Effect of fermentation time on the quality of modified gadung flour from gadung tuber (*Dioscorea hispida Dennst.*)

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Abstract. Gadung (*Dioscorea hispida Dennst.*) is one of the tubers in Indonesia that has the potential as a food source. However, due to the low nutritional value and high content of cyanide acid, these tubers cannot be consumed directly. Therefore, proper processing techniques are needed to improve the nutritional value of gadung tubers. The aim of this study was to investigate the effects of fermentation time on the quality of modified gadung flour in a reasonably short time. Soaking and submerged fermentation processes were applied. It was found that modified gadung flour can be used as a wheat flour substitution under the following conditions: water to gadung mass ratio of 50 for 120 min (soaking process) and initial bacteria cell number of $1.21 \times 10^{11}$ cells of *Lactobacillus plantarum* at the temperature of 32 ºC for 36 h (fermentation process). The result showed that protein, cyanide acid, starch, amylose, and amylopectin contents were 7.63%, 6.66 ppm, 75.27%, 38.18%, and 61.82%, respectively, while physicochemical properties, such as swelling power, water solubility, and flour whiteness degree were 6.75 g/g, 0.56%, and 71.43%, respectively.

1. Introduction

Food is a basic need for humans. In the last 10 years, there has been an increase in the Indonesia population of 32.8%. At present, there are many kinds of ready-to-eat foods in Indonesia which are made from wheat flour, such as bread and instant noodles. Wheat flour is produced from the grinding process of wheat seeds [1]. Wheat seed is not a native plant from Indonesian, so their fulfillment is carried out on an import. This condition has a bad impact on Indonesia's food security. In order to reduce imported wheat from other countries, efforts to optimize the use of local food source are needed. One of carbohydrate source food commodities found in Indonesia is gadung tubers. These tubers can be used as alternative raw material for substituting of wheat flour.

Gadung tuber (*Disocorea hispida Dennst.*) is one of the Indonesia native tubers that has the potential as a food source. This food source contains resistant starch (RS) that can cause the digestion in the small intestine to become slower than it will be fermented in the large intestine. RS can produce short-chain fatty acids, which can provide good benefits, such as reducing the risk of diabetes, obesity and other related diseases. Gadung tubers can be used as a substitute for wheat-based foods that cause celiac disease (CD) because gadung tubers do not contain gluten [2].

Gadung tuber has antinutritional substances contents, such as dioscorine and cyanides. In order to prevent negative effects on the body due to these substances, efforts are needed to treat gadung tubers by reducing their cyanide content to reach a safe limit for consumption. According to WHO, the safe level of cyanide for humans consumption is a maximum of 10 ppm [3].
Fermentation was used to improve the nutritional content of gadung flour [4] and cassava flour [5], resulting in modified gadung flour and modified cassava flour (mocaf), respectively. Modified gadung flour had better properties than non-fermented gadung flour, e.g. protein content, cyanide levels, etc [4]. However, the previous works of modified gadung flour had longer fermentation time due to the lower Lactobacillus plantarum concentration. Moreover, the results of that study have not met the SNI standard of wheat flour so that it cannot be used as a substitute for wheat flour. The SNI standards of wheat flour are water (max. 14.5%), ash (max. 0.7), and protein (min. 7%) [6]. Therefore, the objective of this work was to investigate the effects of fermentation time for improving the quality of modified gadung flour in a reasonably short time and it can be used as a substitution for wheat flour. In this study, pretreatment before fermentation was used to decrease HCN content in gadung tuber. The effects of fermentation time on nutritional value and physicochemical properties were also systematically investigated.

2. Materials and methods
2.1. Materials
Gadung tubers were obtained from gadung plantation in Nganjuk, which is located in East Java, Indonesia. The L. plantarum bacteria was obtained from the “Chemical Laboratory of Microbiology” of Chemical Engineering Department, Institut Teknologi Sepuluh Nopember (ITS) Surabaya. Chemical reagents were obtained from commercial sources.

2.2. Production of modified gadung flour
2.2.1. Pretreatment of gadung tubers. Gadung tubers were sliced with a thickness of 0.1-0.5 mm. Gadung tubers were soaked in water with the ratio of water to gadung tubers of 50 for 120 min.

2.2.2. Starter preparation. Preparing L.plantarum inoculum was according to the method used by Hawashi et al. [7].

2.2.3. Fermentation process. A 400 g of fresh gadung tubers were used for the fermentation process. The starter amount of L. plantarum cells of 1.21 x 10^{11} cells were added. The fermentation was performed for 12, 24 and 36 h. The temperature was kept constant at 32 °C during fermentation.

2.2.4. Milling process. After fermentation, gadung tubers were dried in an oven at 45 °C for 24 h, and then milled in a crusher. After milling, gadung flour was sieved to obtain a granulometric fraction of 70 mesh.

2.3. Proximate composition.
The proximate composition of gadung tuber was determined for ash, fat and protein contents as described by Hawashi et al [7].

2.4. pH analysis.
The pH of the filtrate obtained from fermentation was determined by pH meter.

2.5. Starch content analysis.
The starch content was determined as described by Hawashi et al [7].

2.6. Amylose and amylopectin content analyses.
The content of amylose and amylopectin were analyzed according to the method used by Hawashi et al [7].

2.7. Cyanide acid content.
The cyanide acid content of gadung tuber was determined by SNI standard [8].
2.8. Swelling power and water solubility analyses.
The swelling power and water solubility analyses were measured according to the method used by Sumardiono et al. [9].

2.9. Flour whiteness degree.
Flour whiteness degree of gadung tuber was determined by SNI standard [8].

3. Result and Discussion
3.1. Chemical composition of gadung tuber
In this study, gadung tubers contain 3.08% ash, 0.28% lipids, 1.65% protein, and 94.99% nitrogen-free extract. Nitrogen free extract consists of non-fibrous carbohydrates, such as sugars, fibre, vitamins, and starches. In this study, the starch content of gadung tuber was 81.83%. Starch contains amylose and amylopectin. Amylose is composed of glucose with linear structure. Amylopectin is a polysaccharide composed of α-glucose monomers [7]. The content of amylose and amylopectin in this study was 23.46% and 76.54% respectively. These results cannot be compared due to no other study has been conducted on the proximate composition of gadung tubers.

Moreover, the cyanide acid content of gadung tuber was 161.83 ppm. However, Winarti et al. [10] reported that cyanide acid content in gadung tuber was 411.65 ppm. This result was lower than reported by previous work [10]. It could be due to the gadung cultivar, diversity of agronomic factors, and harvesting age [11].

3.2. Microbial growth
The fermentation process in this study was performed by using L. plantarum bacteria. The microbial growth curve was employed to determine the concentration of bacteria that used in this study. It was found that the lag, exponential, and stationary phases were occurred at 0 to 4 h, 4 to 6 h, and 6 to 12 h, respectively. In the lag phase, the increase in the number of bacteria is slow because this bacterium carries out an acclimatization process to environmental conditions (pH, temperature, and nutrients) [12]. The exponential phase is a phase of bacterial growth that takes place very quickly. The stationary phase is a phase where there is no addition of bacteria because the number of cells that grow is the same as the number of cells that die.

The results were different from the previous work of Setiarto and Widhyastuti [4]. They reported that the exponential phase was occurred at 24 to 48 h and the stationary phase was occurred at 48 to 72 h. The different type of media used in those study could influence the results. In the previous study, the media used was de Man Rogosa and Sharpe (MRS) while in this study, the media used was Nutrient Broth.

3.3. Cyanide acid content of gadung tuber during pretreatment
There are many methods to reduce cyanide levels, such as boiling, drying, soaking, and adsorbent. A combination of several methods also gave better results in reducing levels of cyanide acid [13]. The pretreatment was done by slicing gadung tuber with a thickness of 0.1-0.5 mm and soaking in water with a ratio of water to gadung tubers of 50 for 120 min. This pretreatment was carried out to reduce the cyanide acid content so that when the fermentation occurred, cyanide acid levels can decrease to a safe level (maximum of 10 ppm) and in a shorter time.

Cyanide acid content decrease from 161.83 ppm to 53.21 ppm. It was successful to reduce the cyanide acid content of 67.12%. In the process of soaking in water, linamarin compounds were hydrolyzed to form cyanide acid which has the properties of being easily soluble in water so that the cyanide acid content can be reduced through the soaking in water process [14].
3.4. pH value
A pH is one of the factors that influence the growth of microorganisms and the formation of products in the fermentation process because each microorganism has an optimal pH range for its environment. pH value decreases with increasing fermentation time. The initial pH level of fermentation was 6.03 and decreased to 4.35 within 36 h of fermentation. The results of this research are in accordance with the research conducted by Gunawan, et al [5], where there was a decrease in pH from 5.6 to 3.9. Another study, Setiarto and Widhyastuti [4] also reported the decrease of pH value during fermentation from 7.77 to 5.41. The decrease in pH during the fermentation process was influenced by the increased production of lactic acid by microorganisms. The reaction of the production of lactic acid was:

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\text{Glucose} + 2\text{Pi} + 2\text{ADP} \rightarrow 2 \text{Lactic Acid} + 2\text{ATP} + 2\text{H}_2\text{O}
\]

3.5. Effect of Fermentation time on the protein content of modified gadung flour
Proteins are polypeptide macromolecules composed of a number of L-amino acids that are connected by peptide bonds. A protein molecule is composed of a number of amino acids with a certain composition and is derivative. Amino acids consist of hydrogen, nitrogen, carbon, and oxygen [15]. The results are illustrated in figure 1.

![Figure 1](image_url)

**Figure 1.** Effect of fermentation time on the protein content of modified gadung flour.

Figure 1 shows that the longer the fermentation time, the higher the level of protein obtained. The result showed that protein levels increase from 1.65% to 7.63% for 36 h of fermentation time. According to Gunawan, et al. [5], the increase in protein levels in fermented products was due to the activity of microorganisms to change the substrate that contains carbon and nitrogen to protein. In addition, in that study using sweet cassava explained that proteinase enzyme was produced in the fermentation process by lactic acid bacteria (*L. plantarum*). Another study reported that microbes in the fermentation process utilized food substances in the substrate as an energy source to grow and multiply. The more microorganisms that grow, the higher the protein content [16].

3.6. Effect of fermentation time on cyanide acid content of modified gadung flour
In gadung tubers, there are cyanogenic glucosides which are cyanide precursors and β-glucosidase (linamarase) enzyme. Because of the cutting or crushing of gadung tubers, the linamarase enzyme plays an important role in converting cyanogenic compounds to hydrocyanic acid [17]. Figure 2 shows that the longer fermentation time, the lower cyanide acid content is gained. The lowest level cyanide acid in modified gadung flour using *L. plantarum* was 6.66 ppm for fermentation time of 36 h. It can reduce cyanide acid levels by 87.48% from 53.21 ppm to 6.66 ppm. The type of fermentation used in this study was submerged fermentation. The submerged fermentation can reduce cyanide acid levels because linamarin and cyanide acid are very soluble in water [7]. Another, during fermentation, glucose was converted to organic acid by microorganisms, so that the pH decreased into ± 4.2. This
can reduce linamarase enzyme activity in degrading linamarin to cyanide acid due to the lower pH because the linamarase enzyme has an optimum pH of 6 [5]. In this study, pH value decreased to 4.35 that made linamarase enzyme inactive and cyanide acid was not produced anymore, so cyanide acid value reduced.

Figure 2. Effect of fermentation time on cyanide acid content of modified gadung flour.

3.7. Effect of fermentation time on the starch content of modified gadung flour

Starch is composed of amylose and amyllopectin. Amylose is composed of glucose that has a (1,4) glycosidic bond. Amylopectin is composed of glucose with (1-6) glycosidic bonds in the branches with a (1,4) glycosidic bond [18]. Starches are generally classified according to the digestive level and rate as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) [19]. RS is starch that is digested very slowly in the small intestine, can be fermented in the large intestine and to produce short-chain fatty acids, and has benefits for health such as increasing satiety, increasing insulin response, reducing the risk of colon cancer, diabetes, obesity, and inflammatory diseases intestine. Research has shown that levels of RS increase when starch has higher amylose content [20].

Figure 3. Effect of fermentation time on the starch content of modified gadung flour.

The effect of fermentation time on the starch content of modified gadung flour is shown in figure 3. It can be seen that the longer the fermentation time, the lower the starch content obtained. This occurs because microorganisms use organic matter from starch to provide energy for the growth of microorganisms. In fermentation, the starch content in gadung flour decreases due to the hydrolysis of the enzyme produced by L. plantarum. It was reported that L. plantarum bacteria have high amylase enzyme activity. The amylase enzyme hydrolyzed the α-1.4 glycosidic linear bond in amylose which
produces a mixture of dextrin, maltose, and glucose [21]. Furthermore, glucose products were converted into fatty acids, namely lactic acid [5].

The effect of fermentation time on amylose and amylopectin content on modified gadung flour is shown in figure 4. The result showed that the longer fermentation time, the more amylose content increases, while the amylopectin content decreases. Gadung tuber was fermented for a long time until it reached an acidic pH value. This is caused by bacterial activity during fermentation and acid conditions at low pH resulting in faster hydrolysis of starch in α- (1,4) bonds thus increasing the amylose group [7]. Besides producing amylase enzymes, L. plantarum bacteria also produce pullulanase enzymes. This enzyme was able to hydrolyze the branching α- (1,6) glycosides in amylopectin to produce short-chain amylose [21]. Increased amylose levels were caused by the breakdown of the branch chain amylopectin in α- (1,6) glycosides. Therefore, the amount of amylopectin decreased and amylose content increased as a result of the removal of the bond chain in amylopectin [18].

![Figure 4. Effect of fermentation time on amylose and amylopectin content of modified gadung flour.](image)

The highest amount of amylose from this study was 38.18 for fermentation time of 36 h. according to Luna et al, starch with high amylose content is starch which contains amylose more than 25% [22]. Therefore, the product from this study can be used as raw material for resistant starch.

3.8. Effect of Fermentation time on Physicochemical Properties of modified gadung flour

Physicochemical properties of flour consist of swelling power, water solubility, and flour whiteness degree. The longer the fermentation time, the higher the swelling power of modified gadung flour obtained. It was associated with an increase in amylose content. In addition, the swelling power value was caused by the weakening of intermolecular hydrogen bonds in starch granules (inter-amylose bonds in the crystalline region and the bond between amylose in the crystalline region with amylopectin in the amorphous region) as fermentation time increases, so that when the flour was hydrated with water and heated, high water kinetic energy causes high water bonds with starch granules.

According to Kustiyawati, et al. [23] the amylose content in flour absorbs more water so that the volume development was also greater. The increase of amylose during the fermentation process can occur because of the presence of the pullulanase enzyme produced by L. plantarum to hydrolyze the branching bonds α-1.6 glycosidic linking amylopectin and produce an amylose straight chain. In this study, the swelling power of a commercial wheat flour was 4.57 g/g while the swelling power of modified gadung flour was 6.75 g/g so that the swelling power value of modified gadung flour was greater than that of wheat flour.

The increase in solubility caused by fermentation was causing the starch granules to break so that when dried, the flour was porous and easily absorbed water. The heating process of flour causes a lot of amyloses to come out of starch granules. This was due to the development of power which
suppresses starch granules from the inside until the granules break and break down amylose [24]. Amylose that comes out of starch granules, dissolves in water and becomes a supernatant. In this study, the water solubility of a commercial wheat flour was 1.06%, so the water solubility obtained in this study in 36 h (0.56%) was lower than that of wheat flour.

Figure 5. Effect of fermentation time on swelling power of modified gadung flour.

The value of flour whiteness degrees tends to increase, along with the length of time of fermentation. The fermentation process is able to inhibit the Maillard reaction which can cause brownish-colored products by overhauling reducing sugars into organic acids [25]. In this study, the flour whiteness degree of a commercial wheat flour was 80.32%. From this study, the flour whiteness degree of modified gadung flour (71.43%) at 36 h fermentation time which was close to the characteristics of wheat flour.

3.9. Analysis of modified gadung flour quality standards

From the research that has been done with the variable of fermentation time, the best results were obtained at 36 h of fermentation time which approached the wheat flour quality standard according to SNI 3751 [6]. The comparison between modified gadung flour and wheat flour based on SNI 3751 can be seen in table 1.

Table 1. Comparison of modified starch flour quality standards with wheat flour.

| Parameter                      | Wheat Flour | Modified gadung flour |
|--------------------------------|-------------|-----------------------|
| Protein (%)                    | Min. 7.0    | 7.63                  |
| HCN (ppm)                      | Max. 10     | 6.66                  |
| Swelling power (g/g)           | 4.57a       | 6.75                  |
| Water solubility (%)           | 1.06a       | 0.56                  |
| Flour whiteness degree (%)     | 80.32a      | 71.43                 |
| Moisture (%)                   | Max. 14.5   | 12.48                 |
| Ash (%)                        | Max. 0.7    | 0.24                  |

a does not include SNI parameters
Based on table 1, it can be seen that using L. plantarum bacteria concentration of 1.21 x 10^{11} cells and 36 h fermentation time for SNI parameters, such as protein, HCN, water, and ash indicate that modified gadung flour meets SNI standards of wheat flour. But for values that do not belong to SNI parameters, such as solubility and flour whiteness degrees, the value of modified gadung flour is still below wheat flour while for swelling power has exceeded wheat flour.

4. Conclusions
Modified Gadung Flour can be produced by the fermentation process using L. plantarum to increase the nutritional value of gadung tuber. The best results in this study were at fermentation time of 36 h with a concentration of 1.21 x 10^{11} cells L. plantarum. The cyanide acid content decreased during pre-treatment from 161.83 ppm to 53.21 ppm. The content of cyanide acid, starch, and amylopectin decreased while the content of protein and amylose increased with increasing time fermentation and the results are in accordance with standards of wheat flour (SNI). The physicochemical properties of modified gadung flour in the form of swelling power have a higher value than wheat flour, whereas the solubility and white levels of flour are lower than that of wheat flour.

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