

# Effect Of Rhizopus Oryzae Fermentation On Characteristics Of Fermented Cassava Flour

Ellyas Alga Nainggolan

**Abstract:** The aim of this study was to determine the effect of fermentation using *Rhizopus oryzae* for 24, 48, and 72 hours on characteristics of fermented cassava flour. The characteristics of fermented cassava flour determine through analysis of moisture content, starch content, yield, color, and microstructure. The results showed that the properties of fermented cassava flour were decreased after 72 hours fermentation. The yield of fermented cassava flour using *Rhizopus oryzae* for 24, 48, and 72 hours were 36.16%, 35.64% and 34.88%, respectively. The decrease in water content of fermented cassava flour was 8.57%, 8.45% and 8.36% during fermentation time 24, 48, and 72 hours, respectively. Starch content analysis results obtained in fermented cassava flour during fermentation for 24, 48, and 72 hours were 50.49%, 49.52 % and 49.15, respectively. The brightness level of fermented cassava flour using fermentation *Rhizopus oryzae* for 72 hours was 97.69, while unfermented cassava flour had brightness level 102.46. SEM (Scanning Electron Microscopy) was used to analysis microstructure, the results showed an alteration in the starch granules of fermented cassava flour for 72 hours fermentation.

**Index Terms:** Fermentation time, *Rhizopus oryzae*, characteristics, fermented cassava flour.

## 1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important carbohydrate food sources in the tropical regions [1]. Cassava tubers can be used for processed foods, flour products and for animal feed [2]. It can be processed into tapioca, cassava flour for human consumption and various forms, while the leaves are cooked and eaten especially in Sumatera island, Indonesia. Cassava is cultivated on 1.4 million hectares of land plantation and the annual production average of 16 million tons in Indonesia [3]. Indonesia also has the potential of cassava tubers as well as a source of carbohydrate and also raw materials for local flour. However, cassava tubers contain cyanide acid (HCN) which is toxic to man as they restrict to the functioning of specific organs and enzymes [4]. Cassava tubers is also regarded as a raw material with low in protein, minerals and vitamins contents [5] [6]. It is an obstacle in the processing of cassava broader and also decreasing the economic value of cassava. The perishable cassava also affects the low price of cassava tubers. This is caused by high moisture content of fresh cassava tubers, so the cassava tubers need to handle with post-harvest process. One of the derived products from cassava tubers is fermented cassava flour which is processed using fermentation to modification the cassava cells [7]. Some researchers have focused on the fermentation of cassava with various microorganism and additional nutrients for reducing toxin and improving the quality of fermented cassava tubers [8] [9]. Fermented cassava flour is one of commodity cassava tuber which is produced using fermentation process. The most common microorganism for fermented cassava flour production is lactic acid bacteria. In the current study, the production of fermented cassava flour was done by Gunawan et al [10] using *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Rhizopus oryzae*. The results showed that level protein content of fermented cassava flour was increasing. Moreover, *L. plantarum* could reduce the

cyanogenic glycoside up to 80% during fermentation process [11] [12]. This study was conducted to determine the effect of fermentation time on characteristics of fermented cassava flour of fermented cassava flour using *Rhizopus oryzae*. The characteristics of fermented cassava flour determine through analysis moisture content, starch content, yield, color, and microstructure.

## 2 METHOD

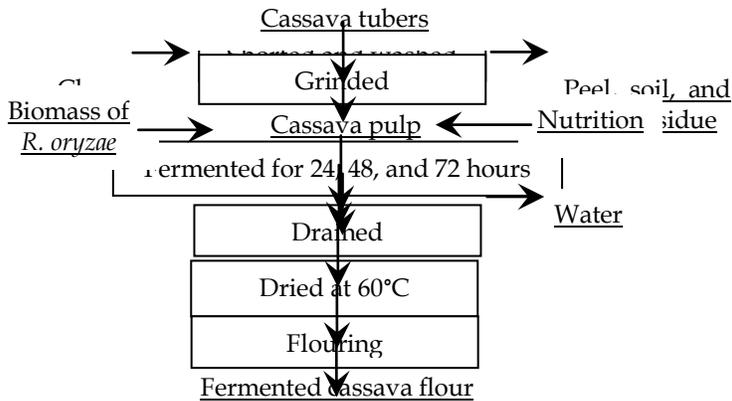
### 2.1 Inoculum Preparation

*Rhizopus oryzae* were obtained from Laboratory of Microbiology and Molecular Genetics, Institut Teknologi Del, Situluama, North Sumatera, Indonesia. The media for inoculum contained (g/l): glucose, 50; yeast extract, 5; urea, 7.5;  $K_2HPO_4$ , 3.5;  $MgSO_4 \cdot 7H_2O$ , 0.75;  $CaCl_2 \cdot 2H_2O$ , 1 and 0.05 M citrate buffer (pH 5.5). 50 ml media were sterilized using autoclave at 121°C. Inoculum cultures were grown anaerobically in 250 ml Erlenmeyer flasks for 30 h, 30°C, and 130 rpm. Samples from cultivation were centrifuged to get the biomass of *Rhizopus oryzae*.

### 2.2 Cassava Pulp Fermentation

Cassava tubers were obtained from Pasar Balige, a local market at Toba Regency, North Sumatera, Indonesia. The cassava tubers were peeled, washed, and grinded using grinder. The cassava pulp (100 g) were put into 250 ml-fermenter. The biomass of *Rhizopus oryzae* and 73 ml nutrition which contained urea (8.0 g),  $MgSO_4 \cdot 2H_2O$  (7.0 g),  $KH_2PO_4$  (1.3 g) and citrate acid (2.0 g) were added into fermenter. The fermentations were conducted for 24, 48, and 72 h, at 30°C, and the relative humidity was 90 - 93%. The fermented cassava flour was analyzed covering moisture content [13], starch content [13], yield [13], color using color reader [14], and microstructure using SEM (Scanning Electron Microscope) ZEISS. The flow process for fermented cassava flour production using *Rhizopus oryzae* can be seen in Fig 1.

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**Fig 1.** Flow chart of fermented cassava flour production using *Rhizopus oryzae*

### 3 RESULTS AND DISCUSSION

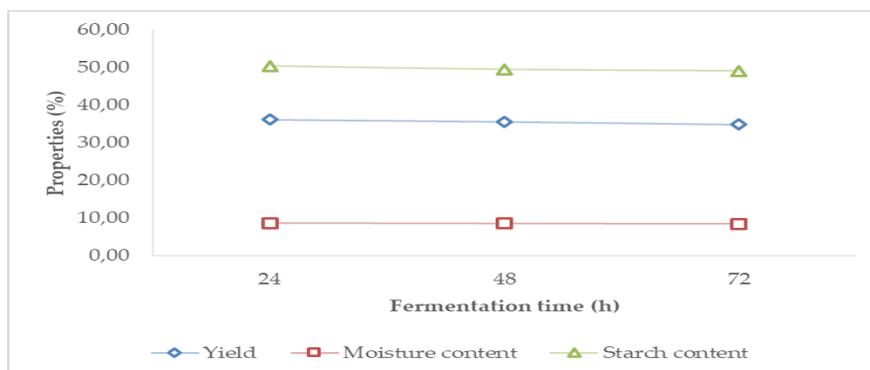
Fig 2 showed the properties of fermented cassava flour that produced using *Rhizopus oryzae*. The yields of cassava flour were obtained from the mass of fermented cassava flour which were formed from the mass of processed cassava. The yield of fermented cassava flour using fermentation *Rhizopus oryzae* for 24, 48, and 72 hours were 36.16%, 35.64% and 34.88 %, respectively. The results of moisture content in fermented cassava flour using fermentation *Rhizopus oryzae* for 24, 48, and 72 hours were 8.57, 8.45 and 8.36, respectively. Julianti et al [15] reported that unfermented cassava flour had 9.51% of moisture content. The moisture content of produced flour has met the quality requirements of flour by SNI [16] that the standard for moisture content of flour is 12%. The moisture content of fermented cassava flour is lower than unfermented cassava flour, it is caused by during fermentation process the starch is degraded and water released from the cell of cassava tubers [17]. The other factor that causes the reducing of moisture content is the pressing and drying [18]. The decrease in moisture content of fermented cassava flour is necessary because it can affect the process storage of flour. Gunawan et al [10] reported that the starch content of fermented cassava flour was 48.20% after 120 hours fermentation process with *Rhizopus oryzae*. Starch content analysis results obtained in fermented cassava flour using fermentation *Rhizopus oryzae* for 24, 48, and 72 hours were 50.49%, 49.52 % and 49.15, respectively. The decrease in starch content during fermentation could be caused by the

microbes breaking down starch into simple sugars (glucose) as carbon sources for protein or fat synthesis [19]. Furthermore, glucose was converted into lactic, acetic, butyric, propionic acids, and ethanol [20] [21]. The starch content of fermented cassava chip using *L. plantarum* was decreased from 76.86% to 70.72% after 96 h fermentation [22].

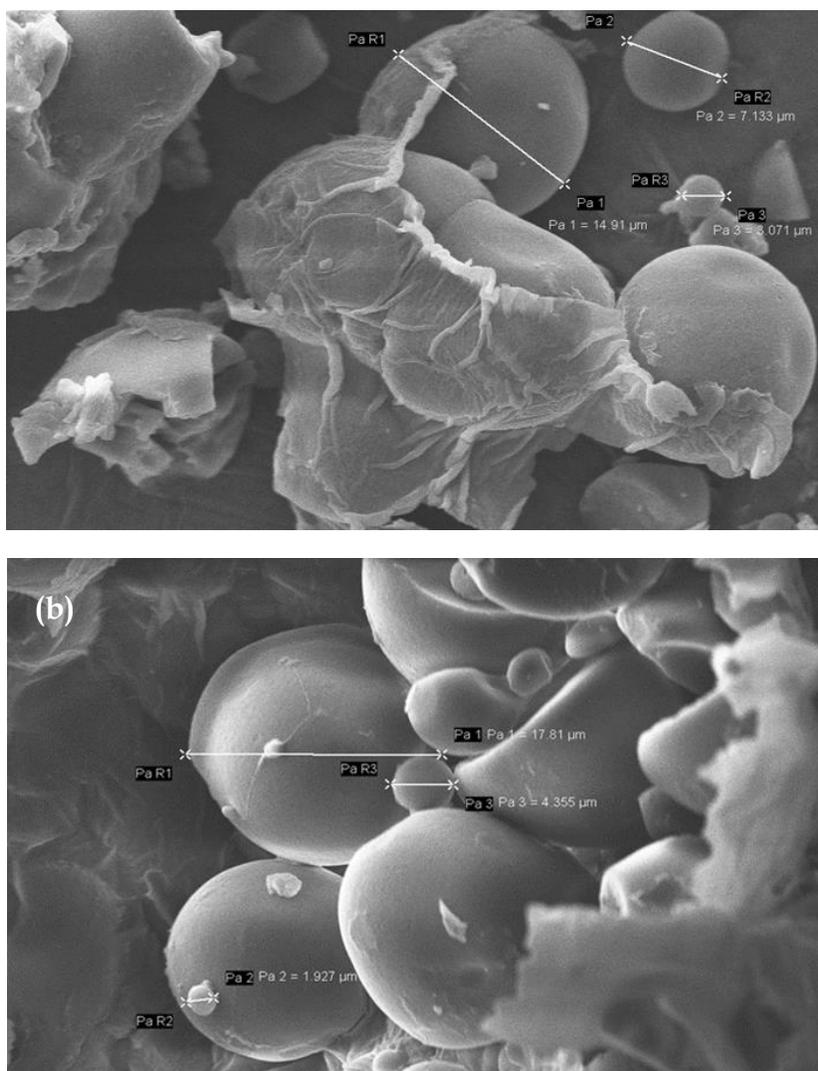
**TABLE 1**  
**COLOR READER RESULT ANALYSIS WITH L\*, A+, AND B+**

Fermentation time (h)	L*	a+	b+
0	102,46	0,63	6,25
24	95,30	0,12	21,17
48	96,92	0,15	19,12
72	97,69	0,06	17,36

The color of fermented cassava flour was measured by color reader (L\*, a+, b+). L value of color reader is from 0 to 100, where value 0 is darkness level and value 100 is brightness level [23], while a+ is magenta and b+ is yellow. The brightness level of fermented cassava flour using fermentation *Rhizopus oryzae* for 72 hours was 97.69, while unfermented (fermentation time 0) cassava flour has brightness level 102.46 (Table 1). Degradation process of pigment during fermentation and browning reaction during drying process affected brightness level of produced flour [24] [25]. Color in food and beverage is one of the most important factors to make them more interesting [26] [27]. The results of microstructure of produced flour using *Rhizopus oryzae* for fermentation time unfermented cassava flour and 72 hours is shown in Fig 3. The microstructure for fermented and unfermented cassava flour are seen having the starch granules with 2000 X magnification. Figure 3a shows that the fermented cassava flour granule has a round shape with a granule diameter from 3.07-14.91  $\mu\text{m}$ , while diameter of unfermented cassava flour was ranging from 1.92-17.81  $\mu\text{m}$ . Micrograph of starch granules have different sizes between fermented and unfermented cassava flour. The difference of granule diameter is caused by fermentation process. Elkhalfifa et al [28] explained that the proteolytic activity removed the protein matrix that envelops starch that affect releasing of its granules during fermentation. Liu et al [29] reported that modifications of starch could affect the functional properties of starch.



**Fig 2.** The properties of fermented cassava flour during fermentation for 24, 48, and 72 hours



**Fig 3.** Microstructure of fermented cassava flour for fermentation time (a) 72 hours and (b) unfermented cassava flour

#### 4 CONCLUSION

Fermentation of cassava pulp using *Rhizopus oryzae* was shown to modify the characteristics of fermented cassava flour (the produced flour). The properties of fermented cassava flour were decreased after 72 hours fermentation. The yield of fermented cassava flour using *Rhizopus oryzae* for 24, 48, and 72 hours were 36.16%, 35.64% and 34.88 %, respectively. The decrease in water content of fermented cassava flour was 8.57%, 8.45% and 8.36% during fermentation time 24, 48, and 72 hours, respectively. Starch content analysis results obtained in fermented cassava flour during fermentation for 24, 48, and 72 hours were 50.49%, 49.52 % and 49.15, respectively. The brightness level of fermented cassava flour using fermentation *Rhizopus oryzae* for 72 hours was 97,69, while unfermented cassava flour has brightness level 102,46. SEM (Scanning Electron Microscopy) was used to analysis microstructure, the results showed an alteration in the starch granules of fermented cassava flour for 72 hours fermentation.

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#### REFERENCES

- [1] FAO. 2004. A cassava industrial revolution in Nigeria: The potential for a new industrial crop. The Global Cassava Development Strategy. Rome.
- [2] FAO dan IFAD. 2001. A review of cassava in Asia with country case studies on Thailand and Viet Nam Volume 3. Proceedings of The Validation Forum on the Global Cassava Development Strategy. Rome, 26-28 April 2000.
- [3] Ginting, E. dan Hartojo, K. 2002. Cassava Processing Technologies Used in Indonesia. Malang. Indonesian Legumes and Tuber Crops Research Institute.
- [4] Rocha-e-Silva, R.C, Cordeiro L.A.V, Soto-Blanco B. 2010. Cyanide toxicity and interference with diet selection in quail (*Coturnix*). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*. 151. 3: 294-297. <https://doi.org/10.1016/j.cbpc.2009.12.001>
- [5] Onwueme, I.C. 1978. The tropical tuber crops. UK: John Wiley and Sons Ltd.
- [6] Aletor, V.A. 1993. Allelochemical in plant food and feeding stuffs: Nutritional, biochemical and physiopathological aspects in animal production. *Veterinary and Human Toxicology Journal*. 35: 57-67
- [7] Subagio A. 2007. Industrialisasi Modified Cassava Flour (MOCFA) sebagai Bahan Baku Industri Pangan untuk

- Menunjang Diversifikasi Pangan Pokok Nasional. Jember: Fakultas Teknologi Pertanian, Universitas Jember.
- [8] Kimaryo, V.M. and Massawe, G.A. 2000. The use of a starter culture in the fermentation of cassava for the production of "Kivunde", a Traditional Tanzanian Food Product. *International Journal of Food Microbiology*. 56: 179-190. [https://doi.org/10.1016/s0168-1605\(00\)00159-8](https://doi.org/10.1016/s0168-1605(00)00159-8)
- [9] Muzanila, Y.C., Brennan, J.G. and King, R.D. 2000. Residual cyanogens, chemical composition and aflatoxins in cassava flour from Tanzanian villages. *Food Chemistry*. 70: 45-49. [https://doi.org/10.1016/S0308-8146\(00\)00062-5](https://doi.org/10.1016/S0308-8146(00)00062-5)
- [10] Gunawan, S., Widjaja, T., Zullaikah, S., Ernawati, L., Istianah, N., Aparamarta, H. W. and Prasetyoko, D. 2015. Effect of fermenting cassava with *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Rhizopus oryzae* on the chemical composition of their flour. *International Food Research Journal*. 22 (3), 1280-1287.
- [11] Iwuoha GN, Ubeng GG, Onwuachu UI. 2013. Detoxification effect of fermentation on cyanide content of cassava tuber. *Journal of Applied Science & Environmental Management*. 17(4):567
- [12] Obilie EM, Tano-Debrah K, Amoa-Awua WK. 2004. Souring and breakdown of cyanogenic glucosides during the processing of cassava into akyeke. *International Journal of Food Microbiology*. 93(1):115-121. <https://doi.org/10.1016/j.ijfoodmicro.2003.11.006>
- [13] AOAC. 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists. Washington, D.C.
- [14] Yuwono, S.S. dan Susanto, T. 1998. Pengujian Fisik Pangan. Fakultas Teknologi Pertanian, Universitas Brawijaya. Malang
- [15] Julianti E, Lubis Z, Ridwansyah, Era Y, and Suhaidi I. 2011. Physicochemical and functional properties of fermented starch from flour cassava varietas. *Asian Journal of Agricultural Research*. 5(6): 292-299. <http://dx.doi.org/10.3923/ajar.2011.292.299>
- [16] SNI, 1992. Syarat Mutu Tepung Ubi Kayu SNI No. 01. 2997. 1992. Dewan Standar Indonesia, Jakarta
- [17] Oktavian, P. 2010. Perubahan Karakteristik Fisiko Kimia Mocaf (Modified cassava flour) Selama Fermentasi (Kajian Lama Proses Fermentasi). Skripsi Sarjana. UB. Malang
- [18] Amin H. 2006. Improvement of Quality and Self-life of Kasoami, a Traditional Cassava Based Food from South East Sulawesi. *Forum Pascasarjana*. 29(4): 301-319
- [19] Lehninger, A.L., Nelson, D.L. and Cox, M.M. 1982. Principles of biochemistry. New York: Worth Publish.
- [20] Phruksawan, P., Kulpreecha, S., Sooksai, S. and Thongchul, N. 2012. Direct fermentation of L (+)-lactic acid from cassava pulp by solid state culture of *Rhizopus oryzae*. *Bioprocess and Biosystem Engineering Journal*. 35: 1429-1436. <https://doi.org/10.1007/s00449-012-0731-3>
- [21] Jennings, D.H. 1995. The physiology of fungal nutrition. Cambridge: Cambridge University Press.
- [22] Sobowale, A.O., Olurin, T.O. and Oyewole, O.B. 2009. Effect of lactic acid bacteria starter culture fermentation of cassava on chemical and sensory characteristics of fufu flour. *African Journal of Biotechnology*. 6:1954-1958. <https://doi.org/10.5897/AJB2007.000-2297>
- [23] Pomeranz Y dan Meloan CE. 1994. Food Analysis: Theory and Practice. Chapman and Hall, New York.
- [24] Garnida Y, Turmala, dan Yusviani. 2000. Pembuatan makanan tradisional gatot dengan variasi ketebalan dan lamanya perendaman ubi kayu. Prosiding Seminar Nasional Makanan Tradisional. Malang.
- [25] Julianti E, Lubis Z, Ridwansyah, Era Y, and Suhaidi I. 2011. Physicochemical and functional properties of fermented starch from flour cassava varietas. *Asian Journal of Agricultural Research*. 5(6): 292-299.
- [26] Manurung A, Nainggolan EA, and Kisno. 2017. Aspergillus niger Fermentation Effect on MOCAF Properties (Modified Cassava Flour). *Journal of Engineering and Applied Sciences*, 12: 2160-2163. [10.36478/jeasci.2017.2160.2163](https://doi.org/10.36478/jeasci.2017.2160.2163)
- [27] Kartika, Bambang, Puji Hastuti dan Wahyu Supartono. 1988. Pedoman Uji Inderawi Bahan Pangan. UGM. Yogyakarta.
- [28] Elkhalfifa, A.E.O., Bernhardt, R., Bonomi, F., Iametti, S., Pagani, M.A. and Zardi, M. 2005. Fermentation modifies protein/protein and protein/starch interactions in sorghum dough. *European Food Research and Technology*. 222(5), 559. <https://doi.org/10.1007/s00217-005-0124-9>
- [29] Liu H, Ramsden, and Corke. 1999. Physical Properties and Enzymatic Digestibility of Phosphorilated and Normal Maize Starch Prepared at Different pH Levels. *Cereal Chem*. 76(6): 938-943.