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The Effect of Fermentation Time on The Quality of MOCAF (Modified Cassava Flour) Using Raw Material of Bokor Genotype Cassava

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ABSTRACT

Cassava genotype Bokor has a fairly high content of beta carotene, which is beneficial for health and can also support the development of the functional food industry. MOCAF (Modified Cassava Flour) is a product made from cassava flour which is processed by modifying cassava cells through fermentation. This study aims to analyze the effect of long fermentation time on the characteristics of MOCAF from beta-carotene-rich cassava genotype Bokor. In this study, three variations of fermentation time were used, namely 15 hours, 24 hours and 40 hours. The research results showed that the time of fermentation affected the physicochemical properties of MOCAF. NIR analysis showed that the fermentation time affected the chemical composition of MOCAF. At 40 hours (last time of fermentation), there was a decrease in fat content with a value of 1.59% and an increase in water, protein and fiber content with a value of 13.71%, 3.94% and 2.28%, respectively. Proximate analysis showed that there was an effect of the length of fermentation time on the MOCAF content, namely at 40 hours, there was an increase in water content with a value of 10.10%, a decrease in dry matter, ash, protein, fat, fiber with a value of 89.90%, 0.60%, 0.78% respectively, 0.45%, and 1.29%. Scanning Electron Microscope (SEM) analysis shows the effect of fermentation time on the morphological properties of MOCAF starch granules. The longer the fermentation time, the more perforated or damaged the surface of the starch granules.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important agricultural product for Indonesia. Since ancient times, Indonesians have used cassava as a staple food, which can also be processed into typical Indonesian food products. Current technological developments have made cassava a basic ingredient in the food industry (Asmoro, 2021). One part of cassava that can be used is the tuber. Cassava root is widely known as a carbohydrate-producing food ingredient (Tuhenay, 2018). The carbohydrate content in cassava is 34%, but cassava is very poor in protein, which is only 1.2% (Wulandari *et al*., 2021). In Indonesia, LIPI has identified several variations of cassava with yellow roots, including Carvita 25, Mentega Cibanon, Mentega 1, Mentega 2, Lombok 1, Adira 1, Nangka, and Bokor. This variation of yellow tuber cassava has a fairly high beta carotene content which is beneficial for health and can also support food development, one of which is supporting the MOCAF industry as a rich source of beta carotene (Sudarmonowati *et al*., 2020). The problems to provide MOCAF (*Modified Cassava Flour*) is a product made from cassava flour processed by modifying cassava cells through fermentation (Anindita *et al*., 2019). Processing cassava with a fermentation process is an effort that can be made to increase the protein contained in cassava so that cassava flour produced from the fermentation process has advantages over ordinary cassava flour. The quality of the MOCAF flour produced is affected by the length of fermentation time. A fermentation process generally makes MOCAF modify cassava cells. Fermentation, which mostly involves lactic acid bacteria (LAB), usually produces pectinolytic and cellulolytic enzymes that can damage the cellulose cell walls in MOCAF fermented cassava so that the starch granules decompose completely. Using LAB for cassava fermentation is a way to produce MOCAF, which is relatively easy and inexpensive (Seveline *et al*., 2020). One of the efforts that can be made to increase the protein contained in cassava is through a fermentation process so that fermented cassava flour has advantages compared to ordinary cassava flour. MOCAF flour is fermented for 24 hours using a mixed culture of LAB. The time the fermentation is used also determines the quality of the MOCAF flour that will be produced (Wulandari *et al*., 2021). Therefore, the resulting MOCAF flour can be optimized by selecting the best fermentation time.

Many studies have been conducted regarding the effect of fermentation time on the quality of MOCAF. Nur'utami *et al*. (2020) reported that the longer the fermentation time, the higher the swelling power of the bread produced from MOCAF flour. Bread made using MOCAF flour by fermentation produces sensory quality characteristics, such as soft texture, yellow to white, sweet taste, and a typical bread aroma. Wulandari *et al*. (2021) reported that the length of time for fermentation had an effect on the water content and protein content with the results obtained, namely the fermentation time of 48 hours with a protein content of 2.06%, a pH value of 5.44, water content 2.55%, yield 25.32%, total LAB 6.2 CFU/g, normal aroma and white color. The longer fermentation time also affects the characteristics of the resulting MOCAF flour. Aini *et al*. (2009) used variations in fermentation time of 0, 12, 24, 36, 48, 60 and 72 hours to obtain the best treatment, namely 72 hours. The longer the fermentation time, the more microbial activity in degrading cassava starch will increase so that it will increase the viscosity and solubility. Aini *et al*. (2009) also reported that fermentation time affects the characteristics of the resulting MOCAF flour. This study aims to analyze the effect of the length of fermentation time on the quality of MOCAF (*Modified Cassava Flour*) from the basic ingredients of cassava rich in beta carotene genotype Bokor through a comparison of several variations of fermentation time.

2. METHODOLOGY

2.1. Materials

The production of MOCAF flour uses cassava with Bokor genotypic cassava that is 8-12 months old and taken from the Boyolali area, Central Java, in the fermentation process, using a starter type Bimo-CF. After the fermentation process, the soaking process is carried out using 0.3% sodium metabisulfite to protect the beta-carotene content in cassava. The tools used in this study were knives, scales, slicers, fermentation containers, pH meters, thermometers, spinners, hammer mills, aluminium foil standing puch packaging and equipment for analysis in the laboratory. The manufacture of MOCAF flour is carried out at PT. MOCAF Flour Solusindo, Surakarta, Central Java. Meanwhile, the physicochemical properties of MOCAF flour were analysed at the Chemical Engineering Laboratory, Muhammadiyah University, Surakarta. NIR and proximate analysis on MOCAF flour was conducted at the Research Center for Applied Microbiology BRIN Cibinong Bogor, West Java. SEM analysis on MOCAF flour was conducted at the Research Center for Food Process Technology BRIN Gunungkidul, Yogyakarta.

2.2. Methods

2.2.1. Production of MOCAF flour

The production process of MOCAF includes several stages: preparing ingredients, stripping, washing, size reduction, fermentation, drying, flouring, sifting and packaging (Putri *et al*., 2018). The first step is material preparation. Sorted cassava of the Bokor genotype at the age of 8-12 months was peeled. Then washing process with water at a temperature of 30 ºC. Clean cassava is sliced thinly to form chips with a chip thickness of 0.2-0.3 cm. Furthermore, the cassava chip fermentation process was carried out with three-time variations, namely 15, 24, and 40 h (Table 1), to get the best time to produce quality MOCAF. Fermentation was carried out using a plastic container filled with water, and then 10 g of Bimo CF starter is added to 10 kg of cassava. After fermentation, a soaking process was carried out in 0.3% sodium metabisulfite for 30 minutes to protect against beta-carotene content during the drying process. Before drying, the cassava chips are put into a spinner machine to reduce the water content. Cassava chip drying is done naturally using sunlight. In flouring, cassava chips are ground using a hammer mill. Flour is done until the cassava chips become flour, and then the flour is sifted. Using a 100-mesh sieve to get fine flour quality. MOCAF flour is packaged using aluminum foil standing pouch packaging.

Table 1. Treatment of the fermentation time for the production MOCAF flour

Treatment	Fermentation time (h)	Starter	Drying methods
1	15	Bimo CF	Solar heat
2	24	Bimo CF	Solar heat
3	40	Bimo CF	Solar heat
Control 1	15	-	Solar heat
Control 2	24	-	Solar heat
Control 3	40	-	Solar heat

2.2.2. Viscosity Test

Viscosity test was carried out based on the method of Wukirsari *et al*. (2022). Amount 5 grams of MOCAF flour sample dissolved in 500 mL of distilled water. The solution was heated using a hot plate at 500°C and stirred for 20 minutes to raise the temperature until it reached boiling. The temperature is controlled using a thermometer. When it reaches boiling, the hot plate is turned off, and the gel formed is cooled to 50°C. After that, the viscosity measurement was carried out using a viscometer with spindle number 4.

2.2.3. Solubility Test

Solubility test in this research was carried out using the method used by Hersoelistyorini (2015).

2.2.4. Syneresis Test

In the syneresis test, the freeze-thaw method refers to the study of Putri *et al*. (2018).

2.2.5. Near Infrared (NIR) Analysis

This study used a MicroNIR 2200 spectrometer (JDSU, Santa Rosa, California, USA) with a spectral range of 1150 – 2150 nm. The NIR spectrometer is an ultra-compact handheld spectrometer with physical dimensions of 45-42 mm and a weight of less than 60 g. The spectrometer consists of a linear variable filter (LVL) as the dispersing element, a pair of integrated vacuum tungsten lamps, a mini USB 2.0 as a connector and an uncooled 128-pixel InGaAs (indium Gallium Arsenide) photodiode array. The microNIR spectrometer is small because it is used for LVF as a dispersive element, a dielectric thin-film Fabry-Perot bandpass filter deposited using an energetic process (O'Brien *et al*., 2012). The layer filter in the LVL is intentionally clamped in one direction because the wavelength of the bandpass filter is a function of the thickness of the layer, and the transmitted wavelength will vary continuously along the clamping direction. According to (O'Brien *et al*., 2012), the working principle of this spectrometer is to record data every 8.1 nm with a spectral resolution of about 1.25% of the central wavelength.

Before collecting the spectra from the sample, the white and dark reference spectra are collected to fix the environment and the instrumental effect. Collecting a 99% diffuse reflectance panel obtained the white reference spectrum. Meanwhile, the dark reference spectrum (~0% reflection) is obtained by completely covering the sapphire glass with an opaque cap. In this study, white and dark reference spectra were collected once for every 6 MOCAF samples. When collecting the spectrum from the sample, polyvinyl chloride film was used to cover the test sample to avoid possible contamination of the sampling window. Next, the handheld MicroNIR spectrometer is placed directly over the film for spectral collection. The spectra were collected using the absorbance mode for all samples with an integration time of 10 ms and were set for all spectra. Some noise is observed from the original spectrum; the 5-point SavitzkyeGolay (SG) spectral pre-processing method is applied via the control software to refine the original spectrum. Three positions were selected from each sample petri dish to obtain the spectrum; the average spectrum from the three positions represented each sample to follow the data analysis.

2.2.6. Proximate Analysis

The following are several stages of proximate analysis such as moisture, ash, fat, protein, crude fibre content and dry matter was carried out based on the method from Yusran *et al*. (2022).

2.2.7. Scanning Electron Microscopy (SEM) Analysis

A sputter coating (Hitachi E102 Ion Sputter, Japan) was used to cover the sampled MOCAF flour. An accelerating voltage of 20.0 kV was used to analyze all MOCAF flour samples. A Hitachi S 2400 Scanning Electron Microscopy (Hitachi, Japan) was used to record and analyze the MOCAF flour. Then the image of the MOCAF flour was captured with a magnification of 10,000x (Ying *et al*., 2013). The particle size of MOCAF flour was analyzed using the free Image-J software functioning to process digital images. This was conveyed by researchers in the Java program at the Research Services Branch, National Institute of Mental Health, Bethesda (Maryland, USA) (Collins, 2007).

3. RESULTS AND DISCUSSION

3.1. MOCAF Flour Viscosity

The viscosity test was carried out to determine the viscosity level of the MOCAF flour produced in each treatment. A material that flows easily can be said to have a low viscosity. Meanwhile, materials that are difficult to flow can be grouped into high viscosity (Regina *et al*., 2018). The results of measuring the viscosity values for all samples are presented in Figure 1. From the figure, the viscosity value of MOCAF flour with the addition of Bimo CF starter and at different fermentation times is 1.95 – 2.45 mPa.s, while the control viscosity value is 1.93 – 2.10 mPa.s. If the two data are compared, it can be seen that the viscosity of MOCAF flour with the addition of a Bimo CF starter has a higher viscosity value than the control. This also occurs in the research of (Khasanah *et al*., 2021), which states that the viscosity with the addition of a starter is higher than the viscosity without a starter. This is possible because there is still a lot of amylose in control MOCAF, which has not been broken down into simple sugars by the α-amylase enzyme, so it has a lower viscosity value than MOCAF flour was fermented using the Bimo CF starter.

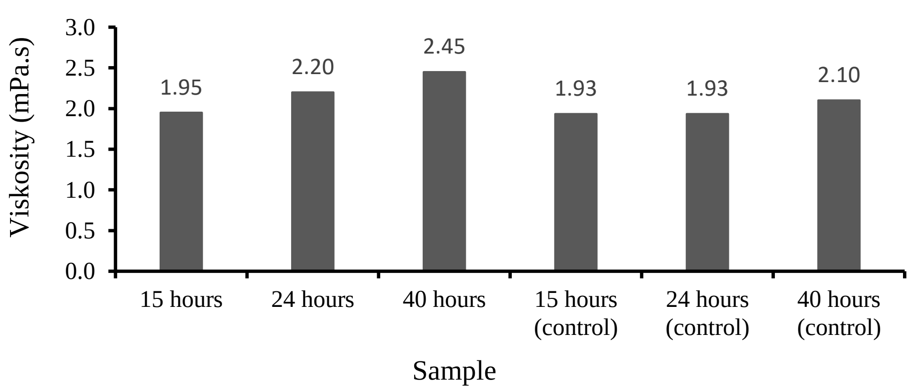


Figure 1. The effect of fermentation time on the viscosity of MOCAF flour

From the obtained data, it can also be seen that the viscosity value is getting higher along with the longer fermentation time. This is the opinion (Widyasaputra & Setyo Yuwono, 2013) that during the fermentation process, there is a behavior of starch granules which increases in initial volume during the fermentation time of 15 hours to 40 hours. This initial increase in volume results in greater swelling during gelatination. This swelling causes the viscosity to increase. Large starch granules tend to have a greater viscosity and faster swelling of the granules. The longer fermentation process will also cause the starch to lose amylose. The less amylose content will make starch have greater amylopectin levels, so the viscosity will also be higher if the amylopectin level is higher.

Aini *et al*. (2009) reported that the length of fermentation is one factor affecting the physical quality of fermented flour. So that the longer the fermentation process, the greater the microbial activity in degrading starch, resulting in an increase in the viscosity value and solubility level. This is comparable to research conducted by (Subagio, 2006) which stated that the increase in the viscosity value of MOCAF flour was affected by cellulotic enzyme activity along with the duration of the fermentation process, and this enzyme activity can change starch granules. The longer the fermentation time, the more intensive cellulotic enzyme activity will degrade the cellulose cell wall, so the cell wall is damaged and the starch granules experience liberation. With the activity of extracellular amylolytic enzymes, the free granules are then partially hydrolyzed on the surface of the granules so that the starch granules are perforated. This allows for the liberation of starch from within the granules, which can change the chemical properties, viscosity and morphology of the resulting starch.

3.2. MOCAF flour solubility

This solubility test was carried out to determine the level of solubility of MOCAF starch with water in each treatment. Solubility is the ability of a material to be absorbed in water so it does not form an emulsion (Hersoelistyorini, 2015). The results of measuring the solubility values for all samples can be seen in Figure 2. The figure showed that the solubility value of MOCAF flour with the addition of Bimo CF starter and at different fermentation times was 1.182 – 1.482%, while the control solubility value was 1.130 – 1.353%. If the two data are compared, it can be seen that the solubility of MOCAF flour with the addition of a Bimo CF starter has a greater solubility value compared to the control. From the data obtained, it can also be seen that the longer the fermentation time, the greater the solubility value. This is consistent with research conducted by (Diniyah *et al*., 2018a), which states that the existence of fermentation can affect the water solubility of MOCAF starch. Cell liberation during the fermentation process will cut the bonds in starch to make its structure simpler. Some of it will also change to its basic structure, namely glucose, so it becomes water-soluble. In addition, the modification can also increase the solubility of starch. The viscosity that increases along with the fermentation time can also occur because microbes that grow during the fermentation process will produce pectinolytic and cellulolytic enzymes that can destroy cassava cell walls resulting in the liberation of starch granules. This liberation process will cause changes in the characteristics of the resulting flour, namely in the form of increased viscosity, gelation ability and increased solubility (Kurniawan, 2010).

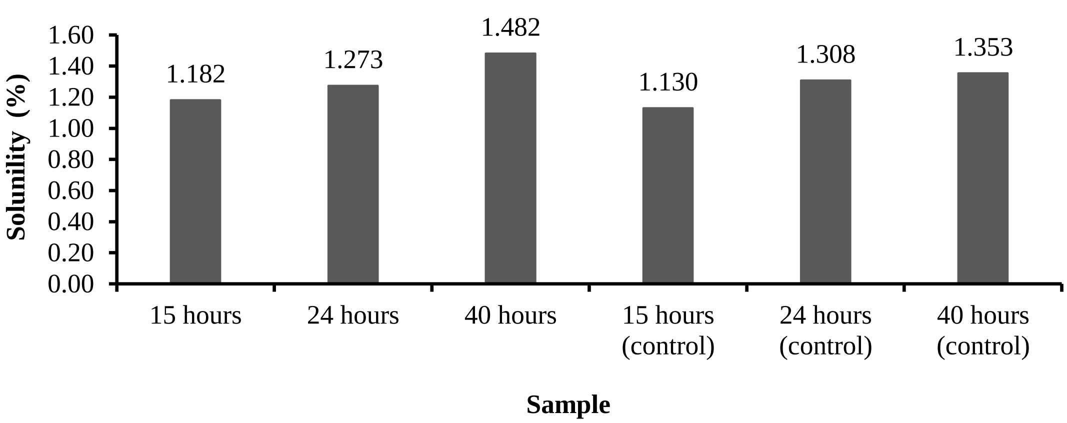


Figure 2. The effect of fermentation time on the solubility of MOCAF flour

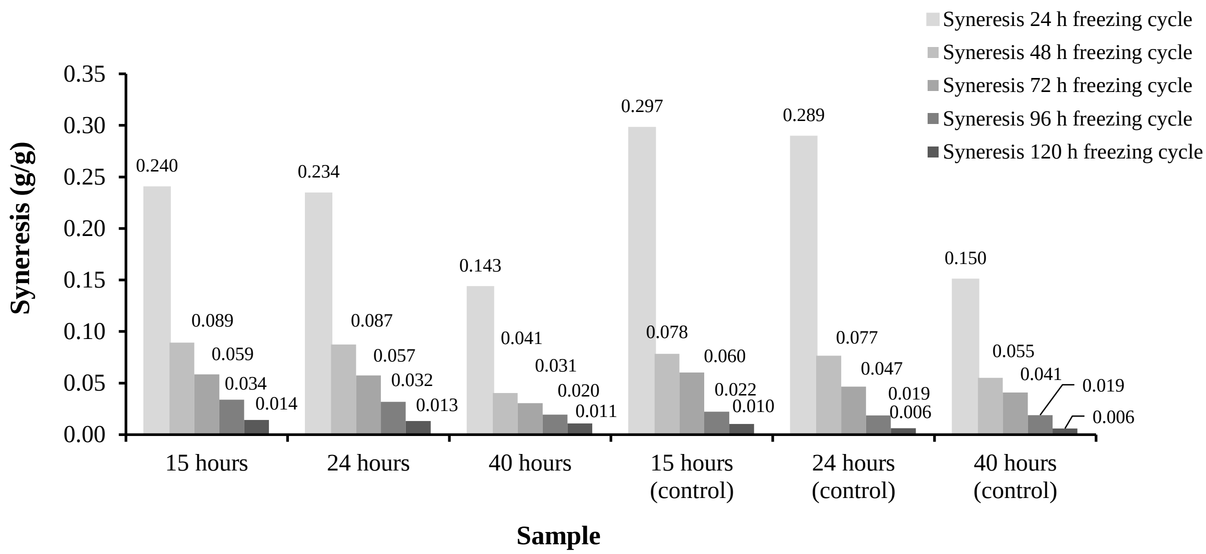


Figure 3. The effect of fermentation time on the syneresis of MOCAF flour

The length of time of fermentation has a significant effect on flour solubility. Aulia *et al*. (2018) reported that the longer the fermentation time, the more starch degradation by microbial activity into simple sugars so that these simple sugars interact more easily with water because some of the amylopectin branch chains are broken due to the hydrolysis process so that solubility increases. The solubility value indicates the ease with which flour can dissolve. A high solubility value indicates that flour dissolves more easily and vice versa.

3.3. Syneresis of MOCAF Flour

This test was carried out to analyze the release of water during the cooling process (separation of water from the gel structure) in MOCAF flour. Syneresis is the release of water from the food where the water is not tightly bound by the components of the existing ingredients (Dipowaseso *et al*., 2018). The results of measuring the syneresis values for all samples can be seen in Figure 3. From this figure, it can be concluded that the syneresis value decreases with the length of fermentation time. This is also directly proportional to the length of time of freezing (storage). The longer the freezing (storage) time, the syneresis value will decrease. This is by research (Putri *et al*., 2018), which shows that the longer the storage process, the less water will come from the starch paste. In syneresis, the lower the syneresis value, the smaller the chances of starch retrogradation occurring, which can affect the shelf life of food products. Amylose has an important role in this case because if the amylose content is less (lower), it can result in fewer opportunities for retrogradation. Conversely, if the amylose content increases, it can result in a greater chance of retrogradation because the amylose will bind again after the gelatinization process at low temperatures (cooling) (Winarno, 2004). In other words, if amylose can bind strongly, then the amount of water that comes out will also increase.

The syneresis value at 15 hours and 24 hours of fermentation was higher than the 40 hours. This is presumably because the amylose content in the sample tends to form a stable gel (Putri *et al*., 2018). This is also comparable to research (Kuncari *et al*., 2014), which states that a high syneresis rate indicates a physically unstable gel. Research conducted by (Dewi *et al*., 2022) also states that syneresis is a condition in starch when water in the gel comes out because the gel shrinks. Hence, it tends to squeeze water out of the gel, and this syneresis value also indicates whether the gel is physically stable. Syneresis testing is important in the manufacture of flour because it determines the quality of the flour to be made later and can affect the shelf life of the flour. A low syneresis value means that the flour has good quality because it shows that the gel is physically stable. However, if the syneresis value is high, it indicates that the gel is unstable, so the flour quality decreases. The best syneresis value is at 40 hours of fermentation because it has the lowest syneresis value, which means the quality of the flour is good.

Table 2. Result Near Infrared (NIR) Analysis of MOCAF Flour

Treatment	Fermentation time (h)	NIR analysis			
		Moisture content (%)	Total fat content (%)	Protein content (%)	Fiber content (%)
1	15	12.95	1.77	3.87	2.21
2	24	13.01	1.63	3.72	2.21
3	40	13.71	1.59	3.94	2.28
Control 1	15	13.39	1.69	4.10	2.46
Control 2	24	13.07	2.10	3.96	2.56
Control 3	40	13.81	1.52	4.04	2.67

3.4. Near Infrared (NIR)

Based on the NIR analysis (Table 2), there are several ingredients in MOCAF flour, including water, fat, protein and fiber. NIR test results on MOCAF flour showed the value of moisture content in MOCAF flour with the addition of a Bimo CF starter and at different fermentation times, namely 12.97 - 13.71%. At the same time, the moisture content value in MOCAF flour for the control (without adding a starter) at 15 hours of fermentation is 13.39%. The water content at 24 hours of fermentation was 13.71%, and at 40 hours of fermentation was 13.81%. Based on the data obtained, it can be concluded that the longer the fermentation time, the greater the moisture content in MOCAf flour. The low water content in both starter and control samples at the 15 and 24-hour fermentation time was due to the fermentation process, which could degrade starch by microorganisms, causing a decrease in the material's ability to retain water. The longer the fermentation time, the activity of enzymes in degrading starch will increase so that more bound water is released and causes the material's texture to become porous and soft. This condition can increase water evaporation during the drying process so that the water content decreases during the same drying period (Widhiastiti *et al*., 2022). Whereas at the 40-hour fermentation time, in both the sample with starter and the control sample, the water content value was quite high; this was due to the possibility that the drying process was not optimal because it was carried out in an open area and also the weather was not conducive during the drying process.

Overall it can be seen that the length of fermentation time affects the flour's moisture content. Along with the length of time of fermentation, the water content tends to increase. This is because the length of fermentation is related to the growth phase of microbes, where the longer the fermentation time means, the more opportunities for microbes to grow and develop, including microorganisms that use carbohydrates as an energy source and then produce water molecules and carbon dioxide. Two types of water are produced, namely water that comes out of the material and water that remains in the material. The water in the material causes the resulting water content to be higher. Therefore, along with the time of fermentation, the water content also increases (Sari *et al*., 2016; Kasmiran, 2011).

The fat content in MOCAF flour with fermentation treatment for 15 hours using the Bimo CF starter is 1.77%. The fat content value at 24 hours of fermentation was 1.63%, and the value of fat content at 40 hours of fermentation was 1.59%. Meanwhile, the value of fat content in MOCAF flour for control (without adding a starter) at 15 hours of fermentation was 1.69%. The fat content at 24 hours of fermentation was 2.10%, and at 40 hours of fermentation was 1.52%. Based on the data obtained, it can be concluded that the longer the fermentation time, the lower the fat content. This is due to the fermentation process, which causes a decrease in the fat content in MOCAF. This statement is the opinion of (Tandrianto & Mintoko, 2014).

Along with the length of the fermentation time, the fat content tends to decrease, which means that the length of the fermentation time affects the fat content of the flour produced. This was also conveyed by (Aisah *et al*., 2021). A decrease in fat content can occur from the overhaul of fatty acids in cassava caused by the microbes' secretion. Research by (Diniyah *et al*., 2018b) found that a decrease in fat content is possible because, during the fermentation process, microbes need energy obtained from fat. So the longer microbial fermentation time requires more and more energy. Therefore, the fat content decreased with the length of fermentation time. Lactic acid is the main product of carbohydrate metabolism by LAB, but its contribution to lipolysis is still there, even in small amounts.

The protein content in MOCAF flour with fermentation treatment for 15 hours using the Bimo CF starter is 3.87%. The value of protein content at 24 hours of fermentation was 3.72%, and protein content at 40 hours of fermentation was 3.94%. At the same time, the value of protein content in MOCAF flour for the control (without adding a starter) at 15 hours of fermentation is 4.1%. The protein content at 24 hours of fermentation was 3.96%, and at 40 hours of fermentation was 4.04%. Based on the data obtained, it can be concluded that the lowest protein content was found at 24 hours of fermentation, both with starter and control, while the highest protein content was found at 40 hours of fermentation, both samples with starter and control. The high protein content produced is also because, during the fermentation process, the protein can also increase along with the increasing mass of the microorganism cells that grow during the fermentation process to increase the protein content of the MOCAF flour produced (Hidayat *et al*., 2009). This is different from the results of the analysis on the proximate test because the proximate analysis and NIR analysis use different analytical method principles to determine different protein levels.

Meanwhile, the low protein content during the 24-h and 40-h fermentation, both with starter and control, was because the fermentation process was carried out using water as the medium (wet fermentation) so that soaking cassava in water can reduce protein levels (methionine acid) due to the type of protein contained in the fermented cassava. Cassava can dissolve in water. The source of protein found in cassava is the amino acid methionine (Badriani *et al*., 2020).

The fiber content in MOCAF flour with fermentation treatment for 15 h using the Bimo CF starter is 2.21%. The fiber content at 24 h of fermentation is 2.21%, and the fiber content at 40 h of fermentation is 2.28%. At the same time, the value of MOCAF flour fiber content for the control (without the addition of a starter) at 15 hours of fermentation is 2.46%. The fiber content at 24 h of fermentation was 2.56%, and at 40 hours of fermentation was 2.67%. Based on the data obtained, it can be concluded that the fiber content during the fermentation process tends to be constant or there is no significant effect. This is to research conducted by (Tandrianto & Mintoko, 2014), which states that fiber content does not change significantly because microbes in the fermentation process only use fiber as a nutritional composition in their growth. There was a slight increase in fiber content, probably because the microbes present during fermentation did not easily degrade hemicellulose and cellulose when breaking down the cassava cell walls. Hence, the hemicellulose and cellulose content increased. This causes fiber levels to increase (Badriani *et al*., 2020).

3.5. Proximate Analysis

Based on the results of the proximate test (Table 3), it shows that the dry matter value varies at each fermentation time. The highest dry matter was at the 15 h fermentation time of 90.56% and the lowest at the 24 h fermentation time was at 89.82%. While the dry matter value in the control ranged from 90.04 – 89.17%. From the results obtained, it can be concluded that the longer the fermentation time, the MOCAF dry matter tends to decrease. This is because the large amount of water that comes out during the fermentation process can result in a decrease in the dry matter content in the substrate. In accordance with the opinion (Kasmiran, 2011) that the longer the fermentation time the lower the dry matter content because during the fermentation process the growth of microorganisms uses carbohydrates as an energy source and produces water molecules and carbon dioxide. Most of the water will remain in the product and some will escape from the product. The water left in the product causes the water content to be large and the dry matter to be small.

Table 3. Result proximate analysis of MOCAF flour

Treatment	Fermentation time (h)	Parameter					
		Raw materials (%)	Moisture content (%)	Ash content (%)	Total protein (%)	Total Fat (%)	Crude fiber (%)
1	15	90.56	9.45	0.76	1.04	0.51	1.46
2	24	89.82	10.19	0.65	0.87	0.79	1.50
3	40	89.90	10.10	0.60	0.78	0.45	1.29
control 1	15	90.04	9.96	1.12	1.21	0.67	1.41
control 2	24	89.13	10.87	0.68	0.94	0.72	1.35
control 3	40	89.17	10.83	0.88	0.78	0.61	1.25

Fermentation time is one of the factors that must be considered. The length of time of fermentation has a significant effect on the dry matter value of the sample. The short fermentation time results in limited opportunities for microorganisms to continue to grow, so the substrate components that can be broken down into cell mass will also be small, but a longer fermentation time means providing opportunities for microorganisms to grow and multiply (Fardiaz, 1992). Then (Rahayu, 1990) added that the longer the fermentation time, the more substances are broken down by enzymes, such as dry and organic matter.

The proximate analysis results showed that the moisture content of MOCAF flour ranged from 9.45 – 10.19%. The highest water content in the 24-h fermentation time was 10.19%, and the lowest in the 15-h fermentation was 9.45%. At the same time, the water content in the control ranged from 9.96 – 10.87%. The water content obtained from the research results meets the quality standards of MOCAF flour according to SNI 7622-2011, where the water content is below 13%. The low water content during the 15 and 40-h fermentation was due to the cassava chips being fermented using BAL, resulting in the breakdown of the cell walls and the overhaul of the starch granules, which caused the water holding capacity to decrease and caused the water to evaporate freely during the flour drying process (Diniyah *et al*., 2018a,b).

Meanwhile, when viewed as a whole, the longer the fermentation time, the higher the moisture content of MOCAF flour. This relates to the explanation of dry matter by (Kasmiran, 2011), where during the fermentation process, the growth of microorganisms uses carbohydrates as an energy source and produces water molecules and carbon dioxide. There is water that comes out and also water that remains in the material. The water in the material causes the resulting water content to be higher than the dry matter content. The length of time of fermentation affects the moisture content of the resulting flour. This was also proven by the results of the NIR analysis (Table 2), where there was an increase in water content along with the fermentation time. The increase in water content is thought to be caused by two things, namely the first is that the drying process is not optimal because it is done in an open space with unfavorable sunlight. The second is because the fermentation process uses water as a medium (wet fermentation). Along with the duration, the fermentation time of soaking cassava in water is getting longer, and the growth of microorganisms is also increasing. More and more and more water that remains in the ingredients results in high water content in the flour. (Kasmiran, 2011; Badriani *et al*., 2020). When compared with the results of the NIR analysis (Table 2), the water content in the NIR analysis results is directly proportional to the water content in the proximate analysis results, where the water content both increases with the length of fermentation time.

The ash content is the mineral elements that remain after the material is burned until it is carbon-free. Ash content can also be defined as a non-volatile component that persists during combustion (Widhiastiti *et al*., 2022). The proximate analysis results showed that the ash content of MOCAF flour ranged from 0.6 to 0.76%. The lowest ash content was in the 40-hour fermentation time treatment of 0.6%, and the highest in the 15-hour fermentation time treatment was 0.76%. The control treatment had the lowest ash content, namely the 24-hour fermentation time treatment of 0.68% and the highest in the 15-hour fermentation time treatment of 1.12%. The ash content obtained from the research results has met the quality standards of MOCAF flour according to SNI 7622-2011, where the ash content is below 1.5%. Based on the results obtained, the ash content tends to decrease with the length of fermentation time. This is due to the effect of soaking during fermentation. The minerals originally bound in the material matrix dissolve in the fermentation liquid when the granules are overhauled (Diniyah *et al*., 2018a,b). In addition, in the opinion of (Herman et al., 2011), the ash content indicates the number of minerals contained in the food, so it can be concluded that the resulting MOCAF flour has a fairly high mineral content. The high ash content in MOCAF is due to the presence of mineral content in cassava which is used as a raw material for making MOCAF which is quite high.

The length of fermentation affects the ash content of the flour. This is comparable to research (Kasmiran, 2011), along with the longer the fermentation time, the lower the ash content in the flour. This is due to the dissolving of the mineral contents in the sample, whereas in the ash, there are some mineral contents. Fermented cassava can cause some minerals to dissolve, reducing the ash content because ash consists of several mineral contents.

The proximate analysis results on MOCAF showed that the highest crude protein value was found in the 15-hour fermentation time treatment, which was 1.04%, and the lowest protein value was in the 40-hour fermentation time, which was 0.78%. This also occurred in the control, where the highest crude protein value was found in the 15-hour fermentation time treatment, 1.21%, and the lowest crude protein value at 40 hours, 0.78%. Based on the results obtained, it can be concluded that the longer the fermentation time, the lower the crude protein value. Badriani *et al*. (2020) found that the low crude protein content is due to the fermentation process in the manufacture of MOCAF flour. The fermentation that takes place is wet, using water as the medium. Soaking cassava in water can reduce protein levels (amino acid methionine) in MOCAF because the type of protein contained in cassava can dissolve in water.

When compared with the results of the NIR analysis (Table 2), the protein content in the NIR analysis results is inversely proportional to the protein content in the proximate analysis results. In the NIR analysis results, the protein content tends to increase with the length of the fermentation time. In contrast, in the proximate analysis results, the protein content decreases with the length of the fermentation time. It can be seen that the length of time of fermentation can have a different effect on the samples produced. Protein levels also increase along with the length of fermentation time, this is by the opinion of (Hidayat *et al*., 2009) states that along with the length of time of fermentation, protein can also increase along with an increase in the mass of microorganisms cells that grow during the fermentation process. This takes place to increase the protein content of the resulting MOCAF flour. In addition, a decrease in protein levels is comparable to research results (Mirzah & Muis, 2015). The longer the fermentation time, the bacterial growth will reach a stationary phase, a phase where the growth rate will decrease. If it reaches the stationary phase, it results in a reduced nutrient supply, resulting in the accumulation of metabolic substances that inhibit growth. The growth rate will continue to decrease until the value equals zero (the number of cells that grow equals the number of cells that die). The total cell mass will be constant, and the number of living cells will decrease due to lysis, so the cell mass will continue to decrease. Microorganisms need nutrients during the fermentation process. The nutrients usually used as carbon sources are carbohydrates, fats, and proteins. So that the length of time for protein fermentation can decrease because it is used as a nutrient for microbial metabolism (Puspadewi *et al*., 2011; Khasanah *et al*., 2021).

The crude fat content in the proximate analysis results of MOCAF flour ranges from 0.45 – 0.79%. The highest crude fat content was in the 24-hour fermentation time treatment of 0.79, and the lowest was in the 40-hour treatment of 0.45%. At the same time, the crude fat content of the control ranged from 0.61 – 0.72%, with the highest crude fat content at 24 hours of fermentation of 0.72% and the lowest crude fat content at 40 hours of fermentation of 0.61%. Overall crude fat content values vary from one to another. Fat content decreases because the microbial fermentation process requires energy obtained from fat (Diniyah *et al*., 2018b). Therefore, the fat decreases along with the fermentation time.

This was also proven by the results of NIR analysis (Table 2), where there was a decrease in fat content along with the time of fermentation. The decrease in fat content is thought to be because some of the fat is used for microbial metabolism, which plays a role during the MOCAF fermentation process. Fats, carbohydrates and proteins are nutrients often used for microbial metabolism. So that along with the length of time for fat fermentation will decrease because it is used as a nutrient for metabolism (Puspadewi *et al*., 2011; Khasanah *et al*., 2021). Compared with the results of the NIR analysis (Table 2), the fat content in the NIR analysis results is directly proportional to the fat content in the proximate analysis results, where the fat content decreased with the length of fermentation time. It can be concluded that the length of time of fermentation affects the fat content in the resulting flour.

The crude fiber in MOCAF flour produces values ranging from 1.29 – 1.50%. The results showed that the lowest crude fiber value was obtained at the 40-hour fermentation time of 1.29%, and the highest was obtained at the 24-hour fermentation time of 1.50%. At the same time, the control of crude fiber MOCAF flour ranges from 1.25 – 1.41%, with the lowest crude fiber value obtained in the 40-hour fermentation treatment equal to 1.25% and the highest crude fiber obtained in the 15-hour fermentation time treatment that is equal to 1.41 %. The crude fiber obtained from the research results has met the quality standards of MOCAF flour according to SNI 7622-2011, where the crude fiber value is below 2%. The low crude fiber is because LAB in the fermentation process produces cellulolytic enzymes that can break down cell walls and use fiber components as carbon sources in their metabolism so that the crude fiber in MOCAF becomes lower (Diniyah *et al*., 2018a,b). At the same time, the high crude fiber may occur at 24 hours of treatment because the fermentation time is still too short, different from MOCAF at 40 hours of fermentation. The short fermentation time can result in hemicellulose and cellulose not being easily degraded when the cassava cell wall is broken, so the hemicellulose and cellulose content will increase. The increased levels of hemicellulose and cellulose during this fermentation process can increase crude fiber levels in MOCAF (Badriani *et al*., 2020).

Compared with the results of the NIR analysis (Table 2), the fiber content in the results of the NIR analysis tends to be constant, only experiencing a slight increase. At the same time, the results of the proximate analysis of fiber content tend to decrease. It can be seen that the length of time of fermentation can have a different effect on the samples produced. This difference can occur because, in samples with decreased fiber content, the nutrients available in the material have been broken down and utilized by microbes. This was also conveyed (Kasmiran, 2011) that microbial growth is closely related to the length of fermentation time. The longer the fermentation, the better, more even and compact the microbial growth will be to the availability of nutrients in the ingredients. Microbes that grow more actively make changes to the material. Laksin & Hubert (1973) said that the microbial population greatly determines the quality of the final product.

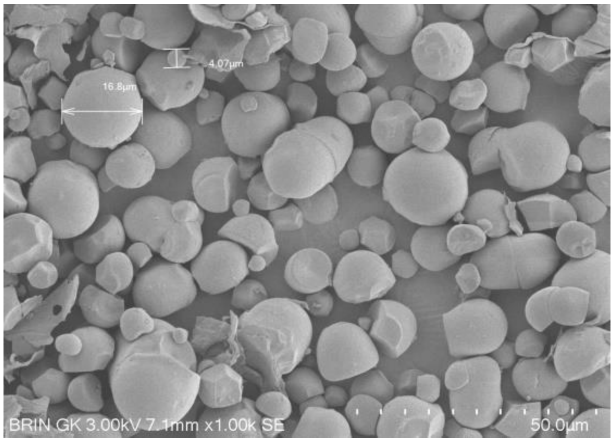
In contrast, the length of time for fermentation, the microbial population will be higher and produce a higher amount of enzymes so that the quantity of crude fiber broken down by enzymes is also higher. The longer the fermentation time, the more nutrients are hydrolyzed and digested (Kasmiran, 2011). Whereas for samples that experienced an increase in fiber content, it was comparable to the opinion of (Ginting & Krisnan, 2006), which stated that the increase in fiber content occurred because, along with the length of time of fermentation, microbial development consistently increased according to the fermentation period, which could contribute crude fiber through the cell walls. In addition, the longer the fermentation time, the higher the crude fiber content in the substrate. The presence of this coarse fiber causes the MOCAF particle grains to have different sizes and resulting in MOCAF being less fine (Sulistiadi *et al*., 2021).

Differences in moisture, fat, protein and fiber content between the results of the NIR and proximate tests are due to the different principles of the NIR analysis method from the principles of the proximate analysis method. The principle of the NIR analysis method in knowing the sample's chemical composition is by measuring the spectrum of the sample (Lengkey *et al*., 2013). NIR technology is also a non-destructive method that can analyze the chemical content of a material at high speed, does not cause pollution and does not require additional chemicals (Karlinasari *et al*., 2012). In contrast, the principle of the proximate analysis method is to separate food components into groups or fractions of food value, including moisture, ash, protein, fat, and carbohydrate (Muza'ki *et al*., 2022). Proximate analysis methods include moisture content using the oven method, ash content using the dry ashing method, fat content using the Soxhlet method, and protein content using the Kjeldahl method (Yusran *et al*., 2022). Some methods of proximate analysis also use the addition of chemicals. This difference in the principle of the analysis method causes the difference in the results of the NIR and Proximate analysis.

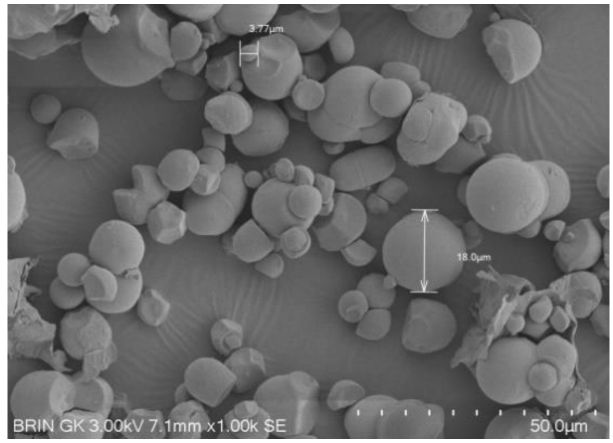
3.6. Scanning Electron Microscopy (SEM) Analysis

SEM is a microstructural test performed to analyze the shape and surface of the particles in the sample. Scanning Electron Microscopy (SEM) is an electron microscope with high magnification that can image surfaces on materials (Kustomo, 2020). The results of the SEM test for each sample conducted at the Gunung Kidul National Agency for Research and Innovation can be seen in **Figure 4**. Based on the picture above, it can be seen that the smallest starch granule size ranges from 3.77 µm – 4.67 µm and the largest starch granule size ranges from 16.8 µm – 18 µm. In sample A, the sample that was fermented using the Bimo CF starter for 15 hours, changes began to occur in the starch granules where the starch granules had started to separate, were not too clustered and started to have a few holes in some parts, of the granules. In sample B, the sample that was fermented using the Bimo CF starter for 24 hours. The starch granules had more holes so that the shape looked irregular, and the size of the starch granules also got bigger. In sample C, the sample was fermented using the Bimo CF starter for 40 hours. The starch granules had increasingly irregular shapes and sizes that were increasingly non-uniform or inhomogeneous. Whereas in the control samples, namely D, E and F, it can be seen that the size of the smallest starch granules ranged from 3.87 µm – 4.96 µm and the largest starch granule sizes ranged from 14.4 µm – 17.1 µm. The results of the SEM test on the control sample showed that there were still many starch granules clustered or bound, not too many holes and many of which were regularly round.

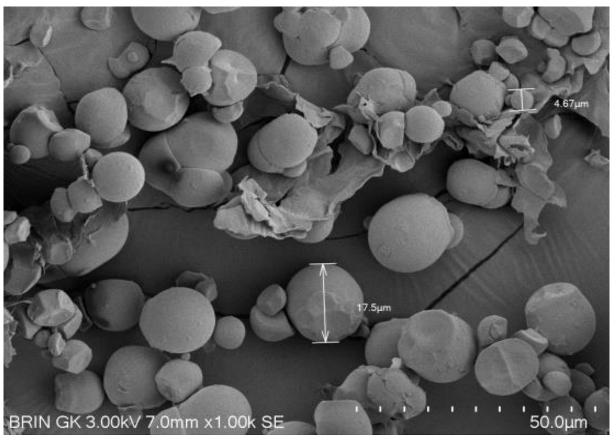
Changes in starch granules in samples A, B and C occurred due to cellulosic enzyme activity, which began to intensively degrade cellulose cell walls, resulting in damaged cell walls and granularity undergoing liberation. Then the activity of extracellular amylolytic enzymes resulted in partially hydrolyzed free starch granules on the surface of the granules so that the starch granules became perforated (Subagio, 2006). The results of SEM photos on MOCAF at 15 hours, 24 hours and 40 hours of fermentation showed structural changes on the surface of the starch granules or holes resulting



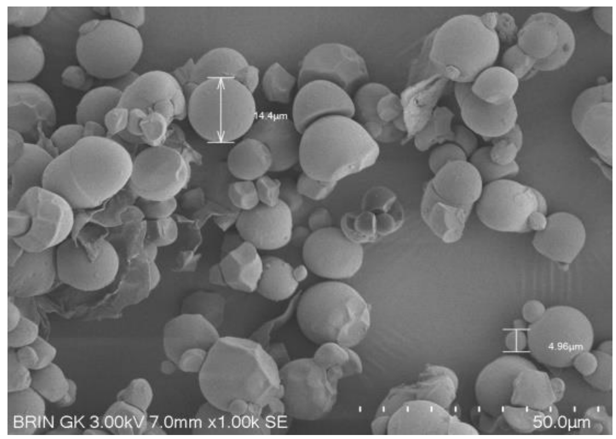
(A)



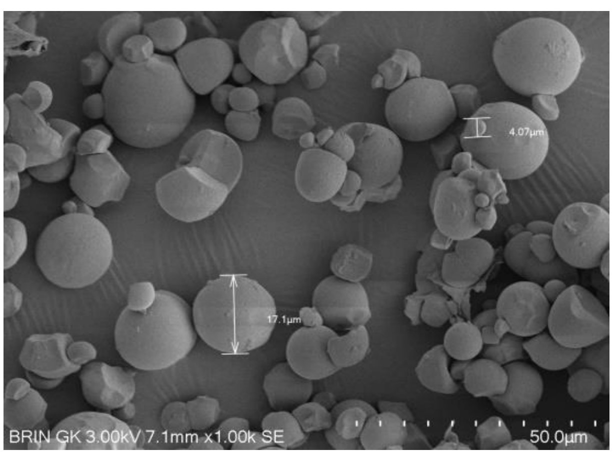
(B)



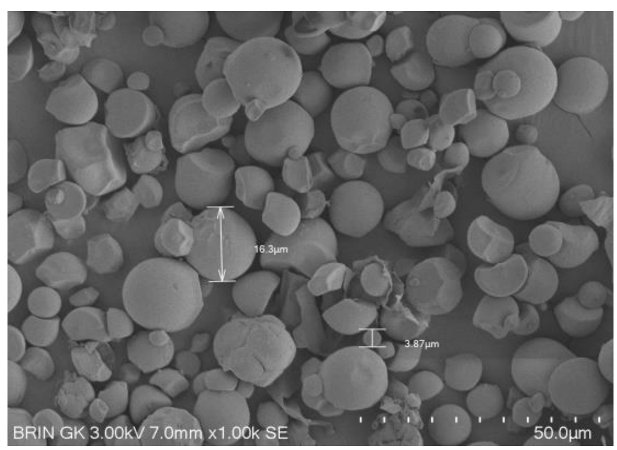
(C)



(D)



(E)



(F)

Figure 4. Result of SEM analysis of MOCAF flour dengan 10.000 perbesaran (A: MOCAF flour was fermented using the Bimo CF starter for 15 hours, B: MOCAF flour was fermented using the Bimo CF starter for 24 hours, C: MOCAF flour was fermented using the Bimo CF starter for 40 hours, D, E and F was the control sample)

from the fermentation process. It can be seen in Figure 4 that the 40-hour fermentation time produces more starch holes than 15 hours. This is possible because the 40-hour fermentation time is when microbes (Suhery *et al*., 2013). The holes that occur during the fermentation process are caused by an enzymatic attack from the enzymes produced during the fermentation process (Putri *et al*., 2018).

4. CONCLUSIONS

The length of fermentation affects the physicochemical properties of MOCAF flour, namely increasing viscosity, solubility and decreasing syneresis values. The chemical composition, such as moisture content, fat content, protein content and fiber content in MOCAF flour, can be predicted by the NIR (Near Infrared) test. Based on the results of the NIR analysis, it is known that there is an effect of the length of fermentation time on the chemical composition of MOCAF where at 40 hours (the last time of fermentation), there is a decrease in fat content with a value of 1.59%, an increase in water, protein and fiber content with a value of 13.71% respectively, 3.94% and 2.28%. In the proximate analysis, the results showed that there was an effect of the length of time of fermentation on the nutrient content, namely at 40 hours, there was an increase in water content with a value of 10.10%, a decrease in dry matter, ash, protein, fat, fiber with a value of 89.90%, 0.60 respectively %, 0.78%, 0.45%, and 1.29%. Based on the results of the Scanning Electron Microscopy (SEM) test, it is known that the length of fermentation time affects the morphological properties of MOCAF starch granules. The longer the fermentation time, the more starch granules get damaged.

AUTHOR CONTRIBUTIONS

Erfa Kurnia Prastiwi, Rois Fatoni, Ahmad Fathoni, R. Haryo Bimo Setiarto are main contributors for this original research paper manuscript. Ema Damayanti is member contributor for this manuscript.

CONFLICT OF INTEREST

There is no conflict of interest for writing this manuscript.

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