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Changes of Gembili (*Dioscorea esculenta*) Tuber Flour Characteristics During Fermentation Process with *L. plantarum* B1765 Starter Culture

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Abstract: Gembili (*Dioscorea esculenta*) is a plant that contains inulin, which can be degraded into fructooligosaccharides known to have advantages and are widely used as food ingredients. The fermentation process can hydrolyse inulin into FOS, which is known to have better stability for food ingredients. Additionally, fermentation can produce Short-Chain Fatty Acids (SCFAs), improving consumer health and enhancing flavor, resulting in better processed products. The objective of this study was to determine changes in the characteristics of gembili tuber flour, including solubility and color, during the fermentation process, supported by data such as pH, Total Titratable Acid (TTA), and Total Lactic Acid Bacteria (TTA). Gembili tubers were cut into pieces and fermented for 0, 2, 4, and 6 days using a 3% *L. plantarum* B1765 starter culture and incubated at 37°C. pH measurements were taken using a pH meter, TTA using acid-base titration, total LAB using total plate count, and solubility determined by gravimetric principles. Total LAB, TTA, pH, solubility, and color were mutually correlated during the fermentation process. As more bacteria grew, pH decreased, TTA increased, solubility increased, and color became whiter. Optimal flour results were obtained at a fermentation time of 6 days, where total LAB reached 7.60 x 10^7, pH was 3.40, TTA was 0.989%, solubility was 51.30%, and the color was the greatest white color. Fermented gembili tuber flour has more water-soluble FOS content and has better stability than inulin in non-fermented gembili flour. In addition, the colour produced from the fermentation process is also whiter than that of fermented gembili flour, which can affect the quality of the product. The FOS content in this flour can provide texture or creaminess, maintain moisture in food products, and lower the freezing point of ice cream. However, further research is still needed to determine the effect of fermentation duration on the degree of polymerisation (DP) of FOS produced during the fermentation process and the addition of fermentation time to improve the flour solubility in water.

Keywords: Gembili Tuber Flour (*Dioscorea esculenta*); Fermentation; Frukto-Oligosaccharide; Solubility.

Introduction

Gembili tubers (*Dioscorea esculenta*) are a type of tuber widely found in Indonesia. However, the community still does not fully utilize the potential of gembili tubers. Most people only use gembili tubers by frying, boiling, or using them as a staple food substitute for rice, or even making them into chips [1]

In 100 grams of gembili tubers, there are 131 kcal of energy, 1.1 grams of protein, 0.2 grams of fat, 1.1 grams of fibre, 0.6 mg of iron, 14 mg of calcium, 91.3 grams of carbohydrates, 56 mg of phosphorus, 4 mg of vitamin C, and 0.08 mg of vitamin B1 [2]. Gembili tubers also contain bioactive compounds such as stigmasterol, furostanol, spirostanol, cholestanol, ergostanol, pregrostanol, diosgenin, dioscorin, tannins, and flavonoids [3]. According to research by [4] methanol extract from gembili contains alkaloids, phenols, tannins, glycosides, and flavonoids. In addition, gembili tubers also contain antioxidants with an IC_{50} of 38.33 µg/mL [5] and 14.77% inulin [6]

One processed product that can be developed is gembili tuber flour. Gembili tuber flour is commonly used as an additive in the production of yoghurt and ice cream because it can be used as a stabiliser and nutritional supplement due to its inulin content, as found in studies by [7] and [8].

Inulin is a water-soluble dietary fiber indigestible by human digestive enzymes because it has a β-(2-1) fructofuranosyl glycosidic bond and a terminal glucose at the end [9].The characteristics of inulin are that it is less soluble and less stable, so it will affect the texture of the product, making it less soft. Inulin is soluble in hot water, but insoluble in cold water [10]**.** This solubility is a concern if gembili flour is to be utilised as flour. The second problem is that gembili contains the enzyme polyphenol oxidase (PPO), which can affect the color of gembili flour to become brownish due to PPO enzyme activity that converts phenolic compounds into quinones that are brown in color [11].

An effort that can be used as a solution is the fermentation of gembili tubers. During fermentation, inulin will be metabolised by microorganisms with inulinase activity and put into FOS. *L. plantarum* B1765 is known to have inulinase activity with an activity of 0.047 U/ml [12]. In research [13], inulin in gembili tubers can be metabolised by *L. plantarum* B1765 into FOS with a DP (degree of polymerisation) of 3.47 with a fermentation time of 24 hours. The DP is suitable for industrial use [14], stating that commercial FOS is FOS with DP 2, 3, and 4. FOS is known to have higher solubility properties than inulin. This high solubility can provide a smoother texture and can function as an emulsifier in processed food products [15]. Fermentation is also expected to improve the color quality of gembili tuber

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flour as the PPO enzyme can be inactivated by the low pH formed during fermentation. The fermentation process in gembili tubers can inhibit the work of the enzyme polyphenol oxidase (PPO), which can cause the browning process. The PPO enzyme can convert phenolics in gembili into quinones and produce a brown color called o-quinone [16]. But this can be inhibited by decreased pH value during the fermentation process. PPO enzymes have maximum activity at pH 7.0 [17]. Therefore, fermentation will produce a lighter flour color than unfermented flour. So, the smaller the pH is, the more enzyme phenolase activity decreases because it is far from the optimum pH [18].

This study aims to determine the effect of fermentation time on the solubility and color of the resulting product. As supporting data, data on LAB growth, pH and TTA are needed, which greatly affect the solubility and color characteristics of fermented gembili tuber flour. The results of this study are expected to benefit the development of fermented gembili tuber flour as a source of FOS, which has various advantages over inulin.

FOS provides a sweeter flavour than inulin, which can be used as a low-calorie sugar substitute. FOS tends to be more stable at various pH, at acidic pH, 2.7-3.3, FOS can survive up to 100℃ [19], while at neutral pH conditions, 5, 6, 7, FOS can be stable up to 120℃ [20] Meanwhile, inulin can only survive up to 60℃ in acidic atmosphere (pH ≤ 4). At neutral pH (≥ 5), inulin can survive up to 100℃ [21]. This better stability of FOS is needed in processed foods to maintain flavour and texture.

FOS has various functions in food products. In dairy products, FOS helps to provide texture or creaminess. In soft bread products, FOS helps to maintain moisture. In frozen desserts, FOS lowers the freezing point, resulting in a smoother texture. FOS also provides crunch in low-fat cookies and serves as an adhesive in nutritional foods such as granola bars, similar to sugar. However, the advantage of FOS is that it is lower in calories, contains fibre, and has other nutritional benefits [15].

FOS is also a prebiotic. Prebiotics are substances specifically utilised by good microorganisms in the body, thus providing health benefits. Regular consumption of FOS is also recommended because it can prevent or delay osteoporosis by helping the absorption of ions into the body, such as Ca^{2+} and Mg^{2+} [22]. FOS is also known to relieve constipation, reduce sugar levels in hyperglycaemia, and reduce the risk of colon cancer [23]

Although FOS and dietary fibre are indigestible by enzymes in the small intestine, FOS can be digested by gut microbiota. In the large intestine, FOS will be broken down by the enzyme β-fructofuranosidase into glucose and fructose, which are then utilised by microbes through heterofermentative fermentation pathways into short-chain fatty acids [24]. These SCFAs support the health of epithelial cells in the large intestine and also serve functions such as acting as anti-inflammatory and anti-tumour agents, helping regulate glucose levels, controlling appetite, and supporting the circulatory system [25].

Several studies on fermented preparations of gembili tubers had been conducted, such as fermentation of yoghurt from gembili flour with starter cultures of *L. acidophilus* IFO 13951 and *Bifidobacterium Longum* ATCC 15707, but were limited to testing sensory quality and growth of LAB [26]. Another research by [27] examined the effect of adding types of bacteria on physicochemical properties, including colour, aroma, texture, through organoleptic tests and bacterial activity in fermented gembili tuber flour. However, previous studies have not obtained data on the effect of fermentation duration on pH, TAT, total LAB, color and solubility.

Research Methods

Material

L. plantarum B1765, obtained from a private collection, was originally isolated from fermented fish (*bekasam*). The materials used include MRS Broth (Merck), distilled water, 70% alcohol (Onemed), NaCl, $CaCO_3$, phenolphthalein, methanol (Merck), and NaOH (Merck).

Culture Preparation

A 1000 µL aliquot of the *L. plantarum* B1765 isolate is transferred into 9 mL of MRS broth and incubated at 37°C for 20 hours. Once the starter culture has grown, it is centrifuged at 3500 rpm for 5 minutes to separate the cells. The supernatant is discarded, and the resulting pellet is washed by suspending it in 10 mL of sterile 0.85% NaCl solution, followed by another round of centrifugation to remove residual MRS broth. The final pellet is then resuspended in 10 mL of sterile 0.85% NaCl, mixed thoroughly using a vortex until homogenized, and used as the starter culture [28].

Sample preparation

Fresh *Dioscorea esculenta* were peeled and cleaned. After cleaning, the *Dioscorea esculenta* were then cut into small pieces. After that, the *Dioscorea esculenta* that have been cut are weighed as much as 300 grams for each variable. Blanch for 5 minutes using boiling water. Then, it was drained and put in a glass jar sterilised using an autoclave. Next, 1% NaCl was added in a ratio of 1:1 (b/v). Then, the *Dioscorea esculenta* tuber pieces were fermented by adding *L. plantarum* B1765 bacteria as much as 3% (v/v). Then, the jar was tightly closed and fermented for 0, 2, 4, and 6 days in an incubator at 37℃. After fermentation, the saline water and pickles were separated by filtering. Afterwards, the fermented pieces of gembili tubers were dried using an oven at a temperature of 50℃ until the weight was constant. After drying, the pieces of gembili tubers were then mashed until they became flour. After that, the flour was sieved using an 80 mesh sieve [29].

Determination pH

The pH measurements were performed by pH meter that was standardized first with the pH 4.01 and pH 6.86 of buffer solutions. The first pickle was blended. For pH measurement, 20 mL of the mixture was placed into a beaker, and the pH was measured using a calibrated pH meter until a stable result was obtained. Repeated three times [30].

Determination of Total Titratable Acidity (TTA)

TTA is determined by the acid-base titration method. The TTA measurements were performed by blending the

pickle. After that, took 10 mL of the sample and diluted it in a 100 mL volumetric flask. Next, pipette 10 mL into an Erlenmeyer flask. After that, and added three drops of phenolphthalein (PP) indicator and titrated with NaOH solution. The titration was stopped if a stable pink color change occurred. Titration was repeated three times [31].

Total Lactic Acid Bacteria (LAB)

The total plate count (TPC) method in deMan Rogosa Sharpe (MRS) agar media and 1% CaCO3 determined total lactic acid bacteria. The sample was diluted with NaCl 0,85% from concentration 10^1-10^8. Sampling and Examining were done by taking a test employing a micropipette of as much as 1 ml of sample in a petri dish. Moreover, the MRS media is poured as much as ± 12 ml. The media within the petri dish was at that point hatched upside down at 37ºC for 48 hours. At that point, the number of lactic corrosive microscopic organisms was calculated utilizing CFU (colony forming unit)/mL units [30]

Determination of Water Solubility of Gembili Flour

Gravimetric principles determine solubility. The flour of gembili tuber was taken in as much as 1 gram and then dissolved in distilled water in as much as 20 ml. After that, it was heated in a container at 60ºC for 30 minutes, while stirring slowly. Then the solution was put into a centrifuge at 3000 rpm for 20 minutes to separate the supernatant from the residue. Residue was separated, and 10 ml was taken for oven drying. Drying the paste on a porcelain cup in the oven at 105ºC. After drying, the sample was weighed to obtain a constant paste weight. Solubility can be calculated using the formula [32]:

$$\% \text{ solubility} = \frac{\text{weight of dry precipitate}}{\text{volume of supernatant}} \text{ x } 100\%$$

Results and Discussion

Effect of Fermentation on pH, Total Titratable Acid (TTA), and Total Lactic Acid Bacteria (LAB)

The fermentation process on gembili tubers is carried out to improve the quality of gembili flour products in terms of solubility and color. These characteristics are strongly influenced by the pH produced during the fermentation process. Therefore, data on total LAB, pH, and TTA values are needed as supporting data to determine the effect of fermentation on the color and solubility of gembili tuber flour.

The one-way ANOVA analysis on Total LAB showed an effect of fermentation time on total LAB ($p<0.05$). The results of further tests with post hoc tests with LSD showed that total LAB at fermentation times of 0 and 2 were significantly different. However, no significant difference was observed during the fermentation time of 2 to 6 days. Meanwhile, the TTA and pH data did not fulfil normality, so the analysis was carried out using the Kruskal-Wallis Test. The result showed the effect of fermentation time on TTA and pH ($p <0.05$). Then, we analysed to show the difference between each treatment using the Mann-Whitney test. The result of TTA showed a significant difference between fermentation times of 0, 2, 4, and 6 days. Besides that, the pH value had different significance on all treatments, 0, 2, 4, and 6 days. Data on total LAB, pH, and TTA are listed in Fig. 3.

Figure 3 showed that the optimal growth of LAB occurred at a fermentation time of 2 days, which was 2.5 x 10^8 CFU/mL. Phase 0 to 2 days is called the logarithmic or log phase because there is a significant increase in LAB growth by 2 log cycles from $6.6x10^6$ to $2.5x10^8$. Other results showed that in the fermentation process by *L. plantarum* B1765 carried out on sweet potato, there was also an increase of log 2 cycles from 2.93×10^6 to 2.81×10^8. The increase in total LAB in the log phase was followed by a decrease in pH value and an increase in TTA. In fermented sweet potato pickle, the pH decreased from 5.52 to 3.84, and the TTA increased from 0.07% to 0.15% on the log phase [33]. During the fermentation time of 0 to 2 days, the pH decreased from 7.87 to 5.80 while the TTA increased from 0.179% to 0.403%. This is also in line with several other studies that showed a decrease in pH when LAB growth entered the log phase. In [34], who conducted research on sweet potato drinks, it was also known that there was a decrease in pH from 6.46 to 5.37 and an increase in TTA from 0.099% to 0.127% during the log phase of fermentation. During the fermentation process in Jicama, *L. plantarum* B1765 was able to reduce the pH from 5.79 to 3.92 and increase the TTA from 0.127% to 0.223% [35]

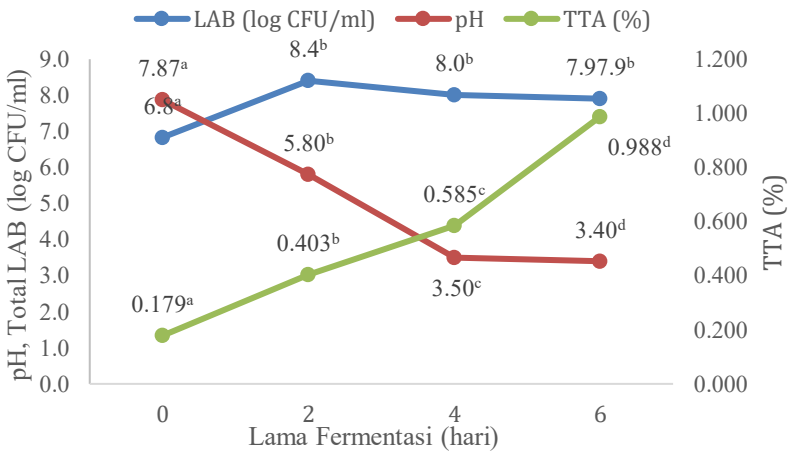


Figure 1. Total Lab, pH, and TTA of gembili flour during fermentation time
Different letters in a column denote significant differences ($p<0.05$)

During the log phase, the maximum activity of the inulinase enzyme occurs [36]. *L. plantarum* B1765 had an inulinase activity of 0.047 units/ml. This enzyme is able to degrade inulin into fructose and glucose, which are then further metabolised into SCFA. This was proven in the research [37]**.** *L. plantarum* B1765 is able to produce inulinase, which metabolises inulin into SCFA. The decrease in pH value in gembili fermentation was higher than in sweet potato and jicama pickle. It is suspected that this is due to the higher inulin content in gembili (14.7%) compared to sweet potato (8.8%) and jicama (12.32%) [38]. The higher the inulin content, the higher the SCFA from inulin metabolism, resulting in a higher pH reduction.

Meanwhile, the growth of LAB during 2 to 6 days of fermentation showed an insignificant difference. This shows the growth phase of LAB entering the stationary phase, where bacteria have the same amount of growth and death. Although the total LAB showed no significant difference, during the stationary phase, there was still a decrease in pH and an increase in TTA value. This is thought to be because the enzyme inulinase is still active, although its activity is not as high as in the log phase. This is in line with research conducted on Jicama pickle, that in the stationary phase, there is still a decrease in pH and an increase in TTA. According to [39], during the stationary phase, the pH decreased from 4.56 to 3.92, and the TTA increased from 0.185% to 0.223%.

Solubility of Gembili Tuber Flour In Water

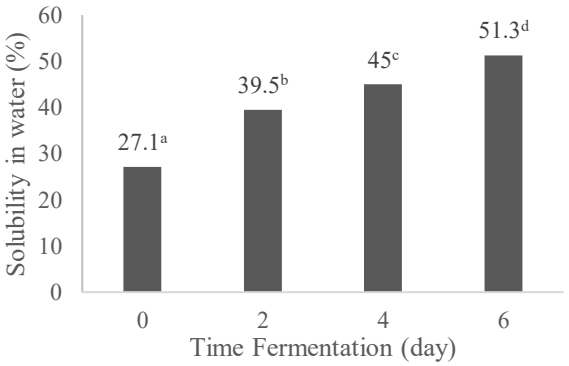
Flour solubility is one of the important factors in food processing. The utilisation of unfermented gembili flour has a disadvantage in its solubility properties. In the form of unfermented gembili flour, inulin content is an issue in the solubility of gembili flour. Inulin has a lower stability and solubility than FOS, so a fermentation process is needed to hydrolyse inulin into FOS to make it more stable and soluble in water to produce better processed food. FOS with DP 4 has >75% solubility in water at 25℃. While inulin with DP 25 only has a solubility of 2.5% at the same temperature. This higher solubility occurs due to the different molecular weight; inulin has a higher molecular weight, so it is less soluble in water [40]

This high solubility can provide a smoother texture and can function as an emulsifier in processed food products. FOS helps provide texture or viscosity. In soft bakery products, FOS maintains moisture [41]. In ice cream, FOS also lowers the freezing point, replaces sugar, reduces fat content, and provides a creamy texture [42].

Fructooligosaccharides (FOS) is a type of oligosaccharide consisting of 2 to 10 fructose monomer units connected via β-(2→1) glycosidic bonds, with one glucose monomer at the end of the chain bound via α-(2→1) glycosidic bonds. FOS can be produced from the hydrolysis of the enzyme inulinase. Inulinase produced by microorganisms is able to hydrolyse inulin into FOS. Endoinulinase (β-2-1-D-fructan hydrolase) randomly cuts inulin into fructooligosaccharides with different chains. Meanwhile exoinulinase (fructan β-fructosidase) enables the hydrolysis of inulin vertically from the nonreducing terminal non-reducing end to produce fructose[43]. The production of FOS from inulin using the enzyme inulinase resulted in a high yield of 90% [44]. Inulin in gembili tubers can be metabolised by *L. plantarum* B1765 into FOS. The longer the fermentation process is carried out, the smaller the degree of polymerisation will be obtained. FOS produced from the fermentation process has several advantages, such as higher solubility than inulin.

Figure 4 shows that the longer the fermentation process is carried out, the more soluble the flour becomes. The statistical analysis results showed the effect of fermentation duration on the solubility of gembili flour ($p<0.05$). The post hoc test results showed that each length of fermentation also showed significant differences in the solubility value of flour in water. The solubility of flour increased from 27.1% at the beginning of fermentation to 51.3% after fermenting for 6 days. The highest increase occurred during the log phase because the inulinase enzyme that degrades inulin into FOS reached its maximum activity during that phase. However, in the stationary phase, there was still an increase in solubility. Inulin degradation into FOS can still occur when bacteria enter the stationary phase, and the inulinase enzyme from bacteria has activity from pH 6 to 4 [45]. The results of this study showed that the stationary pH ranged from 5.8 to 3.4, so that the process of inulinase activity can still run to produce FOS.

There is no solubility standard regarding FOS flour in water. However, some studies state that FOS with DP 4 has a solubility of >75% in water at 25℃, so that the results of the study are still lower than the results of [40]**.** Therefore, further research is needed to extend the fermentation time to determine the increase in solubility and degree of polymerisation of FOS during the fermentation process.



Noted: Different letters in a column denote significant differences ($p<0.05$)

Figure 4. Water solubility of gembili flour during fermentation time

FOS is also a prebiotic. Prebiotics are substances specifically utilised by good microorganisms in the body, thus providing health benefits. Although enzymes in the small intestine cannot digest FOS and dietary fibre, they can be digested by gut microbiota. In the large intestine, FOS will be broken down into glucose and fructose, which are then utilised by microbes through fermentation into short-chain fatty acids [46].

The use of FOS is also very important for the industry. FOS is known to provide a sweeter taste than inulin, so that it can be used as a low-calorie sugar substitute [47]**.** Before becoming SCFA, during the fermentation process, inulin will be degraded into Fructooligosaccharides (FOS).

Effect of Fermentation on Color of Gembili Tuber Flour

Unfermented commercial gembili flour has a brown color compared to fermented gembili flour. This is caused by the enzyme PPO (polyphenol oxidase). The changes in color produced during the fermentation process are shown in Figure 5. The whiter color of the flour will result in a more interesting processed product.

During the fermentation process, the enzyme PPO will inhibit the browning of gembili tubers. PPO enzymes convert phenol compounds into quinones that are brown in color [48]. The fermentation process can inhibit the work of PPO enzymes in producing quinones because lactic acid bacteria produce acids that reduce pH values, so that the low pH will inactivate PPO enzymes. PPO enzyme has maximum activity at pH 7.0 [49].

Figure 5. The Color of gembili flour during the fermentation process

Based on Figure 5, gembili flour with a fermentation time of 0 days produces a brown color. This happens because the pH is still at the value of 7.87. At fermentation times of 2, 4, and 6 days, the pH decreased from 7.87 at the beginning of fermentation to 3.40 at 6 days of fermentation. This decrease is in line with the color of gembili flour from dark brown to bright white. PPO enzyme has maximum activity in the pH range of 6-7. In [11], at pH 2.60-3.09, there is a reduction in the browning reaction caused by the inactivity of the PPO enzyme in apples. In salak fruit, the lower the pH, the browning reaction by the PPO enzyme can also be inhibited [50].

Conclusion

Based on research, it can be concluded that fermentation affects total pH, TTA, total LAB, solubility and color of gembili tuber flour. Increasing LAB followed by decreasing pH, increasing TTA, solubility and color of flour. From the data obtained, the best fermentation time is 6 days, in which the LAB growth reaches 7,60 x 107, pH at 3,40, TTA at 0,989%, highest solubility at 51,30% and has more whiteness among the other flours. Gembili tuber flour has the potential to be used as a nutritious food processing ingredient and improve the quality of its processed products. Nevertheless, additional studies are required to investigate how the duration of fermentation influences the degree of polymerization (DP) of FOS generated during the process, as well as to evaluate whether extending the fermentation time can enhance the solubility of flour in water.

Author's contribution

Adinda Debita Prihastina: collected and analyse data and wrote the article; Prima Retno Wikandari: contributed to the revision and finalisation of the article

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