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Production of High Protein MOCAF (Modified Cassava Flour) Using Papain and Lactic Acid Bacteria

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ABSTRACT. Wheat is one of Indonesia's primary sources of carbohydrates. However, the need for wheat still depends on imports. So, developing local flour based on Indonesia's natural resources is necessary. One source of carbohydrates easily found in Indonesia is cassava (*Manihot esculenta* crantz). Although there are many in Indonesia, the diversification of cassava-based products is still limited. In this research, MOCAF flour was developed and produced by fermenting cassava tubers with lactic acid bacteria. The enzymatic activity during the MOCAF (Modified Cassava Flour) production process is believed to change the physicochemical properties of cassava so that MOCAF products have characteristics similar to wheat flour and are expected to meet the gap between the demand and availability of flour. Even though it can potentially substitute wheat, MOCAF flour only has about 1% protein content, influencing dough rheology. According to the problem, a process modification was done by adding the enzyme papain (PAP) as a support enzyme to improve the performance of lactic acid bacteria (LAB). In order to study the effect of the papain enzyme, variations were made on the ratio of lactic acid starter: papain enzyme (LAB: PAP) and fermentation time. The ratio of (LAB: PAP) was varied to 40:1, 40:3, 40:5, 40:7, and 40:9, while the fermentation period was varied to 24, 48, 72, 96, and 120 hours. The fermentation was conducted by using cassava varieties of Singkur aerobically. The primary starter contained *Lactobacillus, sp*, developed by PT. Rumah Mocaf Indonesia and the papain enzyme used have an activity of 100,000 U/g. Data analysis showed that the addition of papain enzyme doses with a ratio to the lactic acid starter of 40:7 was able to produce MOCAF with the best characteristics: protein content, starch content, and swelling power of 3.72%, 9.99%, 16.00%, respectively. The data trend of research also showed that the number of papain enzymes only significantly affects starch content and swelling power. On the other hand, fermentation time has a significant effect on these three characteristics (protein, starch, swelling power).

Keywords: Cassava, fermentation, lactic acid bacteria, MOCAF (Modified Cassava Flour), papain enzyme

INTRODUCTION

Wheat is one of Indonesia's primary sources of carbohydrates. However, the need for wheat still depends on imports. Based on BPS-Statistics Indonesia, Indonesia's total wheat imports reached 9.350.400 tons in 2022 (BPS-Statistics Indonesia, 2023). This value increased by 2.6 million tons compared to 2018 because Indonesia has been unable to cultivate the wheat. So, developing local flour based on Indonesia's natural resources is necessary. Indonesia is known as an agricultural country with abundant biodiversity. There are at least 77 types of carbohydrates that can be developed into food products (Tresnady, 2017). One of them is cassava (*Manihot Esculenta esculenta* Crantz). Cassava is a carbohydrate source grown in all the provinces of Indonesia. The total production of cassava reached 17.45 million tons in 2020. This value increased by about 6.73 percent or 1.10 million tons (from 16.35 million tons in 2019 (AFSIS Secretariat, 2020). Cassava has a high potential as an alternative staple food substitution. A literature study shows that 100 grams of cassava contain up to 37.9 grams of carbohydrates. Apart from carbohydrates, cassava also contains essential nutrients such as protein, fat, calcium, phosphorus, iron, vitamin A, vitamin B1, and vitamin C, respectively: 0.8grams, 0.3grams, 33 g, 40grams, 0.7 grams, 385S1, 0.06mg, and 30mg (Widyastuti, 2019). By considering its nutritional content and abundance, cassava can be developed as a carbohydrate product.

One of the cassava-based products developed in this research was Modified Cassava Flour (MOCAF). MOCAF is a flour produced by the fermentation of cassava using Lactic Acid Bacteria (LAB) (Triyono et al., 2019). During the fermentation, LAB produces pectinolytic and cellulolytic, capable of hydrolyzing starch and protein (Kardhinata et al., 2019). The presence of enzymatic activity during MOCAF production causes the bonds in the amylose and

amylopectin chains to break so that the bonds in starch become shorter and easier to digest. In addition, the fermentation process also changes flour's physicochemical and functional properties, which can be observed from gelation ability, rehydration power, and ease of dissolving (Agustia et al., 2016). The organoleptic assessment carried out by Rahman et al. (2020) showed that MOCAF has a texture similar to wheat, and the aroma of cassava is no longer visible. That characteristic is different from the texture of tapioca and cassava flour, which tends to be coarser and has a distinctive cassava flavor. Although MOCAF has similar characteristics to wheat, the application of MOCAF in food production is still limited, especially in bakery and noodle products.

The factor that affects the application of MOCAF is the protein content. The protein content in MOCAF is around 1.1% (Diniyah et al., 2019). This value is far lower than wheat protein, reaching 11.1%. The low protein content in cassava is related to the nature of the gluten present in processed products. Gluten is a protein found in grains, such as wheat and barley. The function of gluten in food is to form a three-dimensional protein network as a viscoelastic matrix. The protein matrix's presence ultimately causes the dough to expand and become elastic (El Khoury et al., 2018). The higher the gluten content, the better the texture of the resulting dough. Because the protein content of gluten in cassava is relatively low, the dough produced by MOCAF is less chewy, so its use is still limited. Therefore, the MOCAF protein should be increased. This research studied the papain enzyme's potency to increase the protein level in MOCAF.

Besides using LAB as a primary starter, this research used papain enzyme to support the LAB activities during cassava fermentation. Papain is a protease enzyme produced from papaya (*Carica papaya* L). The papain enzyme is a class of hydryl sulfi protease enzymes and belongs to the eukaryotic thiol protease group, which has an active cysteine site (Kemigabo et al., 2017). This enzyme can hydrolyze proteins containing arginine, lysine, and phenylalanine into chains of amino acids (Amri & Mamboya, 2012). Dissolving protein in amino acids is expected to increase the amount of dissolved protein in MOCAF. The application of the papain enzyme to increase the amount of dissolved protein has been studied by other researchers. Khodijah et al. (2015) and Rachmawati et al. (2018) examined the effect of adding protein to fish feed. The results showed that the feed enriched with papaya extract increased the growth rate (PGR), protein efficiency ratio (REP), feed conversion ratio (FCR), survival rate (SR), and significant protein digestibility (ADCp). Elsamadony et al. (2015) conducted a study to examine the increase in H_2 yield resulting from the hydrolysis of sugar, fat, and protein with the enzyme papain. The results showed that the hydrogen yield increased from 52.2 ± 7.5 to 130.6 ± 8.5 ml/g protein due to using papain enzymes. Susanto et al. (2018) studied the potential use of the papain enzyme to enhance the antioxidant properties of bioactive peptides from chicken feet. The results showed that the interaction of 3% papain enzyme and 36 hours of curing time resulted in the highest antioxidant activity of 55.10 ± 2.24% in bioactive peptides. This increase in antioxidant properties can occur due to changes in the size and weight of the protein to be smaller.

Based on the research results mentioned, it can be concluded that adding papain enzymes can increase the amount of dissolved protein from a material. However, not much has been studied about the effect of adding the papain enzyme on increasing the protein content of the flour, especially MOCAF. This research focused on the effect of increasing papain enzyme and fermentation time on the protein, starch, and swelling ability of MOCAF.

EXPERIMENTAL SECTION

Material

The research used cassava varieties of Singkur, which were older than six months. As stated before, the fermentation process was conducted using two agents. The primary starter contained *Lactobacillus sp, which* was developed by PT Rumah Mocaf Indonesia as the research partner. The papain enzyme used in this research had an activity of 100,000 U/g (Shaanxi Fonde Biotech Co.Ltd). Other chemicals used in this study were H_2SO_4 (Merck, p.a), K_2SO_4 (Merck, p.a), HgO (Merck, p.a), NaOH (Merck, p.a), saturated H_3BO_3, HCl (Merck, p.a), Acetone (Merck, p.a), Luff Schroll solution, KI (Merck, p.a), $Na_2S_2O_3$ (Merck, p.a), phenolphthalein (Merck), starch indicator (Merck), All of the chemicals were purchased from Merck (Darmstadt, Germany).

MOCAF Production

Figure 1 shows the detailed process flow diagram. The MOCAF production was started with peeling, then washed and weighed to 1 Kg. Clean cassava was grated using a cutting machine to obtain cassava chips with a thickness of ±1.0-1.5 mm. The cassava chips were soaked in distilled water and mixed with a fermentation agent by the ratio of cassava: distilled watered: culture = 1:3:0.03 in the clean container. The mixed material was then fermented aerobically for several days. The MOCAF chips were then cultivated and pressed to reduce the water content. In order to optimize the drying process, the MOCAF chips were dried manually using the sun dryer model. The chips were grinded by machine and were then sifted to a size of 70 mesh. Variables studied in this research were ratio (LAB: PAP) and fermentation time. The effect of the (LAB: PAP) ratio on the characteristics of MOCAF was studied by varying the (LAB: PAP) by 40:1, 40:3, 40:5, 40:7, and 40:9 ($^v/_w$). The effect of fermentation time on MOCAF characteristics was studied by varying

the fermentation to 24, 48, 72, 96, and 120 hours. The control condition was conducted by doing the fermentation using an LAB starter.

MOCAF Analysis

Protein Content Analysis by Kjeldahl Method (Mulyo et al., 2022)

The sample weighed as much as 2 grams and then put into a 100 mL Kjeldahl flask. As much as 5 grams of K_2SO_4, 0.2 grams of $CuSO_4$, and 25 mL of H_2SO_4 98% were added into the flask. The Kjeldahl flask was heated in an oil bath at 350 °C in a fume cupboard for 2 hours. The digested sample was cooled and added with 150 mL of distilled water. The sample was put into a beaker glass, and then ± 80 mL of 50% NaOH was added until the sample solution had a pH value of 13 and the solution changed to olive green. The distillation process was then carried out. The distillate was collected in a 100 mL Erlenmeyer. Three drops of PP indicator were added into 50 mL of 0.1 N HCl solution. The distillate solution obtained was then titrated with 0.1 N NaOH until a pink color was obtained. Total nitrogen content was calculated by Equation (1).

$$N_{content}(\%w) = \frac{(V_{NaOH\ blank} - V_{NaOH\ sample}) x N_{NaOH}\ x 14.007}{mg_{sample}} x 6.25 x 100 gr \quad (1)$$

Starch Contents by Luff Schroll Method (Ifmaily, 2018)

As many as 2 grams of samples were dissolved with 200 mL of 0.1 N HCl. The sample was heated for 3 hours. After cooling, the solution was neutralized with 30% NaOH and filtered. The 5 mL sample was taken and then diluted in a 100 mL volumetric flask. As much as 25 mL of the diluted sample was added with 25 mL of Luff Schroll solution. The solution was heated for 10 minutes. After the solution was cooled, 15 mL of 25% KI solution and 25 ml of 25% H_2SO_4 were added. Then, let stand for 15 minutes until a white precipitate was formed. A ten-milliliter solution was titrated with 0.1N $Na_2S_2O_3$ until the color changed to yellow. Twenty drops of the starch indicator were added to the sample. The titration was continued until the color changed to colorless. The starch content was calculated by Equation (2).

Starch content=mg glucose x 0.9 mg sample x 100% (2)

Where: mg glucose, Fp= mL of filtrate titration

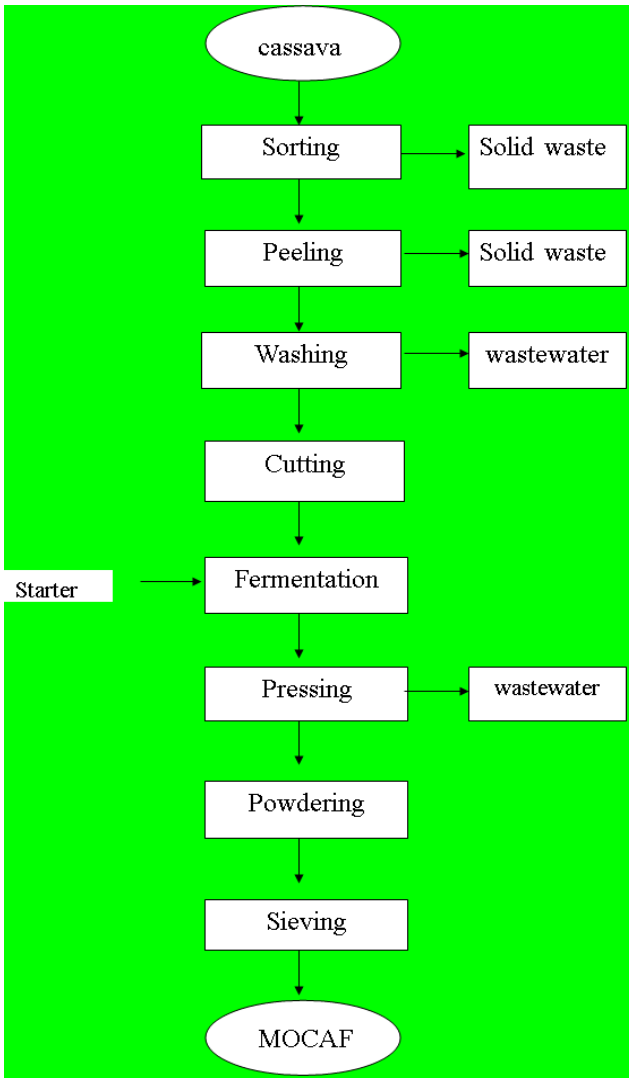

Figure 1. MOCAF Manufacturing proses

Analysis of Swelling and Solubility Levels (Senanayake et al., 2013)

A total of 0.1 grams of sample (A) was mixed with 10 mL of distilled water in a 15 mL centrifuge tube (the weight was known). The sample was then placed in a water bath at 85 °C for 30 minutes with continuous stirring for 10 seconds (after 5, 15, and 25 minutes). The heated sample was cooled to room temperature and centrifuged at 2000 rpm for 30 minutes. The supernatant was taken, and the resulting precipitate was weighed (D). The supernatant was placed in a petri dish whose weight was known (B). The petri dish was dried in an oven at 105 °C to a constant weight and then weighed (C). Swelling power is the ratio between the weight of the precipitate left in the centrifuge tube (D) and the dry weight of the sample. The water solubility index (WSI) is the weight percentage of starch dissolved in water. The swelling power was calculated using Equation (3), and the solubility was calculated using Equation (4).

$$Swelling\ power\ \ (g/g) = \frac{D}{A} \quad (3)$$

$$Solubility(\%) = \frac{C-B}{A} x100\% \quad (4)$$

MOCAF Characterization

Characterization was carried out to evaluate the morphological structure and changes in the bond structure that occurred due to the fermentation process. The morphological structure was observed by Spectroscopy Electron Microscopy (SEM) (HITACHI TM 3000), while the bond pattern was analyzed by Fourier Transform Infra-Red (FTIR) (Shimadzu IRTracer-100)

Data Analysis

The effect of (LAB: PAP) ratio and fermentation time to protein, starch, and swelling point of MOCAF were statistically evaluated using One Way ANOVA test by IBM SPSS Statistic Version 20. If the test result showed that the sample was significantly different at the 0.05% significance level, then a post hoc analysis using Duncan was carried out.

RESULT AND DISCUSSION

Characterization of Modified Cassava Flour (MOCAF)

Scanning Electron Microscopy (SEM) was used to examine the microstructure changes of cassava because of fermentation. The microstructure characteristic was evaluated with 3.000 X magnification for the unfermented cassava, the MOCAF produced by LAB fermentation, and the MOCAF produced by (LAB: PAP) of (40:7). Figure 2 shows that starch granules of unfermented cassava (Figure 2a) and fermented cassava (Figure 2. a.b) have a smooth surface and a spherical shape, in general. The micrograph also shows that some granules of unfermented cassava appeared to have irregular shapes in some portions. This result aligns with the research conducted by Diniyah et al. (2023) that starch granules for Cimanggu and Kaspro Cassava have an oval shape on one side and a polygonal shape on the other. The effect of papain enzyme addition in this research did not appear in the micrograph. The microstructure of MOCAF produced using LAB (Figure 2b) and (LAB: PAP) (Figure 2c) had similar morphological appearance for both particle size and the starch granules shape.

Fourier Transform Infrared (FTIR) spectroscopy observed the functional group of unfermented and fermented cassava. The analyses were evaluated by spectral sorption at region 500-4500 cm^{-1}. The profile of IR Spectra for raw cassava and MOCAF produced using BAL and (BAL: PAP) did not show significant differences (**Figure 3**). Isa et al. (2021) reported that the cassava-based product fingerprint ranged from 800-1500 cm^{-1}, mainly from amylose and amylopectin vibration. The absorbance at 1080.14 cm^{-1} was for C-O stretching, C=O at 1635.64 cm^{-1}, and C-N at 1149.57 cm^{-1} (Raharja et al., 2017) .The region between 3000 and 3600 cm^{-1} is for O-H stretch from starch (Amir et al., 2013).

Fermentation of Cassava using LAB

LAB produces the pectinolytic and cellulolytic enzymes during fermentation, which hydrolyze amylopectin and amylose in the amorphous regions (Yuliana et al., 2014). The partial hydrolysis of amylose and amylopectin short chain causes the deformation of the granule structure. So, the capability to absorb the water improves. In other words, the fermentation increases the swelling power. Besides affecting the swelling power, the water-soluble starch molecules (amylose) will quickly exit and enter the starch granules, leaching amylose. This phenomenon would improve the MOCAF solubility. The increasing swelling power and flour solubility resulted during the cassava fermentation, as shown in **Table 1.** The data shows cassava's starch content decreases by 67.54% after fermentation (from 93.31% to 30.28%). According to the starch content decreases, the swelling power and solubility of MOCAF increase to 10.88g/g and 13.20%, respectively, compared to those of unfermented cassava flour.

Rosida et al. (2020) reported that the degradation of Cocoyam (*Xanthosoma sagittifolium)* starch granules reduces starch's ability to retain water molecules. It happens because the starch loses the carbonyl groups. Thus, the bound water was released into free water because of the heat produced during fermentation. The free water was quickly evaporated during drying, which caused the water content of fermented flour to decrease. Along with the report, MOCAF's water content decreased from 14.65% to 12.87% during the *Lactobacillus sp* fermentation. Flour with lower moisture content is more desirable than flour with high moisture content. The literature mentions that the maximum water content in flour is 13% $^{w}/_{w}$ (Nasir et al., 2003). The higher level of water

will affect the flow and starch's mechanical properties, leading to microbial spoilage and decreasing the shelf life of flour (Kurniadi & Khasanah, et al., 2019).

A Lactobacillus sp is characterized by breaking the starch into simple sugar and producing lactic acid via an enzymatic pathway (Rahma et al., 2017). Besides producing pectinolytic and cellulolytic enzymes, some LABs, like *Lactococcus lactis, Lactococcus cremoris, and Streptococcus thermophilic,* can also produce proteolytic enzymes inside the cell LAB that can degrade proteins. The final products of protein decomposition were then used as a nitrogen source to fulfill the rapid intracellular metabolism (Kieliszek et al., 2021). For example, *Lactococcus* uses free amino acid and nitrogen from glutamic acid, glycine, leucine, isoleucine, histidine, methionine, and valine produced from casein decomposition to grow (Hernandez-Valdes et al., 2020; Laroute et al., 2017). The activity of *Lactobacillus plantarum* to break the peptides was reported by Kusniati et al. (2019). The result shows that the *Lactobacillus plantarum* B110 and *Lactobacillus satsumensis* can increase the protein degradation capacity of the paste flour to 0.084% (purple sweet potato), 1.329% (cassava), 0.584% (corn), 0.749% (rice).

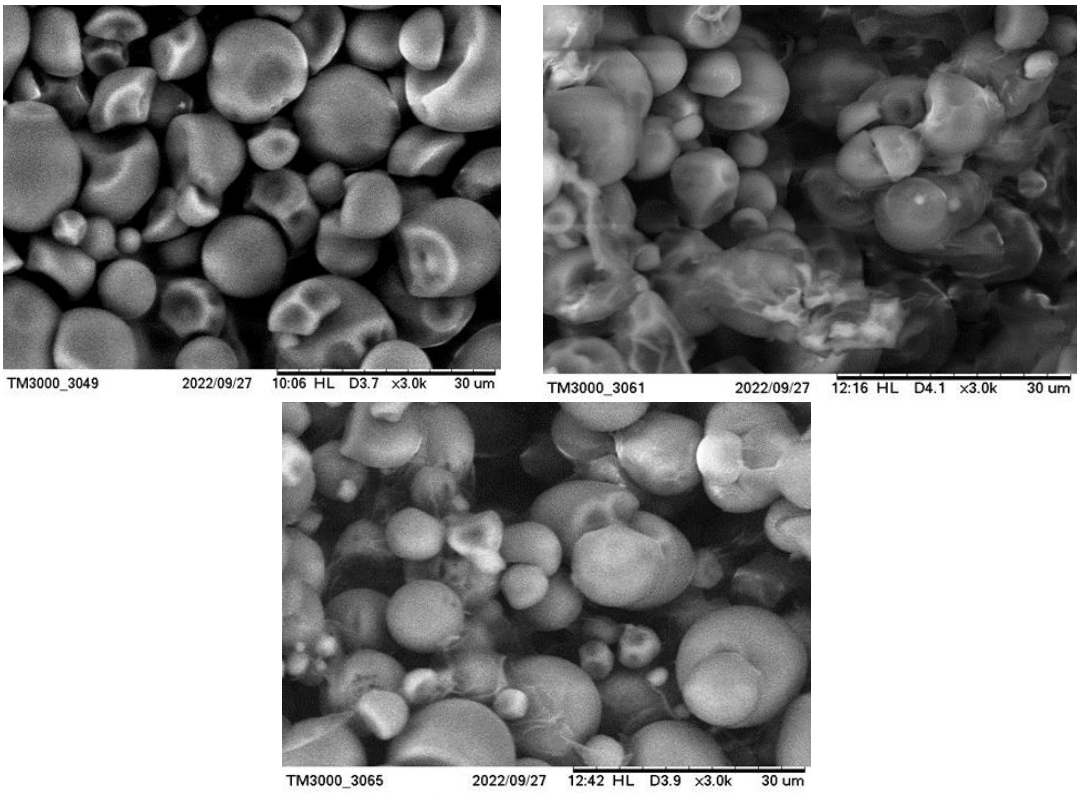

Figure 1. Microstructure of A) Unfermented Cassava B) Fermented cassava flour using Lactobacillus for 72 hours) C)Fermented cassava flour using Lactobacillus: Papain Enzyme (40:3) for 72 hours at Magnification 3.000X

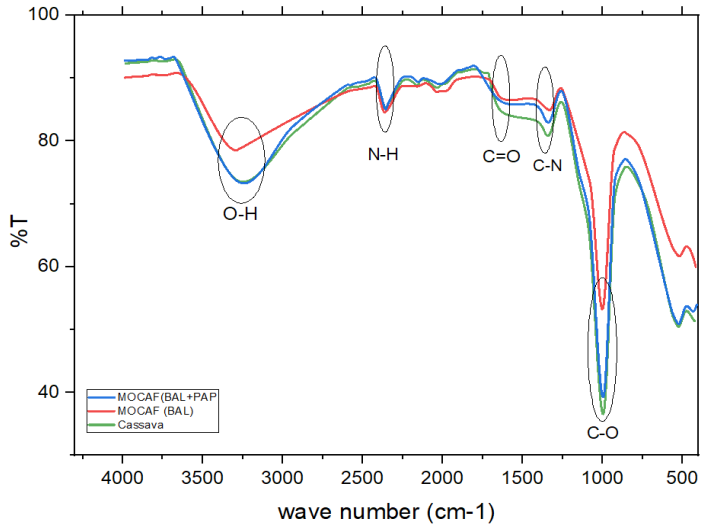

Figure 2. FTIR Spectra of Unfermented and Fermented Cassava

Table 1. Characteristics of Unfermented Cassava and Fermented Cassava Using BAL 40% (w/v)

Characteristic	Unfermented cassava flour	Fermented Cassava Flour (MOCAF)
Protein (%)	0.5	1.93
Starch (%)	93.31	30.28
Water content (%)	14.65	12.87
Swelling power (g/g)	10.88	13.20
Solubility (%)	0.2	0.22
Water Content (%)	14.65	12.87

Table 2. Effect of Papain Enzyme Dose on MOCAF Characteristic

Dose(v/w)	Responses		
	Protein (%)	Starch (g)	Swelling (g/g)
40:0	1.93±0.01[b]	30.28±0.02[a]	13.20±0.01c
40:1	3.39±0.08[a]	19.10±0.70[b]	12.95±0.50[c]
40:3	3.50±0.03[a]	14.80±0.57[b]	16.05±0.07[b]
40:5	3.63±0.06[a]	13.41±0.03[c]	18.75±0.02[a]
40:7	3.67±0.12[a]	10.24±0.05[c]	14.65± 0.07[b]
40:9	3.47±0.05[a]	14.50±0.28[c]	9.50±0.85[d]

Notes: Different letters in the same column indicate a significant difference based on the Duncan Test at the 5% significance level.

Effect of Ratio (LAB: PAP) on MOCAF Characteristic

The effect of ratio (LAB: Papain) on the characteristics of MOCAF was studied by varying the LAB starter: papain enzyme ratio by 40:1, 40:3, 40:5, 40:7, 40:9 (v/w), while the mass of fermented cassava was kept constant as many as 1 Kg. The fermentation process was carried out aerobically for three days (72 hours). The data in **Table 2** shows that adding the papain enzyme had a significant effect ($P < 0.05$) on protein, starch, and the swelling ability of MOCAF. Through the results of this study, it was found that the fermentation treatment with the addition of the papain enzyme dose of 40:3 to the LAB starter was able to increase protein from 1.93% to 3.39%, or in other words, the protein in MOCAF could be increased up to 75.64%. Sulystiyo & Nakahara (2013) stated that the high protein content of MOCAF may be caused by the ability of microbial strains to secrete the extracellular proteins into the cassava starch granules during the fermentation process to form single-cell proteins during the fermentation. When papain comes to lactic acid bacteria (LAB), the effects of papain enhance the LAB activities. Papain helps break down complex proteins into smaller peptides and amino acids as a nitrogen source, improving their growth and metabolic activity.

The enzyme is a specific catalyst. By mean, it only accommodates the reaction involving the particular substrate or a group of closely related substrates. The higher the enzyme concentration, the more substrate will react with the enzyme. This theory was revealed by Wijayanti et al. (2016), who studied the effect of adding bromelin as a protease enzyme on protein hydrolyzate from milkfish (*Chanos chanos* F). The results showed that protein hydrolyzate increased with the addition of bromelain enzyme concentration. This result is reinforced by Rahmalia et al. (2021), who studied the effect of bromelain enzyme on VCO (Virgin Coconut Oil) produced by fermentation using tempeh yeast. The research showed that the proportional amount of enzyme increased the reaction yield.

Contrary to the previous research results, Data in **Table 2** shows that protein content did not increase significantly with the PAP enzyme ratio. It is commonly known that enzyme is highly selective, often recognizing and binding to a particular substrate or a group of closely related substrates. Papain enzyme has proteolysis activity to split positively charged amino acids peptide linkage, mainly lysine, arginine, and phenylalanine residues (Shouket et al., 2020). Amri et al. (2012) stated that the peptides breaking down by papain enzyme are related to the deprotonation of Cys-25 by His-159. In this stage, Asparagine-175 acts to orient the imidazole ring of His-159 to allow this deprotonation. Cys-25 then performs a nucleophilic attack on the carbonyl carbon of a peptide backbone. In contrast, Almasyhuri et al. (1999) reported that the dominant amino acid of cassava was isoleucine, leucine, lysine, phenylalanine, threonine, and valine. In contrast, the content of methionine, cysteine, and tryptophan is very small (Julie & Christopher, 2009). This substrate mismatch causes the amount of protein to not increase significantly with the PAP ratio.

Table 3. Effect of Fermentation time on MOCAF Characteristic using (LAB: PAP) (40:7)

Fermentation Periode (h)	Response		
	Protein (%)	Starch (%)	Swelling(g/g)
24	3.13±0.03[c]	18.60±0.28[a]	14.90±0.14[b]
48	3.26±0.03[b]	16.90±0.00[b]	16.30±0.07[a]
72	3.72±0.01[a]	9.99 ± 0.12[d]	16.00±0.07[a]
96	3.26±0.03[b]	12.90±0.00[c]	14.45±0.00[b]
120	2.94±0.06[d]	14.40±0.00[c]	11.07±0.14[c]

Notes: Different letters in the same column indicate a significant difference based on the Duncan Test at the 5% significance level.

Different from the protein content trend, adding papain enzymes also appeared to significantly affect starch and fiber levels. The data in **Table 2** shows that the starch content decreased with the addition of the papain enzyme. This is in accordance with previous literature, which states that the addition of papain enzyme supports the release of starch granules that previously packed on the protein coated structure. The starch then used by LAB for growth during fermentation process (Elkhalifa et al., 2006). The smallest starch content obtained in this study was 10.24%. This value means adding papain enzymes can reduce starch content by up to 66.18%. The lower the starch content, the MOCAF swelling value will increase.

Effect of Fermentation Time to MOCAF Characteristic

The enzyme is a secondary metabolite produced when microbes are in the stationary phase. At the stationary phase, microbes would be stimulated to produce specific enzymes that could hydrolyze complex compounds into simpler compounds that would be utilized for survival (Tandrianto et al., 2014). Therefore, the fermentation time must be considered to produce products with the desired characteristics. The effect of fermentation time on MOCAF characteristics was studied by varying the fermentation to 24, 48, 72, 96, and 120 hours, while the (LAB: PAP) ratio was set to 40:7. Research data in **Table 3** shows that the fermentation time significantly ($P < 0.05$) affected the protein, starch, and swelling power of MOCAF. The data trend (**Table 3**) shows that increasing the fermentation time up to 72 hours can increase the protein to 3.72% and reduce the complex starch content to 9.99%. According to Tandrianto et al. (2014), this happens because there is an increase in the number of *Lactobacillus* cells over time. The increase in the number of cells increased the number of amylase enzymes used to break down starch. As a result, protein will be maximum, and starch content will be minimum.

Jamilah et al. (2009) stated that α-amylase production reached the maximum activities at the end of the logarithmic growth phase. Then, it decreased gradually as the time of incubation was extended. The lower amount of nutrients can cause a decrease in microbial microbiological activity after 72 hours. Moreover, microbes have entered the death phase, resulting in a drastic drop in cell numbers.

CONCLUSION

The addition of the papain enzyme to the MOCAF production process significantly affected the protein, starch, and swelling power of MOCAF flour. Although it appears to affect these characteristics significantly, the increased ratio (LAB: PAP) more than (40:1) only appears to have a significant effect on starch and swelling levels, while on the other hand, it does not significantly affect MOCAF protein levels. Fermentation time showed a significant effect on protein content, starch content, and swelling power. Based on the data analysis, it can be seen that cassava fermentation using *lactobacillus* starter and papain enzyme at a ratio of 40:7 for 72 hours can produce the best MOCAF characteristics. The MOCAF produced with that condition was reported to have protein content, starch content, and swelling power of 3.72%, 9.99 %, and 16.00%, respectively. From the data that has been described, it can be concluded that adding papain enzymes can be a promising method to improve the characteristics of mocaf flour, especially protein.

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