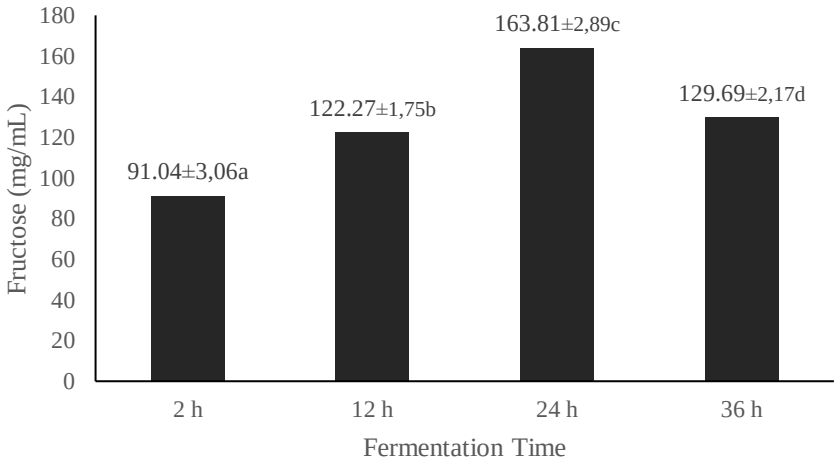

Figure 2. The Production of Fructose from Fermentation of Jicama Extract During Time Fermentation

The Effect of Fermentation Time on The Production of Short Chain Fatty Acid and Lactate Acid

Lactobacillus plantarum is a bacterium commonly found in fermented foods and the intestinal tract, making it potential as a probiotic starter culture in the food industry [34]. *L. plantarum* B1765 is a lactic acid bacteria that can act as a probiotic, characterized by its ability to survive in the gastrointestinal tract and exhibit antagonistic effects against pathogenic bacteria [19]. *L.plantarum* B1765 is also known to have inulinase activity of 0.047 U/ml for 18 hours of incubation time [17].

The result of total inulin degradation by inulinase enzymes is glucose monomers, mainly fructose. Both glucose and fructose undergo glycolysis to form phosphoenolpyruvate (PEP) and further metabolism to SCFA [11]. Acetate, propionate, and butyrate are the main components of SCFA [35], while lactic acid is the primary product of the fermentation process [36]. In this research, ALRP and lactic acid were tested using an HPLC instrument with 0.1% H_3PO4 and 25% acetonitrile as the eluent with a 45:55 ratio. An example of the SCFA and Lactic Acid chromatogram is shown in Fig 3.

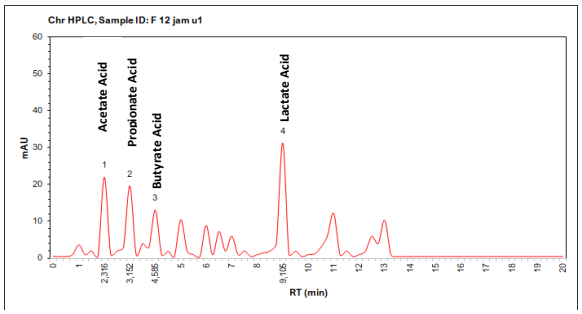

Figure 3. The SCFA and Lactic Acid Chromatogram

The relationship between fermentation time and SCFA formation is visually demonstrated in Fig 4. It can be seen that, as the fermentation time increases, so does the level of SCFAs. Lactic acid was the highest concentration throughout the fermentation process, as it is the main product produced. In fact, at 2 hours after fermentation, its concentration was 1.27 ±0.0093 mg/mL and increased to 3.46 ± 0.0491 mg/mL at the end of fermentation. Lactic acid is the main product in jicama fermentation due to using *Lactobacillus plantarum* B1765 as a facultative heterofermentative starter culture. Besides lactic acid, short-chain fatty acids such as acetic acid, pyruvate, and butyrate are also produced, indicating an increase during the fermentation process. Propionic acid is the highest SCFA grew in this study, reaching 2.01±0.0004 mg/mL at 36 hours of fermentation from an initial concentration of 0.84±0.0258 mg/mL. This result is supported by the research [20-21], which showed that the fermentation of yacon and garlic as inulin sources with *Lactobacillus plantarum* B1765 resulted in the highest propionic acid content among other SCFA types. This is due to propionic acid having three formation pathways: the acrylate, succinate, and propanediol pathways. If the substrate to form SCFA is a hexose, the succinate pathway is the dominant path for propionic acid formation. This is supported by the high fructose content in jicama, as shown in Fig. 4. However, if the substrate used is the acrylate pathway, it dominates lactic acid and propionic acid formation. This is consistent with Fig. 4, where lactic acid shows a significant increase during the fermentation process, indicating that propionic acid formation is likely influenced by the elevated production of lactic acid [10]. Meanwhile, acetic acid and butyric acid can only be formed through one pathway each. Acetic acid is formed via the Wood-Ljungdahl pathway, while butyric acid is created via the acetyl-CoA pathway [37].

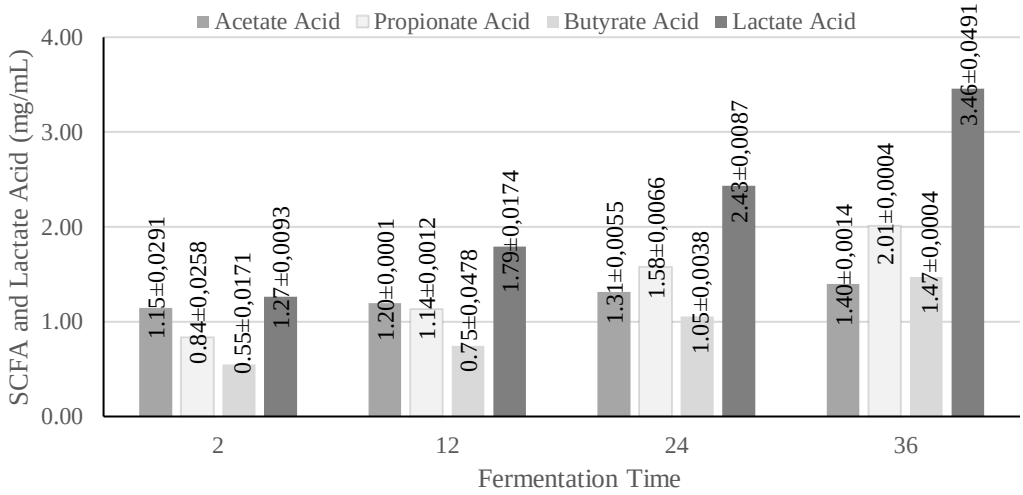

Figure 4. The Production of SCFA and Lactate Acid from Fermentation of Jicama Extract During Time Fermentation

Furthermore, butyric acid also experienced a significant increase from 2 hours to 36 hours of fermentation, from 0.55±0.0171 mg/mL to 1.47±0.0004 mg/mL. Acetic acid is the precursor of butyric acid, so the concentration of acetic acid is the lowest among the SCFA produced during fermentation. At 2 hours of fermentation, the paid acetic acid level was 1.15±0.0291 mg/mL, while at 36 hours of fermentation, the delivered sour acid level was 1.40±0.0014 mg/ml.

CONCLUSION

This study showed that the length of fermentation affected increasing total LAB, TAT, total fructose, SCFA, and lactic acid. Additionally, it also influenced a decrease in pH. The results showed that the optimal growth of Total LAB at 24 hours of fermentation correlated with fructose content, but increasing TTA and SCFA until 36 hours of fermentation and pH decrease to 3,92 at the end of fermentation and decreasing pH due to lactic acid and SCFAproducing. SCFA was identified as consisting of acetic acid (1.31±0.006 mg/mL), propionic acid (1.58±0.0055 mg/mL), and butyric acid (1.05±0.0038 mg/mL), with the highest SCFA was propionic acid. Therefore, fermented jicama extract with *Lactobacillus plantarum* B1765 has the potential to produce SCFA.

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