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# The Effect of Yeast Type and Cassava Age on Hydrogen Cyanide (HCN) and Protein Content in Mocaf (Modified Cassava Flour) of the Local Daplang Variety

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**Abstract:** Cassava (Manihot esculenta Crant.) is a food crop that serves as one of the food sources in Indonesia. One of the cassava processing diversification efforts currently being developed is Mocaf (Modified Cassava Flour). This study aims to determine the method of producing mocaf flour from cassava (local daplang variety) through fermentation that meets quality standards (SNI 01-2997-1992) at various cassava ages. This study used a completely randomized design consisting of two factors, namely yeast type (M-Bio mocaf, pakmaya yeast, and tape yeast) and the age of the daplang cassava plant (7, 8, and 9 months), which was repeated three times to obtain 29 experimental units. The results showed that the yeast type treatment had a significant effect on HCN and protein levels. The best HCN level was found in the tape yeast type at 7.63 mg/kg. The best protein level was found in the M-Bio mocaf treatment at 1.68%. The cassava age treatment had a significant effect on HCN levels but not on protein levels. The best result was obtained in the seven-month cassava age treatment (u1) at 1.27%. In the combination treatment, there was an interaction between yeast type and cassava age on the protein content parameter, with the best result obtained in r3u3 at 1.14%.

**Keywords:** Yeast type, cassava age, HCN, protein

## 1. Introduction

Cassava (Manihot esculenta Crantz) is the fourth most important food source after rice, corn, and secondary crops, and is widely cultivated in Indonesia. Fresh cassava is easily damaged if not processed immediately after harvesting due to its high water content, the presence of polyphenol compounds that cause browning, and the limited availability of cassava processing technology. (Tandrianto et a. 2014). Cassava processing technology through fermentation is one of the efforts to increase protein and reduce hydrogen cyanide (HCN) content. Thus, fermented cassava flour has advantages over regular cassava flour, namely high protein content, lower HCN, wide application, easier dispersion into food products, and easier formation of 3 dimensions between components, resulting in better product consistency Translated with DeepL.com (free version) (Sadjilah, 2011). The use of mocaf flour in the food industry has been widely adopted and has yielded satisfactory results. It is used in the bread, instant noodle, and other food product industries as a substitute for wheat flour (Gaol et al., 2022).

According to Wulandari et al. (2020) Cassava flour that is converted into mocaf flour through a fermentation process that results in the loss of color-producing substances, which can cause brown discoloration in cassava during drying. Mocaf flour undergoes a fermentation process with Lactobacillus bacteria that can secrete extracellular enzymes (proteins) into the cassava during the fermentation process (Khasanah et al. 2024). During the fermentation process, lactic acid bacteria can produce amylase enzymes that are used to modify the amylose substance in mocaf and proteinase enzymes that can hydrolyze proteins into natural peptides (Nazriati et al., 2021). The characteristics of mocaf flour are thought to be influenced by the duration of fermentation, the type of starter used, and the age of the cassava. The longer the fermentation, the more cassava is broken down as the starch is decomposed by microorganisms contained in tape yeast, fermipan yeast, and M-Bio mocaf. The age of the cassava will affect the physical and chemical properties of mocaf, as well as changes in color and aroma. From the above description, the research questions for this study are: how does the use of different types of yeast affect the quality of mocaf produced, how does the age of cassava affect the quality of mocaf, and is there a relationship between the use of yeast types and the age of cassava on the quality of mocaf? The

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purpose of this study is to determine the type of yeast as a fermentation agent and the age of cassava harvest, as well as the interaction between the two on the quality of mocaf. The results of this mocaf study can be used as an alternative to reduce dependence on wheat flour

In addition to improving nutritional value and safety, the fermentation process in mocaf production plays an important role in modifying the physicochemical characteristics of cassava starch. Fermentation alters starch granule structure, reduces amylose crystallinity, and increases water absorption capacity, which directly affects the functional properties of mocaf flour in food processing (Diniyah et al., 2023). These changes enable mocaf flour to exhibit better swelling power, viscosity, and dough-forming ability, making it more comparable to wheat flour in various food applications. Furthermore, the activity of lactic acid bacteria during fermentation contributes to a decrease in undesirable odors and enhances flavor stability, thereby increasing consumer acceptance of mocaf-based products.

## 2. Illustrations

### 2.1 Material

The tools used in this study were knives, basins, buckets, cutting boards, slicers, blenders, scales, and sieves. The ingredients used in this study were local daplang cassava, lime, clean water, fermifan/pakmaya yeast, M-Bio mocaf, and tape yeast. Chemicals: Na2CO3, H2SO4, NaSO4, NaOH, Na2SO3, and HCL.

### 2.2 Experimental Design

This study used a completely randomized design (CRD) with two factors consisting of yeast treatment with three levels, namely r1 (M-Bio mocaf), r2 (Fermipan yeast), and r3 (tape yeast), and cassava plant age treatment with 3 levels, namely u1 (7 months old), u2 (8 months old), and u3 (9 months old). Thus, there were 9 experimental units and 3 replicates. The use of yeast dosage/measurement refers to the research (Nurjanah et al., 2022), namely the fermentation process of mocaf flour with a ratio of 1 g yeast: 1 kg cassava: 1 liter water.
The observation variables included:

#### 2.2.1 HCN Analysis using (qualitative test method)

The analysis to determine the HCN content was carried out using the argentometric titration method, preceded by distillation. Based on the analysis data, the HCN content in the flour can be measured using the following equation:

$$HCN = \frac{(ml\ AgNO_3)}{material\ weight} x1.000\ mg/kg$$

#### 2.2.2 Protein Content Analysis Using the Kjeldahl Method

1.0 g of red mocaf was destroyed by placing it in a Kjeldahl flask and adding 10 mL of concentrated H2SO4 and 5 g of Na2SO4. The Kjeldahl flask was heated with a Bunsen burner in an acid cabinet until the solution became clear. The destroyed sample was added with 10 mL of distilled water, then placed in a distillation apparatus and added with 35 mL of NaOH Na2SO3. Distillation was carried out with a distillate collector in a 100 mL Erlenmeyer flask containing a saturated boric acid solution and a few drops of indicator mix (methyl blue: methyl blue). Distillation is stopped when the solution turns green. The solution obtained is titrated with 0.1 M HCl until the solution turns purple.

The protein content is calculated using the equation:

$$\textbf{Kadar protein}\ \% = \frac{\mathbf{(V1\ -\ V2)x0,014xfk}}{\boldsymbol{W}}\boldsymbol{x}\ \mathbf{100}\%$$

Explanation:
Fp = dilution factor
Fk = conversion factor (6.25)
V1 = Volume of HCl titrant (ml)
V2 = Normality of HCl (ml)
W = Weight of sample (mg)

### 2.3 Stages of the Mocaf Flour Production Process

The mocaf flour production process begins with the selection of fresh cassava, which is then peeled and thoroughly washed to remove adhering dirt before being soaked in clean water. The washed cassava is subsequently sliced into thin chips to facilitate the drying process. These cassava chips then undergo a crucial fermentation stage using various types of yeast, as fermentation distinguishes mocaf flour from ordinary cassava flour. The starter composition used in the fermentation process is 1 g of yeast per 1 liter of water for every 1 kg of fresh cassava. Fermentation is carried out for 72 hours for each treatment. During this process, color components such as pigments especially in yellow cassava and proteins that cause browning during heating are removed, resulting in mocaf flour that is whiter, odorless, and neutral in taste. Moreover, fermentation improves the functional properties of the flour, making its characteristics and quality similar to wheat flour and thus suitable as a wheat flour substitute in the food industry. After fermentation, the cassava is removed, drained, and manually pressed to reduce its water content, thereby accelerating the drying process. The pressed cassava chips are then dried either by sun-drying for 2–3 days until they become brittle and easily broken, or by oven-drying at 50°C during unfavorable weather conditions, although sun-drying produces the best quality. Finally, the dried cassava chips are ground using a blender and sifted to obtain mocaf flour with a fineness level of 80–100 mesh.

## 3. Results

Variables measured to observe the effect of treatment on HCN content and mocaf flour protein content (Table 1).

**Table 1: HCN and protein content due to yeast type and cassava age treatment**

| Treatment | HCN Content (mg/kg) | | Protein Content (%) | |
|---|---|---|---|---|
| | Types of yeast | | | |
| r1=M-Bio mocaf | 9,91 | a | 1,68 | a |
| r2=fermipan yeast | 9,42 | a | 1,46 | b |
| r3=tape yeast | 7,63 | b | 1,43 | b |
| | Age of cassava (months) | | | |
| u1=7 | 8,66 | d | 1,93 | d |
| u2=8 | 9,02 | d | 1,41 | e |
| u3=9 | 9,28 | d | 1,27 | e |
| Interaksi | (-) | | (+) | |

### 3.1 HCN Level

Table 1 shows that the HCN content of mocaf flour produced by yeast types was 7.63-9.91 mg/kg. The results of the analysis of variance showed that the treatment had a significant effect on the HCN content of mocaf flour ($p < 0.05$). For cassava age, the content was 8.66-9.28 mg/kg. The results of the analysis of variance showed no significant effect (p<05.

### 3.2 Protein Level

The protein content (Table 1) shows that the mocaf flour produced from yeast types was 1.43-1.68% and cassava age was 1.27-1.93%. The results of the diversity analysis show that the yeast type and cassava age treatments had a significant effect on the protein content of mocaf flour ($p < 0.05$). There was an interaction between yeast type and cassava age, resulting in mocaf flour content of 1.14-2.31%. The highest result was obtained from the combination of M-Bio mocaf yeast and 7-month-old cassava (r1u1), which showed the highest content of 2.31% (Table 2).

Table 2. Interaction between yeast type and cassava age on protein content (%)

| Jenis ragi | u1=7 | u2=8 | u3=9 |
|---|---|---|---|
| r1=M-Bio mocaf yeast | 2,31a | 1,50b | 1,22c |
| r2=fermipan yeast | 1,69d | 1,35b | 1,33b |
| r3=tape yeast | 1,78d | 1,36b | 1,14c |

**Note: Numbers followed by different letters indicate significant differences.**

## 4. Discussion

Protein in foodstuffs determines the quality of the foodstuffs themselves. Protein can be found in animal and plant-based foodstuffs. In plant-based foodstuffs, it is found in tubers, leaves, roots, and seeds. The results of the analysis of variance show that there is an interaction between the type of yeast and the age of the cassava plant on the protein content of mocaf flour (Table 2).

The yeast used for cassava fermentation contains different microorganisms. Tape yeast contains Saccharmyces and molds such as Amylomyces rouxi, which are capable of breaking down starch into sugar, producing alcohol and organic acids. Bread yeast (fermipan) is dominated by S. cerevisiae, which plays a greater role in producing CO2 and ethanol, resulting in a reduction in HCN compared to tape yeast. Microbes break down linamarin, and tape yeast is most effective at reducing HCN. Yeast that contains a lot of mold (Rhizopus, Aspergillus) can increase the relative protein content because the microbial biomass increases. Protein is a macromolecule composed of amino acids that are linked together by peptide bonds. This occurs because during the fermentation process, L. casei breaks down the starch substrate and produces large amounts of lactic acid. The lactic acid produced lowers the pH of the growth environment, causing the protein to hydrolyze into amino acids, thereby increasing the protein content (Corsetti dan Settani, 2007). L. casei bacteria are lactic acid- producing bacteria obtained through glucose fermentation and homofermentative lactate formation, producing nearly 85% pure lactate Bakteri (Gaol et al., 2022). Lactobacillus plantarum, The increase in protein levels is caused by the fact that during fermentation, Lactobacillus plantarum lactic acid bacteria produce proteinase enzymes. The increase in protein levels is obtained from the activity of protease enzymes produced by microbes in the fermentation process (Tandrianto et al., 2014). This increase in protein content is due to an increase in the number of microorganisms that act as single-cell protein (SCP), which is protein obtained from microorganisms. (Becker & Venktaraman, 1982).

The age of cassava greatly determines its HCN content, which generally increases from young to medium age, then decreases in old age. Cyanide acid (HCN) analysis was conducted to determine the decrease in cyanide acid levels during the process of making fermented cassava flour. Cyanide acid is highly volatile in air, especially at temperatures above 25°C. Due to its high solubility in water, a washing process is essential to reduce cyanide acid toxicity. HCN levels can also be reduced through soaking, or by sun-drying, which can break down cyanide acid by up to 80% (Nazriati, et al., 2021).

During the fermentation process, the interaction between the type of yeast and the age of the cassava shows that young cassava added to yeast will cause HCN to decrease rapidly. Old cassava added to yeast results in relatively high protein levels due to the microorganism mass added to the natural concentration of old.

## 5. Conclusion

The results of the study can be concluded that the use of different types of yeast has a significant effect on HCN and protein levels on the quality of mocaf produced. The age of cassava has no effect on HCN levels, but it has a significant effect on protein levels. The combination of yeast type and cassava age resulted in an interaction. The best results were obtained from the combination of M-Bio mocaf with 7-month-old cassava, with M-Bio mocaf (r1u1) at 2.34%.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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