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Aspergillus niger Fermentation Effect on MOCAF Properties (Modified Cassava Flour)

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Abstract: This research aims to investigate the fermentation effect of *Aspergillus niger* during the production of MOCAF through cassava fermentation on the MOCAF physicochemical characteristics. The method used in this investigation is completely randomized design through three replications. This research is carried out in 2 phases; the first is producing MOCAF through fermentation using *Aspergillus niger* and the second is determining the effect of the fermentation on MOCAF properties including water content, chemical yield, starch content, color and microstructure. The chemical yield, water content and starch content in MOCAF through fermentation on the third day is 34.62; 8.35 and 48.65% consecutively. This method has effect on the chemical yield, water content and starch content of MOCAF.

Key words: Cassava, MOCAF, *Aspergillus niger*, fermentation, SEM microstructure

INTRODUCTION

Cassava (Manihot esculenta Crantz) is one of the resources for food and it is mostly used for many aims such as processed food, flour product and forage (FAO, 2004, 2006). Indonesia has the 1.4 milllion hectares of cassava land plantation with the annual production average of 16 million tons. Tobasa regency is a part of Indonesia where the people grow cassava and rice for living. However, cassava contains acid cyanide (HCN) which is toxic (Oboh *et al*., 2002; Oboh and Akindahunsi, 2003). This becomes the obstacle of cassava utilization and processing so that many efforts have been carried out for consuming cassava safely.

The other factor that causes the low price of cassava is its low resistance; particularly fresh cassava due to the water content in the fresh cassava is so high that handling this issue should be carried out after harvesting period. One of the derivative products of cassava is Modified Cassava Flour (MOCAF) where the cassava is processed through fermenting cellular modification (Subagio, 2007). MOCAF is the product of cassava flour through fermentation so that the result is alike wheat flour (white, soft and cassava odorless). These similar characteristics make MOCAF possible to be the substitute for wheat flour. Lactic acid bacteria is dominating in the process of fermentation of producing MOCAF (Subagio, 2007) therefore, MOCAF is also called the product of cassava fermentation. The fermentation implemented in this research is using *Aspergillus niger* during the production process. This microorganism is often used nitrate acid production since its growth is directly related to food substance, the organic substance in the food can be used for molecular transport activity, cell structure maintenance and cell mobility (Suganthi *et al*., 2011).

Aspergillus niger can grow up rapidly and it is used commercially in nitrate acid production, gluconate acid and other enzyme production such as amylase, pectinase, glucoamylase and cellulose (Pazur *et al*., 1990). It is also tolerant to the low water activity, able to grow in substrate of high osmotic potency and sporulation in relatively low humidity (Carlile and Watkinson, 1994). The research in producing MOCAF states that lactobacillus plantarum, Saccharomyces cereviseae and rhizopus oryzae which are non-pathogenic and cheap are able to increase the protein content and decrease HCN content from MOCAF. However, this research investigates the effect of fermentation using *Aspergillus niger* on the MOCAF physicochemical characteristics (Kurniati, 2012).

MATERIALS AND METHODS

Experimental: Materials that are used for this experiment are the cassava of 5-6 months harvest age obtained from cassava plantation in tobasa regency, North Sumatera. Those materials are peeled and washed and then mashed using grater. The equipment needed for this process is grater, oven, analytical weigh scale, color reader, SEM, shaking waterbath, 60 mesh sieve and dry blender.

Cassava fermentation method: First, inoculum is produced using media that contain (g/L): glucose (50), yeast extract (5), urea (7.5), K_2HPO_4 (3.5), $MgSO_4.7H_2O$

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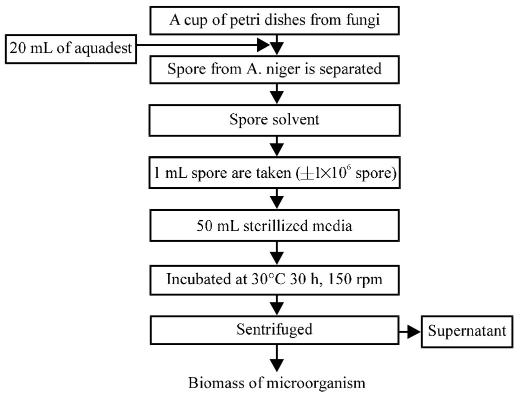

Fig. 1: Inoculum preparation

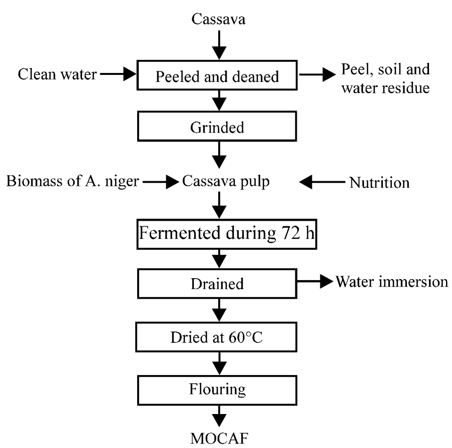

Fig. 2: MOCAF production flowchart

(0.75), $CaCl_2.2H_2O$ (1) and 0.05 M citric buffer with 5.5 pH (50 mL). These media are sterilized with autoclave at 121°C and inoculated in 250 mL erlenmeyer flasks; then the fungi is incubated for 30 h with 30°C and 130 rpm. In the end of incubation process, the content of Erlenmeyer flasks are centrifuged aseptically and used for fermentation for producing MOCAF (Fig. 1).

Approximately 100 g of cassava pulp is fermented and the biomass from fungi and 73 mL of nutrition dissolvent which contain urea (8.0 g), $MgSO_4.2H_2O$ (7.0 g), KH_2PO_4 (1.3 g) and citric acid (2.0 g) are then added to the solid matrix and fermented during 3 days (72 h) at incubation temperature of 30°C and humidity of 90-93%. The control in this research is the cassava pulp which is not fermented and obtained in the initial experiment. Next, both the fermented and unfermented cassava pulps are dried in the oven at temperature 60°C and grinded to cassava flour. This flour or MOCAF is the analyzed (Fig. 2).

Water content analysis: MOCAF flour sample (W1) is dried in the oven at temperature 80°C and refrigerated in the desiccator and weighed. The drying process is carried out repeatedly until reaching constant mass (W2). The moisture content is determined with the following equation:

$$\text{Moisture (\%b/b)} = \frac{W1-W2}{W1} \times 100\%$$

Chemical yield analysis: Chemical yield is obtained through the kg amount of product formed from every kg of the processed materials like in the following equation:

$$\text{Rendemen (\%b/b)} = \frac{\text{End mass} \times 100\%}{\text{Initial mass}}$$

Starch content analysis: The 2-5 g mashed solid sample are weighted and added with 50 mL aquadest and then stirred for one hour. The suspension is filtered with filter study and cleaned with aquadest until filtrate volume is reaching 250 mL. The remaining starch as residue on the filter study is cleaned 5 times with 10 mL ether and it is left to evaporate from the residue. It is then re-cleaned with 150 mL of 10% alcohol content. This residue is then moved quantitatively from filter study to erlenmeyer flasks with the cleansing of 200 mL aquadest and 20 mL HCL±25% (specific weight 1.125). The erlenmeyer flask is then covered and warmed with water bath for 2.5 h. After getting cooled, it is the neutralized with NaOH 45% and diluted until the volume is 450 mL and filtered. The determination of glucose is like the determination of reducing sugar. The mass of starch is obtained through multiplying 0.9 with glucose mass.

Color analysis: This process is carried out through placing sample in the transparent plastic container, patching color reader on the sample surface, setting button L*, a*, b* and pressing target button and recording the result of the reading. L* is contrast level, a* is magenta and b* is yellow.

Microstructure analysis: This stage implements scanning electron microscope. The sample is then sanded with sandpaper and observed with optical microscope to see the sample surface to be continued to preparation stage. The sample must be free from H_2O and then after getting dried it is then patched to specimen holder to be cleaned with hand blower. After the sample is ready, it is then put into specimen chamber to observe the microstructure in the SEM screen and it is then analyzed.

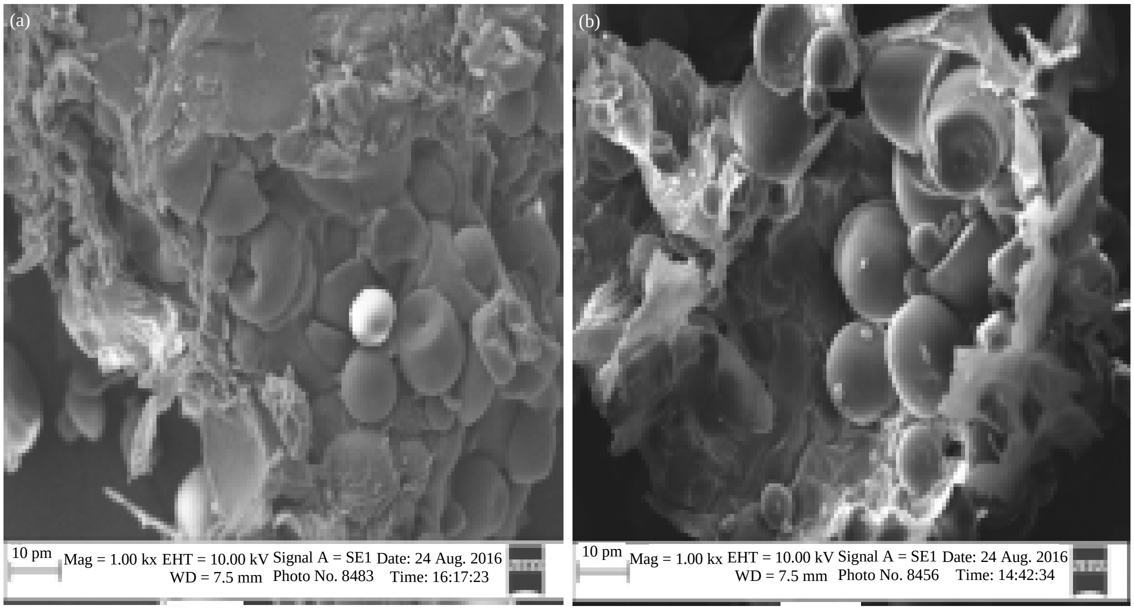

Fig. 3: Granule microstructure of cassava flour with SEM method: a) fermented and b) unfermented

The result from SEM image is the displaying the topography (sample surface texture) and morphology (form and size of the sample constituent particles) (Fig. 3).

Statistics analysis: This process is carried out using completely randomized design through 3 repetitions. Data analysis is carried out using ANOVA and continued to BNT test or DMRT with α = 0.05. The selection of the best treatment is conducted with zeleny method.

RESULTS AND DISCUSSION

From the implemented fermentation, the result can be seen in Table 1.

Cassava flour water content: Water content greatly influences the durability of flour. From the analysis, the water content starting from the first day of fermentation until the third day decreased with 3 repetitions, respectively. The lowest average water content in the cassava flour is 8.35±0.05% with 72 h fermentation. According to Indonesian National Standard (SNI), the recommended qualification of safely consumable cassava flour is the flour that has water content of 12%. This shows that the flour produced with *Aspergillus niger* fermentation meets the requirement of quality determined by SNI.

The data indicates the percentage reduction of water content as the result of fermentation with the treatment period. This shows that the more time fermentation time, the lesser water content is. This has an influence on the texture of the flour as the basic material for making bread; the texture becomes softer and permeable enough to facilitate drying process. As the result, the bread texture is also affected by the flour texture.

Table 1: Water content, chemical yield and starch content on cassava flour with different fermentation duration

Fermentation duration (h)	Water content (%)	Chemical yield (%)	Starch content (%)
24	8.93±0.16	36.28±0.08	50.36±0.07
48	8.36±0.19	35.47±0.14	49.16±0.04
72	8.35±0.05	34.62±0.09	48.65±0.16

Cassava chemical yield: The analysis result shows that fermentation process causes the decrease of cassava flour chemical yield for 34.62%. The highest chemical yield is acquired during 24 h and the lowest one occurs during 72 h fermentation. This is different according to (Kurniati, 2012) stating that the value of chemical yield is affected by the proteins that bind water. The more water bound by the protein, the less water produced as the result, the chemical yield is increasing. The average chemical yield shows that the more fermentation time, the less chemical yield produced.

Cassava flour starch content: Starch content existing in every fermentation shows the difference from one to another. The percentage reduction of starch content in the cassava flour as the result of *Aspergillus niger* fermentation is obtained during 24, 48 and 72 h. The reduction value is 48.65%. During fermentation, cassava

Table 2: Color reader result analysis with L* (contrast level), a* (magenta) and b* (yellow)

Fermentation period (h)	L*	a*	b*
0	103.38	0.57	6.03
24	101.07	0.47	13.54
48	102.31	0.57	10.78
72	99.07	0.09	14.41

that contains starch is separated to simpler glucose by microorganism, particularly *Aspergillus niger* to obtain energy in carrying out activity or metabolism in its body. As the consequence, starch content in the cassava is reducing. The other factor causing starch content reduction is the activity occurs with the aid of amylase enzyme which functions to break down the starch to be simple glucose (Ockermen, 1978; Kombong, 2004).

Cassava flour microstructure: SEM method analysis aims to identify the amilograph characteristics of the flour. This information is really helpful for the consumer to select the variety of cassava which is properly consumable or utilizable for each purpose. From the SEM image (Fig. 3), it can clearly be seen the size of the fermented starch granule and unfermented one is different in term of shape and size. This indicates that fermentation process causes the cell wall to be destroyed due to metabolism process by the microorganism inside (Pelczar and Chan, 1988).

Cassava flour color: As is shown in Table 2, the fermentation duration affects the brightness of the produced cassava flour. The longer fermentation period yields the lower brightness and unfermented cassava flour has the highest brightness. The darker color of fermented flour is not a significant problem since the flour can be adjusted for making certain cake dark-colored bread or cake).

CONCLUSION

From the result, it can be concluded that the fermentation period on the cassava using *Aspergillus niger* has effect on the chemical yield, water content and starch content of MOCAF. Each analysis experiences decreasing trend toward fermentation period. The less percentage of water content is directly proportional with fermentation period and this indicates that the amount of bound water released is getting larger as well as the texture of cassava flour. The same thing occurs with the starch content of the fermented MOCAF that becomes digressing due to the activity of amylase enzyme which splits the starch to simple glucose by *Aspergillus niger*. This research is carried out at laboratory scale. Therefore, it is needed further study of large scale fermentation and the method to increase the cassava chemical yield through fermentation.

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REFERENCES

Carlile, M.J. and S.C. Watkinson, 1994. The Fungi. Academic Press, London, England,.

FAO, 2004. Cassava industrial revolution in Nigeria. Codex Alimentarius Commission XII, Supplementary 4. Rome.

FAO, 2006. Food and agriculture organization of United Nations. Food and Agriculture Organization, Rome, Italy. http://www.fao.org.

Kombong, H., 2004. Evaluation of the hydrolytic power glucoamylase of *Aspergillus niger* culture filtrate. J. Basic Sci., 5: 16-20.

Kurniati, L.I.D., 2012. Making mocaf the fermentation process using Lactobacillus plantarum, Saccharomyces cerevisiae and Rhizopus oryzae. J. Eng. POMITS., 1: 1-6.

Oboh, G. and A.A. Akindahunsi, 2003. Biochemical changes in cassava products (flour and gari) subjected to Saccharomyces cerevisae solid media fermentation. Food Chem., 82: 599-602.

Oboh, G., A.A. Akindahunsi and A.A. Oshodi, 2002. Nutrient and anti-nutrient contents of *Aspergillus niger*-fermented cassava products (flour and gari). J. Food Compos. Anal., 15: 617-622.

Ockermen, H.W., 1978. Source Book of Food Scientist. The Avi Publishing Company, Wesport, Connecticut,.

Pazur, J.H., B.L. Liu and F.J. Miskiel, 1990. Comparison of the properties of glucoamylases from Rhizopus niveus and *Aspergillus niger*. Biotechnol. Appl. Biochem., 12: 63-78.

Pelczar, M.J.D. and E.C.S. Chan, 1988. Fundamentals of Microbiology. Vol. 2, UI Press, Jakarta, Indonesia,.

Subagio, A., 2007. Industrialization Modified Cassava Flour (mocaf) as Raw Materials for the Food Industry Diversification Supporting National Staple Food. Universitas Jember, Jember, Indonesia,.

Suganthi, R., J.F. Benazir, R. Santhi, R.V. Kumar and A.N.M. Hari *et al*., 2011. Amylase production by *Aspergillus niger* under solid state fermentation using agro industrial wastes. Int. J. Eng. Sci. Technol., 3: 1756-1763.