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Antinutritional Content, Protein Profiles, and Flour Characteristics of Taro Tubers (*Colocasia esculenta*) Fermented with *Lactobacillus plantarum*

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ABSTRACT

The purpose of this research is to study the optimum condition, the change of oxalate content, the characteristic of flour, and protein profile of taro tuber that fermented using *Lactobacillus plantarum*. The optimum condition of fermentation was determined based on the lowest oxalate content in the fermented flour. The optimum conditions of fermentation were investigated by using the variation of the pHs (4-6), temperatures (30–50 ^{0}C) and incubation times (6–48 hours). The result showed that the optimum condition of fermentation was achieved at pH 5, 35 $^{\circ}$C in 48 h incubation time. The fermented taro flour characteristics were the increasing level of starch (0.96%), amylose (0.43%), amylopectin (0.52%) and protein (0.99%). The protein profiles of fermented taro showed that the allergenic proteins were not present, suggesting those proteins were missing due to the fermentation process.

Key word: fermentation, *Lactobacillus plantarum*, taro tuber, oxalate, protein profile.

INTRODUCTION

Indonesia is known as a country with high biodiversity. There are many potentials of plants that can grow well and can be used as a source of food. Taro (*Colocasia esculenta*) is a plant of tubers that grow in tropical and subtropics. The taro tuber contains 70-80 g/100 g starch with a tiny granular size between 1.4 μm and 5 μm [1]. Taro tubers contain calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, and proteins such as trypsin, albumin and lectin inhibitors [2,3,4]. However, taro also contains anti-nutrients *i.e* oxalate that causes itching when in contact with skin [3].

Currently, the fermentation process is mostly done to improve the quality of flour characteristics of the tubers. The purpose of fermentation is to enhance the quality of food and reduce or eliminate the anti-nutrients contained in the foodstuff. Fermented tubers with lactic acid bacteria can improve the taste and nutritional value of the resulting flour. Lactic acid bacteria are capable of producing proteinase enzymes that will break down proteins and peptides into simpler compounds [4]. This fermentation activity is able to influence the change in protein profile contained in the food. Changes in protein profiles can occur in the presence of bacterial activity during the fermentation process. Bacteria will use the nutrients present in the food as a source of energy and change the compounds in it, thus, it changes the initial composition of the food. One of the bacteria that can be used is *Lactobacillus plantarum*.

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Lactobacillus plantarum has been used to fermentate cassava. That fermentation can increase protein and also decrease cyanide content in cassava flour [5]. Kobawali in his research proves that fermentation with *Lactobacillus plantarum* bacteria can reduce the cyanide content in the cassava [7]. The utilization of *Lactobacillus plantarum* to reduce anti-nutritional factor and enhance nutritional composition have been applied in cereals [8].

Based on those reasons, this study is investigating taro tuber fermentation with lactic acid bacteria, *Lactobacillus plantarum*. The purpose of this study is to determine changes in the protein profiles, oxalate levels as antinutritional, and characteristics of taro tuber flour.

EXPERIMENT

Chemicals and instrumentation

Materials in this study were isolated from *Lactobacillus plantarum* FNCC 0027 collected from the Food and Nutrition Laboratory, Gadjah Mada University, and taro tuber obtained from Gunung Kawi-Malang.

Equipments used in this study were a set of SDS PAGE electrophoresis, Spectronic Genesys 20, laminar air flow, autoclave (All American Model 20X), and an incubator (Heraeus Type B 5042). All reagents of analytical grade were purchased from Merck or Sigma-Aldrich and were used as received: NaH_2PO_4, $Na_2H_2PO_4$, HCl, NH_4OH, H_2SO_4, $KMnO_4$, tablets, petroleum ether, Nelson reagent, arsenomolybdate reagent, acrylamide or polyacrylamide solution, Tris HCl buffer, ammonium persulfate, SDS, TEMED, and 0.1% Coomassie blue. The growth media for *Lactobacillus plantarum* were MRSA and MRSB (OXOID).

Lactobacillus plantarum preparation

Lactobacillus plantarum in lyophilized form was rehydrated with deMann, Rogosa and Sharpe (MRS) broth and then incubated at 37 °C for 48 hours. Then *Lactobacillus plantarum* was regenerated in deMann, Rogosa, and Sharpe (MRS) agar and incubated at 37 °C for 24 hours.

Sample Preparation

The taro tubers were washed clean to remove the impurities. Tuber was peeled, its outer skin discarded and washed again. Tuber was sliced with 2 mm thickness and ready to be treated.

Optimization of pH

Each 8 g slices of taro tuber were put into 15 erlenmeyer flasks. Furthermore, 16 mL of inoculum and 8 mL of phosphate buffer solution with pHs of 4, 4.5, 5, 5.5 and 6 were added. Erlenmeyer flask was sealed and incubated for 6 hours at 37 °C. After incubation, the tubers were separated from the filtrate and dried at 40 °C. The optimum pH was determined on the basis of the lowest oxalate content of the tuber and filtrate.

Optimization of temperature

The sliced taro tubers were then weighed 8 grams and each of them was put into 15 erlenmeyer flasks. Furthermore, 16 mL of inoculum and 8 mL of phosphate buffer solution of optimum pH were added. Erlenmeyer flask was then sealed and incubated for 6 hours with temperature variations of 30 ^{0}C, 35^{0}C, 40 ^{0}C, 45 ^{0}C, and 50 ^{0}C. After incubation, the tubers were separated from the filtrate and dried at 40 ^{0}C. The optimum temperature was determined based on the lowest oxalate content of the tuber.

Optimization of incubation time

Into 15 erlenmeyer flask were added 8 grams of taro slices, 16 mL inoculum, and 8 mL of phosphate buffer of optimum pH, respectively. Erlenmeyer flasks were closed tightly and incubated for 6 h, 12 h, 24 h, 36 h, and 48 h at the optimum temperature. The filtrate is separated and the tubers are dried at 40 ^{0}C. The optimum incubation time was determined based on the lowest oxalate content of the tubers.

Determination of Oxalate Levels

The treated tuber was separated from the filtrate and dried at 40 °C. The taro tuber that has been dried was crushed and the oxalate content determined. The oxalate content in the flour was determined using the volumetric methods according to AOAC analytical method [9].

Fermentation of Taro Tubers

The 24 g taro tubers were included in a 50 mL Erlenmeyer flask and 24 mL of phosphate buffer pH 5 and 48 mL of *Lactobacillus plantarum* isolate were added. Erlenmeyer was sealed with cotton and brown paper and incubated at 35 °C for 48 hours. The taro tubers were then dried at 40 ^{0}C, mashed, and sieved to obtain 100 mesh flour.

Determination of Fermented Protein Profiles

Flour samples (0.2 g/mL) were dissolved in 0.05 M phosphate buffer pH 7.2, incubated and shaken overnight at 40 ^{o}C. The solid particles were separated by centrifugation at 17,700 rpm for 20 minutes. The protein profile of the sample was determined by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique.

Determination of Characteristics of Taro Flour

The proximate test was applied to fermented and unfermented flour to determine the characteristics of the resulting flour. Characteristics of flour include moisture content, starch content, amylose content, amylopectin levels, and protein content [9].

RESULT AND DISCUSSION

One of the fermentation purposes is lowe anti-nutrient level in food. One of the alternative methods for reducing oxalate level is fermentation using *Lactobacillus plantarum*. There were some conditions of fermentation needed for reducing oxalate content in taro, such as pH, temperature and incubation time.

Optimum Conditions Fermentation

Figure 1 shows the effect of pH, temperature, and incubation time during the fermentation process. The first is the effect of pH on fermentation. In this condition, pH 5 was selected as the optimum pH to perform the fermentation process on taro tubers. On the graph, there was an increase in oxalate levels at pH 4-4.5, but at pH 5 there was a decrease. But, after pH 5.5-6 the oxalate level increased again. The oxalate content comprised in the fermented at pH 5 is the lowest with a content of 0.031 mg/100g. It may occur because *Lactobacillus plantarum* may grow in acidic conditions and reach the best condition at pH 3 to 5.6 [12]. At the appropriate pH, the bacteria will produce enzymes that have maximum activity to reduce the oxalate levels in taro. Furthermore, Figure 1.b shows the effect of temperature on fermentation.

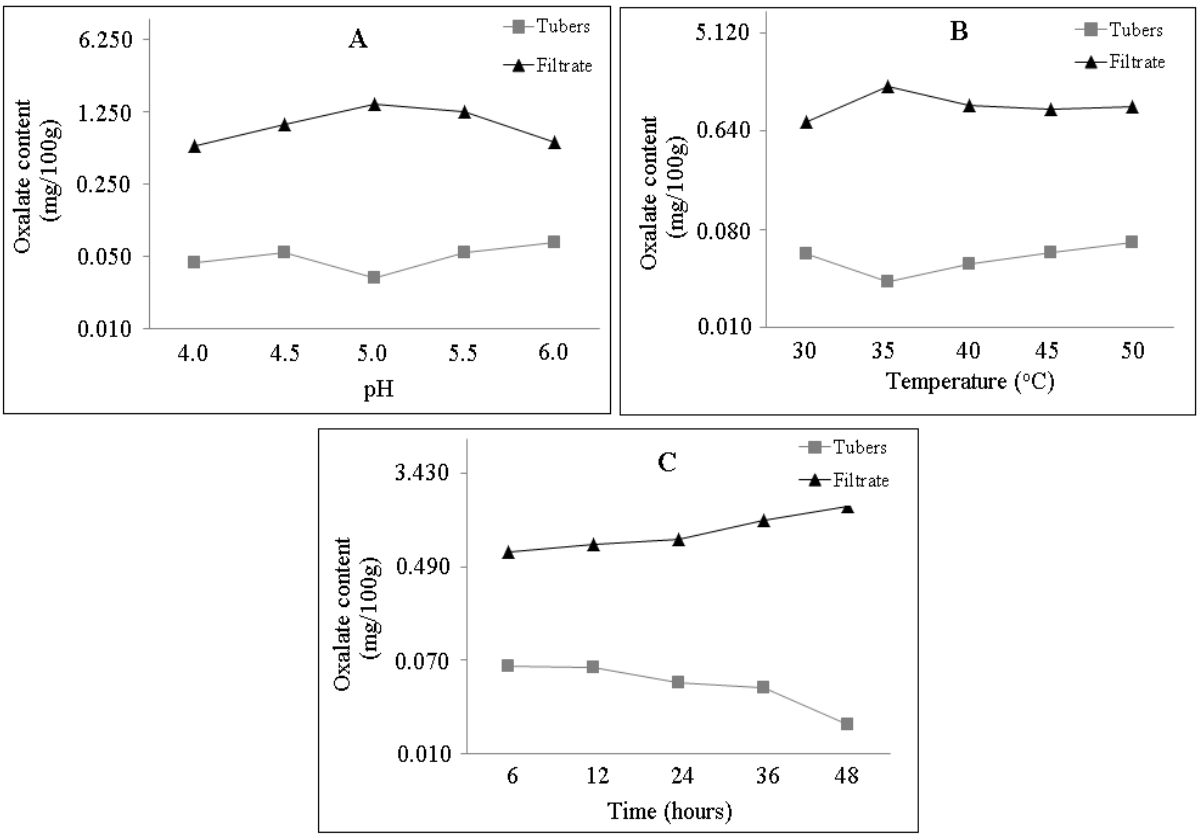

Figure 1. Optimum condition of taro tuber fermentation with *Lactobacillus plantarum* bacteria on the variation of pH (a), temperature (b), and time incubation (c).

On the graph, there was a decrease in oxalate levels at 35^{0}C but again increased to 40-50^{0}C. At temperature 35^{0}C, oxalate content contained in taro tuber flour reached the lowest level of 0.026 mg/100g. Temperature 35 ^{0}C is the best temperature as living conditions of bacteria *Lactobacillus plantarum* in this fermentation process. Each bacterium has a different life tolerance to stay alive and active. Lactic acid bacteria can usually live at a temperature of 15^{o}C to 37^{o}C [12]. The last condition is the incubation time. From graph 1.c it is seen that the longer the incubation time the smaller the oxalate content in the tuber. At 48 hours the oxalate level reaches its lowest point of 0.018 mg/100g. At the 48 h incubation time, the *Lactobacillus plantarum* reaches the stationary phase, whereas nutrient equilibrium occurred. During that time some enzymes were synthesized suggesting protease and α-galactosidase. These enzymes will hydrolyze oxalate in the complex form with proteins or carbohydrates, to be a soluble oxalate. This is shown in Figure 1, the oxalate content in the filtrate is higher than in the tubers in all of the fermentation conditions that had been studied. The enzymes will hydrolyze oxalate, antinutritional, which is similar to a study conducted by Otunola *et al.* on Bambara nut (*Voandzeia subterranean* L.) [13]. The results of this study are similar to that of previous researchers in reducing cyanide on cassava and tannin and phytic acid in sorghum [9,10]. Products of oxalate protein and oxalate carbohydrate complex hydrolysis will induce synthetase enzymes in *Lactobacillus plantarum* to produce the nutritional component in taro for improving the flour character (Table 1).

Protein Profiles of Flour Taro

Taro tuber fermentation was carried out under optimum conditions at pH 5, temperature 35 ºC, and for 48 h. Sliced taro tuber fermented at optimum conditions then dried at 40 ºC, mashed, and isolated the protein. The result of separation using SDS PAGE is shown in figure 2. Determination of molecular weight was carried out with the aid of the marker. According to the Fatchiyah *et al.*, to determine the molecular weight of the protein is performed by calculating Rf of each of the sample bands, from markers of known molecular weight by using the formula Rf [15].

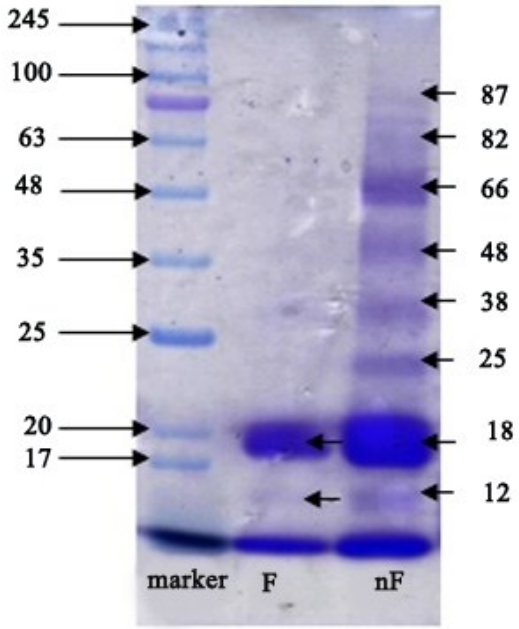

Figure 2. Protein profiles of taro (F = fermented and nF = unfermented)

The protein profile analysis of figure 2 shows a change in the profile of fermented and unfermented proteins. In unfermented taro tuber flours, there are protein bands of 12 kDa, 18 kDa, 25 kDa, 38 kDa, 48 kDa, 66 kDa, 82 kDa, and 87 kDa. However, after fermentation, there are some missing proteins and the only remaining protein with molecular weights of 12 kDa and 18 kDa. The missing protein is thought to be a protein that forms an allergenic complex with oxalates that often cause itching when taken. The loss of some protein bands is suspected because during the process of fermentation the irritant protein is converted to another form or substrate. *Lactobacillus plantarum* produces proteases that can hydrolize proteins of high molecular weight to smaller molecular weights [14]. It is thought to cause protein complex bonds with allergenic oxalates to also hydrolyze.

Flour Characterization

Taro flour also conducted the proximate test to see the quality of flour produced. Sliced taro tuber fermented at optimum conditions then made into flour for later analyzed to know its characteristics of taro fermented tuber flour compared to unfermented. Table 1 shows the comparison of flour characters before and after fermentation. After fermentation showed an increase in starch, amylose, amylopectin, and protein. While the oxalate content as an antinutritional in taro tubers decreased. The increase of starch content is 0.96%. this increase was also followed by an increase in amylose levels of 0.44%, amylopectin by 0.52%.

Tabel 1. Characteristics of unfermented and fermented taro tuber flour

Parameters	Unfermented (%)	Fermented (%)
Moisture	0.580 ± 0.06	0.510 ± 0.030
Starch	36.23 ± 0.59	37.19 ± 3.620
Amylose	12.44 ± 0.14	12.87 ± 1.020
Amylopectin	23.80 ± 0.60	24.32 ± 4.440
Protein	7.080 ± 0.22	8.070 ± 0.370
Oxalate (x 10^{-4})	27.40 ± 1.35	0.180 ± 0.003

In addition, from the comparison of amylose and amylopectin levels, it can be seen that the fermented taro has higher amylopectin content. The ratio of amylose and amylopectin to starch showed a greater amylopectin content than amylose. The ratio of both has an effect on solubility and degree of starch gelatinization. Higher amylopectin content will produce a softer, crisp, and better-tasting texture in the processed products.

The protein content also increased after fermentation by 0.99%. The increase in protein levels is the same as in previous studies conducted on cassava and Tacca (*Tacca leontopetaloides*) fermentation [5,11]. The increase in protein levels is thought to be due to the growing effect of cell mass that increases with the length of the fermentation process [5]. In addition, during the fermentation process, lactic acid bacteria isolates will produce peptidoglycan on the cell wall composed of glycoprotein and lipoprotein components that caused an increase in protein levels [11].

CONCLUSION

This research has described the fermentation process of taro tuber using *Lactobacillus plantarum*. The optimum conditions for fermentation are at pH 5, the temperature of 35 ^{0}C, and for 48 hours. The result of SDS PAGE shows that there is the difference of protein band on fermented and unfermented taro. Therefore, it is suggested that fermentation process alters the protein structure of taro flour. Fermentation with *Lactobacillus plantarum* can reduce oxalate as an antinutritional in taro tubers and can increase starch, amylose, amylopectin, and protein levels. Fermentation by utilizing *Lactobacillus plantarum* bacteria can be done with other food ingredients to reduce the levels of antinutrients contained therein.

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