The Effect of Variation of Types of Microbial Starters on The Physical Characteristics of Modified Cassava Flour (Mocaf) Rich in Beta-Carotene

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ABSTRACT

The development of beta-carotene-rich mocaf flour as a functional food aims to increase its nutritional content and functional properties as an antioxidant for health. The development of beta-carotene-rich mocaf flour can be optimized through good fermentation methods, especially with the use of starter. This study aims to further analyze the effect of using a type of starter on the mocaf characteristics of cassava species rich in beta-carotene through a comparison of several types of starter. In this study, three types of starter were used, namely the Bimo-CF starter, the *Berhasil* starter and thse BRIN starter. The results showed that the use of starter during fermentation affected the physicochemical properties of mocaf flour in the form of differences in solubility, increased viscosity and decreased syneresis. Scanning Electron Microscope (SEM) was used to analyze the microstrutural of mocaf flour using starter during fermentation resulted in a more massive reshuffling of starch granules and causes changes in the structure of starch granules that become irregular round in shape with increasingly non-uniform sizes. NIR (Near infrared) and proximate analysis of the content of mocaf flour, showed that the use of the Bimo-CF starter, the *Berhasil* starter and the BRIN starter during fermentation affected the decrease in moisture content, protein content, ash content, fiber content, phosphorus, and increased fat and dry matter content.

Keywords: beta carotene; type of microbial starter; NIR (Near-infrared); proximate; SEM (Scanning Electron Microscope); mocaf flour

INTRODUCTION

Cassava is one of the root crops from the food crop sub-sector, which generally grows in the tropics, including Indonesia. Cassava is an energy source that contains many carbohydrates and is one of Indonesia's most widely grown food crops because of its ease of cultivation. Cassava has broad adaptability and a good ability to grow in various soil conditions, which makes cultivation opportunities quite good (Sudarmonowati et al., 2020). Based on data from the Indonesian Directorate General of Food Crops (2022), the realization rate of cassava cultivation production capacity in Indonesia reaches 99.82% of the initial target,

with a production achievement of around 14.95 million tons. With this large production capacity, cassava is important in maintaining food security.

Cassava is an alternative food ingredient that stores food reserves in the roots that can be consumed and diversified into various processed food products. Efforts to utilize cassava to support food diversification, among others, can be developed into food products as a source of carbohydrates through the development of technology for making modified cassava flour (mocaf) as a substitute for wheat flour which has great potential in the manufacture of various processed food products, such as cookies, bread and noodles (Hadistio and Fitri, 2019). Processing cassava into mocaf flour aims to modify the characteristics of the flour produced through fermentation (Seveline et al., 2020). During fermentation, cassava's starch content will undergo physical, chemical and microbiological changes (Asmoro, 2021). One of the weaknesses of mocaf flour is its low antioxidant content, which antioxidants are very important for body health because of its activity in protecting cells from damage caused by free radicals (Rahman et al., 2020; Arina et al., 2021). The development of mocaf flour in foodstuffs can be carried out through functional food innovations that are applied as mocaf flour rich in beta-carotene. Beta-carotene is included in the carotenoid group, acting as provitamin A and an antioxidant (Purwanti et al., 2019). Several studies on beta-carotene content in cassava have described the types of cassava containing beta-carotene found in yellow-fleshed cassava varieties where yellow-fleshed cassava variants with deeper colour intensity having higher carotene content (Moorthy et al., 1990; Ayetigbo et al., 2018). The yellow-fleshed cassava varieties are indicated as a source of vegetable provitamin A in the form of beta-carotene which can act as an antioxidant (Rahman et al., 2020).

Starter is an additional ingredient used in the early stages of the fermentation process. The starter can be a microbial culture grown on a substrate or media. The development of beta-carotene-rich mocaf flour can be optimized by selecting a good type of starter. The selection of a starter is an important criterion for the bioprocess of making mocaf to ensure that microorganisms efficiently use cassava as a growth substrate so that mocaf is obtained with good quality and by quality requirements. Starter performance is influenced by several environmental factors that affect microbial growth, such as substrate, temperature and pH (Kusumaningati et al., 2013). In the mocaf fermentation process, a starter is used to ferment cassava in the form of chips with a faster fermentation time (Hadistio and Fitri, 2019). Enzyme and microbial activity in the starter produce lactic acid and organic acids, which ultimately affect the characteristics of mocaf in terms of structure, physicochemical properties and the scent of flour after fermentation (Astuti and Setyawati, 2016).

Novitasari and Arief (2018), reported that using a starter in mocaf fermentation made from cassava casessart variety resulted in a moisture content value of 8.10%, ash 1.44% wb, protein 1.11%, fat 0.73%, crude fibre 0.90%, and carbohydrates 87.69%. Meanwhile, (Hersoelistyorini et al., 2015) reported that using a starter in fermentation using cassava of the Indu variety from the Sumowono area, Semarang Regency produced swelling power and solubility values of 13.05 (g/g) and 1.43 respectively %. (Hartati et al., 2017) reported that using a 10-gram BIMO-CF starter to ferment 10 kg of yellow cassava with 60 grams of sodium metabisulfite before drying in the sun produced a beta-carotene value of 2.65 mg/Kg containing protein reached 1.33%. Meanwhile, (Fathoni et al., 2016) reported that in mocaf flour made from yellow cassava in the same variety with the addition of 0.3% sodium bisulphite at a drying temperature of 40°C-50°C, the beta-carotene content was 9.85 mg/Kg and 9.44 mg/Kg with the resulting protein content reaching

2.02% and 2.41%. In addition to cassava varieties, fermented types of treatment especially using a starter, can produce different characteristics of mocaf flour. This study aims to analyze further the effect of using a type of starter on the characteristics of the mocaf of cassava species rich in beta-carotene to obtain optimal mocaf fermentation results by comparing several types of starter.

METHODOLOGY

Materials

This study used samples of cassava genotype Bokor aged 8-12 months from Boyolali, Central Java. Three types of starter were used for the fermentation process: the Bimo-CF starter, the *Berhasil* starter and the BRIN starter. The Bimo-CF starter is a commercial starter that contains active ingredients of various lactic acid bacteria microbes which are safe for food and enriched with nutrients. The *Berhasil* Starter is a industrial starter made by PT. Tepung Mocaf Solusindo, is a certain starter/enzyme that is added to the fermentation process. The BRIN starter is a starter produced from research that has been carried out by the National Research and Innovation Agency (BRIN) containing lactic acid bacteria (*Lactobacillus plantarum* and *Pediococcus pentosaceus*) and other additives. Then, as an ingredient for post-fermentation soaking, 0.3% sodium metabisulfite was used to protect the beta-carotene content of the sample before entering the drying process.

The equipment used is a scale (CAS), thermometer (Taffware), pH meter (Hanna HI-98103), some of the production equipment from PT. Tepung mocaf Solusindo such as knife, slicer, fermentation tub, hammer mill, spinner, low-density polyethylene (LDPE) aluminum packaging and equipment for analysis. This research was conducted at PT. Tepung Mocaf Solusindo, Surakarta, Central Java. Meanwhile, analysis of the physicochemical properties of mocaf flour at the UMS Chemical Engineering Laboratory and flour quality at the Research Center for Food Process and Technology BRIN Gunungkidul, Yogyakarta and the Research Center for Applied Microbiology (PRMT) BRIN Bogor, West Java.

Methods

Production of mocaf flour rich in beta-carotene

The sorted cassava of the Bokor genotype at the age of 8-12 months was separated from the skin by peeling and washing thoroughly using water and then thinly sliced using a slicer to form chips. To carry out the fermentation process, the chips are soaked in water for 24 hours at ambient temperature (30°C) with the addition of a starter according to a predetermined design (Table 1) with 2 repetitions for each treatment. Next, the chips were immersed in water containing 0.3% sodium metabisulfite solution for 30 minutes The chips are drained and then put into a spinner to reduce the moisture content before the drying process is carried out. In the drying process, the chips are naturally dried using sunlight. After that, the chips were ground using a hammer mill and then sieved using a 100-mesh sieve. After the flouring process, mocaf flour is packaged in aluminium (LDPE) packaging.

Table 1. Design of fermentation starter treatment for mocaf flour production

Treatment	Inoculum starter
1	*Berhasil*
2	Bimo CF
3	BRIN
Control	-

Viscosity Analysis

Viscosity analysis was carried out according to (Wukirsari et al., 2022) with modifications. In the viscosity test, 5 grams of the sample was dissolved in 500 mL of distilled water and then heated for 20 minutes while stirring. After that, the solution was cooled to 50°C, and the viscosity was measured using a Viscotester Rion VT-03F with spindle number 4. The viscosity value is expressed in millipascal seconds (mPa.s).

Solubility Analysis

Solubility analysis was carried out using the Kainuma et al., (1967) in (Hersoelistyorini et al., 2015). One gram of sample was dissolved in 10 mL of distilled water and then heated in a water bath at 60°C for 30 minutes. After that, the suspension was cooled in to 30°C, and then the supernatant was separated from the paste formed using a centrifuge at 3000 rpm for 20 minutes. 5 mL of the supernatant was taken and then dried in an oven at 100°C until the weight was constant and the dry residue was recorded. The solubility value is calculated using the formula:

$$\% \text{ Solubility} = \frac{\text{weight of dry sediment}}{\text{supernatant volume}}$$

Syneresis Analysis

Syneresis testing was carried out using the freeze-thaw method was carried out according to (Putri et al., 2018) with modifications. A sample of 5 grams was dissolved in 100 mL of distilled water and then heated at 100°C for 20 minutes. After that, the sample was cooled in to 30°C. Furthermore, the samples were stored in the freezer, frozen for 24 hours, and thawed at room temperature. The paste and water that came out were separated using a centrifuge at 3500 rpm for 15 minutes. The water that comes out is weighed, and the sample is frozen under the same conditions for up to five times freezing. The syneresis value is calculated using the formula:

Syneresis (g/g)

$$= \frac{\text{weight of the water comes out}}{\text{weight of initial pasta}}$$

Near-Infrared (NIR) Analysis

NIR analysis was performed on all mocaf flour samples using a MicroNIR 2200 spectrometer (JDSU, Santa Rosa, CA, USA) in the spectral range 1150–2150 nm, referencing the study of Tao et al. (2021).

Proximate Analysis

The proximate analysis for mocaf flour, such as moisture, ash, fat, protein, crude fibre content, and dry matter carried out based on the method from AOAC (2010).

Scanning Electron Microscope Analysis

Microstructural analysis of all mocaf flour samples was performed using a Hitachi S 2400 Scanning Electron Microscope (SEM) (Hitachi, Tokyo, Japan) referring to the method of Setiarto et al. (2020). Digital image processing and mocaf particle sizes were analyzed with Image-J software developed by the Research Services Branch, National Institute of Mental Health, Bethesda (Maryland, USA States) (Collins, 2007).

Data Analysis

ANOVA analysis was performed to determine if there were any differences in the variables tested in this case: viscosity, solubility, synthesis, near-infrared (NIR) and instant analysis. The test was performed in triplicate at 95% significance level ($\alpha = 5\%$) with Duncan's additional test (Multiple Range Test). This analysis of variance used SPSS (Social Science Statistical Package) software version 26.00.

RESULTS AND DISCUSSION

Solubility of Mocaf Flour

Solubility is a parameter that indicates the ability of a substance to dissolve in water. In this case, the solubility in water indicates the amount of mocaf flour in grams

dissolved in one milliliter of solvent (water) (Hidayat et al., 2009). Based on the analysis, the solubility value of mocaf flour with a starter ranged from 1,214% -1,391%, while the control solubility value was 1,308% (Figure 1). The use of the BIMO-CF starter type obtained a solubility value of 1.273%. The lowest solubility level was found using the *Berhasil* starter type, with a solubility level of 1,214%. The highest solubility level was found using the BRIN starter type, with a solubility level reaching 1,391%. Compared with the control data, the results obtained from using the BRIN type of starter resulted in a higher solubility than the control (without a starter). Diniyah et al. (2018) reported that modification increases starch solubility.

The difference in solubility is thought to be influenced by the ratio of the basic structure of starch (amylose and amylopectin) which can be decomposed by the activity of each starter during fermentation (Sukma et al., 2019). Cell reshuffling during fermentation will cut the bonds in starch into a simpler structure and some of it will also change into its basic structure, namely glucose, so it becomes water-soluble (Diniyah et al., 2018).

Viscosity of Mocaf Flour

Viscosity is a parameter that determines the thickness of a fluid and expresses the size of the friction in the fluid. The results of measuring the viscosity of mocaf flour using a starter ranged from 1,967 mPa.s to 2,200 mPa.s. The highest viscosity value was found in using the Bimo CF starter type, which reached 2,200 mPa.s, and the lowest viscosity value was found in using the BRIN starter type, which was 1,967 mPa.s. In comparison, using the starter type produced a viscosity value of 2,033 mPa.s (Figure 2). Compared with the control viscosity of 1,933 mPa.s, using the starter type in the fermentation process has resulted in a higher viscosity than the viscosity results in the control fermentation process (without a starter).

This difference in viscosity is thought to be influenced by microbial activity during fermentation which causes changes in the characteristics of the flour. The presence of pectinolytic and cellulolytic enzymes produced by microbes during fermentation increased the number of starch granules released. The activity of pectinolytic and cellulolytic enzymes, which began to intensify in degrading cellulose cell walls, resulted in damage to the cell wall (Subagio et al, 2008). Finally, the starch granules would begin to separate. Due to the activity of extracellular amylolytic enzymes, the separated granules will experience hydrolysis on their surface, resulting in holes in the starch granules.
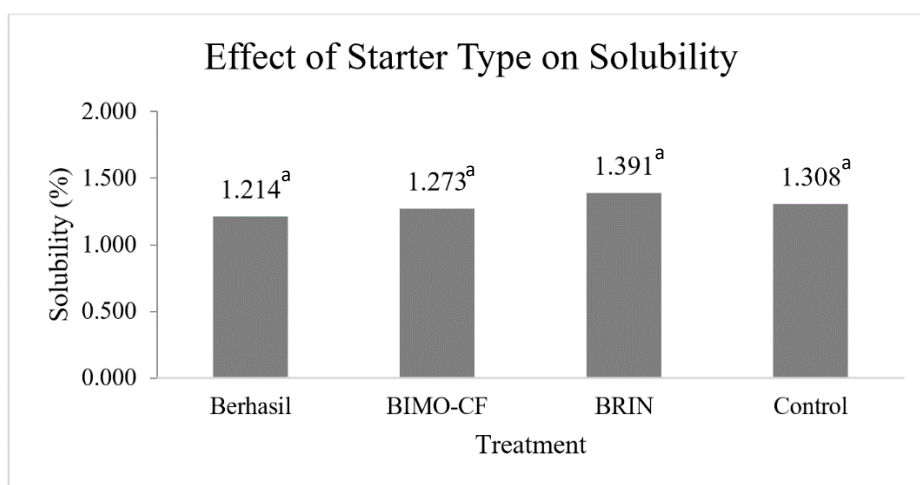

Figure 1. The effect of using lactic acid bacteria starter on the solubility of mocaf flour

Notes: The same letters in the bar chart show values that are not significantly different with a significance level of 95% (α = 5%), after statistical testing with Duncan on SPSS 26.0

Figure 2. The effect of using a starter on the viscosity of mocaf flour

Notes: The same letters in the bar chart show values that are not significantly different with a significance level of 95% ($\alpha = 5\%$), after statistical testing with Duncan on SPSS 26.0
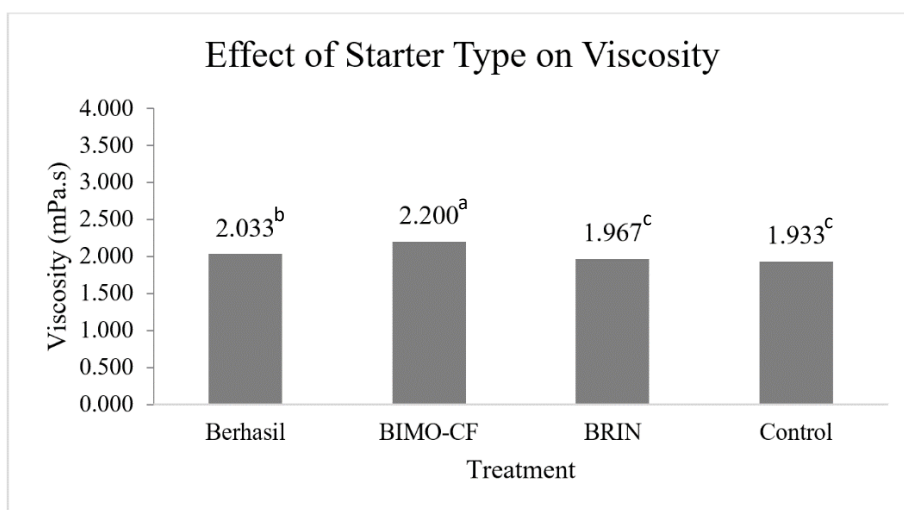
This is in accordance with the results of SEM analysis (Figure 4) from the results of fermentation with the use of starter which shows that the majority of starch granules underwent a significant overhaul which caused the formation of holes on the surface of the granules. The holes strengthen the bond between the grains so that the material is not easily broken and is sticky so that it can change the characteristics of the resulting flour in the form of increased viscosity (Subagio, 2007; Kartikasari et al., 2016; Assalam et al., 2019).

Syneresis of Mocaf Flour

Syneresis is the release of water or seepage of liquid from inside the food where the components in the material do not tightly bind to the water. Freeze-thaw syneresis testing is important because it can be used as an indicator to evaluate flour quality related to the resistance of the physical properties of starch gel to unwanted changes during freezing and thawing, thus affecting the shelf life of flour (Charoerein et al., 2008; Putri et al., 2018).

The results of measuring the syneresis of mocaf flour (Figure 3) show that the amount of water released decreases as the storage time increases. This is to the results of a study conducted by (Putri et al., 2018),

which stated that the syneresis value decreased as the storage process took longer because the quantity of water that came out of the starch paste decreased. Using a starter in fermentation gives a lower syneresis value than the control results (without a starter). This shows that using a starter in the fermentation process can reduce the syneresis value of mocaf flour. According to (Putri et al., 2018), the low syneresis value is caused by the low amylose content in the sample, where the chance of retrogradation will be lower. This happens because when the temperature drops, the amylose will bind again after gelatinization (Winarno, 2004). The existence of strong bonds between amylose during retrogradation causes more water to separate from the starch gel and increases the syneresis value (Dewi et al., 2022). The best syneresis value was obtained by using the BRIN starter because it has the lowest syneresis value. A low or decreasing syneresis value indicates that the flour is of good quality because a high syneresis value indicates that the gel is physically unstable, which can reduce the product's quality (Kuncari et al., 2014). The higher the syneresis value indicates, the greater the chance of starch retrogradation which can affect the shelf life of food products (Putri et al., 2018).
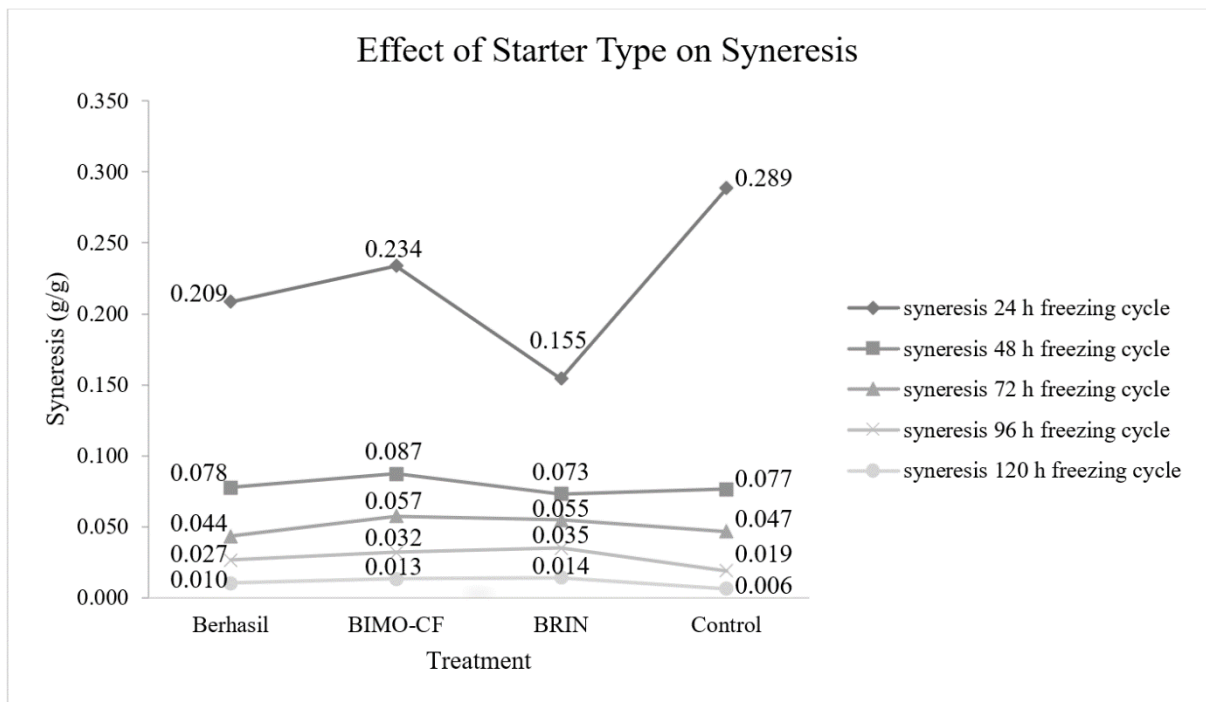
Figure 3. The effect of using a starter on the syneresis of mocaf flour

Near-Infrared (NIR) of Mocaf Flour

NIR analysis is a spectroscopic analysis method that measures the sample spectrum with a relatively fast measurement time and does not damage the sample (Mechram et al., 2021). NIR spectroscopy has been widely used in agriculture and the food industry to identify compounds in food products (Lengkey et al., 2013). From the NIR analysis, it can be seen the value of moisture content, fat content, protein content and fiber content contained in mocaf flour (Table 2).

The results of the NIR analysis of the moisture content of mocaf flour in fermentation using a starter ranged from 12.76% -13.01% (Table 2). This result is lower than the control moisture content of 13.39%. From this, it can be seen that using a starter during fermentation reduces the moisture content in mocaf flour. This decrease in moisture content is possible due to starch degradation during fermentation, which causes the formation of simple sugars to the release of components, including bound water, which has an impact on changing the texture of the material to become soft and porous so that it can increase water evaporation during drying

(Gaol et al., 2023). The lower the moisture content, the better the quality of the flour because, in these conditions, microbial growth can be minimized to extend the shelf life of the flour.

The results of the NIR analysis for the fat content of mocaf flour in fermentation using a starter ranged from 1.63% -1.70% (Table 2). Using the BIMO-CF starter type resulted in a decrease in levels of 0.06% of the control data. However, the BRIN and *Berhasil* starter types increased fat content by 0.01% from the control fat content of 1.69%. Based on the fat content analysis results, it shows that the use of all starters during fermentation gives results in the form of increased fat content when compared to control data (without starter). The increase in fat content is caused by microorganisms being able to produce microbial oil during the fermentation process, like every other living cell system, which produces lipids or fats (Kurniati et al., 2012). In this case, the oil obtained is called single-cell oil (SCO), which is a euphemism similar to single-cell protein, which is commonly used to denote proteins derived from single-cell microorganisms (Kurniati et al., 2012; Wulandari et al., 2017). The longer

the fermentation process, the more oil accumulates due to the number of microbes producing oil. Meanwhile, according to (Khasanah et al., 2021), the decrease in fat content is considered part of the fat component used for microbial metabolism in fermentation because fat is a nutrient usually used as a carbon source besides carbohydrates and protein. In addition, a decrease in fat content is also possible because, during fermentation, the fatcomponent is converted into fatty acids and glycerol by the lipase enzyme produced by the microbes contained in the starter (Ray et al., 2017).

The results of NIR analysis of mocaf protein content in fermentation using a starter ranged from 3.80% -4.10% (Table 2). Using the *Berhasil* starter type gave the same results as the control protein content data, which was 4.10%. However, the use of BIMO-CF and BRIN starter types resulted in a decrease in levels of 0.07% and 0.3% of the control data. From this, it can be seen that using a starter during fermentation tends to reduce the protein content of mocaf flour. The decrease in protein levels is due to the activity of lactic acid bacteria in hydrolyzing proteins through proteolytic enzymes, which can convert protein complexes into shorter peptide fractions and several types of amino acids (Diniyah et al., 2018; Astuti & Setyawati, 2016). Then the results of breaking down the protein will be partly

utilized by lactic acid bacteria to multiply by consuming some of the amino acids and peptides used as a nitrogen source for growth (Nisa et al., 2008). This was confirmed by Mitsuoka (1989), who stated that during its growth, lactic acid bacteria require amino acids and peptides as a source of nitrogen. although it was low. Furthermore, some of the results of other protein overhaul will continue to be degraded to produce an amino acid that quickly oxidizes to produce ammonia (NH_3) or NH_2, which is volatile, causing a decrease in the fermented protein (Shintawati et al., 2022; Muthmainna et al., 2016).

The protein reshuffling during this fermentation was proven by identifying amino acid contents such as alanine, glycine, glutamic acid and melatonin from the NIR test results of mocaf flour (Table 2). Analysis of the alanine content of mocaf flour in fermentation using a starter ranged from 0.05% -0.10%, with an alanine content of 0.07% as a control. Analysis of the glycine content gave different results for each starter, whereby using the Starter *Berhasil* and BIMO-CF, the glycine content could be identified as 0.12% and 0.11%, respectively. Meanwhile, using the BRIN starter and control glycine content was not identified. This also happened in the analysis of methionine content, which was only identified using the *Berhasil* starter and BRIN,

Table 2. Results of NIR analysis of mocaf flour

Parameter	Treatment			
	Starter *Berhasil*	Starter BIMO-CF	Starter BRIN	Control
Moisture content (%)	12.92±0.24[a]	13.01±0.18[a]	12.76±0.13[a]	13.39±0.17[a]
Fat (%)	1.70±0.08[a]	1.63±0.04[a]	1.70±0.14[a]	1.69±0.10[a]
Protein (%)	4.10±0.11[a]	4.03±0.09[a]	3.80±0.06[a]	4.10±0.14[a]
Alanin (%)	0.10±0.01[a]	0.06±0.01[a]	0.05±0.01[a]	0.07±0.01[a]
Glisin (%)	0.12±0.02[a]	0.11±0.01[a]	-	-
Glutamic acid (%)	0.49±0.08[a]	0.51±0.06[a]	0.52±0.04[a]	0.32±0.05[b]
Methionine (%)	0.14±0.02[a]	-	0.14±0.01[a]	0.16±0.02[a]
Fibre (%)	2.23±0.12[a]	2.21±0.10[a]	2.15±0.09[a]	2.46±0.08[a]
Phosphor (%)	0.16±0.02[a]	0.21±0.04[a]	-	-

Notes: The same letters in the row table show values that are not significantly different with a significance level of 95% (α = 5%), after statistical testing with Duncan on SPSS 26.0

which produced the same value of 0.14%, which was still lower than the control data, which was 0.16%.

Meanwhile, methionine content was not identified when using the BIMO-CF starter. Analysis of the glutamic acid content of mocaf flour in fermentation using a starter ranged from 0.49% -0.52%. This result was higher than the control glutamic acid content, which was 0.32%, indicating that using a starter could increase protein breakdown because a high glutamic acid content resulted in lower protein content. This is proven when compared with the identified protein content; the glutamic acid content negatively correlates with the protein content of mocaf flour. The higher the glutamic acid content, the lower the protein content in mocaf flour because many proteins have been hydrolyzed to glutamic acid by proteolytic enzymes during fermentation. Generally, it can be observed that using a starter during fermentation gives different results in protein breakdown. This is thought to be related to the difference in the ability of each bacteria in the starter to overhaul the protein complex. In this case, the decrease in the quantity of protein in mocaf flour can also be understood that a decrease in protein content does not mean a total loss of protein content. However, the protein has been transformed into a simple fraction that is still beneficial for the body.

Proximate Analysis of Mocaf Flour

One of the assessments of food quality can be done through proximate analysis.

Proximate analysis is a method of chemical analysis based on the chemical composition contained in the material. The proximate analysis shows the value of the dry matter, moisture content, ash content, crude protein and crude fat contained in mocaf flour. The results of the proximate analysis of dry matter mocaf flour in fermentation using a starter ranged from 88.91% -89.96% (Table 3). Using the *Berhasil* starter type, there was a decrease in the quantity of dry matter by 0.22%. However, when using BIMO-CF and BRIN starter types, there was an increase in dry matter quantity of 0.68% and 0.83% from the control data. From this, it can be seen that using a starter during fermentation tends to increase the quantity of mocaf flour dry matter. According to (Balo et al., 2022), the results of a high dry matter content indicate that the mechanism for decomposing nutrients can be minimized during the fermentation process to prevent material damage. This can happen because, basically, during fermentation, microbial respiration will break down the nutrient content so that it will reduce the dry matter, while cell liberation activity followed by the release of bound water components in the material will increase the moisture content, which causes a lot of nutrients to decompose so that it has an impact on decreasing the quantity of the dry material (Kuncoro et al., 2015; Seran et al., 2020).

The results of the proximate analysis of the moisture content of mocaf flour in fermentation using a starter ranged from 10.04% -11.09% (Table 3).

Table 3. Proximate analysis of mocaf flour

Treatment	Starter	Parameter				
		Raw materials (% dry basis)	Moisture content (% dry basis)	Ash (% dry basis)	Raw Protein (% dry basis)	Raw Fat (% dry basis)
1	*Berhasil*	88.91±0.21[a]	11.09±0.14[a]	0.51±0.08[a]	0.88±0.09[a]	0.88±0.04[a]
2	BIMO-CF	89.81±0.16[a]	10.19±0.08[a]	0.65±0.05[a]	0.87±0.07[a]	0.79±0.02[a]
3	BRIN	89.96±0.18[a]	10.04±0.18[a]	0.40±0.07[a]	0.79±0.05[a]	0.77±0.05[a]
Control	-	89.13±0.24[a]	10.87±0.16[a]	0.65±0.04[a]	0.94±0.04[a]	0.72±0.03[a]

Notes: The same letters in the column table show values that are not significantly different with a significance level of 95% ($\alpha = 5\%$), after statistical testing with Duncan on SPSS 26.0

When compared with control data, the use of starter *Berhasil* can increase the moisture content by 0.22% from the control data. However, the use of BIMO-CF and BRIN starter types resulted in a decrease in moisture content of 0.68% and 0.83% of the control data. From this, it can be seen that using a starter during fermentation tends to reduce the moisture content of mocaf flour. This was also proven by the results of the NIR analysis (Table 2), where there was a decrease in the moisture content of 0.38% until 0.63% when using a starter during fermentation. This decrease in moisture content is probably caused by changes in the structure of starch granules due to starch degradation during fermentation, which in turn causes the granule structure to become more porous/perforated, thereby increasing water evaporation during drying (Gaol et al., 2023).

The results of the proximate analysis of mocaf flour ash content in fermentation using a starter ranged from 0.40% -0.65% (Table 3). The BIMO-CF starter type gives the same results as the control ash content data, which is 0.65%. However, the use of the *Berhasil* and BRIN starter type resulted in a decrease in levels of 0.14% and 0.25%. From this, it can be seen that adding a starter tends to decrease the ash content of mocaf flour. According to (Dewi et al., 2022), the decrease in ash content is caused by an increase in organic matter during fermentation due to the process of degradation of the material (substrate) by microbes which will affect the ratio of organic matter in the flour. The less organic matter can be decomposed, the lower the ash content obtained. Conversely, the more organic matter that can be decomposed, the higher the ash content proportionally.

The results of the proximate analysis of the crude protein content of mocaf flour in fermentation using a starter ranged from 0.79% -0.88% (Table 3). This result is lower than the crude control protein content of 0.94%. From this, it can be seen that using a starter during fermentation reduces the crude protein content in mocaf flour. This was also

proven by the results of the NIR analysis (Table 2), where there was a decrease in protein content of 0.07% and 0.3% in the use of a starter during fermentation. This decrease in protein content is possible due to the activity of proteolytic bacteria, which produce extracellular protease enzymes which can then hydrolyze proteins into their simpler parts, namely shorter peptide fractions and amino acids. Then, some of the decomposition results will be used for microbial respiration, and the rest will continue to be broken down into volatile compounds such as NH_3 or NH_2 wich canbe lost through evaporation (Shintawati et al., 2022; Muthmainna et al., 2016; Diniyah et al., 2018).

The results of the proximate analysis of the crude fat content of mocaf flour in fermentation using a starter ranged from 0.77% -0.88% (Table 3). This result is higher than the control crude fat content of 0.72%. From this, it can be seen that using a starter during fermentation increases the crude fat content in mocaf flour. This is also proven by the results of the NIR analysis (Table 2), where using a starter during fermentation tends to increase the fat content by 0.01%. However, this increase is not so significant. The increase in fat content is thought to be caused by the ability of microorganisms to produce microbial oil during the fermentation process, which is called single-cell oil (SCO), which will then increase with the length of fermentation time due to an increase in the number of microbes in producing oil (Kurniati et al., 2012).

Scanning Electron Microscope (SEM) of Mocaf Flour

SEM is a sensing instrument used to obtain microstructural images of a sample through the principle of electron reflection. In this case, SEM analysis was used to further observe changes in the shape of cassava starch granules after the fermentation process. The results of SEM analysis of mocaf flour showed that the largest starch granule size ranged from 16.5 µm-18.5 µm,
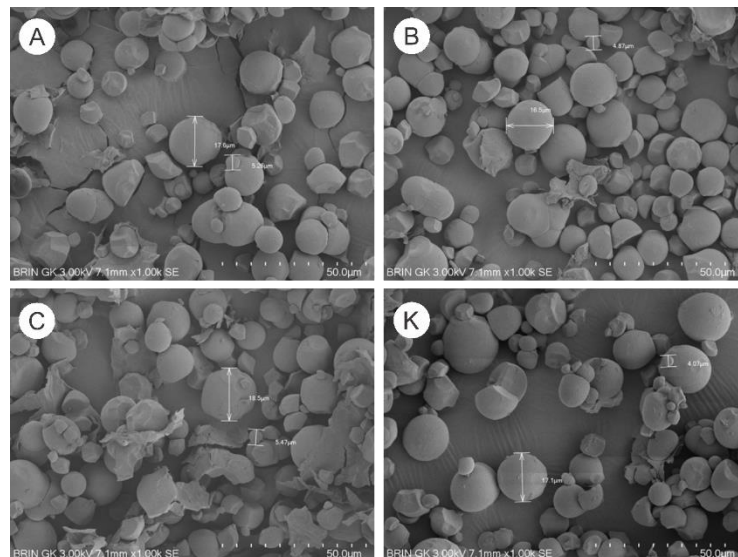
Figure 4. Results of SEM analysis of mocaf flour with starter type (A) *Berhasil*, (B) BIMO-CF, (C) BRIN and (K) Control

while the smallest starch granule ranged from 4.07 µm-5.47 µm (Figure 4). Starch granules generally form in the fermentation method using a starter, which begins to separate. Some parts of the granules begin to have holes with an irregular round granule structure. In more detail, this change can be seen in the use of *Berhasil* starter (A), where the starch granule forms separate from each other, and the sizes are increasingly non-uniform or inhomogeneous. This also happened in using the BIMO-CF (B) starter, where it was found that the size of starch granules had a non-uniform size with smaller granule sizes and found starch granules with many holes so that their shape looked irregularly round. When using the BRIN (C) starter, the shape of the starch granule changes began to appear more significant. This can be seen from the shape of many starch granules, which were already irregular and began to separate.

Compared with the control (K), the starch granules in control appear to be bound to each other and are still clustered on the cassava cell walls with a granular shape that looks more irregularly rounded than in the sample using the starter. From this, it can be seen that using a starter during fermentation gives a more massive overhaul of starch granules characterized by significant

changes in the morphology and structure of starch granules. This can happen because, during the fermentation process, microbial activity in the starter will produce pectinolytic and cellulolytic enzymes, which will degrade the cell wall cellulose and result in the liberation of starch granules, which practically will also make the starch granules separate from each other (Subagio et al., 2008; Kartikasari et al., 2016). Furthermore, in the presence of extracellular amylolytic enzyme activity, the free starch granules will hydrolyze on some of their surfaces, resulting in perforated starch granules (Meutia et al., 2021). As a result, this can also trigger the liberation of starch from the granules so that it can cause changes in the chemical properties, viscosity, and morphology of the resulting starch.

CONCLUSION

The results showed that using a starter during fermentation affected the physicochemical properties of mocaf flour, namely differences in solubility, increase in viscosity and decrease in syneresis. The results of NIR and proximate analysis of mocaf flour content showed that using a starter during fermentation affected the decrease in moisture content, protein

content, ash content, fibre content, phosphorus, and an increase in fat content and dry matter. The results of the microstructural analysis of mocaf flour on the use of starter during fermentation gave a more massive overhaul of the starch granules, characterized by changes in the morphology and structure of the starch granules, which were more separated from each other, hollow, and had an increasingly irregular shape.

AUTHOR CONTRIBUTORSHIP

Habib Ra'uf Alfansuri, Rois Fatoni, Ahmad Fathoni, R. Haryo Bimo Setiarto is main contributors for this original research paper manuscript. Ema Damayanti is member contributor for this manuscript.

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