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The Effect of Fermented Jicama Extract with *Lactobacillus plantarum* B1765 as the Culture Starter on the Product Quality and Flavonoid Contents

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Abstract: Jicama (*Pachyrhizus erosus*) is a functional food containing phenolic compounds, with the main compounds being flavonoids. However, the presence of polyphenol oxidase and the structure of flavonoid compounds are still bound in the form of glycosides, so the bioactivity of jicama is not maximized. This study aims to determine the effects of jicama extract fermentation on product quality and increased flavonoid content in jicama. Fermentation was performed for 0, 12, 24, and 36 hours with 5% (v/v) of the starter culture, *Lactobacillus plantarum* B1765, at 37ºC. The result showed that fermentation times could increase Total Lactic Acid Bacteria (LAB), Total Tertitrable Acid (TTA), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). It also influenced a decrease in pH. The total LAB count was determined using the Total Plate Count method, the Total Tertitrable Acid was determined using acid-base titration, and the pH was determined using a pH meter. Total Phenolic Content was measured using the Folin-Ciocalteu method, and Total Flavonoid Content was measured using $AlCl_3$ and potassium acetate. The analysis of the data revealed that jicama extract fermented optimum after 24 hours, with a total LAB count of $9.7x10^7$±0.31 CFU/mL, a pH value of 4.21±0.22, a TTA of 0.376±0.025%, but TPC and TFC still increasing until 24 hours of fermentation to 16.22±0.312 mg GAE/g, and of 29.01±0.641 mg QE/g respectively. Fermentation of jicama extract with *Lactobacillus plantarum* B1765 increased total phenolic and total flavonoid contents and could be used as a functional food product.

Keywords: Fermentation; Jicama Extract; *Lactobacillus plantarum* B1765; Product Quality; Total Flavonoid Content.

Introduction

Functional food has gained significant attention in nutrition and health due to its potential to promote well-being and reduce the risk of chronic diseases. Functional foods are innovative foods created to include ingredients or live microorganisms that may improve health or prevent disease, as long as the concentration is safe and high enough to provide the desired benefit. Ingredients that are added could be vitamins, dietary fibre, phytochemicals, or probiotics [1].

For example, studies have demonstrated that consuming foods rich in antioxidants, such as fruits and vegetables, can help reduce oxidative stress and inflammation in the body, which are underlying factors in the development of chronic diseases like cardiovascular disease and cancer [2]. Probiotics, another group of functional foods, have been associated with improvements in gut health, digestion, and the immune system [3].

One of the functional foods is jicama (*Pachyrhizus erosus*). The jicama tuber contains dietary fibre, protein, carbohydrates, inulin, vitamin C, folate, riboflavin, pyridoxine, pantothenic acid, thiamine, saponin, and phenolic compounds such as flavonoid [4, 5]. Flavonoids are among all green plants' most significant natural phenolic compounds [6]. Flavonoids, a class of polyphenolic compounds, have properties such as free radical carriers, inhibition of hydrolytic enzymes, oxidative, anti-inflammatory, and antibacterial [7, 8]. Jicama extract contains the flavonoids quercetin and camphor, which have vigorous antioxidant activity [9]. Quercetin also has significant antibacterial activity against several types of bacteria, including *S. aureus* [10].

The problem with using phenolic compounds is that phenolic compounds cause a browning reaction in jicama. The activity of polyphenol oxidase (PPO) enzymes changes phenolic compounds into quinones to reduce their activity as bioactive compounds [11]. Phenolic compounds with adjacent orthodoxy or trihydroxy types are the potent substrates for browning reactions. The phenolic substrate in plants is hydroxylated to 3,4-dihydroxyphenylalanine (dopa) and oxidized to quinones by the phenolase enzyme [12].

The activity of phenolase enzymes can be prevented by using fermentation methods. Fermentation produces lactic acid and some organic acids that can lower the pH. Fermentation in jicama can lower the pH from 6.9 to 3.8 [13]. This decrease in pH would inhibit the activity of the enzyme phenolase from maintaining total phenolics in jicama. PPO enzymes have an optimum pH range between 5.0 and 7.0 [14]. With pH lowered in jicama, the activity of the PPO enzyme is expected to be obstructed, and the browning effect on phenolic will be reduced. Nurdjannah [15] stated that phenolase activity has the optimum pH at pH 7. So, the smaller the pH is, the more enzyme phenolase activity decreases because it is far from the optimum pH.

Fermentation by Lactic Acid Bacteria (LAB) also plays a role in hydrolyzing isoflavone glucosides. The low

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pH produced by the fermentation process can hydrolyze the isoflavone glycoside bonds. Thus, free phenolic compounds will be released, increasing the concentration of total phenolic compounds [16]. Isoflavone glycoside bonds are also hydrolyzed by β-glucosidase enzyme [17]. Salar et al. [18] state that lactic acid bacteria can produce β-glucosidase enzymes that hydrolysis isoflavone glucoside compounds. The β-glucosidase enzyme hydrolyzes isoflavones glucosides by severing the β-D-glucoside bonds so that the availability of isoflavones increases and glycones (glucose) and aglycones (isoflavones) are produced. Lactobacillus strain bacteria (*L. plantarum, L. casei*, and *L. brevis*) has the ability to secrete β-glucosidase [19, 20].

The Lactic Acid Bacteria (LAB) that will be used as a starter culture in this study is *Lactobacillus plantarum* B1765. Based on research conducted by Huda and Wikandari [21], *Lactobacillus plantarum* B1765 can produce β-glucosidase with an activity of 0.868 U/mL. *L. plantarum* B1765 exhibited higher than *L. plantarum* SMN *025*, obtained at 0.653 U/mL.

This study aims to determine the effects of jicama extract fermentation on the quality of products and increased flavonoid levels in jicama. The fermentation of jicama juice by *Lactobacillus plantarum* B1765 is expected to increase the total LAB, thereby metabolizing reducing sugar to lactic acid, lowering pH, and increasing TTA. Low pH would inhibit the activity of the PPO enzyme and thus maintain total phenolics. In contrast, a decrease in pH and β-glucosidase secretion by *L. plantarum* B1765 is expected to increase free flavonoids. The fermented jicama juice is expected to increase the functional value of jicama as an available food.

Research Methods

Materials

Lactobacillus plantarum B1765 was got from a private collection, MRS Broth (Merck), MRS Agar (Merck), distilled water, alcohol 70% (Onemed), NaCl (Pudak), CaCO3, Na2CO3 (Pudak), gallic acid (Sigma-Aldrich), phenolphthalein, methanol (Merck), NaOH (Merck), Folin-Ciocalteu reagent (Merck), Quercetin (Sigma), $AlCl_3$, and potassium acetate. The jicama, with a harvest age of 4-6 months, were purchased from East Java, Indonesia.

Preparation of Jicama Extract

Jicama is peeled and washed thoroughly. To simplify the smoothing process, cut it into small pieces. Jicama is then blanched for 5 minutes and smoothed using a blender. The results are then shaken for 1 hour and then filtered three times. The obtained jicama extract is then dispensed with 12.5% sugar (w/v) and pasteurized for 15 minutes at 70°C. The pasteurized jicama extract was placed into a sterile glass jar, and 10% (v/w) of culture starter *Lactobacillus plantarum B1765* was fermented at 37°C in 12, 24, and 36 hours and subsequently used as a sample.

Determination of pH

The pH was determined using a pH meter that was standardized first with the pH 4.01 and pH 6.86 of buffer solutions. Then, place the electrode dipped into ± 20 mL of jicama extract samples until the value on the pH meter appears in the display [22].

Determination of Total Titratable Acid

A sample of 10 ml is diluted to 100 ml in a volumetric flask, then take 20 ml of it before adding 2-3 drops of phenolphthalein indicator. The sample is then titrated with a 0.1 N NaOH solution until the color changes to pink. NaOH is standardized by 0.1 gr of oxalic acid nitrated using a NaOH solution with a phenolphthalein indicator. Total Titratable Acid (TTA) is expressed in the percent of lactic acid.

Determination of Total Lactic Acid Bacteria

Total LAB was conducted using the Total Plate Count method [23]. The jicama sample was diluted using 0.85% NaCl solution to a 10^{-1}-10^{-8} dilution. Sampling was done by taking a sample using a micropipette of as much as 1 ml of the dilution into a petri dish. Furthermore, the mRS gelatine medium is poured as much as ± 12 ml. The media in the petri dish was then incubated upside down at 37ºC for 24 hours. Then, the number of lactic acid bacteria was calculated using CFU (colony forming unit)/mL units.

Determination of Total Phenolic

The Folin-Ciocalteu method, as described by Myo *et al*. [24], was used to determine the total phenolic (TPC) content. Approximately 2.5 g of concentrated jicama extract was dissolved in methanol in a 25 mL volumetric flask. The test was conducted under dark conditions by combining 2.5 mL of Folin-Ciocalteu reagent and 0.5 mL of the extract solution, followed by vortexing for 1 minute until a uniform mixture was obtained. The mixture was allowed to stand for 10 minutes. Subsequently, 2 mL of a 7.5% $NaCO_3$ solution was added, and the solution was shaken until homogeneous and then placed in a water bath at 45°C for 15 minutes. The absorbance of the resulting solution was measured at a wavelength of 746.5 nm using a UV-Vis Spectrophotometer. The TPC content was quantified based on the gallic acid standard curve, and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

Flavonoid Content Analysis

Preparation of Quercetin Standard Curves.

Set the quercetin's maximum wavelength first. Dissolved in 25 mL of methanol after being weighed at up to 25 mg of the standard form of quercetin. Pipetting 1 mL of the stock solution into 10 mL of methanol resulted in a concentration of 100 ppm. A 100 ppm quercetin common solution created several concentrations, including 20 ppm, 35 ppm, 50 ppm, 65 ppm, and 80 ppm. Pipette 1 mL of the quercetin standard solution from each concentration. Then,

1 mL of 120 mM potassium acetate and 2% AlCl3 were added. The samples were incubated at room temperature for one hour. UV-Vis spectrophotometry measures absorption at its most excellent wavelengths [25].

Determination of Total Flavonoid.

A sample solution is prepared from a fermented extract of jicama cider at 100,000 ppm concentration for all samples. From each sample solution pipette, 1 mL. Then, 1 mL of 120 mM potassium acetate and 2% AlCl3 were added. The samples were incubated at room temperature for one hour. Utilizing UV-Vis spectrophotometry at the maximum wavelength, the absorbance was calculated. Each sample was created in three replicates for each analysis, and an average absorbance value was obtained [25].

Results and Discussion

The Effect of Fermentation Time on Total LAB, pH, and TTA

The fermentation process of jicama juice with *L. plantarum* B1765 starter culture produced a milky white beverage, resulting in a different taste and aroma. These differences were caused by the growth of LAB during fermentation, which resulted in a decrease in pH and an increase in TTA.

Based on the study results, there was a significant difference ($p<0.05$) in total LAB between the fermentation times of 0 hours and 12 hours but significantly different between 12 and 36 hours. The results of TTA analysis showed a significant difference between 0 hours and 24 hours and no significant difference between 24 hours and 36 hours, while the results of pH test data showed a significant difference at 0, 12, and 36 hours of fermentation, while between 12 hours and 24 hours of fermentation, there was no significant change (Fig. 1).

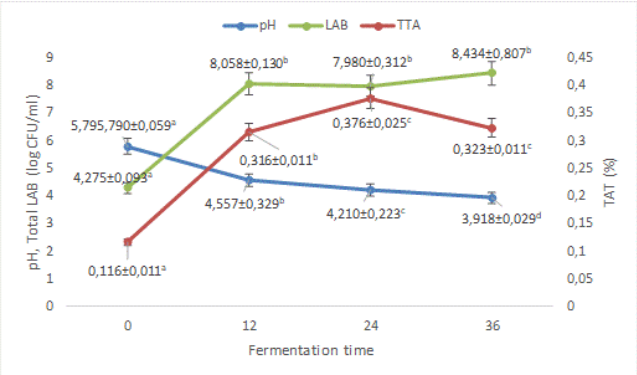

Figure 1. Total LAB, pH, and TTA of Extract Jicama during Fermentation Time.

The dates were reported as the mean value ± standard deviation, and the dates with different superscript letters (a–e) were significantly different ($p < 0.05$).

The increase in total LAB is influenced by the fermentation time; the longer the fermentation time, the higher the resulting total LAB count [26]. The jicama juice as the source of inulin fermented for 12 hours had the highest total LAB at $1.15x10^8$±0.13 CFU/ml, increasing by 2 log cycles from the beginning of fermentation, indicating that the occurrence of LAB entered the logarithmic phase. Following 12 hours of fermentation, LAB entered the stationary phase, during which no discernible change in the overall number of LAB between 12 and 36 hours of fermentation could be seen. Inulin, present in 6.52% of the jicama extracts, also impacted the rise in total LAB [27]). A probiotic dietary fibre called inulin can be used by gut microbes as a source of energy even though it cannot be digested by digestive enzymes, making it an ideal environment for their growth [28]. Inulin in jicama is a nutrient source for LAB growth during fermentation. It is possible to hydrolyze inulin further to create monosaccharides and oligosaccharides. Glucose and fructose comprise most of the monosaccharides produced, where fructose can act as a source of nutrients for LAB. LAB development can occur due to the hydrolysis of sugar into monosaccharides [29].

Another study showed that yakon as a source of inulin fermented with *L.plantarum* B1765 10% showed an increase of up to 2 log cycles after incubation for 48 hours [30]. *Dioscorea esculenta* tuber flour incubated for 72 hours with a concentration of *L. plantarum* B1765 5% (v/v) managed to increase total LAB by 2 log cycles [31]. LAB grows quicker on jicama extract than pickle yacon, despite yacon having a greater inulin concentration than jicama. This is because inulin is already present in the jicama extract as soluble fibre, making it easier for inulinase to break it down and release glucose and fructose, which serve as LAB growth's energy sources. *L. plantarum* B1765 ferments inulin more effectively because it secretes the enzyme inulinase, which converts inulin to glucose, fructose, and short-chain fatty acids. In the meantime, during the 24-hour fermentation period, the total LAB count in the tomato extract fermented with *L. plantarum* B1765 grew by 1 log cycle [32]. Compared to the three products, fermented jicama extract has the advantage of being able to grow LAB faster due to the difference in nutrient content.

The length of fermentation showed a significant difference in pH ($p<0.05$) on the pH value. The longer the fermentation time, the lower the pH value produced. In this study, the pH value decreased from 5.79±0.059 to 3.918±0.029 at 36 hours of fermentation, and TTA increased from 0.116±0.011% to 0.376±0.025% at 24 hours of fermentation, then at 36 hours of fermentation showed no significant difference. This was due to the activity of Lactic Acid Bacteria *L. plantarum* B1765, which hydrolyzes glucose into lactic acid and other organic acids. *L. plantarum* B1765 is a facultative heterofermentative Lactic Acid Bacteria that can produce lactic acid and other organic acids.

According to the International Food Standard [33], the total LAB and TTA test results for the fermentation of jicama extract met the minimum quality criteria for fermented beverage products. IFS has set the minimum limit for TTA at 0.3% and the minimum for total LAB in fermented drinks at 10^6 cfu/mL.

Total Phenolic Content

The total phenolic content (TPC) was calculated using the Folin-Ciocalteu method, with gallic acid standard for phenolic compounds. The outcomes demonstrated that fermentation can raise the overall phenol content of jicama extracts.

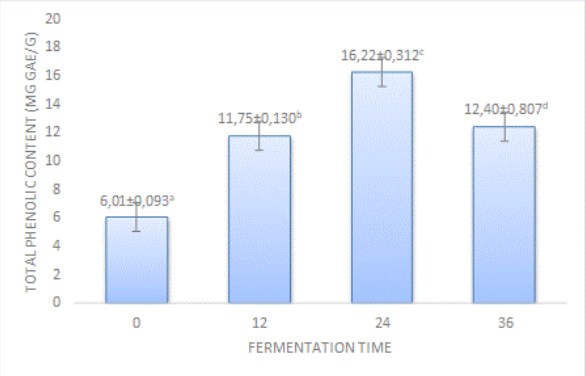

Figure 2. Total Phenolic Content of Jicama Extract during Fermentation Time.
The dates were reported as the mean value ± standard deviation, and the dates with different superscript letters (a–e) were significantly different ($p < 0.05$).

Based on the results of the study, 24-hour fermentation was able to produce higher TPC (16.22±0.312 mg GAE/g) than the unfermented control (6.01±0.093 mg GAE/g) but then decreased to 12.4±0.807 mg GAE/g at 36 hours fermentation time. The increase in TPC can be caused by the growth of bacteria that produce organic acids that reduce pH and inactivate PPO enzyme activity, which inhibits phenolic components into quinones so that phenolic components can be maintained. Fermentation by *L. plantarum* B1765 produces β-glucosidase, which can hydrolyze isoflavone-glycosides into glycones and aglycones, free phenolics. Jicama contains polyphenols bound by isoflavone-glycosides, such as daidzein and genistein [34]. Polyphenols can be broken down into free phenolic compounds by the enzyme β-glucosidase produced by LAB [35]. In the form of aglycones, 3-hydroxy anthranilic acid or hydroxy-genistein can be hydrolyzed by the enzyme activity of β-glucosidase from isoflavone-glycosides [36, 37].

The TPC in fresh jicama varied between 0.63 and 0.928 mg GAE/mL [38]. Meanwhile, the results revealed that the fermented jicama extract had the highest TPC of 16.22±0.312 mg GAE/g. This demonstrates that the jicama extract's TPC can be raised through fermentation. Chen *et al*. [39] also stated that after 24 hours of fermentation, *Rhizoma dioscoreae* extract fermented with *L. plantarum* increased TPC from 201.27 µg GAE/ml to 281.27. According to the results, fermented jicama extract has a greater TPC than fermented extract from *Rhizoma dioscoreae*. The phenolic compounds' glycosidic linkages can be broken down and released into free phenolic compounds by the enzyme β-glucosidase in *L. plantarum* B1765, utilized as a culture starter in the fermented jicama extract. The greater TPC of the finished product is also a result of this enzymatic activity during fermentation.

Lactobacillus, *Bifidobacterium*, and *Propionibacterium* are a few LAB that can create enzymes [40]. According to Lodha *et al*. [41], Lactobacillaceae demonstrated a high prevalence of β-glucosidase enzyme activity, and *L. plantarum*, in particular, significantly increased the potential to release the free phenolic compounds. Jicama is well known for its polyphenol content bonded by isoflavone-glycosides, such as daidzein and genistein [34]. According to Huda and Wikandari [21], the *L. plantarum* B1765 strain tested in this study was known to have β-glucosidase activity.

The findings also revealed a reduction in TP after 36 hours of fermentation. LAB fermentation treatment was shown to have lower TP but improved antioxidant activity 24 hours after fermentation [42]. The reduced solubility of phenolic chemicals or possible degradation during fermentation may be responsible for decreased TPC at 120 hours of fermentation in coffee grounds extract [23]. According to Svensson *et al*. [43], decarboxylase, reductase, esterase, and the capacity of LAB in fermentation all contribute to the reduction and degradation of phenolic compounds.

According to Adebo *et al*. [44], the phenolic compounds were polymerized and interacted with other macromolecules (such as amino acids and starch). They may have been converted into other health-beneficial compounds like quercetin, pro-anthocyanidins, catechins, gallic acid, and other phenolic compounds that were not investigated, which could have reduced the ability of the phenolic compounds to be extracted. This work focuses on the reduction of TPC after fermentation, which may be impacted by L. plantarum B1765 activity through decarboxylation, reduction, or polymerized processes that are continuously changed into other components. The result after 24 hours of fermentation had the highest TP.

Total Flavonoid Content

Determination of total flavonoids using $AlCl_3$ and potassium acetate [24]. The results showed that fermentation can increase the total flavonoid content of jicama extracts.

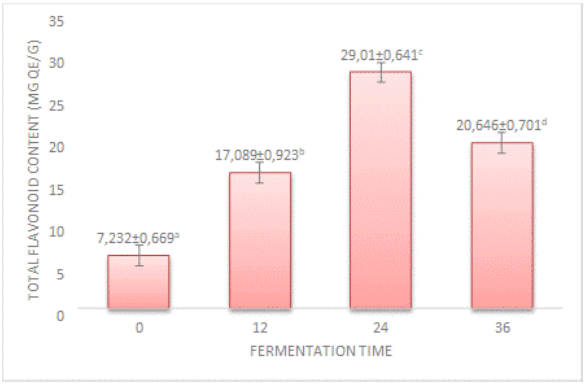

Figure 3. Total Flavonoid Content of Jicama Extract during Fermentation Time.
The dates were reported as the mean value ± standard deviation, and the dates with different superscript letters (a–e) were significantly different ($p < 0.05$).

There was a significant difference between the fermentation duration of each treatment on the TFC of

jicama extract ($p<0.05$). Fermentation was able to increase the TFC from 7.232±0.669 mg QE/g in the unfermented control, increased to 29.01±0.641 mg QE/g at 24 hours fermentation time, then decreased to 20.646 ± 0.701 mg QE/g at 36 hours fermentation time. This outcome is consistent with the Total Phenolic Content that was found. The result showed a correlation with the TPC data, which reached the maximum amount at 24 hours and then decreased, as listed in Fig. 4

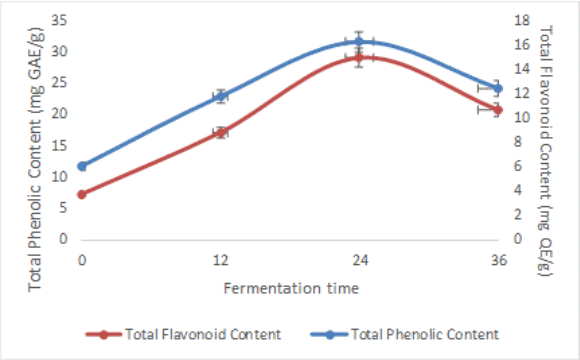

Figure 4. The Correlation Between Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Jicama Extract during Fermentation Time

The rise in flavonoid levels in fermented jicama extract could be attributed to the elevation in acidity during fermentation, which releases bound flavonoid constituents and enhances their bioavailability. Ademiluyi & Oboh [45] reveal that fermentation can potentially increase the liberation of various groups of phenolic phytochemicals, including flavonoids in the polyphenolic compounds category.

According to research by Adetuyi and Ibrahim [46], fermentation affects the increase of okra seeds, where during 24 hours of fermentation, there was an increase in total flavonoids by 492.10% and further decreased after 72 hours of fermentation. Dwiputri and Feroniasanti [47] also reported that the flavonoid content in Butterfly Pea kombucha increased as fermentation progressed. This indicates that the fermentation process can result in higher TFC. Similarly, the fermentation of *A. Malaccensis* leaf tea, after 48 hours of fermentation, increased to 5.555 mg/g [48]. Nazarni [35] also reported that the highest flavonoid content from fermented *tigarun* flower extracts after 48 hours of fermentation is 6.53±0.00 mg QE/g. This indicates that compared to other products, fermented jicama extract has a greater TFC, perhaps due to the fermented jicama extract's enzymatic activity from the enzyme β-glucosidase in *L. plantarum* B1765, which was utilized as a culture starter.

L. plantarum B1765 is known to secrete β-glucosidase enzyme, thereby increasing the TFC in fermented jicama extract by breaking down the glycosidic bonds and releasing free flavonoid compounds. The release of aglycones from flavonoid glycosides by the catalytic action of β-glucosidase during fermentation increased the TFC of the extracts.

Microbial enzymes such as glucosidase can hydrolyze glucosides and break down starch or plant cell walls during fermentation. These enzymes contribute to the plant cell wall matrix's breakdown, facilitating flavonoid extraction [49]. The β-Glucosidase of microbial origin could also be used as a secondary mechanism and fermentation to hydrolyze the phenolics and flavonoids. Vigorous glucosidase activity in *L. plantarum* has been observed [50], suggesting that the enzymatic cleavage of matching lucosides may produce more active chemicals. *L.plantarum* B1765, used in this study, is known to show β-Glucosidase activity of 0.868 U/mL [21].

However, TFC decreased after 48 hours of fermentation. According to Karimi *et al*. [51], who investigated the fermentation of pistachio hulls, the total flavonoid content of okra seed reduced as the fermentation period lengthened. The decrease in TFC was due to the sample concentration of flavonoid components or the length of the fermentation process. The findings of this research prove that the size of fermentation can increase TPC and TFC, but prolonged fermentation time will reduce TPC and TFC.

Conclusion

This study showed that the length of fermentation increased total LAB, TAT, Total Phenolic Content, and Total Flavonoid Content. Additionally, it also influenced a decrease in pH. However, prolonged fermentation can also decrease total LAB, TAT, Total Phenolic Contents, and Total Flavonoid Contents. The findings indicated that a 24-hour fermentation period has the optimum result with a total LAB of $9.7x10^7$±0.31 CFU/mL, a pH value of 4.21±0.22, a TTA of 0.376±0.025%, a TPC of 16.22±0.312 mg GAE/g, and a TFC of 28.930±0.650 mg QE/g. This value complies with the International Food Standard's requirements for fermented beverage products, and the fermented jicama extract with *L. plantarum* B1765 could increase the TPC and TFC.

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