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Study on Antioxidant Activity of Purple Sweet Potato *(Ipomoea batatas)* Juice Fermented with *Lactobacillus plantarum* B1765

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Abstract: Purple sweet potatoes are a source of anthocyanins, which are antioxidants. However, the anthocyanin compounds in purple sweet potatoes are naturally bound in glycosidic form, so their potential as antioxidants is still limited. Fermentation is one method that can be used to degrade glycosidic bonds to liberate free phenolics so that it can increase the antioxidant potential of purple sweet potato. This research aims to study the growth of lactic acid bacteria (LAB), pH, total titratable acid (TAT), phenolic content, anthocyanin content, and antioxidant activity of sweet potato juice (*Ipomoea batatas*) fermented with *Lactobacillus plantarum* B1765 starter culture for 0.2, 4,6,8,10,12 and 14 hours. Total LAB was measured using the Total Plate Count (TPC) method, pH was measured using a pH meter, TAT was measured using acid-base titration, total phenolic content (TPC) was measured using the Folline-Ciocalteu method, anthocyanin content used the differential pH method and antioxidant activity was measured using the radical scavenging method 2, 2-diphenyl-1-picrylhydrazyl (DPPH) which is expressed in IC50 values. The results showed that fermentation time affected ($p<0.05$) total LAB, pH, TAT, phenols, anthocyanin levels, and antioxidant activity in purple sweet potato juice. Total BAL increased to reach an optimum of 2.6 x 108 CFU/mL within 6 hours. However, until the end of fermentation for 14 hours, there was still a decrease in pH to 3.73, an increase in TAT reaching 0.426%, an increase in total phenolics up to 324.21 mg GAE/g, a reduction in anthocyanin levels (13.68 mg/L) and an increase in antioxidant activity with an IC50 of 47 .05 ppm which is classified as very strong. This product meets the Indonesian National Standards for fermented drinks and can potentially be a source of antioxidants.

Keywords: Anthocyanin; Antioxidant; *Lactobacillus plantarum* B1765*;* Product Quality; Purple Sweet Potato Juice.

Introduction

Changes in society's lifestyles have led to a large number of cases of degenerative disease. One of the triggers of many degenerative diseases is the result of free radicals. Free radicals can trigger oxidative stress that triggers diseases such as cancer, diabetes, metabolic disorders, and cardiovascular diseases [1]. Oxidative stress can be overcome by providing antioxidants [2]**.** Antioxidants can be obtained from anthocyanins, which are a secondary metabolite compound. Anthocyanins are flavonoid compounds found in purple, blue, magenta, and purple flowers, fruits, and vegetables [3].

Purple sweet potatoes are one of the plants that are rich in anthocyanin. A study conducted [4] found that 51.5-174.7 mg/100 g of anthocyanin [5] also stated that the purple sweet potato varieties of Ayamurasaki have a high anthocyanin content of 282 mg/100 grams. [6] . But all this time, purple sweet potatoes, chips, and flour are still limited for daily use. Not much effort has been made to develop purple sweet potato products as a source of antioxidants. Nutritional sources of antioxidants have the potential to be used as functional foods that have recently been developing in a significant way, along with public awareness of the importance of food intake and health.

One of the problems faced in developing purple sweet potato as a functional food source of antioxidants is that anthocyanins in purple sweet potato are still present in a bound state as glycoside compounds [7]. Anthocyanin glycolates reduce its activity as an antioxidant. According to [8], anthocyanins in aglycone form have higher antioxidant activity. It is, therefore, necessary to attempt to break the glycoside binding of anthocyanins into anthocyanidins (aglycones), thereby enhancing its antioxidant potential.

The solution to the problem is fermentation. Fermentation can decrease the pH, which is known to degrade glycoside bonds in anthocyanins and form anthocyanidins. [9]. When in a low pH state, the amount of anthocyanin in the product will decrease because it has been degraded to anthocyanidin, which has a lower stability than anthocyanin, so it is more reactive as an antioxidant. The methanol extract of purple sweet potato at a low pH showed higher antioxidant activity than at a high pH [10]. the antioxidant activity in the longan fruit anthocyanin is relatively high at pH 3-4 [11]. Another mechanism in the hydrolysis of glycoside binding in anthocyanins is the presence of the enzyme β-glucosidase produced by the starter culture used in the fermentation process. Research by [12] shows that adding raw extract and pure β-glucosidase enzyme from yeast can lower the anthocyanin content in wine.

Research on purple sweet potato as a source of antioxidants has been carried out but has been limited to the antioxidant activity of non-fermented purple sweet potato. The process of fermenting purple sweet potato using *L.*

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plantarum B1765 is expected to be able to produce glucose and fructose through the hydrolysis of purple starch, which is further metabolized into lactic acid so that it can lower the pH of the product and helps dismantle the glycoside bonding in anthocyanins to form anthocyanidins. Furthermore, the activity of the enzyme β-glucosidase by *L. plantarum* B1765 should be capable of degrading the glucoside binding in anthocyanins, forming anthocyanidins, increasing free phenolic levels, lowering the levels of anthocyanins and increasing antioxidant activity.

This study examines the impact of the fermentation time of sweet potato with L. plantarum B1765 as the culture starter on total LAB, pH, TTA, TPC, total anthocyanin, and antioxidant activity. It is essential to conduct this research as one of the efforts to diversify purple sweet potato-based foods as functional food, considering the increasing number of cases of degenerative diseases and curative actions with drugs with unwanted side effects. Efforts are needed to explore local food ingredients that are safer and easier to obtain. This research is crucial to examine the benefits of purple sweet potatoes as a natural antioxidant source to combat diseases caused by free radicals.

Research on the effect of fermentation time on the anthocyanin antioxidant activity of sweet potato needs to be done, considering that the anthocyanin structure is naturally glycoside bound, which can reduce its potential as an antioxidant. The longer the fermentation, the more the amount of LAB, the lower the pH value of the product, and the higher the TTA and producing the enzyme β-glucosidase of LAB. β-glucosidase activity plays a role in breaking the glycosidic bonds in anthocyanins, resulting in free anthocyanidins. This process reduces anthocyanin levels but increases TPCs and antioxidant activity.

Research Methods

Materials and Equipment

The materials used in this research include Ayamurasaki variety purple sweet potatoes purchased from a local market in Mojokerto, *L. plantarum* B1765 (personal collection), MRSB (Merck), NaCl (Merck), sugar, NaOH, white plain agar powder (Satelit), $CaCO_3$ (Merck), methanol p.a (Merck), DPPH (Sigma), gallic acid (Sigma-Aldrich), 7.5% Na_2CO_3 (Merck), 10% Folin-Ciocalteu reagent (Merck), KCl (Merck), $CH_3COONa \cdot 3H_2O$ (Merck), phenolphthalein indicator (Merck), and sterile distilled water. The equipment used in this research includes glassware, an analytical balance (Denver Instrument), laminar airflow (Thermo Scientific 1300 Series A2), a rotary evaporator (Buchi), autoclave (Hirayama HVE-50), incubator (Memmert), centrifuge (Eppendorf), pH meter (Eutech), micropipettes and blue tips (Eppendorf), a spirit burner, matches, glassware, spray bottles, knives, cutting boards, filter cloth, plastic wrap, basins, pots, and a stove.

Preparation of Starter Culture

An isolate of *L. plantarum* B1765 (1000 µL) is inoculated into 9 mL of MRS broth and incubated for 20 hours at 37°C. After the starter culture grows, it is separated by centrifuging at 3500 rpm for 5 minutes. The supernatant is discarded, and the pellet residue is suspended in 10 mL of sterile 0.85% NaCl solution and centrifuged again to separate any remaining MRS broth. The residue is resuspended in 10 mL of sterile 0.85% NaCl solution, vortexed until homogeneous, and used as the starter culture [13]**.** The starter culture was then used for the fermented purple sweet potato juice.

Preparation of Fermented Purple Sweet Potato Juice

Approximately 800 grams of purple sweet potatoes are peeled, washed, and sliced into ± 2 cm thick pieces. The sweet potatoes are then steamed in boiling water for 15-20 minutes. After steaming, water is added to the sweet potatoes in a 1:5 (w/v) ratio and 5% sucrose (w/v). The mixture is blended for 5 minutes, then shaken for 1 hour. The smooth sweet potato mixture is filtered using a cloth to separate the pulp from the juice. Sixteen sterilized glass bottles are prepared as fermentation containers, each filled with 250 mL of sweet potato juice. The juice is pasteurized at 70 ± 5°C for 10 minutes and allowed to cool to room temperature. The juice is then inoculated with 2.5% (v/v) *L. plantarum* B1765 and incubated for 0, 2, 4, 6, 8, 10, 12, and 14 hours at 37°C [13]. This fermented product then determined the total Lactic Acid Bacteria (LAB), pH, Total Titratable Acidity (TTA), Total Phenolic Content (TPC), Anthocyanin and Antioxidant activities.

Determination of Total Lactic Acid Bacteria (LAB) Quality

The total count of *L. plantarum* B1765 is analyzed using the Total Plate Count (TPC) method. 1 mL of purple sweet potato juice from each fermentation time variation is pipetted into 9 mL of 0.85% NaCl solution at dilution levels of 10^-1 to 10^-8. Then, 1 mL of sample from each dilution (10^-5 to 10^-8) is plated on MRSB medium with 1% CaCO3 and 1.5% agar and incubated for 48 hours at 37°C. The counted LAB colonies produce clear zones around them [13].

Determination of pH

The pH value is determined by taking 20 mL of the fermented drink sample from each fermentation time variation and measuring the pH using a previously calibrated pH meter.

Determination of Total Titratable Acidity (TTA)

Total Titratable Acidity in purple sweet potato juice is measured as a percentage of lactic acid. 10 mL of the juice sample is diluted in a 100 mL volumetriAntocc flask. 20 mL of the diluted sample is pipetted into an Erlenmeyer flask, and 2-3 drops of phenolphthalein indicator are added. The sample is titrated with 0.1 N NaOH until a stable blue-green color change occurs [13]. Total titratable acidity (TTA) is calculated using the formula:

TTA (lactic acid) = (V x N x P x MW x 100%) / B

Explanation:

V: Volume of NaOH for titration (mL)

N: Normality of NaOH

P: Dilution factor
MW: Molecular weight of lactic acid
B: Sample weight (mg)

Anthocyanin Content Testing

Anthocyanin content is tested using the pH differential method [14]. A total of 1 mL of concentrated sample is placed into a 5 mL volumetric flask, followed by the addition of potassium chloride buffer solution at pH one until the volume reaches 5 mL. Another 1 mL of concentrated sample is placed into a different 5 mL volumetric flask, and sodium acetate buffer solution at pH 4.5 is added until the volume reaches 5 mL. The flasks are then incubated in a dark space for 30-60 minutes. Afterward, the absorbance of the sample is measured at λ max-700 nm using distilled water as a blank. The absorbance of the diluted sample (A) is determined using the following equation:

$$A = (A_{\lambda vis-max} - A_{700})_{\text{pH 1,0}} - (A_{\lambda vis-max} - A_{700})_{\text{pH 4,5}}$$

The anthocyanin pigment content of the sample is calculated using the equation:
Total Anthocyanin (mg/L) = (A x MW x DF x 1000) / (ε x l)
Where:
A = Absorbance
MW = Molecular weight
DF = Dilution factor
l = Path length of the cuvette
ε = Molar absorptivity

Determining total anthocyanin concentration is based on the average result from measurements using the pH differential method.

Determination of Total Phenolic Content

TPC is analyzed using the Folin-Ciocalteu method [15], read at the maximum wavelength of gallic acid (λ = 747 nm). The test solution is prepared by dissolving some concentrated extract in methanol. 0.5 mL of the extract solution is transferred into a centrifuge tube, and 2.5 mL of Folin-Ciocalteu reagent is added, then homogenized and left for 4-8 minutes. The test solution is then added with 2 mL of 7.5% Na_2CO_3, homogenized, and left in the dark for 30 minutes. The measurement is then carried out at the maximum wavelength of gallic acid (747 nm). The total phenolic content can be calculated using the following equation:

Total phenolic (mg/g) = (C × V × fp) / m

Explanation:
C: Concentration from the standard curve (mg/mL)
fp: Dilution factor
V: Sample volume (mL)
m: Sample mass (g)

Antioxidant Activity Testing

The determination of antioxidant activity is performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method [10]. 2 mL test solution with concentrations of 25, 50, 100, 200, and 400 ppm is added with 1 mL of 40 ppm DPPH solution, homogenized, and incubated in the dark for 30 minutes. Absorbance is observed at the wavelength (λ) of 517 nm. The free radical scavenging activity is calculated as the percentage of DPPH color reduction using the following equation:

$$\%\ \text{DPPH inhibition} = \frac{A\ blako - A\ sampel}{A\ blanko}\ X\ 100\%$$

Next, the IC50 value is determined by creating a regression curve from the percentage of inhibition at various sample concentrations, with % inhibition as the x-axis and sample concentration (ppm) as the y-axis, resulting in a linear regression equation.

Data Analysis

Statistical analysis is conducted using SPSS software version 22 with One-Way ANOVA followed by LSD post-hoc test ($p<0.05$) and Kruskal-Wallis with Mann-Whitney post-hoc test.

Results and Discussion

In this study, the fermentation of purple sweet potato juice aims to determine the antioxidant activity related to the TPC and anthocyanin compounds. The anthocyanin compounds in purple sweet potatoes are still glycosidically bound, requiring a low pH and the enzyme β-glucosidase to degrade these bonds. Fermentation is necessary to cultivate LAB that produces β-glucosidase and generates acidic products that lower the pH, thus degrading the glycosidic bonds in anthocyanins. This process creates free anthocyanins, which correlate with increased TPC and antioxidant activity.

Growth of LAB, pH, and TTA

Purple sweet potatoes are known as a source of antioxidants. Using *L. plantarum* B1765 as a starter culture for fermenting purple sweet potato juice aims to ferment the starch in the purple sweet potatoes, resulting in a low pH that can enhance antioxidant potential. The research results indicate that *L. plantarum* B1765 can grow in purple sweet potato juice. Data analysis of the total LAB, pH, and TTA in the fermented purple sweet potato juice is presented in Figure 1.

Changes in total LAB, pH values, and TTA of purple sweet potato juice during the fermentation stage are shown in Figure 1. The length of fermentation is expected to increase the growth of LAB by utilizing the nutrients in the growth medium, which will lower the pH of the product and increase the TAT resulting from glucose metabolism into lactic acid and other organic acids. This research indicates that during fermentation, there was an increase in total LAB, followed by a decrease in pH and an increase in TTA in the product. One-way ANOVA analysis showed significant differences ($p<0.05$) in total LAB between fermentation times of 0-6 hours and 8-14 hours but no significant differences between 6-8 hours of fermentation. The optimum growth of LAB occurred at 6 hours of fermentation, where the bacteria reached the logarithmic

phase. The number of cells increased by 1 log cycle from the start of fermentation, from 3.2 x 10^7 CFU/mL to 2.6 x 10^8 CFU/mL at 6 hours of fermentation, followed by a stationary phase at 8 hours of fermentation. These data show that purple sweet potato juice is a suitable growth medium for *L. plantarum* B1765. Purple sweet potato is rich in starch, containing 31.36% -39.39% [16] , which can be used as a nutrient source for the growth of *L. plantarum* B1765 during fermentation. This is supported by [13], who stated that the fermentation of purple sweet potato juice with L. plantarum B1765 increased total LAB to 1.86 x 10^9 CFU/mL after 12 hours of fermentation and then decreased. This study showed faster LAB growth because the logarithmic phase was reached within 6 hours of fermentation. It is suspected that adding 5% (w/v) sugar to the fermented purple sweet potato juice beverage also influenced the growth of LAB. The bacteria utilize carbohydrates from the purple sweet potato, which consists of starch, sucrose, glucose, fructose, dietary fiber, and added sucrose as a nutrient source, which will then be degraded into monosaccharides to support LAB growth (Kasmiyetti et al., 2022). *L. plantarum* has an advantage in fermentation because it produces amylase enzymes that can convert starch into glucose [19]. *L. plantarum* also can degrade sucrose into glucose and fructose [20]. The amylase enzyme ability of *L. plantarum B1765* is unknown, but *L. plantarum* B1765 is known to grow well on cassava, a source of starch [21]. Overall, the increase in LAB is influenced by the duration of fermentation [17] and the nutrients present in the substrate [18].

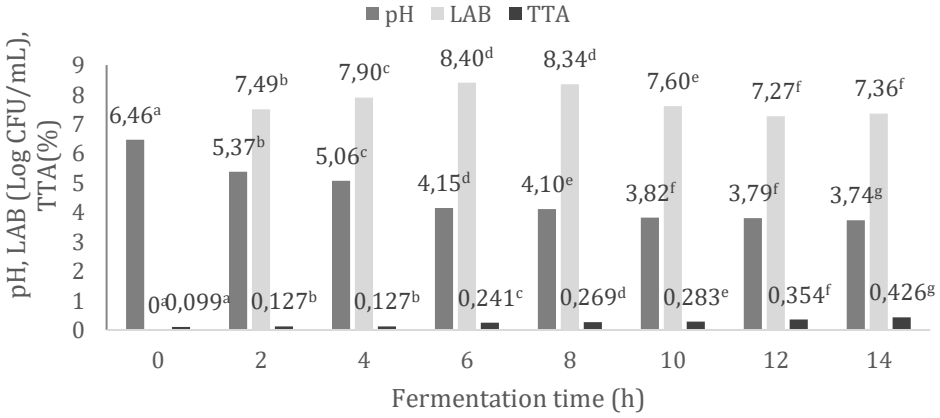

Figure 1. Correlation between Total LAB, pH, and TTA of Purple Sweet Potato Juice during Fermentation Time. Results with different letters indicate significant differences ($p<0.05$)

During the fermentation process, there was a significant decrease in pH from 6.46±0.053 to 3.73±0.042 ($p<0.05$). At the same time, TTA showed a substantial increase during 4-14 hours of fermentation from 0.127% to 0.426%, but not significantly during 2-4 hours of fermentation. The rise in TAT and the decrease in pH are related to the metabolism experienced by *Lactobacillus plantarum* B1765, a facultative heterofermentative bacterium capable of producing lactic acid and other organic acids, such as short-chain fatty acids (SCFAs) [22].

During the exponential phase of bacterial growth, starch or other polysaccharides are broken down into glucose, which is then converted into pyruvic acid through glycolysis, releasing H+ and thus causing a decrease in pH. The reduction in pH during fermentation is also caused by the increased growth of lactic acid bacteria (LAB) and the production of metabolites such as lactic acid and acetic acid. The amount of lactic acid produced during fermentation affects the pH reduction because lactic acid dissociates into H^+ and $CH_3CHOHCOO^-$. The decrease in pH during fermentation is suspected to be due to LAB growth and the production of metabolites such as lactic acid and acetic acid. The amount of lactic acid produced during the fermentation process affects the decrease in pH because lactic acid will dissociate into H^+ and $CH_3CHOHCOO^-$ [23].

The standard used to assess the quality of this fermented beverage product is SNI 7552:2009. Based on the total LAB and TTA values, the fermented beverage product has met the SNI standard, with a total LAB count exceeding 1 x 10^6 CFU/mL and TTA reaching more than 0.2%.

Determination of TPC Content and Anthocyanin Levels

The TPC content in fermented purple sweet potato juice was evaluated using the Folin-Ciocalteu method at a wavelength of 747 nm, and the determination of anthocyanin levels was assessed using the pH differential method at wavelengths of 522-523 nm. The tests were conducted using UV-Vis spectrophotometry. The research data are presented in Figure 2.

One-way ANOVA analysis showed significant differences ($p<0.05$) in TPCs across different fermentation times (0, 2, 4, 6, 8, 10, 12, and 14 hours). The study results indicate that fermentation can increase TPC and decrease anthocyanin levels in purple sweet potato juice.

Impact of total LAB increase and pH decrease on TPC and anthocyanin levels in fermented purple sweet potato juice as depicted in Figure 2; during the fermentation process, there was an increase in TPC in purple sweet potato juice, reaching 324.31 mg GAE/g after 14 hours of fermentation. A decrease followed this increase in TPC in total anthocyanins, and significant differences in pH were observed during fermentation. The increase in TPCs is believed to be due to the decrease in pH and the activity of β-glucosidase enzymes, which can degrade glycosidic bonds of phenolic compounds into phenolic aglycones. By 6-8 hours of fermentation, bacteria had entered the stationary phase.

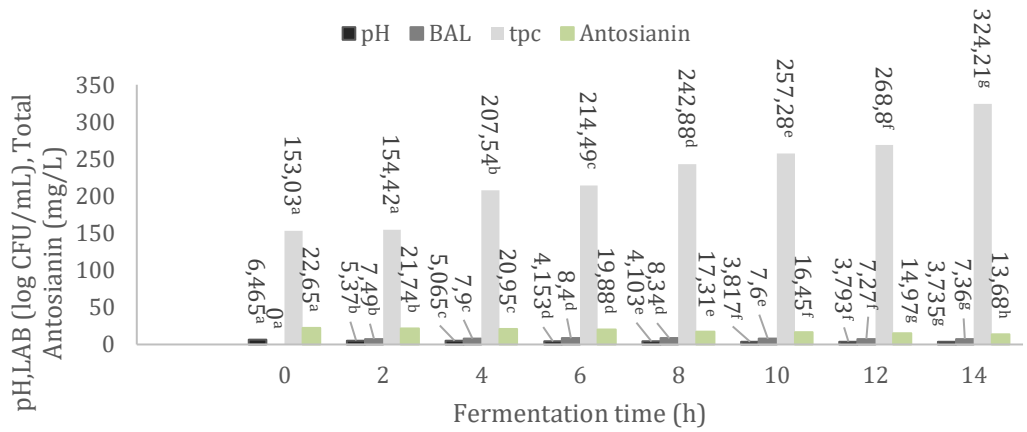

Figure 2. TPC and anthocyanin levels correlate with total LAB and purple sweet potato juice pH during fermentation time. Results with different letters indicate significant differences ($p<0.05$)

This study shows that increased total LAB during fermentation processes decreases pH. At low pH levels, phenolic compounds can degrade into free phenolics. Moreover, when LAB growth reaches the stationary phase, they secrete β-glucosidase enzymes that degrade glycosidic bonds in phenolics into free phenolics. β-glucosidase enzyme production peaks during the stationary phase [24]. The low pH due to amylase enzyme activity and the role of β-glucosidase enzymes contribute to the increase in TPC during fermentation. This finding is supported by [25], who reported phenolic content in non-fermented purple sweet potato porridge at 37.03 mg GAE/g and fermented results at 74.74 mg GAE/g. Research by [26] also reported increased TPC in fermented jicama extract with L. plantarum B1765 from 17.17 mg GAE/g to 40.26 mg GAE/g. This demonstrates that the fermentation process can indeed increase TPC.

Fermented purple sweet potato juice has a higher TPC content compared to fermented jicama extract, possibly due to the inherently higher phenolic content in purple sweet potato. Additionally, purple sweet potato has a higher starch content (31.36% - 39.39%) (Kurnianingsih et al., 2020) compared to jicama extract (10.7%) [27], which affects LAB growth and pH reduction in the product differently. The research results show that fermentation for 14 hours can lower the pH of purple sweet potato juice to 3.73, while fermentation for 36 hours lowers the pH of jicama extract to 3.91 [23]. LAB growth also influences the TPC in the product. β-glucosidase enzymes in *L. plantarum* B1765 used as a starter culture in fermented purple sweet potato juice can degrade glycosidic bonds in phenolic compounds and convert them into free phenolic compounds. This enzymatic activity contributes to higher TPC during fermentation. pH reduction is also known to degrade glycosidic bonds, releasing phenolics into phenolic aglycones.

In contrast to TPC, the anthocyanin content in fermented purple sweet potato juice during fermentation decreased from 22.65 mg/L to 13.68 mg/L after 14 hours of fermentation. Anthocyanins can be converted into other phenolic compounds through microbial metabolism. According to [28], microbial metabolism, bond degradation, and compound destruction during fermentation processes are conducted by microbes to produce more sugar to support their growth. Anthocyanins will undergo microbial metabolism, releasing single sugars that microbes will use as an energy source. Anthocyanins are relatively unstable and will degrade into anthocyanidins due to pH changes. The decrease in anthocyanin content in the product may be due to the pH decrease caused by fermentation, which can degrade glycosidic bonds in anthocyanins into anthocyanidins. Glycosidic bonds in anthocyanins can degrade at pH 2-4 [9]. The LAB (L. plantarum B1765) can also produce β-glucosidase enzymes that degrade glycosidic bonds in anthocyanins into anthocyanidins. These factors contribute to the increase in TPCs and decrease in total anthocyanin content in the product. This study is supported by [29], who reported a decrease in anthocyanin content by 9.41 mg/100g in fermented blueberry yogurt. Research by [30] also reported a decrease in anthocyanin content in pure blueberries by 29%, 46%, and 58% compared to fresh pure blueberries. This indicates that fermentation can increase TPC and decrease total anthocyanin content.

Determination of Antioxidant Activity

The antioxidant activity of fermented purple sweet potato juice was evaluated using the DPPH radical scavenging method and expressed with an IC_{50} value as inhibition against DPPH. DPPH inhibition was determined using UV-Vis spectrophotometry at a wavelength of 516 nm. The research results show that fermentation can increase TPC, decrease anthocyanin levels, and enhance antioxidant activity in purple sweet potato juice, as presented in Figure 3.

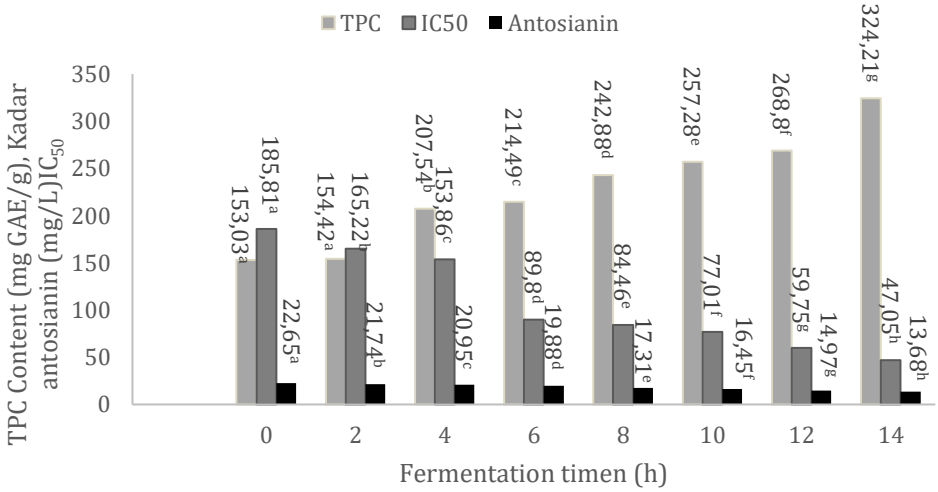

Figure 3. Correlation between antioxidant activity and TPCs and total purple sweet potato juice anthocyanins during fermentation. Results with different letters indicate significant differences ($p<0.05$)

The results of the One-Way ANOVA analysis indicate a significant difference ($p<0.05$) in TPC content, anthocyanin levels, and IC_{50} values at different fermentation times (0, 2, 4, 6, 8, 10, 12, and 14 hours). As shown in Figure 3, fermentation correlates with an increase in TPC content, a decrease in anthocyanin levels, and a decrease in IC_{50}. The increase in antioxidant activity is indicated by the reduction of IC_{50} from 185.81 µg/mL at the beginning of fermentation to 47.05 µg/mL at the end of 14 hours. Fermented purple sweet potato juice exhibits vigorous antioxidant activity with an IC_{50} value of less than 50 ppm.

The study's results are better compared to the research conducted by [26], which reported an increase in antioxidant activity in jicama extract fermented using *L. plantarum* B1765 bacteria, with IC_{50} values decreasing from 143.12 µg/mL to 83.88 µg/mL over 36 hours of fermentation. This improvement can be attributed to several factors, including the higher starch content in purple sweet potato (31.36% - 39.39%) [16] compared to jicama extract (10.7%) [27], leading to different growth rates of lactic acid bacteria (LAB) and pH reduction in the products. In fermented jicama juice, the optimal growth of LAB occurred at 12 hours of fermentation, reaching 1.15 x 10^8 CFU/mL and a pH drop to 3.79. In contrast, fermented purple sweet potato juice reached optimal LAB growth of 2.66 x 10^8 CFU/mL at 6 hours of fermentation, with a pH drop of 4.15. The differences in LAB growth and pH reduction in the samples influence the hydrolysis activity of glycosidic bonds in phenolic compounds. With higher LAB growth, the activity of β-glucosidase enzymes increases, leading to more free phenolic compounds and potent antioxidant activity. The result, supported by another study by [31]**,** reported that the antioxidant activity of kombucha tea increased from 88.88% to 93.79% after seven days of fermentation.

The antioxidant activity of fermented purple sweet potato juice was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, expressed as IC_{50} inhibition against DPPH. The IC_{50} value was determined using spectrophotometric analysis at a wavelength of 517 nm. It is important to note that anthocyanidins have more vigorous radical scavenging activity than anthocyanins, likely because anthocyanidins have more hydroxyl groups than anthocyanins [7]. According to [8], anthocyanins with an extra sugar attached at the C-3 position in the heterocyclic C ring have lower antioxidant activity than anthocyanidins with a single sugar molecule. Fermentation hydrolyzes the glycosidic bonds in anthocyanins through the activity of β-glucosidase enzymes and the low pH resulting from fermentation, producing anthocyanidins with more hydroxyl groups. This is supported by data showing increased free phenolic compounds during fermentation. Microbial enzymatic hydrolysis leads to an increase in phenolic and flavonoid compounds in the product [32].

Based on the conducted research, it can be concluded that the fermentation process increases anthocyanidins due to glycosidic flavonoid bond hydrolysis in anthocyanins, thereby enhancing their inhibition of DPPH and indicating increased antioxidant activity.

Conclusion

This study shows that the duration of fermentation of purple sweet potato juice increases total LAB, TTA, TPCs, and antioxidant activity. Additionally, fermentation also influences the reduction of the product's pH. LAB growth reaches its optimum point at 6 hours of fermentation, achieving 2.66 x 10^8 CFU/mL. However, the decrease in product pH to 3.73, the increase in total acidity to 0.426%, the reduction in anthocyanin content to 13.68 mg/L, the increase in TPCs to 324.21 mg GAE/g, and antioxidant activity with IC50 of 47.05 µg/mL continues until the end of the fermentation process. The IC50 value of the fermented purple sweet potato juice indicates vigorous antioxidant activity. This product meets the Indonesian National Standard (SNI) for fermented beverages and can be used as a functional food source of antioxidants.

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