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EFFECT OF FERMENTED JICAMA EXTRACT WITH *Lactobacillus plantarum* B1765 AS THE STARTER CULTURE ON THE PRODUCT QUALITY AND TOTAL PHENOLIC

Pengaruh Lama Fermentasi Sari Bengkuang dengan Starter Kultur Lactobacillus plantarum B1765 terhadap Kualitas Produk dan Fenolik Total

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ABSTRACT

This research studied the effect of fermented jicama extract with Lactobacillus plantarum B1765 as a starter culture on the product qualities and Total Phenolic (TP). Fermentation was carried out for 0, 12, 24, and 36 hours at 37^{o}C with 5% (v/v) of starter culture then determine the Total Lactic Acid Bacteria (LAB) were measured using the Total Plate Count (TPC) method, pH, and TTA were measured using a pH meter and acid-base titration, and TP was measured using the Folin-Ciocalteu method. These results showed that the length of fermentation affected increasing total LAB, TTA, TP, and decreasing pH. The best fermentation time in jicama extract was fermented is 24 hours with a total LAB of (9.7±0.31)x10^{7} CFU/mL, pH of 4.21±0.22, TTA of 0.220±0.069%, and TP of 16.22±0.31 mg GAE/g. This value is following the criteria for fermented beverage products. Fermented jicama extract with L. plantarum B1765 potentially increases the TP.

Keywords: Jicama extract, Fermentation, *Lactobacillus plantarum*, product quality, total phenolic

ABSTRAK

Penelitian ini bertujuan guna menentukan pengaruh lama fermentasi sari bengkuang dengan kultur starter Lactobacillus plantarum B1765 terhadap kualitas produk dan Total Fenolik (TF). Fermentasi dilakukan selama 0, 12, 24, dan 36 jam pada suhu 37^{o}C dengan kultur starter sebanyak 5% (v/v), total Bakteri Asam Laktat (BAL) dengan metode Total Plate Count (TPC), pH dan TAT dengan metode pH meter dan titrasi asam basa, dan TF dengan metode Folin-Ciocalteu. Hasil penelitian menunjukkan bahwa lama fermentasi berpengaruh terhadap peningkatan total BAL, TAT, TF, dan penurunan pH. Lama fermentasi terbaik pada sari bengkuang yang difermentasi adalah 24 jam dengan total BAL sebesar (9,70±0,31)x10^{7} CFU/mL, pH sebesar 4,21±0,22, TAT sebesar 0,220±0,069%, dan TP sebesar 16,22±0,31 mg GAE/g. Nilai ini sesuai dengan kriteria produk minuman fermentasi. Sari bengkuang yang difermentasi dengan Lactobacillus plantarum B1765 berpotensi meningkatkan TF.

Kata kunci: Sari bengkuang, fermentasi, Lactobacillus plantarum, kualitas produk, total fenolik

INTRODUCTION

Jicama (*Pachyrhizus erosus*) is among the root crops with the potential as a source of carbohydrates and phenolic content. The phenolic content of Jicama is 0.063-0.928 mg GAE/ml including daidzein, 5-OH-daidzein-7-O-β-glucopyranose, daidzein-7-O-β-glucopyranose, and 8,9-furanyl-pterocarpan-3-ol (Lukitaningsih, 2014; Soetan *et al*., 2018). Phenolic compounds are beneficial as anti-inflammatory, antimicrobial, antioxidant, and anticarcinogenic(Kumar and Goel, 2019). However, these phenolic compounds have obstacles to utilization due to the activity of

polyphenol oxidase (PPO) enzymes and phenolic structures in plants that are still bound to phenolic glycosides, thus reducing their health potential.

Jicama reported having PPO activity that oxidizes phenolic compounds into quinones, indicated by a brownish color change (Aquino-Bolanos and Mercado-Silva, 2004). PPO activity will impact the levels of phenolic compounds to reduce the potential of Jicama for health effects. PPO enzyme works optimally at pH 4-7 (Zhang, 2023). So, one to inhibit its activity is with low pH through fermentation. Microbes on fermentation hydrolyze amylum into glucose and then metabolize it to produce lactic acid and other organic acids. The organic acids formed regulate acidity and lower pH to pH 2.9 -3.5 (Pau *et al.*, 2022). The low pH also plays a role in hydrolyzing glycoside bonds in phenolic glycosides, so releasing the free phenolic compounds increases the concentration of phenolic compounds. Not all plant polyphenols are free (Tsao, 2010).

Jicama known a source of inulin with an inulin content of 6.52% (Nurjanah *et al*., 2020). Inulin is a dietary fiber that cannot be digested by digestive enzymes and can only be hydrolyzed by the inulinase enzyme secreted by microorganisms. *L. plantarum* B1765 is a lactic acid bacterium that has been studied to be able to secrete inulinase enzymes (Nabila and Wikandari, 2018). The fermentation process using *L. plantarum* B1765 is expected to hydrolyze inulin into glucose and fructose, which will be metabolized to produce lactic acid and other organic acids, thus reducing the pH of the product.

Hydrolysis of glycoside bonds can also be destroyed by the β-glucosidase enzyme so that the phenolic content will increase (Landete *et al*., 2015). *L. plantarum B1765* is known to have β-glucosidase activity of 0.868 U/mL(Huda and Wikandari, 2016). Jicama is known to have polyphenol content bound by isoflavone-glycosides, including daidzein and genistein (Lukitaningsih, 2014). The role of *L. plantarum* B1765 is to hydrolyze glycoside bonds into daidzein and genistein aglycones, increasing the free phenolic content.

Many studies have been conducted on the benefits of Jicama for health, but there has yet to be further research on fermented jicama extract products. Thus, this study aims to determine the effect of fermentation time on the product quality and total phenolics.

METHODS

Materials

Lactobacillus plantarum B1765 was got from a private collection, MRS Broth (Merck), MRS Agar (Merck), distilled water, alcohol 70% (Onemed), NaCl (Pudak), $CaCO_3$, Na_2CO_3 (Pudak), gallic acid (Sigma-Aldrich), phenolphthalein, methanol (Merck), NaOH (Merck), and Folin-Ciocalteu reagent (Merck). The Jicama, with a harvesting age of 4-6 months, were purchased from East Java, Indonesia.

Experimental Design and Data Analysis

This study used a design to treat fermentation duration for 0, 12, 24, and 36 hours, adding 5% (v/v) *L. plantarum* B1765 as starter culture at 37 ^{0}C. Data analysis was conducted using SPSS with One-way ANOVA and continued by the Post Hoc LSD test. The data from the samples tested showed a normal distribution and homogeneity of variance.

Research Procedure

Preparation of Starter Culture

Starter culture isolates were inoculated into MRS Broth and incubated for 24 hours at 37°C. The growing culture was separated by centrifuge (3.500 rpm for 5 min) and decanted. The residue was suspended in 9 mL of 0.85% NaCl solution and centrifuged. The residue was then re-suspended in 10 mL of 0.85% NaCl solution and vortexed as a starter culture (Montijo-Prieto et al., 2023).

Preparation of Sample

Jicama 500 g that has been sorted, then peeled and washed thoroughly. The Jicama was cut into small pieces and blanched in boiling water for 5 minutes, at 85^{o}C, then blended with the addition of water in a ratio of 1 2 (w/v) until it became a slurry. The slurry was shaken at 300 rpm for 60 min using D-Lab SK 220 Pro, USA, then filtered,

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and the filtrate (jicama extract) was put into a sterile glass bottle (Kamsina, 2014). The jicama extract was then added with 12.5% (w/v) sugar cane and stirred until dissolved, then pasteurized into a double boiler and stirred the jicama extract for 5 minutes at 70°C in a sterile glass bottle and stood until room temperature. About 5% (v/v) of *L. plantarum* B1765 was added as the starter culture and fermented at 37°C for 0, 12, 24, and 36 hours. The jicama extract was enumerated for the total Lactic Acid Bacteria (LAB), pH, and Total Titratable Acid (TTA) and then concentrated using a rotary evaporator with Buchi R-300, USA. The thick extract obtained was calculated for water content and determination of TP.

Procedure of Analysis

pH and TTA testing were carried out by the advice of AOAC (2005), total LAB testing was carried out by the advice of Mailoa *et al*. (2017), and total phenolic testing was carried out by the advice of Myo *et al*. (2021).

The pH and TTA analyses were conducted following the guidelines provided by AOAC (2005). Determining total lactic acid bacteria (LAB) was performed per the recommended procedures outlined by Mailoa et al. (2017). Additionally, total phenolic compounds were quantified following the protocols recommended by Myo et al. (2021).

RESULTS AND DISCUSSION

Characteristics of Fermented Jicama Extract

Fermented jicama extract product by *L. plantarum* B1765 is presented in Figure 1.

Figure 1. Fermented Jicama Extract

The fermentation of jicama extracts affected insignificantly (p>0.05) color changes but affected significantly ($p < 0.05$) the sour taste and aroma, as well as the increasing sediment. Kamsina (2014) reported that the changing quality of taste and aroma due to the ability of microorganisms is in line with the length of storage time on jicama extract. The results are due to the activity of *L. plantarum* B1765, which can break down amylum into glucose to produce lactic acid, reducing the product's pH and increasing the sour taste and aroma of the jicama extract. The length of fermentation also affects the amount of sediment due to the hydrolysis of protopectin into soluble pectin caused by the low pH of the product (Yuliani *et al*., 2017).

Total LAB, pH, and TTA

The results of the analysis carried out on data of total LAB, pH, and TTA in jicama extracts can be shown in Table 1.

The length of fermentation affected significantly ($p<0.05$) the total LAB and TTA. There was a significant difference in total LAB at fermentation time of 0-12 h, but no difference in fermentation time of 12-36 h. Regarding TTA, a difference was observed in fermentation time 0-24 h, but it was not significant in subsequent fermentation time 24-36 h. The pH was not normally distributed and homogeneous, so the pH statistical test was carried out with the Kruskal Wallis test. The results of the Kruskal Wallis test ($p < 0.05$) showed that the length of fermentation affected the pH. The continued test with the Mann-Whitney ($p < 0.05$) showed a significant difference in fermentation times of 0, 12, and 36 hours, but there was no significant difference in fermentation times of 12-24 hours.

The total LAB of fermented Jicama increased from 0-24 h (Table 1.). The highest total LAB in the jicama extract fermented for 12 h was $(1.15\pm0.13)x10^8$ CFU/mL. The length of fermentation influenced the increase in total LAB (Febricia *et al*., 2020). The difference in total LAB in the length of fermentation is influenced by the availability of nutrients in the media and different generation times, so the ability of the log phase in bacteria is different. The maximal growth of LAB on fermentation occurred in the exponential phase at 12 h of

fermentation, with a rapid increase in the number of bacteria up to 2 log cycles. After 12 hours of fermentation, LAB entered the stationary phase, which showed no significance in the total LAB between 12-36 hours of fermentation.

Table 1. Total LAB, pH, and TTA of Jicama extract during fermentation

Properties	Fermentation time (h)			
	0	12	24	36
Total LAB (CFU/mL)	$(1.90\pm0.28)x10^{6\ a}$	$(1.15\pm0.13)x10^{8\ b}$	$(9.50\pm0.31)x10^{7\ b}$	$(2.70\pm0.062)x10^{8\ b}$
pH	5.79 ± 0.06^{a}	4.56 ± 0.33^{b}	4.21 ± 0.22^{b}	3.92 ± 0.03^{c}
TTA (%)	0.127 ± 0.062^{a}	0.185 ± 0.057^{b}	0.220 ± 0.069^{c}	0.223 ± 0.092^{c}

Note: Data (mean ± SD) of triplicates. The data was analyzed by ANOVA. Different letters within the same row denote significant differences (LSD test, $p < 0.05$).

Febriana and Wikandari (2022) reported that the total LAB *L. plantarum* B1765 increased by 1 log cycle in tomato extract probiotic incubated for 0-24 hours with a concentration of *L. plantarum* B1765 of 2.5% (v/v). Rafsanjani and Wikandari (2017)reported that the total LAB *L. plantarum* B1765 increased by 2 log cycles in yacon pickle incubated for 0-48 hours with a concentration of *L. plantarum* B1765 of 10% (v/v). Jicama extract is better than yacon pickle, although it requires less starter culture concentration and a faster time to increase to 2 log cycles. The total LAB *L. plantarum* B1765 in tomato extract is smaller than in jicama extract, and yacon pickle was due to the lower starter culture concentration of only 2.5% (v/v). The second is the relatively low initial pH in tomatoes, so the growth of *L. plantarum* B1765 was lower, while yacon is rich with inulin and FOS, so that growth is faster. The increase in total LAB also occurred in the fermentation of jicama extract, which was also influenced by the presence of 12.5% (v/v) sugar as a nutrient for the growth of *L. plantarum* B1765. Sugar can hydrolyze into monosaccharides to produce LAB growth(Putri *et al*., 2020). The increase in total LAB in jicama extracts was also influenced by the presence of inulin in 6.52% (Nurjanah *et al*., 2020). Inulin is a carbohydrate compound that cannot be digested by humans but can be digested with the help of LAB. *L. plantarum* B1765 produces the inulinase enzyme to break down inulin into glucose and fructose(Nabila and Wikandari, 2018).

The growth of total LAB contained caused a decrease in the pH and an increase in TTA. In the fermented jicama extract, there was a significant decrease in pH from 0-36 hours of fermentation from 5.79±0,059 to 3.92±0,029. While the TTA increased from 0-24 hours from 0.127±0,062% to 0.22±0,689%, it was insignificant at 24-36 hours. The increase in TTA is related to the decrease in pH due to the metabolism of *L. plantarum* B1765, which is a facultative heterofermentative lactic acid bacteria that can produce lactic acid and other organic acids (Suryono and Wikandari, 2019). The results followed the quality product of fermented beverages minimally on Total LAB of (10^{6}CFU/g) based on the International Food Standard(WHO, 2003).

Total Phenolic (TP)

The phenolic compounds in the jicama extract were carried out using $FeCl_3$. The positive result was indicated by a color change to green, blue, purple, and darker green (Harbone, 1996). The test results showed that during the fermentation process, a po, a positive reaction was shown as green and darker green, as shown in Figure 2.

The controlled (nonfermented) gives a light green color, while at 12-36 hours of fermentation, it gives a cheerful darker green color. This shows that fermentation can increase the phenolic content contained in the jicama extracts. The darker green color indicates the formation of phenol derivative

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compounds with $FeCl_3$ (Manongko *et al*., 2020).

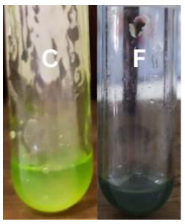

Figure 2. Positive Results using $FeCl_3$ (C: nonfermented and F: fermentation).

Determination of the concentration of TP was carried out by the Folin-Ciocalteu method. The results showed that fermentation can increase the total phenol contained in the jicama extracts. Figure 3 shows the TP with fermentation time.

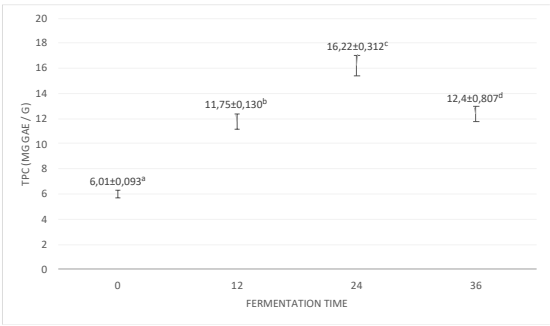

Figure 3. The TP of Jicama Extract during Fermentation Time. All data (mean±SD) are from triplicates. Data followed with different letters on the top of the bar denote a significant difference (p<0.05)

Fermentation affected significantly (p<0.05) on the TP (Figure 3.). TP compounds were expressed as Gallic Acid Equivalent (GAE). The results showed that the TP at control (nonfermented) was 6.01±0.09 mg GAE/g, increased to the optimal at 24 h of fermentation to 16.22±0.31 mg GAE/g, then decreased to 12.4±0.8 mg GAE/g at 36 h of fermentation.

The results of Soetan *et al.* (2018) showed that the TP in fresh Jicama ranged from 0.063-0.928 mg GAE/mL. Meanwhile, the results showed that the highest TP in fermented jicama extract was 16.22±0.31 mg GAE/g. This shows that the fermentation process can increase the TP in jicama extract. Similarly, the fermentation of waterlily seed flour extract with *L. plantarum* JBSxH.6.4 increased TP for 48 hours of fermentation (Rahmi *et al*., 2020). The fermentation of seaweed in *Gelidium* sp. with *L. plantarum* for 24 hours increases TP (Sumardianto *et al*., 2021).

The fermentation process can increase TP due to good microbial growth that can produce organic acids, thus reducing pH and activating the role of enzymes. Enzymes released by LAB can degrade polyphenols into simple phenolic compounds such as β-glucosidase (Nazarni *et al*., 2016). β-glucosidase enzymes can catalyze the hydrolysis of phenolic-glycosidic bonds and release free aglycones that can increase their bioactive potential (Lodha *et al*., 2021). β-glucosidase enzyme activity can hydrolysis of glycoside bonds from isoflavone-glycosides, such as 3-hydroxy anthranilic acid or hydroxy-genistein in the form of aglycones(Cheng *et al*., 2013; Leonard *et al*., 2021).

Some LAB that can produce enzymes are *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium* (Yuksekdag *et al*., 2018). *Lactobacillaceae* showed a high prevalence in the effect of β-glucosidase enzyme activity, including *L. plantarum*, significantly increased the potential to release the free phenolic compounds (Lodha *et al*., 2021). Jicama is known to have polyphenol content bound by isoflavone-glycosides, including daidzein and genistein (Lukitaningsih, 2014). *L. plantarum* B1765, used in this research, was known to have β-glucosidase activity(Huda and Wikandari, 2016). that used in this research was known to have a β-glucosidase activity(Singhvi and Zinjarde, 2020). This can hydrolyze phenolic compounds such as isoflavone-glycoside bound into simple isoflavones such as genistein and daidzein aglycones (Wijayanti *et al*., 2017).

The results also showed that at 36 hours of fermentation, there was a decrease in TP. Similarly, Hunaefi *et al.* (2013) reported that LAB fermentation treatment was detected at 24 h after fermentation decreased in TP but increased antioxidant activity. The decrease in TP content at 120 h fermentation in coffee grounds extract can be related to the reduced solubility of phenolic compounds or can be degraded during

fermentation (Myo *et al*., 2021). Taylor and Duodu (2015) stated that the decrease in TP during the fermentation process is thought to be phenolic compounds stimulating the formation of enzymes forming other components. The decrease and degradation of phenolic compounds are associated with the action of decarboxylase, reductase, esterase, and the ability of LAB in fermentation (Svensson *et al*., 2010). *L. plantarum*, *L. brevis*, and *L. fermentum* are some of the *Lactobacillus* metabolizing phenolic acids through reduction and/or decarboxylation activities (Filannino *et al*., 2015).

The rearrangement of phenolic structures can also cause a decrease in TP due to the influence of low pH, thus reducing the extraction ability of the phenolic compounds that were polymerized and/or interacted with other macromolecules (such as amino acids and starch), which were possibly converted into other health-beneficial such as quercetin, pro-anthocyanidins, catechins, gallic acid, and other phenolic compounds, which were not investigated (Adebo *et al*., 2018). This study reduces TP at the end of fermentation, which may be affected by degradation by *L. plantarum* B1765 activity through decarboxylation, reduction, or polymerized processes that are continuously converted into other components. The highest TP was the result at 24 hours of fermentation.

CONCLUSION

This study showed that the length of fermentation affected increasing total LAB, TTA, TP, and decreasing pH. The results showed that the best fermentation time in jicama extract was fermented for 24 hours with a total LAB of $9.7x10^7$±0.31 CFU/mL, pH value of 4.21±0.22, TTA of 0.22±0.689%, and TP of 16.22±0.312 mg GAE/g. This value follows the criteria for fermented beverage products based on the International Food Standard, and fermented jicama extract with *L. plantarum* B1765 potentially increases the TP.

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