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Effect of Starter Concentration and Fermentation Time on the Characteristics of Modified of Cassava Flour (Mocaf)

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ABSTRACT

Mocaf (Modified Cassava Flour) is a modified cassava flour made through a fermentation process using microbes. One of the microbes that can be used for mocaf fermentation is *Acetobacter xylinum* . In this research, cassava tubers were fermented into mocaf flour with the help of a starter of *Acetobacter xylinum* bacteria. This research was conducted to determine the effect of starter A. *xylinum*'s concentration and fermentation time on the characteristics of mocaf flour. The research method used OFAT (One Factor at A Time). The research consists of the making flour process (sorting, stripping, washing, slicing, fermentation, drying, milling, and sifting) and analyzing samples. The variations of starter *A. xylinum* concentration used were 5%, 10%, 15%, 20%, and 25%. While the variations of fermentation time were 24 hours, 36 hours, 48 hours, 60 hours, and 72 hours. The Parameters analyzed were % mass, moisture content, ash content, acid level, HCN content, and crude fiber content. The results showed that the starter concentration and fermentation time affected the percent mass (P-value <0.05). The mocaf flour that passes SNI is fermented cassava with a starter concentration of 15-25% and a fermentation time of 48-72 hours.

Keywords: Cassava, Modified Cassava Flour, Fermentation, *Acetobacter xylinum*

1. INTRODUCTION

Cassava is one of the staple foods commonly consumed by Indonesian people. This food plant has a high carbohydrate content. Cassava is usually used as a staple food, chemical raw materials, food raw materials, and others. Some food products that can be produced from cassava include tapioca flour, cassava flour, cassava flour, and liquid sugar. Cassava flour can be processed into mocaf flour as a substitute for wheat flour for the manufacture of pastries, bread, noodles, snacks, etc [1].

Modified Cassava Flour (MOCAF) is a product derived from cassava flour that is made using the principle of modifying cassava cells through a fermentation process [2]. This modification process involves the role of enzymes or microbes during fermentation. Several microbes that have been used to produce mocaf flour include *Acetobacter xylinum*, *Rhizopus oryzae, Saccharomyces cerevisiae*, *Lactobacillus plantarum, and Lactobacillus casei*. Mocaf flour processing is technically almost the same as ordinary cassava flour processing. It's just that, in making mocaf flour it is accompanied by fermentation, then it is dried and ground into Mocaf. The resulting flour product is whiter, softer, does not smell of cassava, and has characteristics similar to wheat flour.

Generally, the mocaf industry uses *Lactobacillus plantarum* bacteria and only a small proportion uses *Acetobacter xylinum* bacteria or other microbes. In Salim's experiment [3], acetic acid bacteria, namely the starter *Acetobacter xylinum*, have been shown to produce mocaf flour which is white and does not smell of cassava. However, it is not yet known whether the characteristics of mocaf flour with *A. xylinum* fermentation are following SNI or not, so further research is needed. This research was conducted to find out more to determine the effect of *Acetobacter xylinum* starter concentration and fermentation time on the characteristics of mocaf flour.

2. MATERIALS AND METHODS

2.1 Experimental design

The location of research was carried out in the Basic Instrument laboratory and the Chemical

Engineering Process laboratory at Universitas Muhammadiyah Purwokerto. The research method used is the OFAT (One Factor at A Time) method. This research begins with the process of making Mocaf flour which includes the stages of sorting, peeling, washing, slicing, fermenting, drying, milling, and sifting. After that, it was continued with mass analysis and SNI tests (moisture content, ash content, acid degree, HCN content, and crude fiber content).

2.2 Fermentation of mocaf

Cassava is sorted, peeled, and washed thoroughly. Then cassava is cut into chips (0.2 - 0.3 cm thick) of as much as 500 grams. The chips are fermented with a certain concentration of starter *A. xylinum* and a certain soaking time. After being fermented, the chips were dried in an oven for 24 hours at 40^{o}C. The dry chips are ground to a powder state. The flour is then analyzed.

2.3 Yield analysis

The mass percentage can be known from the yield of flour. The analysis is carried out by weighing the chips before they are fermented and weighing the resulting mocaf flour, then the mass percentage can be determined.

2.4 Water Content Analysis

The sample is weighed in the amount of 1-2 grams in a weighing bottle with a lid whose weight is known. The sample is dried in an oven at 130^{o}C for 1 hour. Samples were cooled in a desiccator for 30 minutes. Samples were weighed dry weight. This step is repeated several times until a fixed/constant weight is obtained.

2.6 Ash Content Analysis

The sample is weighed in the amount of 3-5 grams into a porcelain cup whose weight is known. The sample is charred over a burner flame, then incubated in an electric furnace at 550^{o}C for 1 hour. The sample ash was cooled in a desiccator for 30 minutes, then weighed again until a constant weight was obtained.

2.7 Acid Degree Analysis

Weighed as much as 10 grams of sample then dissolved with 100 ml of 95% ethanol, allowed to stand for 24 hours, and then filtered. 50 ml of the filtered solution was put into the Erlenmeyer and added 3 drops of pp indicator were. Titrate with 0.05 N NaOH solution until the color changes to pink.

2.8 HCN Analysis

Weighed as much as 20 grams of sample then added 100 ml of distilled water in Erlenmeyer and allowed it to stand for 2 hours. Add another 100 ml of distilled water and distilled with steam. The distillate is collected in an Erlenmeyer which has been filled with 20 ml of 2.5% NaOH. After the distillate reaches a volume of 150 ml, the distillation process is stopped. The distillate was then added with 5 ml of 5% KI and 8 ml of NH_4OH. The distillate mixture was titrated with 0.02 N $AgNO_3$ solution until turbidity occurred. Then the cyanide acid content was calculated.

2.9 Fiber Analysis

The sample was weighed as much as 2-4 grams and put into a 500 ml Erlenmeyer, added 50 ml of 0.3 N H_2SO_4 then boiled for 30 minutes using an upright cooler. 25 ml of 1.5 N NaOH was added and then boiled for 30 minutes. When hot, it is filtered using a funnel containing filter paper of known weight. The precipitate on the filter paper was washed successively with hot 1.25% H_2SO_4, hot water, and 96% ethanol. The filter paper and its contents were removed, put into the oven and dried at 105^{o}C, cooled in a desiccator for 15 minutes, and then weighed. If the fiber content is greater than 1%, the filter paper and its contents are incinerated, then weighed again.

3. RESULTS AND DISCUSSION

3.1 The Effect of Acetobacter *xylinum* Starter Concentration on Mocaf Flour Mass

Based on the results of ANOVA calculations, a P-Value of 0.006 is obtained or it can be said that the P-value <0.05. So it can be concluded that the concentration of *A. xylinum* starter has an effect on the yield percentage/mass of mocaf flour produced.

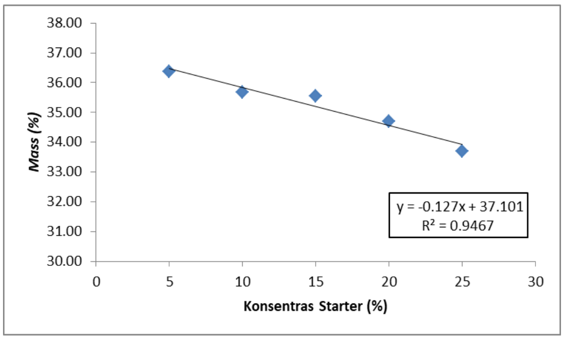

Figure 1 Graph of starter concentration to % the mass of mocaf flour

In **Figure 1** it can be seen that the percentage of mass obtained decreased with increasing concentration of the added *A. xylinum* starter. This decrease in the percentage of flour mass indicates that the starch content in mocaf flour is getting less. This is because the more microbes that play a role in the fermentation process, the more enzymes are produced. These enzymes will degrade starch into simple sugars, namely glucose which will then be converted into organic acids, causing the starch content of mocaf flour to decrease [4]

3.2 Effect of Fermentation Time on Mocaf Flour Mass

Based on the results of ANOVA calculations, a P-Value of 0.001 or a P-value <0.05 is obtained. So it can be concluded that the fermentation time of mocaf flour with *A. xylinum* starter affects the mass percentage of mocaf flour produced.

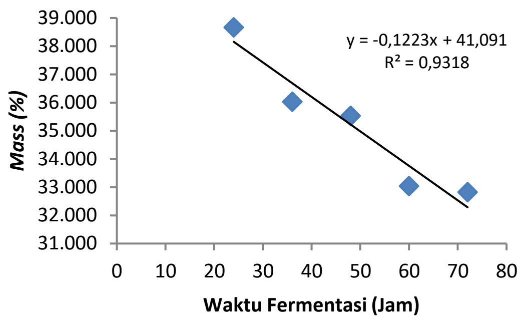

Figure 2 Graph of fermentation time vs % the mass of mocaf flour

Figure 2 shows that the percentage of mass obtained decreases with increasing fermentation time. One of the factors that affect the mass of flour produced is the extraction time, the accuracy of the length of time used affects the efficiency of the process. Mocaf flour mass gain in each sample decreased with increasing fermentation time. This is because the longer the fermentation, the more starch is hydrolyzed into simple sugars so that the mass decreases [5].

3.3 Quality Test of Fermented Mocaf Flour

Water Content Test

The water content greatly affects the quality of mocaf flour. The lower water content makes the quality of mocaf flour better because it is resistant to damage caused by the growth of fungi and bacteria [3].

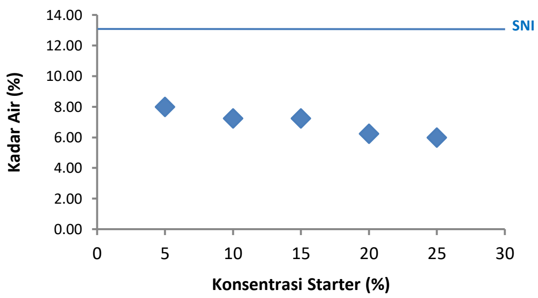

Figure 3 Graph of starter concentration vs. moisture content of mocaf flour

In **Figure 3** it can be seen that the water content in mocaf flour decreased as the concentration of *A. xylinum* starter was added. The water content decreased with the addition of *A. xylinum* starter concentration. Mocaf flour produced by the fermentation of *A. xylinum* complies with SNI 7622: 2011 standards where the maximum permissible moisture content of mocaf flour is 13% [6].

This decrease in water content is due to the decomposition of organic compounds during fermentation by A. *xylinum*. Fermentation produces acetic acid and other products of the *A. xylinum* starter metabolic process, namely free H_2O and energy in the form of heat. With the formation of heat during the fermentation process, the temperature of the food will increase and then the water will evaporate, resulting in a decrease in water content [7]

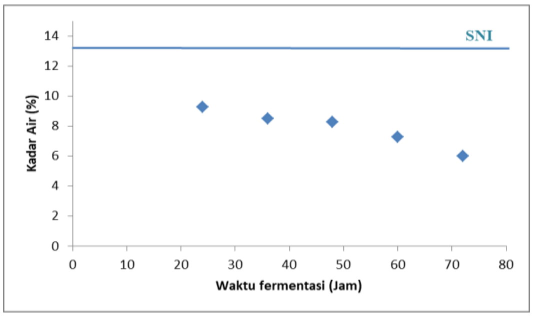

Figure 4 Graph of fermentation time vs moisture content

In **Figure 4** it can be seen that the water content in mocaf flour decreases with increasing fermentation time. The moisture content of mocaf flour decreased because the longer the fermentation time, the more starch was dispersed by water and degraded by enzymes from bacteria. The degraded starch will release bound water in starch to become free water. Enzyme activity in degrading starch also makes the texture of the material soft and porous. This situation can increase water evaporation during the drying process so that the water content will decrease in the same drying period [8]

3.4 Ash Content Test

Ash content shows the total mixture of inorganic or mineral components contained in a food.

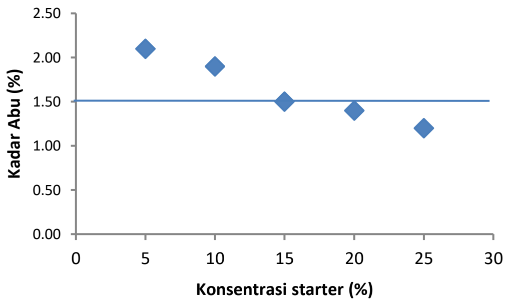

Figure 5 Graph of starter concentration vs ash content of mocaf flour

Figure 5 shows that the ash content of mocaf flour tends to decrease with increasing concentration of *A. xylinum* starter. The ash content of the samples of 5% and 10% did not meet the SNI standard, while the samples of 15%, 20%, and 25% had an ash content that met the SNI, which was a maximum of 1.5%. *xylinum* to sustain life [9][6]

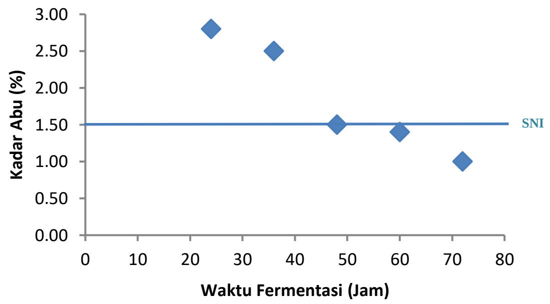

Figure 6 Graph of fermentation time vs ash content

In **Figure 6** it can be seen that the ash content in mocaf flour decreases with increasing fermentation time. Ash content that meets SNI (max. 1.5%) [6] is a sample that is fermented for 48 hours, 60 hours, and 72 hours.

The decrease in ash content is since during fermentation, inorganic minerals are dissolved in the water. The decrease in inorganic compounds is inversely proportional to the increase in organic compounds during fermentation. Besides affecting purity, ash content also affects the color and stability of a food ingredient. This is proven by the difference in the color of mocaf flour which is whiter than the color of unfermented cassava flour. The effect of this color change is due to the removal of color-producing components during the fermentation process, such as pigments (especially in yellow cassava), and proteins which can cause a brown color when drying or heating [7]

3.5 Acid Degree Test

The principle of analysis used is the dissolution of organic acids in the mocaf flour sample using an organic solvent, namely 96% alcohol, then titrated with a basic solution of NaOH. The degree of this acid shows how much organic acids are present in the mocaf flour sample.

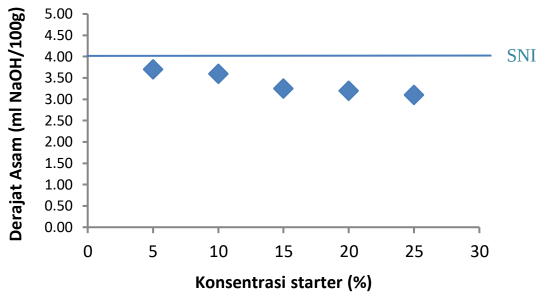

Figure 7 Graph of starter concentration vs degree of acidity of mocaf flour

Figure 7 shows that the acidity of mocaf flour tends to decrease with increasing concentration of *A. xylinum* starter. All samples of mocaf flour are acidic, pH <7, and comply with SNI, namely the degree of maximum acidity is 4 ml of NaOH/100g sample. A decrease in the degree of acidity indicates an increase in the acid content in the flour. The increase in the amount of this acid is due to the microbial activity of *A. xylinum* in the fermentation process. The microbe *A. xylinum* destroys cassava cell walls resulting in the liberation of starch granules.

Furthermore, starch granules will be hydrolyzed into monosaccharides. This monosaccharide is later used as a raw material to produce organic acids, namely acetic acid.

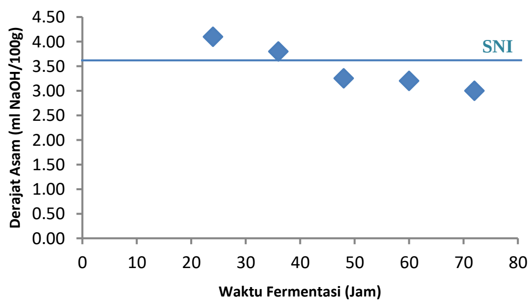

Figure 8 Graph of fermentation time vs degree of acid

In **Figure 8** it can be seen that the degree of acid (pH) in mocaf flour decreases with increasing fermentation time. All samples of acid degree complied with SNI (4 ml NaOH/100g sample) except for 24-hour samples. The decrease in pH occurs because the longer the fermentation time will provide an opportunity for bacteria to produce enzymes and convert glucose into organic acids. If the enzyme activity increases, more organic acids will be produced. These acids will be imbibed into the chip so that it can cover the distinctive taste and aroma of cassava [5].

3.6 HCN Levels Test

The purpose of testing for cyanide levels is to determine the levels of cyanide acid contained in mocaf flour so that it can be known whether the fermented flour is safe and suitable for consumption or not.

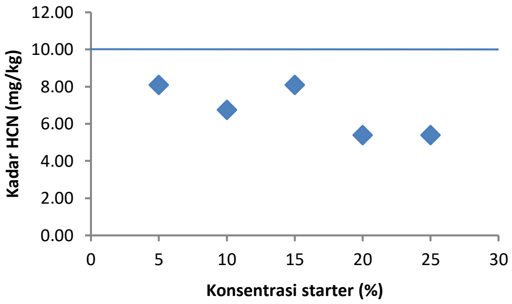

Figure 9 Graph of starter concentration vs HCN content of mocaf flour

Figure 9 shows that the HCN content of mocaf flour seems to decrease but not consistently. Even though the decrease in HCN levels was inconsistent, all samples met SNI, namely HCN levels should not be more than 10 mg/kg.

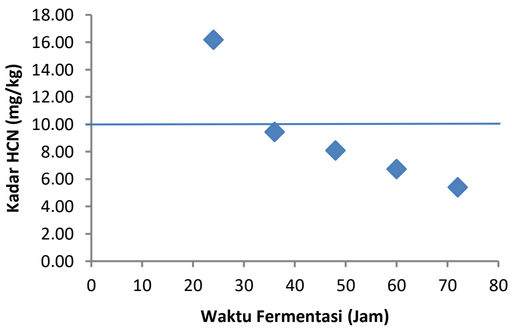

Figure 10 Graph of fermentation time vs HCN content of mocaf flour

Figure 10 shows that the HCN content obtained seems to decrease with increasing fermentation time. All samples complied with SNI (HCN level should not be more than 10 mg/kg) except for 24-hour samples. The decrease in cyanide acid levels was due to the washing and soaking process during fermentation. Cyanide acid has soluble and volatile properties. During the washing and soaking fermentation processes, the cyanide acid will dissolve and be wasted with water [10]. When the fermentation time is longer, more and more HCN compounds in the tubers will diffuse out. This is due to the loosening of network bonds during fermentation so that HCN compounds and other compounds will come out and dissolve in water [11].

3.7 Crude Fiber Level Test

Crude fiber is a non-nutritional compound that cannot be hydrolyzed by acids or alkalis. Crude fiber consists of cellulose, hemicellulose, and lignin.

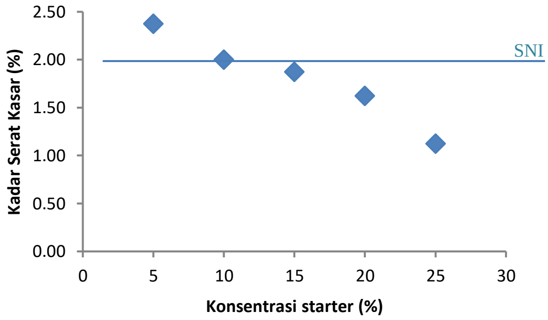

Figure 11 Graph of starter concentration vs crude fiber content of mocaf flour

Figure 11 shows that the crude fiber content of mocaf flour tends to decrease with increasing concentration of *A. xylinum* starter. The 5% sample does not meet SNI while the crude fiber content of samples 10%, 15%, 20%, and 25% have fulfilled SNI, which is a maximum of 2%. The decrease in crude fiber content was due to the activity of *A. xylinum* bacteria which produced several enzymes including pectinolytic and cellulolytic enzymes. These two enzymes play a role in the process of degrading or destroying cassava cell walls. The more microbes that play a role in fermentation, the more the degradation process will increase, causing the crude fiber content in cassava to decrease [3]

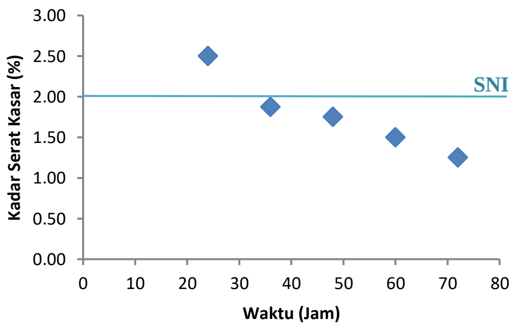

Figure 12 Graph of starter concentration vs crude fiber content of mocaf flour

Figure 12 shows that the crude fiber content obtained decreased with increasing fermentation time. All samples met SNI (maximum crude fiber content of 2%). The longer the fermentation time provides more opportunities for *A. xylinum* microbes to grow and produce enzymes for mocaf flour fermentation. Pectinolytic and cellulolytic enzymes will degrade the tubers, causing fiber in cassava tubers to lyse and break down into glucose. This is what causes the crude fiber content of mocaf flour to decrease with increasing fermentation time [4].

4. CONCLUSION

Based on the results of research on making mocaf flour that has been done, it can be concluded that the concentration of starter *A. xylinum* affects the mass of mocaf flour. The higher the concentration of the starter added, the lower the mocaf flour mass that will be produced. Fermentation time also affects the mass percentage of mocaf flour. The longer the fermentation time, the lower the mocaf flour mass that will be produced. Samples whose characteristics meet the SNI test parameters (moisture content, ash content, degree of acidity, HCN content, and crude fiber content) are samples of starter variations of 15%, 20%, and 25% and samples of fermentation time variations of 48, 60, and 72 hours.

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