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Antioxidant Potential of Jicama (*Pachyrhizus erosus*) Extract Fermented by *Lactobacillus plantarum* B1765

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Abstract: Jicama is a source of phenolic compounds that function as antioxidants. However, the presence of polyphenol oxidase and naturally bound phenolic compounds limits its antioxidant potential. Fermentation is one method to enhance the antioxidant potential of jicama. This study investigates the growth of lactic acid bacteria (LAB), pH, total titratable acidity (TTA), total phenolic content (TPC), and antioxidant activity of jicama extract (*Pachyrhizus erosus*) using *L. plantarum* B1765 as a starter culture which was fermented for 2, 12, 24, and 36 hours at 37°C. Total LAB is measured using the whole plate count technique, pH using a pH meter, TTA by acid-base titration, TPC using the Folin-Ciocalteu method, and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method represented by IC_{50}. The fermentation duration of jicama extract increased the growth of LAB to 10^8 CFU/mL and reached its optimum at 12 hours. However, reducing pH, increasing TTA to 0.223%, increasing TPC, and antioxidant activity still occur up to 36 hours of fermentation. Antioxidant activity was classified as vital. Duration of fermentation increased the growth of total LAB until the defined time, secreted β-glucosidase and inulinase enzymes, and also produced acid as their metabolism product, which hydrolyzed the glycoside bond and reduced pH, both act to free phenolic and increased antioxidant activity. This product has met Indonesian National Standards for beverage-fermented types and could be an antioxidant agent.

Keywords: Antioxidant Activity; Fermentation; Jicama extract; *Lactobacillus plantarum* B1765; Total Phenolic Content.

Introduction

Jicama (*Pachyrhizus erosus*) is a root vegetable with brownish-yellowish roots thick and tough skin that is easily peeled to reveal the crisp and juicy white flesh [1]. Jicama originates from Mexico and North America and has been cultivated in Southeast Asia, South America, and Africa. Jicama is still produced in Indonesia, especially in the West Sumatra region. Generally, jicama is locally used, commonly consumed in the fresh state, used in salads, and occasionally cooked or pickled [1]. Various plant components possess pharmacological and health-enhancing attributes, making them potentially valuable in multiple biological functions and health conditions. These include antioxidant effects, immune system regulation, diabetes, and anti-aging effects [2].

Jicama is also known as a source of antioxidants. Some compounds that function as antioxidants are phenolic compounds such as flavonoids, tannins, phenolic acids, anthocyanins, catechins, and lignans [3]. Phenolic compounds such as daidzein, daidzin, genistin, daidzein-7-o-β-glucopyranoside, and 8,9-furanil-pterocarpan-3-ol have been isolated from jicama tuber and are known to have potential as antioxidants that inhibit DPPH activity [2].

One of the issues encountered is that jicama also contains polyphenol oxidase (PPO) enzyme, leading to brown spots on jicama tubers when damaged during distribution and food processing [4]. PPO is an enzyme that catalyzes monophenol hydroxylase to o-diphenol and o-diphenol to o-quinone, decreasing phenolic compounds that can affect antioxidant activity [2]. Another problem is that most of the phenolic compounds in plants are usually found in a bound or non-free state as glycoside compounds [5]. Glycosylation of phenolic reduces their antioxidant activity compared with phenolic aglycones [6].

The way to solve the issue is by fermentation to lower the pH, inactivate the PPO enzyme, and degrade phenolic glycoside bonds to preserve free phenolic compounds. The optimal pH range for PPO activity is 4.0-7.0, with most PPO enzymes working optimally at neutral pH [7,8]. By reducing the pH below the optimal range, it is expected that the activity of the PPO enzyme can be inhibited. Jicama contains 10.7% starch [9], which will be metabolized by lactic acid bacteria (LAB) into lactic acid and other organic acids. Jicama is a source of inulin with a content of approximately 12.32% [10]. Inulin is classified as a non-digestible carbohydrate. However, with the aid of the enzyme inulinase mainly produced by microorganisms, it can be degraded into glucose and fructose [11]. Glucose and fructose will undergo further metabolism to produce lactic acid and the precursor for short-chain fatty acids (SCFA), which can lower the pH of the product [12], thereby inhibiting the activity of the PPO enzyme.

The second approach is deleting the glycosidic bond in phenolic compounds to produce free phenolic. The bioconversion of phenolic from glycoside form to aglycone can also be carried out using various microorganisms that produce β-glucosidase enzyme. Some organisms known to produce β-glucosidase enzymes include *Lactobacillus plantarum*, *Bifidobacterium pseudocatenulatum* B7003, *Aspergillus oryzae* CCT 4359, *L. plantarum* FSO1, *Candida pelliculosa* L18 [13,14]. Several strains of *L.*

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plantarum have been found to enhance phenolic content and increase antioxidant activity [15]. The fermentation process with LAB can reduce phenolic glycoside compounds, resulting in free phenolic aglycones. Low pH is known to degrade glycosidic bonds, releasing sugars and free phenolic aglycones [16].

Fermentation of jicama extract has been conducted by [17] using *L. plantarum* and *Streptococcus thermophilus*. The research findings showed a decrease in pH, an increase in TPC, and antioxidant activity. In this study, jicama extract was fermented using another strain of *L. plantarum* species using *L. plantarum* B1765, which had been known to produce inulinase [18] and β-glucosidase enzymes [19]. The fermentation process of inulin in jicama by inulinase of *L. plantarum* B1765 is expected to produce glucose and fructose, which will be further metabolized into lactic acid and SCFA, thereby lowering the pH of the product and inactivating PPO to preserve phenolic compounds. Additionally, the activity of β-glucosidase by *L. plantarum* B1765 is expected to hydrolyze phenolic glycosides, forming aglycones and glucose, increasing the levels of free phenolic compounds and enhancing antioxidant activity.

This research assesses how fermentation time impacts the total LAB, pH, TTA, TPC, and antioxidant activity in jicama extract. Fermentation time affects the characteristics of fermented jicama products. The longer the fermentation time, the more LAB will grow, causing a decrease in the pH value, increasing the TTA and secreted β-glucosidase. The low pH and β-glucosidase enzyme activity will degrade the phenolic glycoside bond to free phenolic and increase the antioxidant activity. This research is essential to explore the benefits of jicama, particularly as a source of natural antioxidants and as an effort to diversify the functional foods of jicama. Antioxidants are indispensable today to anticipate the formation of free radicals, which could be correlated with the current lifestyle that triggers the occurrence of various degenerative diseases such as diabetes, arteriosclerosis, cancer, and Alzheimer's.

Research Methods

Chemical and materials

L. plantarum B1765 is a private collection isolated from fermented fish (*bekasam*) [20], 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), Folin-Ciocalteu reagent (Merck), DPPH (Himedia). With a harvest age of 4-6 months from Gresik, East Java, Indonesia, Jicama was preserved at room temperature in wet conditions.

Preparation of fermented jicama extract

Jicama extract was prepared using the *L. plantarum* B1765 as a starter culture developed in deMan Rogosa Sharpe (MRS) broth. Jicama was washed, peeled, and cut into smaller pieces, weighing approximately 500 grams. The jicama pieces were blanched for 5 minutes and then blended with adding 1000 mL of water. The jicama pulp was shaken at 150 rpm for 60 minutes and subsequently subjected to 3 rounds of filtration. The jicama extract was then pasteurized at a temperature of 70°C for 15 minutes. The *L. plantarum* B1765 of 5 % (v/w) as the starter culture was then inoculated and the mixture was incubated at 37°C for 2, 12, 24, and 36 hours [21].

pH and TTA measurement

pH analysis was conducted utilizing a pH meter. Approximately 10 ml of the sample was placed in a beaker glass. TTA was measured using the acid-base titration method. The sample was diluted 10 times. Then, the phenolphthalein indicator was added to 20 mL of the diluted sample. The sample underwent titration using a 0.1 N NaOH solution until the colour changed to pink [22].

Enumeration of total lactic acid bacteria (LAB)

The total LAB test was measured using the Total Plate Count. The total LAB count is determined by counting the LAB colonies grown on deMan Rogosa Sharpe (MRS) agar media by adding 1% CaCO3 (w/v). The sample is diluted using a 0.86% NaCl solution to dilutions of 10-1 to 10-8. The plating is done by transferring 1 mL (1000 µl) of the diluted sample into a petri dish. The dilution media is prepared in triplicate. Then, approximately 12 mL of MRS agar medium is poured into each petri dish. The Petri dishes are then inverted and incubated at 37ºC for 24 hours [22].

Determination of total phenolic content (TPC)

TPC is analyzed using the Folin-Ciocalteu method, and the absorbance is measured at the maximum wavelength of gallic acid (λ = 746.5 nm). A test solution is prepared by dissolving a certain amount of the concentrated extract in methanol. 0.5 mL of the extract solution is transferred to a centrifuge tube, and 2.5 mL of Folin-Ciocalteu reagent is added. The mixture is homogenized and left to stand for 10 minutes. A 2 mL of Na_2CO_3 and the mixture were homogenized and then placed in a water bath at 45ºC for 15 minutes. Subsequently, the measurement is performed with a spectrophotometer at the maximum wavelength for gallic acid [23].

Determination of antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method calculates the antioxidant activity. A 2 mL test solution with concentrations of 10, 20, 40, 80, and 160 µg/mL was added with 1 mL DPPH solution (40 µg/mL), then homogenized and placed in a dark room for 30 minutes. The absorbance was observed at the wavelength (λ) of 515.6 nm [24].

Data analysis

Statistical analysis is performed utilizing SPSS Version 26 software, with One-Way ANOVA, and applying the LSD test ($p < 0.05$).

Results and Discussion

Jicama fermentation was performed in this study to identify its antioxidant activity that correlates with the total phenolic compounds contained. However, since the phenolic is still in the glycoside form, low pH and ß-glucosidase activity are needed to degrade the bonds. This requires fermentation to produce LAB growth that secretes the ß-glucosidase and inulinase enzymes and produces

acidic products, thereby decreasing the pH of the product that functions to degrade phenolic glycoside, producing free phenolics and increasing the antioxidant activity.

Growth of total LAB, pH, and TTA

Data analysis results of total LAB, pH, and TTA in jicama fermentation are presented in Figure 1

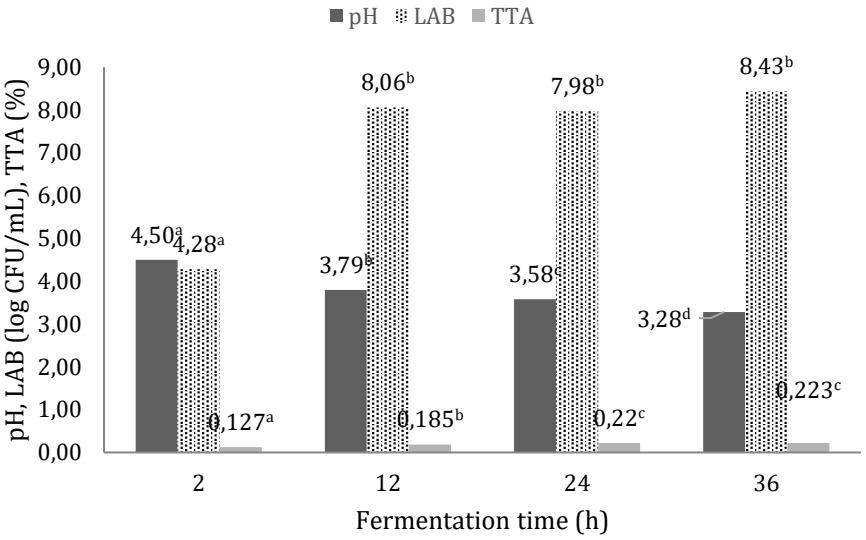

Figure 1. Total LAB, pH, and TTA of jicama extract during fermentation time. Different letters in a column denote significant differences ($p<0.05$)

The research results showed a significant difference ($p<0.05$) in total LAB during the fermentation time of 2-12 hours, but no significant difference was observed during the fermentation time of 12-36 hours. The optimum growth of LAB occurred at 12 hours of fermentation, in which the bacteria reached the logarithmic phase. There was increasing in cell numbers by 4 log cycles from the start of fermentation and reaching 1.15×10^8 log CFU/mL, followed by a stationary phase until 36 hours. The proliferation of LAB exhibits diversity based on environmental conditions, exhibiting a slower expansion rate in limited nutrients and an accelerated growth rate in nutrient-rich environments [25]. This result was supported by [26] that fermentation of yacon pickle with *L. plantarum* B1765 increased the total LAB count by 2 log cycles after 48 hours of fermentation. Jicama contains approximately 12.32% inulin [10], and yacon has a higher inulin content, but LAB grows faster on jicama extract than pickle yacon. This is presumably because, in the jicama extract, inulin is already in the form of soluble fiber, so it is more easily degraded by inulinase to produce glucose and fructose as the energy source for the growth of LAB. *L. plantarum* B1765 has an advantage in fermenting inulin because it secretes the enzyme inulinase, which can convert inulin into glucose, fructose, and short-chain fatty acids. Meanwhile, in fermented jackfruit seed juice with *L. plantarum* B1765, the total LAB count increased by 1 log cycle during 24 hours of fermentation [22]. Purple sweet potato extract fermented with L. plantarum B1765 has improved the entire LAB to 2 log cycles after 12 hours [27]. Fermented jicama extract fermentation was more effective in optimizing LAB growth than jackfruit seed juice and purple sweet potato extract, presumably due to the difference in nutrient content between the three products.

There was a significant decrease in pH from 4.50 to 3.28, while TTA increased significantly during the 2-24 hours of fermentation from 0.127% to 0.220%, but not significantly during the 24-36 hours. The growth of total LAB during fermentation time also affects the decrease in pH and the increase in TTA [28]. *L. plantarum* B1765 is a facultative heterofermentative bacterium that can produce lactic acid and other organic acids. The decrease in pH during the fermentation process is caused by the growth of LAB and the production of metabolites such as lactic acid and acetic acid. During yacon fermentation, there was a decrease in pH from 6.15 to 3.8 and TTA from 0.044% to 0.452% caused by the production of short-chain fatty acids (SCFA), such as acetic acid, butyric acid, and propionic acid by *L. plantarum* B1765 [29]. Based on the Total LAB and TTA value, the product satisfied the minimum quality requirements for fermentation products based on the Indonesian National Standards, with the number of LAB of 1×10^6 CFU/ml and TTA of 0.2% minimally, respectively.

Determination of total phenolic content (TPC)

The research results showed that fermentation can increase the TPC in jicama extract, as presented in Figure 2.

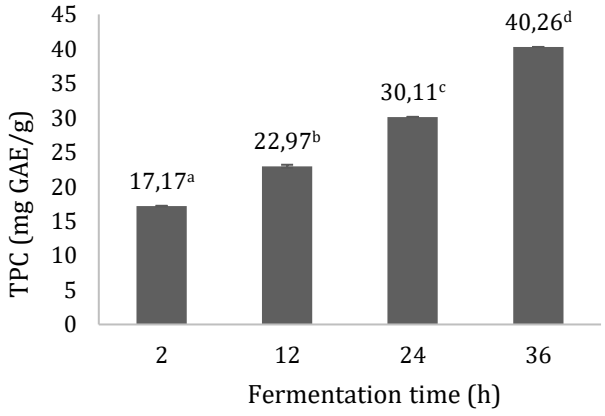

Figure 2. TPC of jicama extract during fermentation time. Different letters in a column denote significant differences ($p<0.05$).

Based on the research data, it is known that LAB fermentation increased the TPC in jicama extract, reaching

its maximum value of 40.26 mg GAE/g at 36 hours of fermentation. This increase occurs due to the inulinase activity from L. plantarum B1765, which causes hydrolyzed inulin to produce glucose and fructose and continue metabolizing to SCFA, causing a decrease in pH. The low pH is known to be able to degrade phenolic glycosides into phenolic aglycones [16]. In addition, there is the role of the enzyme β-glucosidase from *L. plantarum* B1765, which plays a role in degrading phenolic glycosides into phenolic aglycones. At 12-36 hours of fermentation, the LAB had undergone a stationary phase. Research conducted by [30] has shown that inulinase activity increases after the log phase and remains active throughout the stationary phase. In addition to inulinase, LAB also produces β-glucosidase enzyme during this phase. The β-glucosidase enzyme is known to be made at its maximum level during the stationary phase [31,32]. The activities of inulinase and β-glucosidase enzymes influence the increase in TPC during the 12-36 hour fermentation period. The low pH resulting from inulinase activity and the role of β-glucosidase enzymes can degrade phenolic glycosides into aglycon phenolic compounds, leading to higher total phenolic content.

This research is supported by [17], who explained the TPC in non-fermented jicama as 201.27 µg GAE/mL, while jicama fermented with *L. plantarum* increased to 281.27 µg GAE/mL. Research by [33] also reported an increase of TPC in fermented black wheat, from 13.3 to 16.2 and 18.4 mg GAE/g with *S. cerevisiae* and *L. rhamnosus*. This indicates that the fermentation process can result in higher TPC.

L. plantarum B1765 is known to secrete β-glucosidase enzyme [19], thereby increasing the TPC in fermented jicama extract by breaking down the glycosidic bonds and releasing free phenolic compounds. The release of aglycones from phenolic glycosides, such as genistin, by the catalytic action of β-glucosidase during fermentation increased the antioxidant activity of the extracts [34]. Research by [35] observed a reduction in genistein/glycitein/daidzein 7-o-glucosides and an increase in free genistein glycitein and daidzein in yellow soybean flour fermented with *L. plantarum* compared to non-fermented yellow soybean flour.

Fermented jicama extract has a higher TPC than fermented black wheat [33], possibly because the TPC in jicama is already higher. Moreover, the enzyme β-glucosidase in *L. plantarum* B1765, used as a culture starter in the fermented jicama extract, can degrade the glycosidic bonds in phenolic compounds, converting them into free phenolic compounds. This enzymatic activity during fermentation also contributes to the end product's higher TPC.

Additionally, the low pH is also known to degrade glycosidic bonds, resulting in the release of free sugars and phenolic aglycones [16]. Research by [36] explained that the DPPH scavenging effect of all sweet potato varieties is superior in acidic pH compared to other pH conditions, correlating with their phenolic content.

Determination of Antioxidant activity

The research results showed that fermentation can increase the antioxidant activity in jicama extract, as presented in Figure 3.

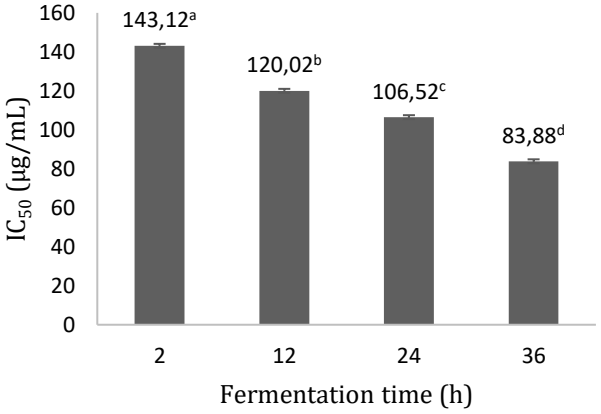

Figure 3. Antioxidant activity of jicama extract during fermentation time.
Different letters in a column denote significant differences ($p<0.05$).

The research data shows that the fermentation process can increase antioxidant activity, as indicated by the decrease in IC_{50} value. At the beginning of fermentation, the IC50 value of jicama extract was 143.12 µg/mL. The maximum DPPH scavenging activity was achieved at 36 hours of fermentation with an IC_{50} value of 83.88 µg/mL.

This result is supported by [37], which reported that *L. plantarum* B1765 has been found to increase the antioxidant activity in single garlic pickles by 11.17% on the 9th day of fermentation. Research by [17] also reported increased antioxidant activity regarding % DPPH inhibition in jicama extract fermented with *L. plantarum*, which was twice as high as non-fermented jicama extract. In [38], ethanolic crude extract of fermented soybean exhibited greater antioxidant activity compared to regular crude soybean extract, with IC_{50} values of 29.468 mg/mL and 41.294 mg/mL.

The antioxidant activity in fermented jicama extract continues to increase as the fermentation proceeds. This is correlated with the increase in TPC during fermentation, which is thought to be influenced by the activity of the β-Glucosidase enzyme from *L. plantarum* B1765 and the decrease in pH during the fermentation process. According to [39], they elaborated on how fermentation can enhance the levels of phenolic compounds and antioxidant capacity. Some research indicates increased antioxidant activity due to the rise in TPC of the fermentation process using *L. plantarum* in cowpeas and olives [34].

A compound is categorized as a highly effective antioxidant when its IC_{50} value is less than 50, classified as vital (50-100), labeled as moderate (100-150), and identified as a weak antioxidant. In contrast, an IC_{50} value falls between 151 and 200 [40]. The findings from this research demonstrate that the fermented jicama extract displays an IC_{50} value of 83.88 µg/mL, indicating intense antioxidant activity.

Conclusion

Based on the research, it can be concluded that the fermentation duration of jicama extract increased the growth of LAB to 10^8 CFU/mL and reached its optimum at

12 hours, but reducing pH, increasing TTA to 0.223%, increasing TPC and antioxidant activity still occurs up to 36 hours of fermentation. Antioxidant activity was classified as vital. Duration of fermentation increased the growth of total LAB until the defined time, secreted β-glucosidase and inulinase enzymes, and also produced acid as their metabolism product, which hydrolyzed the glycoside bond and reduced pH, both act to free phenolic and increased antioxidant activity. This product has met Indonesian National Standards for beverage-fermented types and could be used as an antioxidant agent. Further research is needed to determine whether the antioxidant activity of jicama extract can continue to increase after 36 hours. In addition, further research is also necessary to resolve the antioxidant effect of fermented jicama extract in inhibiting oxygen radical species in *vivo.*

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