The Use of Three Species of Lactic Acid Bacteria in the Mocaf (Modified Cassava Flour) Production

Penggunaan Tiga Spesies Bakteri Asam Laktat dalam Pembuatan Tepung Mocaf (Modified Cassava Flour)

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Abstract

The use of cassava as foodstuffs has been widely developed. Modifying it into a mocaf (modified cassava flour) is one of the cassava utilization. This research aimed to process cassavas into mocaf utilizing lactic acid bacteria and to obtain a better mocaf in terms of its physicochemical parameters. Bacteria used were L. plantarum, L. fermentum, and L. paracasei, which can ferment cassava to mocaf. The fermentation process was carried out by two fermentation duration of 48 hours and 72 hours, followed by draining and drying using the oven at 50 °C for 6 hours. This research analyzed mocaf’s physicochemical properties such as water content, fat content, protein content, ash content, carbohydrate content, whiteness, and acidity. A Factorial Randomized Block Design with two replications was applied as the research design. If the test result showed that the tested sample has a significant difference at the level of significance of 0.05, it then subjects to the further Duncan test, using SPSS. The result showed that the use of L. paracasei produced best characteristics mocaf with a high protein content of 1.44%, an ash content of 0.31%, a white degree of 102.20, and a low degree of acid of 3.66.

Keywords: cassava, fermentation duration, L.fermentum, L.paracasei, L.plantarum

INTRODUCTION

The diverse and sufficient food availability is essential for humans to meet their food needs. Indonesia has extensive areas and various kinds of food sources. Therefore, an effort to develop and identify the foods to become food products that can be consumed by all society is required. Carbohydrates derived from plants are varied and diverse, i.e., from tubers such as cassava, sweet potato, ganyong (Canna edulis Kerr), and many others. The cassava (Manihot esculenta Crantz) is

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one of the tubers that are abundantly available in Indonesia, easy to cultivate because it has diverse varieties, easy to grow in Indonesia tropical air, and provides bountiful harvest (Ginting, 2009).

The society frequently consumed several cassava varieties such as Adira, Mangi, Betawi, and so forth. The cassava's tuber is easily spoiled due to its high water content, which is classified into perishable foods. Besides, it also needs further treatment to be used in other food processing. The cassava's tuber is widely used as raw materials of food processing, even in industry. It can be made into snacks, tapioca flour, cassava flour, and mocaf (modified cassava flour). Mocaf is different from starch flour in general; it is fermented to modify cassava's cell. The fermentation, which mostly involves lactic acid bacteria usually produces pectinolytic and cellulolytic enzymes that can destroy cellulose cell walls in the mocaf fermentation in the fermented cassava so that the starch granules break down completely (Subagio, 2006).

The mocaf texture is generally softer and smoother. It has low water content and a typical aroma. Products that use mocaf flour are also more tasteful and crunchy. The mocaf production is widely made, but most of them are used as commercial starters as they are quite expensive and must be ordered well in advance. It is necessary to conduct research using several microorganisms capable of fermenting the cassava. Some investigations have been conducted using various microorganisms that are presumed to convert the cassava into mocaf flour. The mocaf production can be carried out using simple fermentation using yeast, mold, and bacteria (Gunawan et al., 2015). According to the National Standardization Agency of Indonesia, in terms of mocaf flour, a good mocaf must have a smooth form, a standard aroma of mocaf, a maximum water content of 13%, and a minimum whiteness of 87% (Badan Standardisasi Nasional, 2011).

Lactic acid bacteria are utilized in several fermented products such as yogurt, cheese, pickled vegetables, sour milk, and mocaf flour. Lactobacillus is included in the lactic acid bacteria group with several beneficial properties and is often used as a probiotic (Tannock, 2004). Lactobacillus has properties that are not much different from the general lactic acid bacteria properties that produce lactic acid as the final product from carbohydrate fermentation. Lactobacillus is usually aerotolerant or anaerobic, acidic, acid-loving, and requires complex nutrients (carbohydrates, amino acids, peptides, fatty acids, salts, nucleic acid derivatives, and vitamins). Some Lactobacillus can break down carbohydrates into lactic acid; they are Lactobacillus, Lactococcus, and Streptococcus (Petrova, Petrov, & Stoyancheva, 2013). The use of lactic acid bacteria for cassava fermentation is one way to produce a relatively easy and cheap mocaf production. It is expected to be applied either on a small scale, home, large scale, or industrial scale. Also, Lactobacillus in mocaf production is expected to produce excellent physical and chemical mocaf characteristics under the intended standard. Thus, it needs research on mocaf fermentation using other microorganisms to produce fermented mocaf products with quite good physicochemical properties in terms of water content, protein content, low fat, and high yield.

**METHODS**

The research used cassava varieties of Adira 1 (Biotechnology LIPI) which were older than six months, L. plantarum InaCC B146, L. paracasei InaCC 143 and L. fermentum FNCC 0322 and MRS B Himedia analysis materials, NB Oxoid, technical Sodium metabisulfite (Na₂S₂O₃), K₂SO₄ from Merck, HgO, H₂SO₄ from Merck, Na₂S₂O₃.5H₂O from Merck, H₃BO₃ from Merck, HCl 0.02 N from Merck and BaSO₄ from Merck.

The equipment used were basin, sieve, oven pan, test tube, chopper, Kern analytical balance, Memmert oven, Excalibur dehydrator, Fomae ZT100 flouring machine, Pyrex desiccator, FB 1310M Benchtop kiln, Kjeldahl flask, Agilent pH meter, filter paper, Erlenmeyer Schott Duran, micropipette Thermo, Duran burette, distillator, Schott Duran fat flask, and Duran soxhlet.

Lactic acid bacteria culture preparation was carried out by refreshing 10 ml culture into the MRSB media, taking 1 ml from the stock culture, then inoculated into the new MRSB culture media as much as 10 ml, then incubated for 24 hours. The culture used must be fresh and new.

The mocaf production was according to the method used by Tandrianto, Mintoko, & Gunawan (2014) with modifications. One kilogram (net weight) of cassava was used. The first step was cleaning the cassava, then washed and weighed it to 1 kg. The cassava was then grated into small shreds, soaked into distilled water and mixed lactic acid bacteria culture by the ratio of cassava: distilled water : culture = 1 : 1 : 0.01 in the clean basin/container. The mixed materials were then...
covered with plastic wrap with little holes to obtain 1 kg of cassava, and added with 1 liter of clean water and 10 ml of lactic acid bacteria. It was then fermented for 48 hours and 72 hours, drained, and soaked again into the water with the addition of metabisulfite 0.02% for 15 minutes to reduce the color change. It was dried through a dehydrator at the temperature of 50 °C for 6 hours subsequently, and the mocaf flour was ready to analyze. Figure 1 shows the detailed process flow diagram.

After the mocaf flour is produced, a physicochemical analysis is carried out. The research analyzed the following parameters; water content, protein content, fat content, ash content, carbohydrate content, acidity, whiteness, and yield. The analysis was applied to 6 treatments with twice repetitions using factorial Randomized Block Design by L. plantarum treatment with fermentation lengths of 48 hours and 72 hours, L. fermentum treatment with fermentation lengths of 48 hours and 72 hours, and L. paracasei treatment with fermentation lengths of 48 hours and 72 hours. The data are processed using SPSS 22 and ANOVA test was carried out. If the test result showed that the sample was significantly different at the 0.05% level of significance, then a further test, Duncan, was carried out.

Data Analysis
This research performed water content analysis by oven method (AOAC, 1995), protein content analysis by Kjeldahl method (AOAC, 2012), fat content analysis by soxhlet method (AOAC, 2012), ash content analysis by furnace method (AOAC, 2012), carbohydrate content analysis by carbohydrate difference, whiteness analysis by using KETT digital whiteness meter, pH measurement (AOAC, 2006) and the yield analysis.

Water Content Analysis by Oven Method (AOAC, 1995)
The aluminum cup was dried in the oven for 15 minutes, cooled in a desiccator for 10 minutes, and then weighted (A). The sample was weighted to 1-2 grams (B) and put into the cup. Cup with its content was dried in the oven at the temperature of 105 °C for 6 hours, cooled in a desiccator for 15 minutes, and then weighted. They were re-dried till a constant weight was obtained (C). The following equation was used to calculate the sample's water content.

$$WC\text{ (%wb)} = \frac{B - (C - A)}{B} \times 100\%$$

(1)

$$WC\text{ (%db)} = \frac{WC\text{ (%wb)}}{100 - WC\text{ (%wb)}} \times 100\%$$

(2)

Description:
WC = water content
wb = wet basis
db = dry basis

Protein Content Analysis by Kjeldahl Method (AOAC, 2012)
A total of 0.1 gram - 0.25 gram sample was weighed in a Kjeldahl flask, added with 1.0 + 0.1
gram K₂SO₄, 40 + 10 ml HzO, and 2.0 + 0.1 ml H₂SO₄, the sample was boiled until the liquid was clear, and then cooled.

The clear liquid was moved quantitatively to the distillation apparatus. The Kjeldahl flask was rinsed by 1-2 ml of distilled water, and the rinse water was put into the distillation apparatus; the rinse was carried out 5-6 times. Then, 8-10 ml of 60% NaOH – 5% Na₂S₂O₃,5H₂O solution was added into the distillation apparatus. An Erlenmeyer containing 5 ml of saturated H₂BO₃ solution and 2-4 drops of indicator (a mixture of 2 parts 0.2% methyl red and 1 part 0.2% methylene blue in 95% ethanol) was placed under the condenser. The condenser tube tip had to be immersed in H₂BO₃ solution and then distilled, until about 15 ml of distillate was p. The distillate obtained was then titrated with 0.02 N HCl until the color changed from green to gray. The following equation was used to calculate the crude protein content:

\[ \text{N Content (\%wb)} = \left( \frac{V \times \text{HClsample} - V \times \text{HClblank}}{\text{N} \times \text{Fk}} \right) \times 100\% \] (3)

\[ \text{Protein content (\%wb)} = \% \text{N} \times \text{Fk} \] (4)

Description:
Fk: Conversion factor (6.25 for flour and noodle)

**Fat Analysis by Soxhlet Method (AOAC, 2012)**

A total of 1 - 2 gram sample was put into the filter paper. The filter paper containing the sample was dried in the oven at temperature 105 °C until dried. The dried filter paper was put into the sleeve with a cotton plug. The sleeve was then inserted into the Soxhlet extraction tool and connected with the condenser and fat flask. The condenser tool was put on it, and the flat flask was placed under it. A sufficient amount of hexane solvent was then added into the fat flask. Then, the extraction was carried out for 6 hours. The solvent in the fat flask was distilled and collected again. The fat flask filled with extracted fat was dried in the oven at a temperature of 105 °C, cooled in the desiccator, and weighted. The drying was repeated until constant weight was obtained. The following equation was used to obtain fat content.

\[ \text{Fat content (\%wb)} = \left( \frac{W_1 - W_2}{W} \right) \times 100\% \] (5)

Description:
\( W = \) Sample weight (gram)
\( W_1 = \) Flask weight + fat (gram)
\( W_2 = \) Flask weight (gram)

**Ash Content Analysis (AOAC, 2012)**

The porcelain cup prepared for ashing was dried in the oven for 15 minutes, then cooled in the desiccator and weighted (A). The sampel with weight of 2-5 grams (B) was put into the cup, then burned in the smoke room till no smoke was observed. After that, the ashing was carried out in the electric furnace at 400-550 °C for 4 – 6 hours till white ash was produced and had a constant weight. The ash and the cup were cooled in the desiccator and then weighted (C). The following equation was used to calculate the sample ash content.

\[ \text{Ash content (\%wb)} = \left( \frac{(C-A)}{B} \right) \times 100\% \] (6)

\[ \text{Ash content (\%db)} = \left( \frac{\text{Ash content (\%wb) \times 100\%}}{100 - \text{Ash content (\%wb)}} \right) \] (7)

**Acidity Analysis (AOAC, 2006)**

The filtrate pH measurement was carried out before and after the fermentation. A total filtrate of 25 ml was put into a 50 ml goblet. The pH meter is firstly calibrated using pH buffers of 4 and 7. Sample measurement was then conducted by dipping the electrode into the sample solution until the stable reading was obtained.

**Whiteness Analysis (AOAC, 2006)**

The whiteness measurement was carried out using a KETT Digital Whiteness Meter Model C-100 tool. The sample was put in the sample cup until slightly exceeding the cup's lip. The cup filled with the sample was placed into the sample container. The sample container was then inserted into the measuring place so that the tool was turned on. The LED then showed the whiteness value and the measurement serial numbers. The whiteness value was measured by comparing the whiteness value read on the tool and the whiteness of BaSO₄ as the standard, which is 84.3.

\[ \text{Whiteness (\%)} = \left( \frac{\text{Whiteness sample value}}{\text{Standard value BaSO₄}} \right) \times 100\% \] (8)

**Yield**

The yield was stated by the percentage resulting from the final product weight divided by processed materials weight. The formula is described as follows.

\[ \text{Yield (\%)} = \left( \frac{\text{Product Weight After drying}}{\text{Materials weight before fermentation}} \right) \times 100\% \] (9)

**RESULTS AND DISCUSSION**

Lactic acid bacteria (LAB) has been used for a long time as beneficial bacteria in food processing. Moreover, the LAB's role has been widely used in food products such as yogurt, cheese, and even further development leads to the dried or encapsulated bacterial product preparations bearing a commercial brand.
The cassava of Adira 1 is harvested, sorted, and removed from its skin before passing the fermentation process. Through the fermentation treatment, it is known that the treatment used *L. paracasei* bacteria was the best treatment. The mocaf flour was produced by a treatment using different lactic acid bacteria and different immersion times. Thus, the research obtained mocaf flour with the following characteristics: smoother, white, soluble, with a water content of 6-8%. Figure 1 presents the result of the white and smooth-textured mocaf flour.

**Proximate Analysis**

**Water content analysis**

The water content is a crucial factor in the material. The water content of fresh cassava is about 62.5% (Ginting, 2009), indicating a perishable food. The mocaf production causes changes in the cassava's characteristics. Table 1 presents the statistical analysis result. The result shows that treatment with the immersion time of 48 hours and 72 hours significantly affected mocaf’s water content. On the other hand, the immersion with *L. plantarum*, *L. fermentum*, and *L. paracasei* give no significant effect to the mocaf's water content.

Drying is another crucial aspect of mocaf production. The flour's high content water was due to the more extended immersion in the fermentation process during mocaf production. Mweta et al. (2008), stated that starch flour of cassava and Japanese taro produced water content of about 10% - 11%. According to the Indonesian National Standard (SNI) 01-7622:2011, the maximum water content of mocaf flour is 13%; thus, the research result showed suitability with the regulation (Badan Standardisasi Nasional, 2011). This research result also shows that the material’s water content is lower, about 6.10% - 8.89% compared to the research result by Aini, Wijonarko, & Sustriawan, (2016) which shows that the cornstarch’s water content is 7.4% - 9.3%. It is due to the fermentation time of up to 80 hours. Furthermore, it is in line with research by Richana, Budiyanto, & Mulyawati (2010) in the modified cornstarch production with the immersion time for one and two days, which resulted in 8.56% - 12.02% of water content.

Apart from drying time, the drying method and device also provide different results. A drying process using a dehydrator with hot air blowing produced a better result in terms of color and material's water content than drying with an ordinary oven at high temperatures. In the previous research, the drying with an oven was carried out, and the flour's color became browner compared to the one produced by drying using the dehydrator. The lower content of water will also produce lower material's water content and affect the flour's shelf life. The lower material water content, the less perishable the product is.

The immersion and drying process are crucial aspects of mocaf flour production. The more extended immersion will produce flour with higher...
water content. On the contrary, the overlong drying will produce flour with lower water content, yet cause a change in color into darker and even damage the flour color. Moreover, the drying type and time will also affect the flour’s water content. In the mocaf production, the difference occurred in immersion time treatment, namely 48 hours and 72 hours. Thus, the water content will affect the shelf life of flour or foodstuffs. The foodstuff which has lower water content will be perishable and quickly smells unpleasant or rancid. The lower water content is preferred in mocaf flour production.

The analysis of protein content

Protein is a crucial indicator in the food product. The protein content is used to determine mocaf flour quality as the substitution of imported flour, such as wheat flour. Good quality flour contains higher protein content. However, mocaf flour resulting from the fermentation process in immersion treatment for 48 and 72 hours does not have different protein content. The process of lactic acid fermentation commonly uses carbohydrate and transform it into lactic acid.

This research used total protein and from Table 1, it can be seen that the protein obtained is ranging from 1.07% - 1.44%. The result is similar to the research performed by Aini et al., (2016), which used L. bulgaricus, L. casei, and yeast. That previous research produces relatively high dissolved protein content in the fermented corn flour, which is around 1.30% – 2.51%. However, if we compare this current research and the previous research conducted by Gunawan et al. (2015) on mocaf flour, this research shows a significant increase in protein content, about 2% - 8%. This phenomenon occurs because during the fermentation, L. plantarum produces proteinase enzyme. According to Petrova et al. (2013), L. paracasei is a strain included in L. casei group that can make the amylopullulanase enzyme. Likewise, L. plantarum produces amylase and amylopullulanase enzymes, while L. fermetum produces more amylose enzymes. It is predicted that those three lactic acid bacteria cannot produce protein enzymes, yet it can break down more carbohydrate into amylase. The study performed by Darmawan et al. (2013) stated that the 5% addition of starter and 2 mm thick cassava chips produces 3.68% of protein content. Meanwhile, in this current research, the starter added was only 0.01% and the cassava cut in an irregular shape with a length of 3-4 cm and 0.02 mm thick instead of chips. Therefore, the result obtained was also slightly different.

Comparing the duration of the fermentation process of corn flour with 20 hours difference, a longer immersion process resulted in an increase of protein content up to 1% (Aini et al., 2016). Meanwhile, the research of Richana et al. (2010) shows a slightly different result on the total protein content, that is about 5.07% - 6.84% compared to the fermented corn flour with different fermentation duration, with the fermentation result by using mocaf bacteria immersed for two days which resulted in 6.84% protein content. The protein result of all the same treatments is not significantly different; however, the treatment that used L. paracasei for 72 hours produced a relatively higher protein content value, which is 1.44%, than the treatments that used L. plantarum and L. fermetum. Some lactic bacteria acid can change protein content in the ingredient into simpler amino acid. Nonetheless, this process's mechan

| Description | Water Content (%) | Fat Content (%) | Protein Content (%) | Carbohydrate Content (%) | Ash Content (%) |
|-------------|------------------|----------------|---------------------|--------------------------|----------------|
| L.p 48      | 6.11±0.68a       | 1.15±0.27ab    | 1.24±0.19a          | 91.10±0.12b              | 0.42±0.09ab    |
| L.p 72      | 8.21±0.95b       | 1.47±0.15b     | 1.31±0.22a          | 88.53±1.35b              | 0.51±0.03b    |
| L.f 48      | 6.21±0.27a       | 1.19±0.04ab    | 1.09±0.15a          | 91.10±0.09b              | 0.42±0.02ab   |
| L.f 72      | 8.89±1.20b       | 1.16±0.06ab    | 1.07±0.12a          | 88.47±1.37a              | 0.42±0.01ab   |
| L.pc 48     | 6.10±0.01a       | 0.87±0.05a     | 1.18±0.14a          | 91.59±0.04b              | 0.31±0.05a    |
| L.pc 72     | 8.84±0.63b       | 1.27±0.04b     | 1.44±0.03a          | 87.99±0.68a              | 0.47±0.02b    |

Description: degree of whiteness and acidity degree; L.p 48 = fermentation with L. plantarum for 48 hours; L.p 72 = fermentation with L. plantarum for 72 hours; L.f 48 = fermentation with L. fermetum for 48 hours; L.f 72 = fermentation with L. paracasei for 72 hours; L.pc 48 = fermentation with L. paracasei for 48 hours; L.pc 72 = fermentation with L. paracasei for 72 hours. The number followed by the same letter shows non-significant difference of among treatments, while the number followed by different letter shows significant difference among the treatment.

Table 1. Proximate result of mocaf flour by using lactic acid bacteria

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ism is positively related to the species and strain used in the lactic acid fermentation. A type of lactic acid bacteria which only has some enzymes to break down amylose and starch, and certain lactic acid bacteria which is only able to break down protein into simpler substances were used since proteolysis and peptidase is commonly produced by lactic acid bacteria that work in fermented milk products, e.g., some types and strains from Lactococcus (Pertrova, 2013; Liu et al., 2010).

The analysis of fat content

The determination of fat content was carried out through the fat separation process from the food product. This separation was performed based on extraction and distillation. Fat content in the food product in the form of flour is not used much since it causes a rancid and musty smell in a short time. A statistic test with a 95% confidence level states that the highest fat content is found in mocaf flour with the treatment of 72 hours immersion by using L. plantarum as many as 1.47%. Simultaneously, the lowest fat content of mocaf flour was found in the treatment of 48 hours immersion by using L. paracasei as many as 0.87%. This is almost similar to the fat content of mocaf flour with the starter of encapsulated lactic acid bacteria, which produces relatively high-fat content in mocaf flour with 1.60% of encapsulated bacteria of L. lactis subsp. lactis and the lowest fat content was obtained from the use of 0.60% encapsulated L. plantarum bacteria (Loebis & Meutia, 2012). Research on fat content executed by Allung, Hartini, & Cahyanti (2016) where the fat content increases significantly to 3.66% from the mocaf flour that uses 22% of red fermented rice as a starter. Meanwhile, for the fermentation process without the addition of red fermented rice, the fat content obtained is only 1.34%. Ginting (2009) also stated that the fat content of cassava flour without any treatment yield 0.65% of fat content.

Starter type and the amount of starter added will also bring impact to the fat content. Research performed by Nusa, Suarti, & Alfiah (2012) shows that adding more bacteria means more active bacteria, and enzyme activity will also increase. Lactic acid bacteria used here is only 0.01% of the whole bacteria in the fermentation process. Those bacteria are previously activated in the MRS broth media and then dissolved in the cassava immersion water. The fat content of fermented mocaf flour will not be much affected. In terms of nutrition factors, excessive fat content is considered harmful to consume. More than that, too much fat content is also less profitable in the flour's storing process since it can cause rancidity, decreasing the flour quality. Fat content in starch and flour will interfere gelatinization process since fat can build a complex structure with amylose to inhibit the release of amylose from starch granule (Aini et al., 2016).

The analysis of ash content

The ash content analysis can be leveraged for various purposes, such as the parameter determinant of nutrition facts of an ingredient of a particular food, determining how appropriate certain food processing is, or figuring out the ingredient type used. Ash content is the inorganic component of a compound passing through the ashing process due to high-temperature ignition. Cassava containing high phosphor and calcium, and these substances contributes to significant ash content (Ginting, 2009). Gadd (2010) classified some microorganisms that can turn some natural materials into organic and inorganic salt, where these types of salt will further affect the ash content of certain food products.

The highest ash content was found in mocaf flour under the 72 hours immersion treatment by using 0.51% of L. plantarum. Meanwhile, the lowest ash content was found in mocaf flour under 48 hours of immersion treatment using 0.31% of L. paracasei. Lactic acid bacteria can transform carbohydrates into organic acids that will influence a fermentation product's ash content. As we know, as the microorganisms more effectively change the carbohydrate, some organic acids, such as lactic acids, will also increase. Moreover, this condition is undoubtedly associated with the duration of the fermentation. More extended immersion will cause a more optimum process in turning carbohydrates into lactic acids. This situation will affect ash content, and it can be seen from the long fermentation duration that yields higher ash content. This condition is different from the ash content of 48 hours of flour fermentation that results in low ash content.

This result is in line with the outcome of a study by Gunawan et al. (2015), which obtained 0.69% ash content. In the research performed by Loebis & Meutia (2012), it is known that the process of mocaf production by using a variety of encapsulated lactic acid bacteria had resulted in an
The Use of Three...

average of 0.50% of ash content. Besides, the result from a study by Richana et al., (2010) indicated that the ash content of modified corn flour is ranging from 0.25% to 0.47% and this result is not significantly different from this current research. The product of obtained ash content of mocaf flour in this research is almost similar to the ash content of mocaf flour as defined in the requirement of SNI 7622:2011, which is 1.5% w/w at most (Badan Standardisasi Nasional, 2011).

The Analysis of Carbohydrate

The carbohydrate content of mocaf is determined by the difference method. Carbohydrate is the main component of root vegetables (Mweta et al., 2008). Carbohydrate is a kind of organic compound found abundantly in nature and its amount is also more considerable than fat and protein. Cassava is one of the high carbohydrate content products. The result of carbohydrate analysis shows that immersion treatment of 48 hours and 72 hours brings a significantly different impact on the carbohydrate content in mocaf flour. The immersion in various lactic acid bacteria does not bring significantly different implications.

In this research, the carbohydrate content increases significantly from 48 hours up to 72 hours fermentation, using the immersion treatment with L. plantarum, L. fermentum and L. paracasei. The highest carbohydrate content is found in mocaf flour under the immersion treatment for 48 hours, using 91.59% of L. paracasei. Meanwhile, the lowest carbohydrate content is found in the mocaf flour under the immersion treatment for 72 hours by using L. paracasei 87.99%.

The Analysis of Whiteness

Color is an essential component in determining the quality of a specific product. Consumers' acceptance of a product highly depends on the color it presents. According to SNI 01-7622:2011 standard, the color of mocaf is at least 87. The result of the color analysis can be seen in Table 2. The whiteness analysis of mocaf flour resulting from three types of lactic acid bacteria indicated an almost similar result. The whiteness value of the treatment using L. fermentum gains the highest value of 108.55. Yet, the color testing value of all treatments is not different.

The color of the product is usually derived from the original/natural color of that food product. Since the color of cassava is white to yellowish, then the color of the product will not be much different. The fermentation process will lead to a pigment degradation process occurs in the food product. However, the immersion step will cause color components to decay so that the resulted flour will be whiter (Amanu & Susanto, 2014). The element of color in cassava is the food's natural component color. Loebis & Meutia (2012) stated that mocaf flour made of encapsulated lactic acid bacteria had generated better flour color and white color than common cassava flour.

Acidity (pH) Analysis

pH value indicates the acidity level of a product. Lower pH indicates more acidic characteristics; otherwise, higher pH indicates more alkaline characteristics. The longer the fermentation duration, the lower the pH value of mocaf flour. This phenomenon is predicted due to a longer immersion process that causes lactic acid bacteria's optimum activity. 48 hours to 72 hours of fermentation does not indicate any different results.

Lactic acid bacteria used in this research include L. plantarum, L. fermentum and L. paracasei bacteria categorized in Lactobacillus. These bacteria are actively turning carbohydrates into organic acids and one of them is lactic acids. This condition leads to changing the pH of the ingredient that will decrease the pH by leveraging the nutrition it contains, even though those bacteria's ability is not affected by the low acid content (Reddy et al., 2008). Nuryana et al. (2019) stated that the organic acids resulting from the lactic acid bacteria could be measured using HPLC and the

| Description | Whiteness   | pH          | Yield         |
|-------------|------------|-------------|---------------|
| L.p 48      | 100.20±2.19a | 3.73±0.22a  | 26.65±3.13a   |
| L.p 72      | 101.25±0.92a | 3.94±0.27a  | 33.02±4.11a   |
| L.f 48      | 108.55±3.32a | 3.83±0.15a  | 26.59±2.22a   |
| L.f 72      | 102.65±1.63a | 3.98±0.30a  | 28.04±2.42a   |
| L.pc 48     | 104.35±2.19a | 3.66±0.17a  | 28.04±0.88a   |
| L.pc 72     | 102.20±3.11a | 3.88±0.11a  | 29.58±3.62a   |

*Description: The number followed by the same letter shows the non-significant difference among treatments, while the number followed by different letters shows the significant difference among the treatments.
result will be acetate, lactic, and formate.

In this case, lactic acid is a type of acid that includes in the GRAS (generally recognized as safe) category that undoubtedly changes the condition and acidity of an ingredient or product (Martinez et al., 2013). Change in acidity will also affect the character of the flour, both from its aroma and taste. Among those treatments, the pH of mocaf is not significantly different, around 3.66-3.98. As seen in Table 2, although L. fermentum pH in that treatment is almost 4, that value is not different from other treatments. The same pH in mocaf flour indicates that mocaf flour has relatively high acid content. This condition will also affect the taste of the mocaf flour.

**Yield Analysis**

The yield amount was measured based on the mocaf flour weight percentage divided by raw cassava weight and multiplied by 100%. The yield of mocaf flour under the treatment of fermentation duration and lactic acid bacteria is ranging from 26.59% - 33.02%. In this research, mocaf flour yield decrease from 48 hours to 72 hours fermentation in the immersion process by using L. plantarum, L. fermentum and L. paracasei, although statistical testing shows the same result for the yield value. The water content of the ingredient can also affect flour yield value. In higher water content, the water loss tends to be higher, unlike in low water content (Gunawan et al., 2015). The water content of the ingredient decreases during the drying process as a result of the evaporation process. If the ingredient’s water content is high, then the yield value will be lower since more of the ingredients evaporate.

In her research, Aini et al. (2016) suggested that fermentation media and timing also affect the yield. The use of water from fermentation media will dissolve some ingredient components so that some solids will be wasted. Additionally, some other components will also be wasted due to scattered ingredients adhering to drying tools and any ingredients that stick in the grinder during the flour grinding process.

**CONCLUSION**

The utilization of L. plantarum, L. fermentum, and L. paracasei can yield mocaf flour which has a good characteristic, especially in terms of its physical aspect and proximate aspect. The use of L. paracasei has successfully produced the best mocaf flour, with a relatively good protein content of 1.44%; low-fat content of 1.27%; and low carbohydrate content of 87.99%. Referring to SNI standard which encompasses ash content, whiteness, and acidity, the best whiteness result is 102.20, and its acidity is 3.88, which is not too low and will affect the mocaf flour taste. Besides, its ash content is also meeting the requirement, which is around 0.31%. It is suggested that the future research will further include mocaf made of fermented L. plantarum, L. fermentum and L. paracasei in food production, for example, in production of noodle, cookies, bread, or other products, as well as the likeness level on flour product and any processed product made of mocaf flour.

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