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Effect of fermentation duration and $Ca(OH)_2$ concentration for immersion on the characteristics of modified cassava flour (mocaf) of bitter cassava variety (*Pandemir* L-2)

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Abstract. *Kurniawan S, Amanto BS, Utami R. 2019. Effect of fermentation duration and Ca(OH)2 concentration for immersion on the characteristics of modified cassava flour (mocaf) of bitter cassava variety (*Pandemir *L-2). Bioteknologi 16: 53-61.* Due to the high HCN content (>100 ppm) in bitter cassava varieties, a technology is needed to remove the content so that this cassava starch can be used as a food ingredient. One technology to remove HCN is by processing bitter cassava into mocaf (modified cassava flour). Mocaf is cassava flour made by modifying the cassava cell using the fermentation method. The fermentation process of cassava results in flour with a neutral (tend to be aromatic) smell characteristic, soft texture, and whiter color. In addition, the fermented cassava flour also has an advantage over the ordinary cassava flour, including digestibility, viscosity, gelation ability, rehydration power and solvability, the capability of binding water, lower HCN, wide application, easier dispersion to the food product, and easily forming 3 dimensions between the components so that the product consistency become better. The objective of the research is to find out the effect of Calcium Hydroxide $Ca(OH)_2$ and fermentation duration on the chemical (water, acid, protein, HCN levels, and viscosity) and physical (whiteness degree) characteristics of bitter cassava mocaf. The experimental design employed was a Completely Random Design (CRD) with two factors: $Ca(OH)_2$ concentration variation (0%, 5%, 10%, 15%) and fermentation duration (0 hour, 24-hours, 48-hours, 72-hours). The research data was analyzed using ANOVA at a significance level $\alpha = 5\%$ and followed by DMRT with a significance level of 95%. The research results show a higher concentration of Ca(OH)2, lower total acid, protein, and HCN content and higher water containt, viscosity levels, and whiteness degree of mocaf. Longer fermentation duration, higher water content, total acid levels, whiteness degree intensity, and lower HCN content and protein levels of mocaf.

Keywords: $Ca(OH)_2$, fermentation duration, HCN, mocaf, modified cassava flour

INTRODUCTION

Indonesia has tubers that can potentially be a source of carbohydrates and raw materials for local flour, which is equivalent to wheat, namely *canna*, *gembili*, sweet potato, arrowroot, cassava, and so on (Subagyo 2006). Until now, the use of cassava in Indonesia is still very limited. Cassava is generally planted simultaneously at the beginning of the rainy season on dry or rainfed land. Therefore, the harvest time is also simultaneous, from July to September. As a result, overproduction occurs, which is repeated every year so that cassava prices become very low at harvest time. The low price of cassava is also influenced by the nature of fresh cassava, which is easily damaged if post-harvest handling is not immediately carried out due to the high moisture content of fresh cassava, the presence of cyanide acid (HCN), which causes poisoning, the presence of polyphenol compounds that cause browning, and the limited technology of cassava processing. Flour technology is one of the recommended alternative processes for semi-finished products because it is more resistant to storage, easy to mix (made composites), can be enriched with nutrients (fortified), easy to shape, and can be cooked faster according to the demands of modern life which wants practicality.

The cassava processing technology is generally traditional: boiling, frying, *gaplek* making, flouring (cassava flour, *tiwul* flour), and extracting tapioca starch. However, one of the diversification efforts in cassava processing currently being developed is mocaf (modified cassava flour). Mocaf is cassava flour made using the principle of cell modification of cassava by fermentation. The cassava fermentation process produces flour with a characteristic neutral smell (which tends to be fragrant), soft texture, and whiter color. In addition, fermented cassava flour also has advantages over ordinary cassava flour, namely digestibility, viscosity, gelation ability, rehydration and ease of dissolving, water binding ability, lower HCN, wide application, easier dispersion into food products, and easy to form 3 dimensions between components, so that product consistency is better. The technology of the making process of fermented cassava flour was first introduced in West Africa, especially in Nigeria. The flour produced is used as a staple food known as *gari* flour (Wahjuningsih et al. 2009).

However, some cassava contains cyanide compounds that can cause poisoning for those who consume them. Cyanide compounds decompose to produce cyanide acid (HCN), which can inhibit oxygen absorption in the respiratory system, resulting in throat spasms followed by shortness of breath, loss of consciousness, and even death.

The lethal dose of cyanide is 0.5-3.5 mg per kg of body weight. Types of cassava that contain cyanide compounds generally have tightly-packed large tubers with no stem on the tree and contain more starch.

HCN in bitter cassava is a solid material that is more than 100 ppm in size. The process of peeling and washing cassava does not eliminate all HCN toxins. Therefore, to remove the HCN content in cassava, it is necessary to carry out special treatments, including fermentation and adding $Ca(OH)_2$. Hydrogen cyanide present in wood tubers is a weak acid, which, theoretically, an acidic compound can be neutralized with an alkaline solution, which will form salt and water. The addition of alkaline $Ca(OH)_2$ is expected to reduce or eliminate the HCN content in cassava. According to Wahjuningsih (1990), cassava fermentation can help hydrolysis of cyanogenic glucosides and HCN.

Bitter cassava is usually only used as animal feed. However, increasing utilization of bitter cassava can be done by turning it into mocaf. Therefore, it is necessary to research to determine the effect of the addition of $Ca(OH)_2$ and fermentation on the chemical characteristics of the resulting mocaf. In this study, the type of cassava used was *Pandemir* L2 variety with fermentation duration of 0-hour, 24-hours, 48 hours, and 72-hours and used $Ca(OH)_2$ solution with concentrations of 5%, 10%, and 15%. This research is expected to provide scientific information to the public on processing bitter cassava into mocaf as a safe food for consumption.

This study aims (i) to determine the effect of adding calcium hydroxide ($Ca(OH)_2$) to the chemical characteristics (moisture content, acid content, protein, HCN, viscosity) and physical characteristics (degree of whiteness) of mocaf produced from bitter cassava. , (ii) to determine the effect of fermentation duration on chemical characteristics (moisture content, acid content, protein, HCN, viscosity) and physical characteristics (degree of whiteness) of mocaf produced from bitter cassava.

MATERIALS AND METHODS

Materials

This research was conducted at the Food and Nutrition Laboratory of the Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia, and the Food Laboratory, Universitas Gajah Mada, Yogyakarta, Indonesia.

The main material in this study was cassava of the *Pandemir* L2 variety aged 10-12 months obtained from a farmer from Nglengkong Hamlet, Sendang Village, Purwantoro Sub-district, Wonogiri District, Central Java, Indonesia, with specifications of 10-12 months of age, the water content of 63.2961% w/w, the starch content of 28.875% w/w, HCN content of 186.55 ppm. Meanwhile, the materials for soaking and fermenting are well water (whose hardness has been identified) and a solution of $Ca(OH)_2$ with concentrations of 5%, 10%, and 15%.

Research design

This study used a completely randomized design with two factors, namely the effect of the use of $Ca(OH)_2$ (P) and the effect of variations in fermentation duration (F) and the design was as follows (i) Factor I: Immersion concentration of $Ca(OH)_2$ (P) consisting of 4 levels, namely: P1 = 0%, P2 = 5%, P3 = 10%, P4 = 15%, (ii) Factor II: Fermentation duration (F) consisting of 4 levels, namely: F1 = 0 days, F2 = 1 day, F3 = 2 days, F4 = 3 days, so there would be 16 treatments.

Research procedure

Material preparation

This sample preparation stage begins by sorting cassava according to the freshness state (not deformed/rotten), the size uniformity (not too small), and the color.

Preparation of $Ca(OH)_2$ solution

$Ca(OH)_2$ is dissolved using water (well water of identified hardness). The concentrations of $Ca(OH)_2$ were 5%, 10%, and 15%.

Mocaf making

The process of mocaf making in this study was according to Wahjuningsih et al. (2009). In this study, the manufacture of mocaf was carried out by pretreatment with 5%, 10%, 15% $Ca(OH)_2$ immersion and then followed by the fermentation for 0-hour, 24-hours, 48-hours, and 72-hours.

Mocaf analysis

All mocaf samples were then analyzed chemically (HCN, protein, viscosity, moisture content, acid content) and physically (whiteness). Each analysis was carried out on samples of fermented mocaf at 0, 24-hours, 48 hours, 72-hours, and at immersion concentrations of 5%, 10%, and 15% $Ca(OH)_2$. The methods for analyzing the chemical properties and physical properties of the mocaf are as follows: for the moisture content test using thermogravimetry (Sudarmadji et al. 1997), for the acid level test using titration (AOAC 1995), for the protein content test using the Kjeldahl method (AOAC 1995), for the HCN test using a Spectrophotometer (AOAC 1995), for the Viscosity test using a Stromer viscometer (Fardiaz et al. 1992), for the degree of whiteness using a photometer (Fardiaz et al. 1992).

Data analysis

To determine the effect of each treatment on mocaf, statistical test analysis of variance (ANOVA) was used. If there were a significant difference between treatments, it was continued with Duncan's Multiple Range Test (DMRT) with a significance level of 95%.

RESULTS AND DISCUSSION

Chemical properties of mocaf

Water content (%) db

Water content is one of the most important characteristics of foodstuffs because water can affect the appearance, texture, and taste of foodstuffs. The water content in foodstuffs determines the freshness and durability of these foodstuffs. High water content makes it easier for bacteria, fungi, and yeasts to breed, so food ingredients will change (Winarno 2002). In this study, with the treatment of fermentation duration (0. 24, 48, 72-hours) and immersion concentration (0%, 5%, 10%, 15% $Ca(OH)_2$), the water was calculated as the water content (%) in the material, and it can be seen in Table 1.

The purpose of mocaf water content analysis is to determine the water content in the final product because it relates to the product's resistance to microorganism attacks (Winarno 2002). When the free water content is reduced, the growth of microorganisms can be controlled.

The results of statistical analysis in Table 1 show that the variation of the immersion concentration of 5%, 10%, and 15% $Ca(OH)_2$ give no significantly different effect on the water content of mocaf at 0-hour, 24-hours, 48-hours and 72-hours when compared to the control / 0% $Ca(OH)_2$. It is shown in the mocaf sample with Ca(OH)2 immersion variations having the same notation behind the numbers.

The treatment of fermentation duration in the manufacture of mocaf had a significantly different effect on the water content. Based on Table 1, the length of fermentation duration increases the moisture content of the mocaf. Mocaf samples with 24-hour, 48-hour and 72-hour fermentation on variations of 0%, 5%, 10% and 15% $Ca(OH)_2$ immersion did not show a significant difference, but at 0-hour fermentation on variations of 0%, 5%, 10% and 15% $Ca(OH)_2$ immersion showed significant differences when compared with 72-hours fermentation.

Table 1 shows the water content of mocaf with variations in the $Ca(OH)_2$ immersion treatment and fermentation duration. In this study, the water content of mocaf tends to increase from 0-hour to 72-hours of fermentation, both with the initial treatment of 0%, 5%, 10%, and 15% $Ca(OH)_2$ immersion.

Table 1 shows that the length of fermentation treatment affects the water content of the mocaf. The length of time of fermentation affects the water content of the mocaf. This result is from research carried out by Wahjuningsih et al. (2009), which shows that the water content will increase in proportion to the length of fermentation in the making of *gari*.

Total acid

The degree of acidity is a certain concentration required to neutralize an alkaline solution. According to Kusmanto (2009), microbes that grow during fermentation will produce enzymes that hydrolyze starch into sugar and convert it into organic acids, especially lactic acid. According to Wahjuningsih (1990), microbes that grow during gari fermentation will produce pectinolytic and cellulolytic enzymes that can destroy cassava cell walls so that starch granules' liberation occurs. In this study, with the treatment of fermentation duration (0, 24, 48, 72-hours) and immersion concentration of Ca(OH)2 (0%, 5%, 10%, 15%), the acid content was calculated as the acid content contained in the ingredients. The amount of this content can be seen in Table 2.

Based on Table 2, the total acid content of mocaf with the treatment of fermentation duration and concentration of $Ca(OH)_2$ immersion ranged from 0.11072% to 0.49472%. From the results of statistical analysis, it can be seen that the $Ca(OH)_2$ immersion treatment with concentrations of 5%, 10%, and 15% had no significant effect on acid levels in the mocaf sample but was significantly different from the control / 0% $Ca(OH)_2$ at 0-hour, 24-hours and 48-hours of fermentation. While at 72-hours fermentation, variations of immersion of 5%, 10% and 15% $Ca(OH)_2$ gave no significant effect on the control / 0% $Ca(OH)_2$.

The long fermentation treatment had a significantly different effect on the total acid content of mocaf. Based on Table 2, all treatments of 0%, 5%, 10%, and 15% $Ca(OH)_2$ samples showed a significant increase in total acid after 24-hours of fermentation. The increase in total acid will increase with the longer fermentation. The mocaf sample with variations of 5%, 10%, and 15% $Ca(OH)_2$ immersion at 48-hours of fermentation did not significantly differ from 24-hour fermentation, but 72-hours of fermentation had a significant effect on the total acid content of mocaf. While the mocaf sample with 0% $Ca(OH)_2$ / control immersion will significantly affect fermentation from 24-hours to 72-hours. It can be seen from the different notations on the numbers (Table 2).

Table 2 shows the total acid content of mocaf with variations of $Ca(OH)_2$ immersion treatment and fermentation duration. In this study, the total acid content of mocaf tended to increase from 0-hour fermentation to 72-hours fermentation, both with pre-soaking treatments of 0%, 5%, 10%, and 15% $Ca(OH)_2$. From Table 2, it can be seen that the highest total acid content was in the mocaf within 48-hours of fermentation treatment and 0% $Ca(OH)_2$ immersion or with no immersion, namely, 0.49472. On the contrary, the lowest acid content was in mocaf with 0-hour of fermentation or no fermentation and immersion of 15% $Ca(OH)_2$, namely, 0.11072 (Table 2).

In Table 2, it can be seen that the acid content of the mocaf with 0% $Ca(OH)_2$ immersion treatment was higher than the 5%, 10%, and 15% immersion; it was due to the presence of $Ca(OH)_2$, which is a strong base. When used for immersion, it will inhibit the growth of acid-producing bacteria, and disturb the environmental conditions in the fermentation location, because these acid-producing bacteria can only grow and develop in an acidic environment with a pH range of 3.6. These bacteria have an optimum pH of about 6.5-7.5 (Fardiaz et al. 1992). The process of cassava tuber fermentation produces lactic acid (pH 3.8), and the dominant microbe is lactobacillus (Kobawila et al 2005), so the amount of acid produced will be inversely proportional to the concentration of $Ca(OH)_2$ used in the immersion. $Ca(OH)_2$ solution is also a binder of vegetable acids.

Table 1. Results of water content analysis (%)

Fermentation duration	$Ca(OH)_2$ concentration			
	0% (P1)	**5% (P2)**	**10% (P3)**	**15% (P4)**
0 hour (F1)	7.028^{ab}	7.023^{ab}	7.016^{a}	7.015^{a}
24-hours (F2)	7.186^{abc}	7.188^{abc}	7.185^{abc}	7.185^{abc}
48-hours (F3)	7.227^{c}	7.198^{abc}	7.208^{bc}	7.214^{c}
72-hours (F4)	7.327^{c}	7.261^{c}	7.279^{c}	7.283^{c}

Note: *) different notation indicates significant difference ($p< 0.05$)

Table 2. Results of analysis of total acid levels (%)

Fermentation duration	$Ca(OH)_2$ concentration			
	0% (P1)	**5% (P2)**	**10% (P3)**	**15% (P4)**
0 hour (F1)	0.151^{b}	0.114^{a}	0.111^{a}	0.110^{a}
24-hours (F2)	0.406^{d}	0.334^{c}	0.332^{c}	0.332^{c}
48-hours (F3)	0.494^{f}	0.363^{c}	0.358^{c}	0.353^{c}
72-hours (F4)	0.458^{e}	0.469^{ef}	0.436^{de}	0.435^{de}

Note: *) different notation indicates significant difference ($p< 0.05$)

Table 2 shows that the length of fermentation will increase the acid content of mocaf. EL Tinay et al. (1984) state that fermented bitter cassava resulted in a decrease in pH from 6.0 to 3.8 and an increase in acidity from 0.111% to 0.802% during 192 hours (8 days) fermentation. Gari usually only undergoes fermentation for approximately 96 hours (4 days), so the pH reaches around 4.75, and the acidity reaches 0.422%.

Wahjuningsih (1990) added that the gari flavor was due to lactic acid produced in the first stage of fermentation. In contrast, ketones and aldehydes were produced in the second stage. Also mentioned by Akinrele in Wahjuningsih (1990) is that two types of organic acids have been identified in cassava fermentation, namely lactic acid, and formic acid, but only lactic acid is dominant in mocaf. It is due to the breakdown of formic acid to form carbon dioxide and possibly hydrogen. The gas will cause an anaerobic atmosphere on the substrate. Further research has found lactic, oxalic, and succinic acids in *gari*. However, lactic acid is still dominant in the *gari* (Dougan et al. 1983).

Furia (1980) stated that lactic acid is not a volatile acid, so it can be assumed that the low acidity of mocaf produced by drying can be caused by evaporation of other organic acid components contained in mocaf. Dougan et al. (1983) added that lactic acid (CH_3CH (OH)COOH), succinic acid ($HOOCH_2C$-CH_2COOH), and oxalic acid (HOOCCOOH) in mocaf were 0.4%, 0.04%, and 0.04% respectively. Oxalic acid begins to evaporate at temperatures below 100^{o}C (Kirk and Othmer 1967), while succinic acid evaporates at room temperature (Furia 1980), and lactic acid has a boiling point of 122^{o}C (Anonymous 2010).

Lactobacillus plantarum bacteria produce lactic acid, which becomes dominant after 48-hours of fermentation. On the other hand, oxalic acid is known to be produced by a fungus of the type *Geotricum*, which appears after 3 days of fermentation. In mocaf fermentation, sooner or later, this acid becomes anaerobic. Under these conditions, oxalic acid can be metabolized and depleted as the fermentation continues, so only a small amount is left in the mocaf. Likewise, succinic acid is produced from the metabolic process of fungus (Wahjuningsih 1990).

Further research conducted by Ejiofor and Okafor (1980) stated that it was possible to identify microorganisms that play a role in *gari* fermentation, namely *Leuconostoc*, *Lactobacillus*, *Bacillus* sp., and *Geotrichum* sp. *Corynebacterium* manihot will break down starch into glucose in the early stages of fermentation. Then lactic acid bacteria will convert glucose into lactic acid and other organic acids, aldehydes, and ketones, resulting in the distinctive aroma of *gari*. *Leuconostoc* sp. can be isolated as soon as fermentation begins, and the amount continues to increase until 72-hours of fermentation. After that, the growth was reduced, and no growth was observed after 96 hours of fermentation.

Protein level

In this study, the protein content determination test was carried out using the Kjeldahl method to determine the total protein content, which was calculated as total N. The total protein content of mocaf with variations in fermentation treatment and immersion concentration with $Ca(OH)_2$ can be seen as in Table 3.

Table 3 shows that the treatment variation of the fermentation duration and the concentration of $Ca(OH)_2$ immersion in mocaf gave various effects on the protein content, expressed as N-total. From these data, it can be seen that the protein content of mocaf with various treatment variations of fermentation duration and $Ca(OH)_2$ immersion concentration ranged from 0.9306% to 2.3610% (Table 3).

Immersion treatment with 5%, 10%, and 15% $Ca(OH)_2$ at 0-hour, 24-hours, 48 hours, and 72-hours had a significant effect on the total protein content of mocaf samples when compared to the control (0 % $Ca(OH)_2$).

While the 5%, 10%, and 15% $Ca(OH)_2$ treatment variations did not have a significantly different effect on 0-hour, 24-hours, 48 hours, and 72-hours of fermentation. It is shown in Table 3. The protein content of mocaf samples soaked with 0%, 5%, 10%, and 15% $Ca(OH)_2$ at 0-hour fermentation were 2.3610%; 1.5646%; 1.5528%; 1.5239%, respectively.

The long fermentation treatment had a significantly different effect on the total protein content of the mocaf sample. Long fermentation will lower the protein content of mocaf. Table 3 showed that the protein content of mocaf decreased significantly at the 24 hour fermentation when compared to the control in all samples, namely, 1.5229% (0% $Ca(OH)_2$); 1.3719% (5% $Ca(OH)_2$); 1.3707% (10% $Ca(OH)_2$); 1.3547% (15% $Ca(OH)_2$), respectively. The decrease in protein content occurred along with the increase in fermentation duration. A significant decrease in protein occurred up to 72-hours of fermentation in all samples of 0%, 5%, 10%, and 15% $Ca(OH)_2$. Until 72-hours of fermentation, total protein content in mocaf in sample of 0% $Ca(OH)_2$ was 1.2950%, 5% $Ca(OH)_2$ was 0.9285%, 10% $Ca(OH)_2$ was 0.9314%, 15% $Ca(OH)_2$ was 0.9306%.

Table 3 indicates the total protein content of mocaf decreased in line with fermentation. Wahjuningsih (1990) stated that the protein content of fresh cassava is low. In this study of making mocaf, the value of the protein content produced was also low. In this study, the highest total protein content was shown by mocaf with 0-hour fermentation treatment and 0% $Ca(OH)_2$ immersion or control, which was 2.3610%. Meanwhile, the lowest total protein content was shown by mocaf with 72-hours fermentation treatment and 15% Ca(OH)2 immersion, which was 0.9306% (Table 3).

In this study, a protein content determination test was carried out using the Kjeldahl method to determine the total protein content, calculated as total N. The protein content of mocaf was related to the amount of HCN. Therefore, the smaller the HCN mocaf, the smaller the protein content. Therefore, the N element in HCN will decrease along with the length of fermentation treatment and the variation of $Ca(OH)_2$ immersion.

The fermentation in the manufacture of mocaf is a wet fermentation that uses water as the medium. According to Hidayat (2009), most types of protein can be dissolved in water, especially methionine. Cassava is an energy source rich in carbohydrates but low in protein. The source of protein found in cassava is the amino acid methionine (Panggih 2009). Based on this description, soaking cassava with water can reduce protein content because the type of protein in cassava can dissolve in water.

According to Ezeala (1984), *gari* fermentation can cause a protein reduction of approximately 3%. It is proven by the low value of protein content in the fermentation treatment of 24-hours, 48 hours, and 72-hours and by immersing 5%, 10%, and 15% $Ca(OH)_2$. Even the presence of immersion during the fermentation process greatly influences the reduction of protein content.

HCN Level

HCN is naturally found in cassava as a cyanogenic glycoside. Cyanogenic glycosides are potentially toxic compounds because they can decompose and produce HCN. The cyanogenic glycoside found in cassava is called Linamarin with the chemical name acetone cyanohydrin glycoside (Winarno 2002). Meanwhile, according to Waspodo (1980), cyanogenic glycosides in cassava are linamarin and lotaustralin, with 93% and 7% of the total cyanogenic compound content. Mocaf HCN levels with treatment variations in fermentation and immersion concentration with $Ca(OH)_2$ can be seen in Table 4.

Table 3. Results of analysis of mocaf protein content (%) with various treatments

Fermentation duration	$Ca(OH)_2$ Concentration			
	0% (P1)	5% (P2)	10% (P3)	15% (P4)
0 hour (F1)	2.36^{f}	1.56^{e}	1.55^{e}	1.52^{e}
24-hours (F2)	1.52^{e}	1.37^{cd}	1.37^{d}	1.35^{cd}
48-hours (F3)	1.41^{d}	1.29^{bc}	1.28^{b}	1.27^{b}
72-hours (F4)	1.29^{bc}	0.92^{a}	0.93^{a}	0.93^{a}

Note: *) different notation indicates significant difference ($p < 0.05$)

Table 4. Analysis results of HCN levels (ppm) on mocaf with various treatments

Fermentation duration	$Ca(OH)_2$ Concentration			
	0% (P1)	5% (P2)	10% (P3)	15% (P4)
0 hour (F1)	91.97^{j}	62.63^{i}	50.76^{gh}	47.80^{fg}
24-hours (F2)	65.79^{i}	55.55^{h}	40.20^{de}	37.04^{cd}
48-hours (F3)	47.78^{fg}	42.72^{ef}	34.40^{bcd}	31.69^{bc}
72-hours (F4)	43.09^{ef}	31.69^{bc}	23.91^{a}	19.18^{a}

Note: *) different notation indicates significant difference ($p < 0.05$)

Based on Table 4, it can be seen that the immersion treatment with $Ca(OH)_2$ in the manufacture of mocaf has a significantly different effect on the HCN content when compared to the control. It can be seen from the different notations behind the HCN numbers (Table 4). The high concentration of $Ca(OH)_2$ will lower the HCN content of mocaf. Table 4, showed that HCN levels on mocaf with 0-hour fermentation at immersion of 0%, 5 %, 10 %, 15% $Ca(OH)_2$ concentrations were 91.97 ppm, 62.6349 ppm, 50.7659 ppm, 47.8048 ppm, respectively. A significant decrease in HCN occurred at 5%, 10% and 15% $Ca(OH)_2$ concentrations when compared with 0% $Ca(OH)_2$ concentrations, while at 10% $Ca(OH)_2$ and 15% $Ca(OH)_2$ concentrations was not significantly different in HCN levels.

On the contrary, the length of fermentation treatment also significantly affected the HCN levels of mocaf. HCN levels of mocaf also decreased along with the length of fermentation compared to the control. In 0% $Ca(OH)_2$ treatment, HCN decreased significantly and significantly differed during the 24-hour fermentation compared to 0-hour fermentation. Meanwhile, at 48-hour and 72-hour fermentation, the decrease in HCN was not significantly different, but when compared with control or 0-hour fermentation and 24-hours fermentation, there was a significant difference. In 5% $Ca(OH)_2$ treatment, HCN will experience a significant decrease and give a significant difference in 24-hour, 48-hour, and 72-hour fermentation compared to control / 0-hour fermentation. This condition is shown in Table 4.

Table 4 indicates that in this study, the 10% $Ca(OH)_2$ treatment did not give a significant difference at 0-hour, 24-hours, 48 hours, and 72-hours of fermentation compared to the 15% $Ca(OH)_2$ treatment. The 24-hour fermentation treatment compared to 48-hour fermentation in 10% and 15% $Ca(OH)_2$ immersion did not give a significant difference but gave a significant difference with 0-hour and 72-hour fermentation.

Table 4 shows that the HCN content of mocaf with treatment variations of $Ca(OH)_2$ immersion and fermentation duration tends to experience a significant decrease from 0-hour fermentation to 72-hours fermentation, with the initial treatment of 0%, 5%, 10%, 15% $Ca(OH)_2$ immersion (Table 4). Based on Table 4, it can be concluded that the length of fermentation duration will reduce the HCN level of mocaf. Table 4 shows that the duration of fermentation has a significantly different effect from 0-hour fermentation to 72-hours fermentation. Based on Table 4, the level of HCN at the treatment of 72-hours fermentation with 0%, 5%, 10%, and 15% $Ca(OH)_2$ immersion was 43.0917 ppm; 31.6915 ppm; 23.9138 ppm; 19.1809 ppm, respectively.

The decrease in HCN levels in this mocaf study is in accordance with Wahjuningsih et al. (2009) that hydrogen cyanide is an acid that is easily soluble in water so that it can be reduced. In addition, cyanide compounds will be reduced if they react with one of the basic compounds, for example, calcium hydroxide ($Ca(OH)_2$). This is because it will cause the CN- ions in the HCN structure to bind with calcium hydroxide to form a complex salt, i.e., the cyanide salt. For example, the following is the reaction of the HCN compound when reacted with $Ca(OH)_2$ to form $Ca(CN)_2$:

$$2HCN + Ca(OH)_2 \rightarrow Ca(CN)_2 + 2H_2O$$

Lactic acid fermentation, commonly used in processing cassava products, also aids in the hydrolysis of cyanogenic sugar glucosides and HCN and can be removed further in processing by heating (Akingbala et al. 2005). Winarno (2002) revealed that the processing of bitter cassava is in the form of drying, soaking before cooking, or fermentation for several days. With this treatment, much linamarin was damaged, and the HCN content dropped to 10 to 40 mg/kg of peeled cassava. HCN is easily removed by boiling as long as the lid of the pot is not tightly closed. With heating, the linamarin cleavage enzyme becomes inactive so that HCN is not formed.

Bourdoux et al. (1982) also stated that soaking cassava for one day will reduce HCN levels by 45%; if it is continued for 96-hours (4 days), HCN levels drop to 90%, and if continued for five days, HCN levels will disappear 100%, but the cassava will rot. EL Tinay et al. (1984) in Wahjuningsih (1990) stated that fermentation primarily aimed to reduce or eliminate HCN levels from cassava at low pH.

According to Wahjuningsih (1990), the allowable HCN content in traditional *gari* is a maximum of 19 ppm. However, according to FAO, cassava with a maximum HCN content of 50 ppm is still safe for human consumption (Winarno 2002). Therefore, the mocaf in this study is safe for human consumption.

Viscosity

Viscosity is one of the most important physical properties of flour. Viscosity is the internal frictional force that occurs in a fluid or fluids. The purpose of the viscosity test was to determine the level of mocaf viscosity. Based on the standard method (FAO 1990) cited in Samsuari (2006), flour viscosity was measured at 29^{o}C with a concentration of 2%. Mocaf viscosity was measured using a stormer viscometer. Table 5 exhibits the viscosity of mocaf samples with variations in fermentation duration and $Ca(OH)_2$ immersion concentration ranging from 19.5817 to 26.0733 c poise.

From the statistical analysis results, it can be seen that the immersion treatment with $Ca(OH)_2$ had a significantly different effect on the viscosity of the mocaf sample. It can be seen from the different notations behind the viscosity numbers (Table 5). The high concentration of $Ca(OH)_2$ will increase the viscosity of the mocaf. Table 5 shows that the fermentation treatments at 0-hour, 24-hours and 48-hours with 0% $Ca(OH)_2$ and 5% $Ca(OH)_2$ immersion did not significantly affect the viscosity in mocaf but were significantly different at 72-hours fermentation.

On the contrary, the 10% $Ca(OH)_2$ immersion treatment compared to the 15% $Ca(OH)_2$ immersion treatment mostly differed significantly. The 0-hour fermentation treatment at 10% $Ca(OH)_2$ immersion compared to 15% $Ca(OH)_2$ immersion did not significantly differ, but it would

significantly differ in the 24-hour, 48-hour, and 72-hour fermentation.

The long fermentation treatment also had a significantly different effect on the mocaf. The length of fermentation will make the viscosity higher. It can be seen in Table 5 that compared to 0-hour fermentation, the 24-hours fermentation had a significantly different effect on the 0%, 5%, and 15% $Ca(OH)_2$ treatments except for the 10% $Ca(OH)_2$ treatment. Compared to the 48-hours fermentation, at the 24-hours fermentation, samples with the 5%, 10%, and 15% $Ca(OH)_2$ immersion treatment did not have a significantly different effect, except for the samples with 0% $Ca(OH)_2$ immersion treatment which gave a different effect. Compared with 72-hours of fermentation, 24-hours of fermentation gave significantly different effects on the $Ca(OH)_2$ treatment of 0%, 5%, 10%, and not significantly different from the 15% $Ca(OH)_2$ treatment.

Table 5 shows that $Ca(OH)_2$ immersion treatment will increase the viscosity of mocaf. In this study, the increase in viscosity value will be directly proportional to the immersion treatment with $Ca(OH)_2$. It is because the starch content in cassava tubers, especially amylopectin, will bind to calcium ions and function to strengthen the cell wall structure. Calcium's ability to form insoluble complexes with amylopectin and free carboxyl groups on the amylopectin chain will form a strong formation so that the viscosity value of the mocaf sample will increase.

Table 5 also shows that the viscosity of mocaf with variations in the length of fermentation treatment will increase. In this study, the viscosity of mocaf tended to increase significantly at 0-hour fermentation to 72-hours fermentation, both with the initial treatment of 0%, 5%, 10%, and 15% $Ca(OH)_2$ immersion (Table 5). In addition, it is shown in Table 5 that the viscosity at 0-hour, 24-hours, 48 hours, and 72-hours of fermentation experienced a significant increase at the immersion of 10% $Ca(OH)_2$ concentration.

Wahjuningsih et al. (2009) stated that adding water to the fermentation will make the starch molecules absorb water to break the amylose crystals and break the structural bonds of the molecules. As a result, amylose will begin to diffuse out of the tissue, eventually, the tissue only consists of mostly amylopectin (Harper 1981). Winarno (2002) as well as Widaningrum and Purwani (2006) stated that the amylose content of a food ingredient has an effect on its amylograph properties.

In Table 5, it can be seen that the fermentation treatment has a major influence on the viscosity of the mocaf. It can be seen that the longer the fermentation is carried out, the higher the viscosity value produced (Table 5). It is due to the presence of fermentation, microbes that grow during fermentation will produce pectinolytic and cellulolytic enzymes that can destroy cassava cell walls in such a way that starch granules liberation occurs. This liberation process will cause changes in the characteristics of the flour produced in the form of an increase in viscosity, gelation ability, rehydration power and dissolving easiness (Wahjuningsih 1990).

As a glucose polymer, amylose and amylopectin are the two largest components of starch. Amylose has a linear structure with 1,4-α-D-glucoside bonds and forms the amorphous part of starch, while amylopectin has branched chains that meet with linear chains at 1,6-α-D-glucoside bonds and forms the crystalline part of starch. The composition of amylose and amylopectin is different for each type of starch which also affects the characteristics of starch (Belitz and Grosch 1999; McWilliams 2001).

In the presence of fermentation, microbes will degrade cell walls which cause damage to the structure and integrity of starch granules. Damage to the integrity of starch causes starch granules to absorb water so that some fractions separate and enter the medium (Greenwood 1979). Starch tends to absorb more water with either the smaller the amylose content or the higher the amylopectin content (Tjokroadikusoemo 1986). With this description, the starch fractions in cassava, amylose and amylopectin will also be damaged due to fermentation and water used for the fermentation media. This causes the amylose fraction to be dissolved in water and the amylopectin fraction to be insoluble in water. With the release of the amylose fraction from the medium, the content of the amylopectin fraction increased.

The starch composition, which mostly consists of amylopectin makes the starch structure more open so that water will more easily enter, penetrate the starch granules and cause the starch granules to swell (swollen), which is indicated by the increasing value of viscosity. According to Wuzburg, the presence of branching in amylopectin will hinder the movement and tendency to approach each other in forming hydrogen bonds. It causes amylopectin to be more stable and more resistant to changes than amylose. It affects the viscosity of the mocaf, namely the more amylose that comes out, the greater the viscosity.

Table 5. Results of mocaf viscosity analysis (c poise) with various treatments

Fermentation duration	$Ca(OH)_2$ Concentration			
	0% (P1)	**5% (P2)**	**10% (P3)**	**15% (P4)**
0 hour (F1)	19.58^{a}	19.85^{a}	23.86^{f}	24.33^{fg}
24-hours (F2)	20.81^{b}	21.19^{bc}	24.20^{f}	25.76^{h}
48-hours (F3)	21.52^{cd}	21.69^{cd}	24.07^{f}	25.98^{h}
72-hours (F4)	21.91^{d}	22.72^{e}	24.80^{g}	26.07^{h}

Note: *) different notation indicates significant difference ($p < 0.05$)

According to Subagyo (2006), when compared to tapioca starch, the viscosity of mocaf is lower. It is because, in tapioca, the starch component covers almost all of the dry matter, while in mocaf, the components other than starch are still in significant amounts. However, within 72-hours of fermentation, a mocaf product with a viscosity close to tapioca will be obtained. Therefore, it can be understood that, with long fermentation, more and more cassava cells will be broken so that the starch granule liberation becomes very extensive.

Physical properties of mocaf

Whiteness level

The whiteness level of flour depends on the basic ingredients that are processed. The color of the *Pandemir* L-2 cassava variety was classified as white so that the whiteness value was higher. In general, consumers prefer white flour.

In this study, the whiteness level on the mocaf was tested using the L*a*b* system using the *Color Reader CR-100* (Minolta, Japan), to determine the intensity of the color produced by mocaf with variations in fermentation duration and immersion concentration with $Ca(OH)_2$ which can be seen in Table 6. In Table 6, the whiteness level of the mocaf samples with variations in fermentation duration and immersion concentration with $Ca(OH)_2$ ranged from 85.195 - 90.1567 (Table 6).

From the results of statistical analysis showed that the treatment of soaking $Ca(OH)_2$ with different concentrations had an effect on the degree of whiteness of the mocaf (Table 6). This is shown in samples with $Ca(OH)_2$ immersion with different concentrations having different notations behind the numbers on the degree of whiteness. The concentration of $Ca(OH)_2$ used will increase the degree of whiteness of the mocaf. This can be seen in Table 6. Mocaf soaked with $Ca(OH)_2$ at a concentration of 5%, 10%, 15%, when compared to the control, the degree of whiteness was greater, namely: 85.1950 for 0% $Ca(OH)_2$, 86.5717 for 5 % $Ca(OH)_2$, 87.0867 for 10% $Ca(OH)_2$, 87.2300 for 15 % $Ca(OH)_2$, respectively (Table 6).

Table 6 shows that 5% $Ca(OH)_2$ treatment, when compared with 10% $Ca(OH)_2$ treatment, mostly showed significant differences. Compared to 10% $Ca(OH)_2$ treatment, the 5% $Ca(OH)_2$ treatment at 0-hour, 24-hours, and 72-hours gave a significant difference, except for 48-hours fermentation, which did not give a significant difference. On the contrary, compared to 15% $Ca(OH)_2$ treatment, the 10% $Ca(OH)_2$ treatment did not have a significant effect on 0-hour, 24-hour and 48-hour fermentation, except for 72-hour fermentation which gave a significant difference.

In Table 6, it is shown that the length of fermentation also has a significantly different effect on the mocaf sample. The length of fermentation will increase the degree of whiteness produced. When compared with the 24-hour fermentation, the 0% $Ca(OH)_2$ treatment with 0-hour fermentation was significantly different, namely 85.9517 for 24-hour fermentation and 85.1950 for 0-hour fermentation, respectively. On the other hand, when compared with control / 0-hour fermentation, the treatment of 5%, 10%, and 15% $Ca(OH)_2$ at 24-hour fermentation was not significantly different. It is shown in Table 6 by the same notation behind the number on the intensity of the whiteness level.

Compared to 24-hours fermentation, the 0% and 5% $Ca(OH)_2$ treatments at 48-hours fermentation gave a significant difference, meanwhile the 10% and 15% $Ca(OH)_2$ treatments at 48-hours fermentation compared to 24-hours fermentation did not give any significant difference. Meanwhile, the 48-hour and 72-hour fermentation for all treatments of 0%, 5%, 10%, and 15% $Ca(OH)_2$ gave significantly different effects.

Table 6 depicts the degree of whiteness of the mocaf with variations in the $Ca(OH)_2$ immersion treatment and the duration of fermentation. In this study, the degree of whiteness of mocaf tended to increase significantly (Table 6) from the 0-hour to 72-hour fermentation, both with the initial treatment of soaking 0%, 5%, 10%, 15% $Ca(OH)_2$. The highest degree of whiteness was in the mocaf sample immersed with 15% $Ca(OH)_2$ and fermented for 72-hours at 90.1567. While the intensity of the lowest level of whiteness was in the mocaf without the treatment of $Ca(OH)_2$ immersion and fermentation (control) at 85.1950 (Table 6).

The duration of immersion in the $Ca(OH)_2$ solution has an effect on increasing the color quality of the flour produced. This means that the longer the $Ca(OH)_2$ solution is immersed, the clearer the resulting color will be. Chemically, quicklime solution emits a lot of heat, is alkaline, and easily attracts carbon dioxide gas from the air, so that the water is easily cloudy. It causes the color pigments in the material to dissolve in the $Ca(OH)_2$ solution. Thus the soaked cassava becomes clear and will allow the resulting mocaf to be white.

Table 6. Whiteness levels of mocaf

Fermentation duration	$Ca(OH)_2$ Concentration			
	0% (P1)	5% (P2)	10% (P3)	15% (P4)
0 hour (F1)	85.19^{a}	86.57^{c}	87.08^{def}	87.23^{efg}
24-hours (F2)	85.95^{b}	86.82^{cde}	87.44^{fgh}	87.52^{fghi}
48-hours (F3)	86.63^{cd}	87.37^{fg}	87.70^{ghi}	88.01^{i}
72-hours (F4)	87.36^{fg}	87.88^{hi}	88.72^{j}	90.15^{k}

Note: *) different notation indicates significant difference ($p< 0.05$); L = brightness of color, L (0) = dark, L (100) = bright

In Wahjuningsih et al. (2009), it is stated that the fermentation in making *gari* uses the wet method, meaning that when the fermentation takes place, soaking in water will prevent the material from browning. According to Kusmanto (2009), the protein content in cassava flour can cause a brown color when drying or heating. Cassava is an energy source rich in carbohydrates but low in protein. Based on this description, soaking cassava with water can reduce protein content because the type of protein contained in cassava can dissolve in water. From this description, the protein content of the ingredients greatly affects the whiteness of the mocaf.

In the manufacture of flour products, the needed cassava is the one that does not contain much protein because flour containing more than 2% protein will become less white in color and has a "musty" smell and cannot be stored for a longer period of time. From this description, the protein content of the ingredients greatly affects the whiteness of the mocaf. Subagyo (2006) stated that the bright color of the mocaf was due to the absence of a protein hydrolysis process, so the browning caused by the Mailard reaction did not take place intensively. While the mocaf without fermentation treatment has a lower color intensity, this is due to the hydrolysis process so that the protease enzyme breaks the peptide bond to produce an amine group which is the Mailard reaction material, where in this condition, the amine group of the protein reacts with the aldehyde or ketone group of reducing sugar to produce a brown color (Subagyo 2006).

The conclusions from the research on the effect of fermentation duration and concentration of $Ca(OH)_2$ for immersion on the characteristics of mocaf (modified cassava flour) of bitter cassava variety (*Pandemir* L2) are as follows: (i) Treatment variation of fermentation duration (0-hour, 24-hours, 48-hours, and 72-hours) and immersion with $Ca(OH)_2$ (0%, 5%, 10%, 15%) had an effect on the chemical properties of mocaf. The higher the $Ca(OH)_2$ concentration, the lower the water content, viscosity, acidity, HCN and protein content. The longer the fermentation, the higher the water content, acid content and viscosity, but the HCN and protein content decreased. (ii) Variations in the treatment of fermentation duration (0-hour, 24-hours, 48-hours, and 72-hours) and $Ca(OH)_2$ immersion (0%, 5%, 10%, 15%) affected the physical properties of mocaf. The high concentration of $Ca(OH)_2$ and the length of fermentation increased the whiteness level of the mocaf.

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