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Improving the Quality of *Gembili* Pickle *(Dioscorea esculenta)* Through Fermentation with Starter Culture *Lactobacillus plantarum* B1765

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Abstract: *Gembili* is a type of tuber that grows widely in Indonesia and has the potential to be developed into functional food, but its utilization has not been optimal. One of the efforts that can be made is the development of *gembili* as pickle, a synbiotic fermented product of *gembili* by a starter culture of *Lactobacillus plantarum* B1765, which has been known as a probiotic. This study examines the effect of fermentation duration on the growth of total lactic acid bacteria (LAB), pH value, Total Titratable Acidity (TTA), and organoleptic quality aspects. The *gembili* tubers were cut into sticks and fermented for 0, 2, 4, and 6 days, using *Lactobacillus plantarum* B1765 starter culture at 37°C. Total LAB was calculated by total plate count (TPC), pH by pH meter, TTA by acid-base titration, and organoleptic test using untrained panellists to assess the preference level using a hedonic scale. The results showed that total LAB increased from the beginning of fermentation (2.37 x 10^7 CFU/mL) to 1.15 x 10^8 CFU/mL fermentation 4 days but decreased to 1.01 x 10^5 CFU/mL at the end of fermentation (6 days). The pH decreased from 6.43 ± 0.02 to 3.07 ± 0.002, and TTA increased from 0.31 ± 0.010% to 0.53 ± 0.008% at the end of fermentation (6 days). Organoleptic test results showed that fermentation time affected the preference level for color, taste, aroma, and texture. The highest preference level of the four aspects was the product with a fermentation time of 4 days with a preference level in the very like category. The results of this study indicate that the optimal time of *gembili* pickle fermentation is 4 days based on the total amount of LAB (> 10^6 CFU/mL), TTA (> 0.20%), pH (3.4-4.3) has met SNI criteria and also shows the highest preference level in color, taste, aroma, and texture. *Gembili* pickles can be recommended to be developed as a safe synbiotic product.

Keywords: *Gembili* Pickle; *L. plantarum* B1765; Product Quality; Synbiotic Food.

Introduction

Gembili (Dioscorea esculenta) is a type of tuber that comes from the *Dioscoreacea* family [1]. The production of *gembili* in Indonesia can reach 60-70 t/ha/year [2]. The high production of *gembili* is not accompanied by its optimal utilization. The utilization of *gembili* is still limited to consumption by steaming and is still processed with a touch of simple technology such as chips and *gembili* flour.

The potential of *gembili* can be improved and developed as a synbiotic food that benefits the body`s health. Synbiotic food is a processed food product that results from the combination of probiotic and prebiotic components. Synbiotic food can provide benefits to the body`s health by providing nutrients in the form of prebiotics that can be utilized by microorganisms that can produce various compounds, including lactic acid [3]. Several studies have shown that *gembili* contains bioactive compound components in the form of inulin, which has the benefit of being a prebiotic agent. The inulin content in *gembili* is 14.77% [4]. The results of the analysis of the effect of ethanol solvents in the extraction process of *gembili* tuber inulin on the yield of *gembili* tuber inulin, which is 26.22% of food fibre [5].

Inulin is a fructan polymer that consists mainly of linear β-2, 1-D-fructofuranose, which is connected to a glucose unit at its terminal end [6]. However the β-configuration may cause the structure of inulin to be unable to hydrolyze through human digestive enzymes. Therefore, inulin is classified as a type of carbohydrate that cannot be digested in the human digestive system [7]. Inulin can be metabolized anaerobically by some digestive microorganisms that possess the enzyme inulinase to produce fructose and glucose, which will then be metabolized to short-chain fatty acid (SCFA). The benefits of SCFA for health include changing cell proliferation and function, having anti-inflammatory, antitumorigenic, and antimicrobial effects and changing intestinal integrity [8]. In addition, the benefits of SCFA include stabilizing glucose levels in the body by increasing insulin secretion and reducing pancreatic glucagon secretion [9].

The enzyme inulinase and intestinal microflora metabolism can degrade inulin. Intestinal microflora in the form of bacteria that can produce the enzyme inulinase, namely the genus Kluyveromyces, Aspergillus, *Streptomyces spp, Achromobacter spp., Paenibacillus spp., Bacillus cereus, Arthrobacter spp.,* and *Clostridium tyrobutyricum* [10]. However, several studies have shown that inulinase can also be produced by microorganisms grown on an inulin source medium. It was found that the inulinase enzyme activity of *Pichia manshurica* DUCC Y-015 yeast. The results of its isolates that inoculate into dahlia tubers showed an increase in inulinase enzyme activity in the log phase with an incubation time of 12 hours, which amounted to 0.574 U/mL, and decreased inulinase enzyme

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activity in the stationary phase with an incubation time of 18 hours at 0.092 U/mL [11].

In this study, *gembili* acts as a source of inulin, which will later be used to produce lactic acid and organic acid compounds through a fermentation process of a pickle using *Lactobacillus plantrarum* B1765 starter culture. Pickle is a processed fruit or vegetable product using salt and acid with or without adding sugar and spices as seasonings. Acid comes from fermentation of fruits or vegetables or adding vinegar [12]. This study is important to determine the suitability of *gembili* pickle product quality with SNI pickle requirements from chemical quality, microbiology and level of liking for aroma, taste, texture and color. The results of this study can be used further for the basis of *gembili* pickle development as an effort to diversify processed *gembili* products.

The use of *L. plantarum* B1765 as a starter culture in this study is based on research showing that it can produce inulinase enzyme with an incubation time of 18 hours and a result of 0.047 Units/mL [13]. The bacteria *L. plantarum* B1765 also acts as a probiotic culture agent based on research that has been conducted, *L. plantarum* B1765 has resistant properties to generic amoxicillin clavulanate antibiotic tablets at a concentration of 50 ppm [14]. The length of fermentation in the *gembili* tuber pickle fermentation process is related to the growth phase of LAB and the secondary metabolites produced, including the enzyme inulinase. Long fermentation results in a decrease in pH and an increase in total acid, affecting organoleptic quality parameters, including aroma, taste, colour and texture. The low pH also has a role in food safety because the low pH condition of the product can inhibit the growth of destructive microorganisms or pathogens, which can cause the product not to last long [15].

Based on the description above, this study aims to evaluate the growth of total lactic acid bacteria (LAB), pH, and total titratable acidity (TTA) as an indicator of the metabolism of inulin along the fermentation process of the *gembili* pickle by starter culture of *L. plantarum* B1765. These parameters were chosen based on the standard quality of fermented food. We also determined the organoleptic characteristic to evaluate the preference level of the product. The results of this study provide new information about the diversification product of *gembili* as *gembili* pickle is a synbiotic agent that is useful for the health and safety of functional food.

Research Methods

Preparation of *L. plantarum* B1765 Starter Culture

The starter culture *L. plantarum* B1765 (isolate of *bekasam*), a fermented fish product) developed in MRS Broth medium, which has been sterilized using an autoclave at 121℃ and 15 Psi pressure for 15 minutes. A total of 1 mL of *L. plantarum* B1765 stock culture was cultured in 10 mL of MRS Broth and incubated at 37 ℃ for 24 hours. Next, the *L. plantarum* B1765 starter culture was centrifuged for 15 minutes at 3,500 rpm, then resuspended in 10 mL of sterile NaCl of 0.85% solution and centrifugated again. The pellet obtained from the centrifugation process was resuspended in 10 mL of sterile NaCl of 0.85% solution and then used as a starter culture [16].

Preparation of *Gembili* Pickles

Gembili is peeled and washed thoroughly, then made into smaller sizes by grating using a grater with a diameter of $\pm$0.5 cm, then weighed as much as $\pm$100 grams and blanching using hot water at 100℃ for 5 minutes. The results of the *gembili* pieces were put into glass jars that had been sterilized and labelled. Sterilization of glass jars was carried out through an autoclave process at 121℃ and 100 mL of sterile aquadest was added. Then, *L. plantarum* B1765 starter culture was added as much as 3% (v/v) and fermented for 0, 2, 4, and 6 days in an incubator at 37℃ [17].

Enumeration of Total Lactic Acid Bacteria (LAB)

Total LAB enumeration was done by calculating the total LAB that grew on culture media (MRS) with the Total Plate Count (TPC) method. MRS agar media was made from 5.22 grams of MRS Broth dissolved in 100 mL of aquadest and added 1.5% agar and 1% $CaCO_3$. Samples were diluted using 0.85% NaCl solution at a dilution value of 10^5-10^8. The dilution was done by taking a sample using a micropipette as much as 1 mL (1000μL) into a petri dish. Furthermore, MRS agar medium was poured into a petri dish as much as $\pm$15 mL. Immediately after pouring, the petri dish was shaken gently so that the inoculated bacteria were evenly distributed and then allowed to solidify. The media in the petri dish was then incubated upside down at 37℃ for 48 hours. Then the number of lactic acid bacteria counted between 25-250 colonies. All treatments were done aseptically [16].

pH Test

The pH value from each fermentation time was measured by crushing *gembili* pickle pieces and the pickle liquid. Then, 20 mL of each pickle sample was taken and the pH value was measured with a pH meter calibrated with a buffer solution of pH 4.0, pH 7.0, and pH 10.0 [17].

TTA Test

The TTA test was measured using the titration method expressed as a percentage of lactic acid. Measurement of TTA values using the acid-alkalimetric titration method by crushing pieces of *gembili* pickle along with the pickle liquid. Furthermore, a sample of 10 mL was diluted in a 100 mL volumetric flask, then pipetted as much as three drops and titrated with 0.1 N NaOH. The titration process is immediately stopped when there has been a stable and permanent pink color change [17].

Organoleptic Test

The organoleptic test effectively includes the preference level for aroma, taste, color, and texture. The testing process is carried out using a hedonic test or level of preference. In this test, 30 untrained panellists were asked to give an impression of the aroma, taste, color, and texture of the *gembili* tuber fermented pickle product in a questionnaire with a hedonic scale from 1 to 4 to express not very like, not like, like and very like [16].

Data Analysis

In this study, the data obtained were processed using statistical tests, namely the Annova test, to determine the effect of fermentation length (<0,05) on the total LAB, pH and TAT test on *gembili* pickle. A Post Hoc LSD test was used to see the difference in each treatment. If the test results do not meet the normality requirements, data analysis will be carried out using another method, the Kruskall Wallis test and the Whitney further test. On the results of organoleptic testing, statistical analysis was carried out of the Kruskall Wallis test in each group of panellists and Mann Whitney as the further test.

Results and Discussion

LAB Growth, pH and TTA

In this study, we determine the effect of fermentation time on gembili pickle and the growth of LAB, pH, and TTA. The research results showed an effect (< 0.05) on total LAB and pH. Further tests on total LAB showed no significant difference in the length of fermentation time between 0 and 2 days, and there was a significant difference in the length of fermentation time from 2 to 6 days. However, the pH and TTA showed a significant difference from the beginning of fermentation (0 days) to the end (6 days). The results of the total LAB growth, pH, and TTA of fermented *gembili* pickle can be seen in Fig. 1

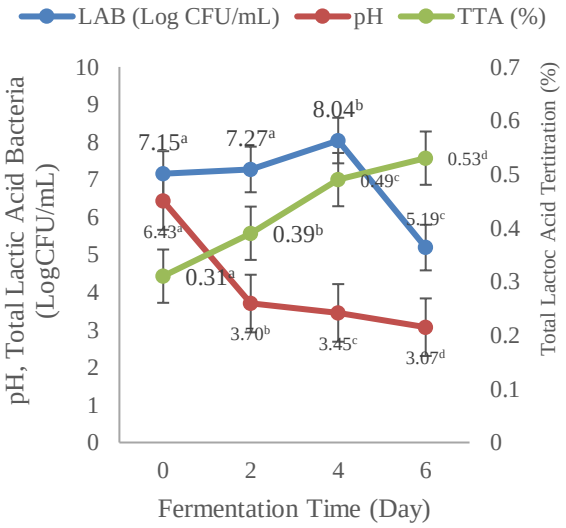

Figure 1. Total LAB, pH and TTA Test Results of *Gembili* Pickle Fermentation
Note: Values followed by the same letter indicate that they are not significantly different (α=0.05)

Figure 1 shows that the fermentation time between 0 days and 2 days did not show any significant difference in the growth of LAB. This indicates that LAB entered the lag phase. Furthermore, the most significant growth of LAB occurred at the fermentation time of 4 days, where LAB increased by 1 log cycle from 2.37 x 10^7 at the fermentation time of 2 days and increased significantly at the fermentation time of 4 days which amounted to 1.15 x 10^8, this indicates that LAB entered the exponential phase. At the length of fermentation time between 4 days and 6 days, there was a significant difference in LAB, namely a decrease. So, LAB had entered the death phase.

The same research results were also carried out by Insani & Wikandari [17] The sweet potato and pickle fermentation showed significant LAB growth at 8 hours of fermentation from 2.93 x 10^6 at 0 hours to 2.81 x 10^8. This indicates that LAB is in the exponential phase and decreased at the end of fermentation (24 hours), indicating that it has entered the death phase. Another research by Wikandari et al., [18] also showed an increase in total LAB in fermented yacon pickle from inulin sources of 3.25 x 10^8 in a fermentation time of 48 hours, which indicated that LAB had entered the exponential phase.

In this research, the highest LAB growth occurs on day 4 fermentation, when the bacteria enter the exponential phase. In this phase, starch and other polysaccharides will be broken down into glucose, maltose, and dextrin by the α-amylase enzyme that begins to be secreted in this phase. The α-amylase enzyme is known to be produced by several bacteria, including *L. plantarum* [19]. Glucose then undergoes further metabolism into lactic acid and other organic acids so that the pH of the product becomes low.

In this phase, there is also an increase in the enzyme inulinase, which hydrolyzes inulin into fructose in the media, which will be used as an energy source [20]. *L. plantarum* B1765 bacteria are known to produce inulinase enzyme activity of 0.047 U/mL during an incubation time of 18 hours [13]. The research of Fitrania et al. [11], it was found that the inulinase enzyme activity of the yeast *Pichia manshurica* DUCC Y-015 isolated from dahlia tubers showed an increase in inulinase enzyme activity in the log phase with an incubation time of 12 hours, which amounted to 0.574 U/mL and decreased inulinase enzyme activity in the stationary phase with an incubation time of 18 hours at 0.092 U/mL. Another research by Wijanarka & Sarsa [21], the inulinase enzyme activity test of Yke yeast isolate showed an increase in inulinase enzyme activity of 0.3977 U/mL in the log phase at 12 hours incubation time. Meanwhile, in the incubation time range of 18 hours and 24 hours, namely in the stationary phase, it showed a decrease in inulinase enzyme activity, which amounted to 0.3634 U/mL and 0.3205 U/mL. Furthermore, the function of adding salt to the fermentation process can also stimulate the growth of lactic acid bacteria, where salt acts as a selective agent that serves to inhibit other microorganisms besides lactic acid bacteria [17].

Amylase and inulinase enzymes play an important role in the growth process of lactic acid bacteria (LAB) during fermentation. Amylase enzymes break down starch into glucose, while inulinase hydrolyzes inulin into fructose. Both glucose and fructose play a role in providing energy sources (substrates) in LAB growth. LAB growth enters the optimum period of 4 days, indicating that amylase and inulinase enzymes provide simple sugar compounds from the *gembili* pickle medium, which are important for LAB metabolism so that it can increase energy production and cause significant growth.

The growth of LAB is also accompanied by a decrease in pH and an increase in TTA due to the accumulation of lactic acid, where the *gembili* pickle product has met the requirements of SNI 01-3784-1995 with the requirement of pH between 3.4 – 4.3 and microbiological quality (total LAB) of >10^6 so that *gembili* pickle can be a prebiotic agent for *L. plantarum* B1765 bacteria. In addition

to producing lactic acid, the inulin content in *gembili* is expected to hydrolyse to produce fructose, then undergo an aerobic metabolism process to produce SCFA that will decrease the pH of *gembili* product. Wijayanti & Wikandari [16], showed that fermentation of yam bean produces lactic acid, which can produce SCFA consisting of acetic acid of (1.31 ± 0.006 mg/mL), propionic acid of (1.58 ± 0.005 mg/mL) and butyric acid of (1.05 ± 0.0038 mg/mL). The formation of SCFA in the study was suspected due to the metabolic process of lactic acid bacteria *L. plantarum* B1765 containing inulinase.

The growth of total LAB during the fermentation process can also affect other product qualities, namely a decrease in pH value and an increase in TTA. Fig. 1 shows that the pH value decreased significantly from the beginning of fermentation, which was 6.43 plus or minus 0.02, 6.43 ± 0.02 to 3.07 ± 0.002 at the end of fermentation at 6 days. Meanwhile, the TTA value increased from 0.31 ± 0.010% at the beginning of fermentation (0 days) to 0.53 ± 0.008% at the end of fermentation at 6 days. Research by Insani & Wikandari [17], showed that the fermented yam pickle product also decreased in pH from 5.52 ± 0.59 at 0 hours to 3.23 ± 0.20 at the end of fermentation (6 hours) and increased in TTA from 0.07 ± 0.001% at 0 hours to 0.56 ± 0.02% at the end of fermentation (6 hours). The decrease in pH and increased TTA values occurred due to the metabolic process by *L. plantarum* B1765 bacteria that can produce acidic compounds, namely lactic acid. The amount of lactic acid produced during the fermentation process affects the decrease in pH because the lactic acid that has been formed dissociates into H^+ ions and $CH_3CHOHCOO^-$ ions. An increase in H^+ ions can reduce the pH value of the *gembili* pickle sample.

Organoleptic Test

In this study, we determine the effect of fermentation time on *gembili* pickles on organoleptic quality, including color, flavour, aroma, and texture parameters. The study results were analyzed using Kruskal Wallis, which showed an effect ($p < 0.05$) on organoleptic tests. The results of the organoleptic test using Kruskall Wallis showed that fermentation times have significant differences in color, flavour, aroma, and texture parameters. Furthermore, a further test was carried out, namely Mann Whitney test to determine the variation of samples that experienced significant differences.

The color parameter did not show a significant difference at fermentation times of 0 days with 2 days fermentation. However, the preference level for taste differs significantly (<0.05) from days 4 to 6 of fermentation, with the highest preference level of 3.93 on day 6 fermentation, categorized as very liked. The results of the organoleptic test of the color parameter can be seen in Figure 2.

There were changes in color of the *gembili* pickle product during the fermentation process. At 0 days of fermentation, the pickle product is brownish and whiter as the fermentation process increases. The color change in *gembili* pickles is caused by the activity of the polyphenol oxidase (PPO) enzyme, which will convert phenolic compounds into brown quinones. *Gembili* contains a phenolic of 0.79 ± 0.07 g / 100 g [22], and the PPO enzyme [23]. PPO activity can be controlled with low pH so that the fermentation process can keep the color of the *gembili* pickle from turning brown. The optimum pH to be able to inactivate the PPO enzyme in soursop juice is 4.40, with the lowest PPO enzyme specific activity of 0.0005 U/mg with 93% enzyme inactivation [24]. Fig.2 shows the highest preference level occurred at 6 days of fermentation with a level of liking of 3.93 with a pH of 3.07 ± 0.002. However, at 4 days of fermentation, the pH had reached 3.45 ± 0.002, so at that time, the PPO enzyme had also experienced inactivation, which caused the color of the *gembili* pickle to become white with a preference level of 3.90 or in the category of very like.

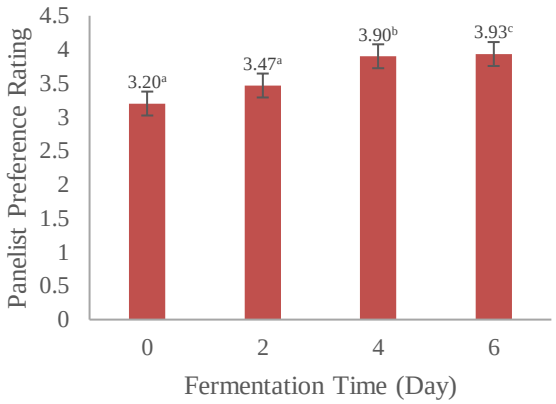

Figure 2. Organoleptic Test Results For *Gembili* Pickle Color

Note: Values followed by the same letter indicate that they are not significantly different (α=0.05)

In the flavour parameter, there is a significant difference ($p < 0.05$) in each fermentation sample variant. There was a significant increase in value in fermentation from 0 days to 2 days fermentation with a value of 2.37, which is not like and increased on the 2 days by 3.00, which is like. Real differences also occurred on 2 days and 4 days, increasing from 3 to 3.96, namely very like to the 4 days fermentation time, and the preference level value showed a real difference between the fermentation time of days 4 and 6 fermentation, a decrease from 3.97 to 3.23. The results of the organoleptic test of flavour parameters can be seen in Fig. 3

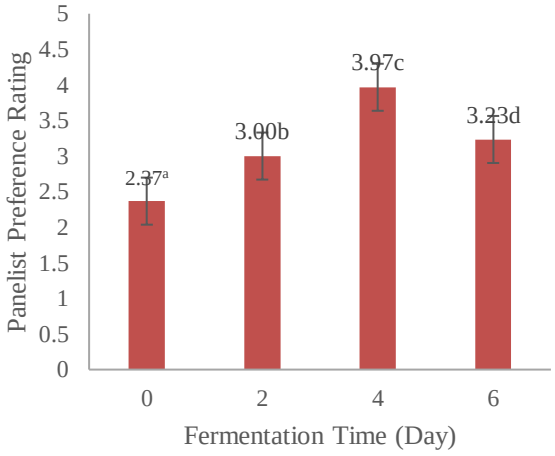

Figure 3. Organoleptic Test Results For *Gembili* Pickle Flavour

Note: Values followed by the same letter indicate that they are not significantly different (α=0.05)

The taste of the *gembili* pickle product is typical of Sauerkraut, the sour taste from the fermentation process. The longer the fermentation time, the more sour the taste of the *gembili* pickle. This can happen because the longer fermentation time will cause a decrease in pH caused by the formation of acidic compounds from the process of bacterial growth during fermentation, as well as an increase in TAT value caused by the presence of acidic compounds such as lactic acid, acetic, propionic, and butyric acids produced by the metabolic process of *L. plantarum* B1765 bacteria during fermentation. On day 4 of fermentation, the pH of *gembili* pickle decreased by 3.45 ± 0.002, and the TTA value increased by 0.49 ± 0.008%. On day 4 of fermentation, there was an increase in the assessment of panellists in the category of very like. However, the 6 days of fermentation showed a decrease in the assessment of panellists in the like category. This was caused by on the 6 days of fermentation, the pH value decreased by 3.07 ± 0.002, and the TAT value increased by 0.53 ± 0.011% caused the taste of *gembili* pickle to be too acidic.

There is a significant difference ($p < 0.05$) in the aroma parameter between the fermentation period on day 0 and the fermentation period on days 2, 4 and 6 where on days 0 and 2 the panellist preference value was 2.73 – 3.13, which is like and increased on day 4 fermentation at a value of 3.90, which is very like and decreased again on day 6 fermentation, which is like with a value of 3.23. the results of the organoleptic test of aroma parameters can be seen in Fig. 4

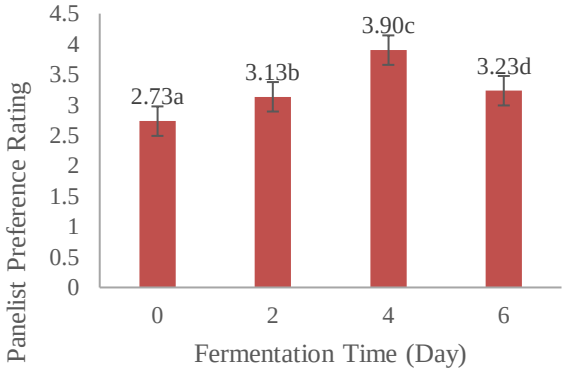

Figure 4. Organoleptic Test Results For *Gembili* Pickle Aroma

Note: Values followed by the same letter indicate that they are not significantly different (α=0.05)

This can happen because the longer the fermentation time, the stronger the acid aroma produced. As the fermentation time increases, the *L. plantarum* B1765 bacteria will produce acid compounds such as lactic acid and SCFA, making the aroma stronger and more complex. On the 6 days of fermentation, the panellists' ratings decreased in the like category because, for 6 days of fermentation, the aroma of *gembili* pickle was too acidic and complex.

In the texture parameter, there were significant differences ($p < 0.05$) in the texture parameter between the fermentation period on day 0 to 4 days, which increased in the range of 3.17 – 3.70 and decreased on day 6 of fermentation with a value of 3.57. The results of the organoleptic test of the texture parameter can be seen in Figure 5.

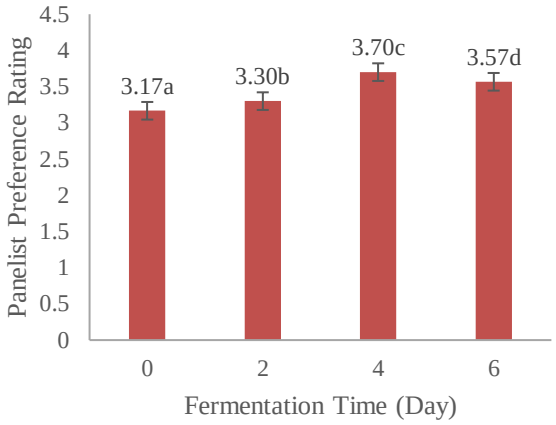

Figure 5. Organoleptic Test Results For *Gembili* Pickle Texture

Note: Values followed by the same letter indicate that they are not significantly different (α=0.05)

This is tough to cause by the degradation process of inulin and cellular components in bacteria because the long fermentation period results in the more significant breakdown of cell walls so that the texture becomes soft. Inulin was degraded to glucose and to be metabolized, then to SCFA. The presence of *L. plantarum* B1765 starter culture in *gembili* pickles can degrade glucose so that it affects the texture of the pickles to become softer because *L. plantarum* B1765 bacteria play a role in softening the tissue in the fermentation process of *gembili* pickle [25].

Conclusion

Gembili can improve quality by changing it into synbiotic food as a *gembili* pickle with a fermentation process using *L. plantarum* B1765 bacteria with an optimum fermentation time of 4 days. Total LAB increased by 1 log cycle from the beginning of fermentation 0 days (2.37×10^7 CFU/mL) and reached 1.15×10^8 CFU/mL at 4 days of fermentation, but pH decreased to 3.07 ± 0.002 until the end of fermentation (6 days), and it was increasing of TTA value from 0.31 ± 0.010% at the beginning of fermentation (0 days) to 0.53 ± 0.008% at the end of fermentation. The organoleptic test showed that fermentation times give a very high preference for color, taste, aroma, and texture. The best panellists' preference in color, taste, aroma, and texture was a product with a fermentation time of 4 days. In this study, the most optimal fermentation time was 4 days, based on the total LAB (>10^6 CFU/mL), TTA (>0.20%), pH (3.4 – 4.3) had met of SNI, and the product had the best preferences on color, taste, aroma, and textures. The *gembili* pickle could be recommended to develop as a safe synbiotic food product.

Author Contribution

Yurita Gita Puspa Nugrainglahi: was responsible for conducting the research, data collection, result analysis and manuscript drafting. Prima Retno Wikandari: served as the corresponding author, providing academic guidance and reviewing and refining the article draft.

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