African Journal of Food Science and Technology (ISSN: 2141-5455) Vol. 2(2) pp. 030-035, February, 2011
Available online http://www.interesjournals.org/AJFST
Copyright © 2011 International Research Journals

Full Length Research Paper

Addition of cellulolytic bacteria to improved the quality of fermented cassava flour

Anja Meryandini[1*],Vitria Melani[1], Titi Candra Sunarti[2],

1. Biology Department, Faculty of Mathematic and Science and Research Centre for Natural Resources and Biotechnology, Bogor Agricultural University, Bogor 16680. Indonesia,
2. Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor 16680. Indonesia

Accepted 19 February, 2011

Cassava is one of the food sources of energy as its main components are starch and fibres. Limitation of its utilization is due to cyanide acid content (HCN). Modification of cassava products should be conducted to increase the value of cassava as food source. One of effort can be done is using lactic acid bacteria, cellulolytic bacteria, and yeast in fermentation of cassava. The objective of this research is to investigate the effect of local cellulolytic bacteria in the fermentation process and product quality of cassava flour produced. Cellulolytic bacterial used was *Bacillus pumilus* C11-1 with the highest activity 1.755 nkat / ml after three days of incubation. Beside that, lactic acid bacteria was isolated from the brine of fermented pickle mustard and commercial baker yeast were added in this fermentation. The combination of culture that has shown good results seen from flour quality tests was the E.1 treatment with the addition of 30 ml cellulolytic bacteria (3.9 x 10^{11} cfu/ml) in 24 hours. This can be seen from the highest whiteness of flour amounted to 88.23% and the low concentration of HCN 0.20 ppm.

Keywords: cellulolytic bacteria, fermented, cassava, flour

INTRODUCTION

Various types of crops grown in Indonesia, one of them is manioc (Cassava). Cassava is the third food source in Indonesia after rice and maize (Barrett and Damardjati, 1984).The advantages of this plant is able to grow in dry and less fertile land, as well as its relative highly resistance to disease. Most people in tropics use the tubers as sources of carbohydrate (Blagbrough et al. 2010).

Limitation of cassava as a food is caused by its high cyanide acid (HCN) content in fresh tubers. According to Djazuli and Bradbury (1999) cassava roots in Indonesia contain total cyanogen about 10 – 60 ppm. Amount of HCN depends on the type of cassava. Sweet cassava has a lower HCN content than bitter cassava. Processing cassava through fermentation process is one of efforts to reduce cyanide acid content. The fermentation processes using a liquid medium containing a defined mixture of microbe. This condition is expected to hydrolyze cyanide contained in the tubers and improve the texture properties of cassava flour produced. Microbes that are commonly used in the process of fermentation are group of cellulolytic bacteria, lactic acid bacteria and yeast (Achi and Akomas, 2006).

Obilie et al. (2003) showed that the groups of lactic acid bacteria such as *Lactobacillus plantarum* can reduced HCN about 80% after 72 hours of fermentation. Using lactic acid bacteria will be able to preserve the product and provide a distinctive flavor to the flour. Previous studies using lactic acid bacteria and yeast produced cassava flour with a softer texture, higher fiber content and whiter than other flour. This research aims to study the effect of local cellulolytic bacterial isolate on the fermentation process and the quality of flour produced.

MATERIALS AND METHODS

Raw materials that used in this study were local varieties of sweet cassava. The microorganism used consisted of cellulolytic

*Corresponding autor Email: ameryandini@yahoo.com

Table 1 The composition of six treatment of fermentation

Codes	Treatments		
	Yeast	LAB	CB
A	-	-	-
B	1 g	-	-
C	1 g	12 ml	-
D	1 g	12 ml	10 ml
E	1 g	12 ml	30 ml
F	1 g	12 ml	50 ml

LAB : Lactic Acid Bacteria
CB: Cellulolytic Bacteria

bacterium *Bacillus pumilus* C11-1 collection of Microbiology Laboratory, Biology Department, Bogor Agricultural University, lactic acid bacteria from salt water immersion mustard, and commercial baker's yeast.

***Bacillus pumilus* C11-1 growth curve in the cassava medium**

B. pumilus C11-1 was cultured as much as four cork borer on cassava medium and kept in an incubator shaking at 125 rpm at room temperature. Enzyme activity measurements performed every 24 hours for seven days. Crude enzyme extract obtained by centrifugation of culture at 3000 rpm for 25 minutes at 4˚ C. Enzyme activity was measured by the formation of reducing sugars using DNS method (Miller, 1959) and the protein content was measured using the method of Bradford (1976).

Isolation of Lactid Acid Bacteria (LAB)

Lactic acid bacteria (LAB) isolated from brine of fermented pickle mustard and streaked on MRS agar medium and grown for 3-4 days at room temperature. Isolates of lactic acid bacteria were grown in MRS liquid medium to determine the growth curve by measuring the turbidity every six hours with a spectrophotometer at wavelength (λ) 620 nm.

Cassava Fermentation

Cassava fermentation process carried out by submerged culture method. A total of 1 kg of cassava tuber slices with a thickness of about 0.5 cm were washed and immersed in 1500 ml sterile water containing a mixture of microorganism. The fermentation were done with three concentration of cellulolytic bacteria: 10 ml, 30 ml, and 50 ml (3.9×10^{11} colonies / ml), 12 ml lactic acid bacterial culture (2.8×10^{7} colonies / ml), and 1g of yeast (2.5×10^{6} colonies / g) and was covered with paper. As a control, cassava is fermented without cellulolytic bacteria. Time variation of fermentation for all treatments was 24 hours and 48 hours. Each treatment was done in three replicates (Table 1).

Cassava Flour Production

Sliced cassava which is fermented then dehydrated by sun drying for seven hours, followed by drying in the oven for 24 hours at 50˚C. and ground and sifted to produce flour with a size of 100 mesh.

Fermented Fluids Analysis

The analysis includes the measurement of acidity (pH) using a pH meter, total of acid by titration method (AOAC, 1995), total of sugar by phenol-H_2SO_4 method according to Dubois et al. (1956), and the calculation of total microorganisms by Total Plate Count (TPC) method. The number of cellulolytic bacteria was analyzed using CMC media, lactic acid bacteria using MRS media, and yeast using PDA media.

Flour Quality Analysis

Parameter analysis of the quality of flour was whiteness using the whiteness meter, flour yield, HCN levels using colorimetric method and moisture content using AOAC methods (1995), and microscopic observation. Microscopic observations on cassava and flour from fresh tubers fermented using light and polarized light microscope at 200x magnification.

RESULT

Growth curve of Bacillus pumilus C11-1

B. pumilus C11-1 has the highest activity of 1755 nkat / ml on third day of incubation (Figure 1).

Isolation of Lactid Acid Bacteria (LAB)

The bacteria that were isolated from brine of fermented pickle mustard were a group of lactic acid bacteria (LAB). LAB growth began to reach the exponential phase at the 30^{th} hours of observation (Figure 2). Therefore LAB as an inoculum was used after 30 hours of incubation.

Cassava Fermentation and Flour Producing

Fermentation of cassava with submerged culture method performed in six treatments and two variations of fermentation time for each treatment. After fermentation, changes in the number of microorganisms are shown in Table 2. The numbers of microorganism in liquid fermented and fermented cassava flour were presented in Table 3.

Microscopic observation

Microscopic observation of flour using polarized light microscopy at 200x magnification. The results showed

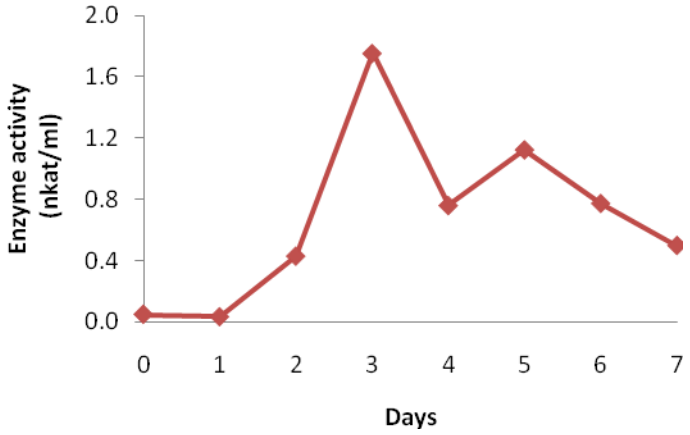

Figure 1. Production curve of *Bacillus pumilus* C11-1 cellulolytic on 1 % cassava medium at 30 °C

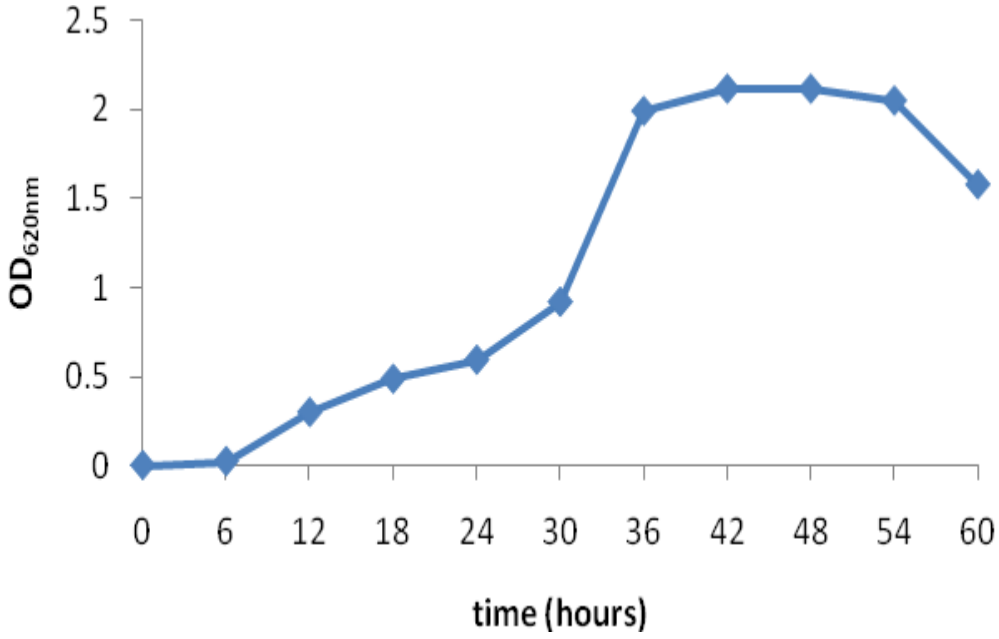

Figure 2. Growth curve from Lactobacillus on MRS medium at 30 °C

the influence of cellulolytic bacteria on starch granule structure and the fiber after the fermentation process.

DISCUSSION

Cassava is a food which is rich in carbohydrates. However, until now the variation of processing cassava for industrial use is still not well developed. Most cassava are used for making flour. Common flour produced from cassava is cassava flour and tapioca flour (Febriyanti, 1990). Fermented cassava flour has advantages over the other types of flour because the manufacturing process is relatively simple and cyanide acid content is lower (Hanif, 2009).

Cassava flour can be produced through spontaneous fermentation, that occurs without the addition of microbes (starter) (Prasetya, 1985). This technique has a

Table 2. Comparison of the number of Cellulolytic. Lactic acid and Yeast in the fermentation liquid before and after fermentation

Treatments	Number of microorganism before fermentation (colony/ml)			Number of microorganism after fermentation (colony/ml)		
	CB	LAB	Yeast	CB	LAB	Yeast
A.1	-	-	-	$2.2x10^{12}$	$3.8x10^{6}$	$6.8x10^{7}$
B.1	-	-	$1.7x10^{3}$	$5.3x10^{11}$	$1.9x10^{6}$	$2.0x10^{8}$
C.1	-	$2.2x10^{5}$	$1.7x10^{3}$	$1.3x10^{12}$	$2.3x10^{6}$	$9.2x10^{5}$
D.1	$2.6x10^{9}$	$2.2x10^{5}$	$1.7x10^{3}$	$4.3x10^{12}$	$4.1x10^{6}$	$2.5x10^{8}$
E.1	$7.8x10^{9}$	$2.2x10^{5}$	$1.7x10^{3}$	$5.0x10^{12}$	$4.1x10^{6}$	$4.2x10^{8}$
F.1	$1.3x10^{10}$	$2.2x10^{5}$	$1.7x10^{3}$	$4.2x10^{12}$	$3.6x10^{6}$	$6.5x10^{7}$
A.2	-	-	-	$4.7x10^{12}$	$2.4x10^{6}$	$8.6x10^{7}$
B.2	-	-	$1.7x10^{3}$	$3.2x10^{12}$	$2.9x10^{6}$	$2.9x10^{7}$
C.2	-	$2.2x10^{5}$	$1.7x10^{3}$	$1.9x10^{12}$	$2.9x10^{6}$	$1.4x10^{7}$
D.2	$2.6x10^{6}$	$2.2x10^{5}$	$1.7x10^{3}$	$2.7x10^{12}$	$4.3x10^{6}$	$2.9x10^{7}$
E.2	$7.8x10^{9}$	$2.2x10^{5}$	$1.7x10^{3}$	$3.6x10^{12}$	$2.9x10^{6}$	$6.8x10^{6}$
F.2	$1.3x10^{10}$	$2.2x10^{5}$	$1.7x10^{3}$	$2.9\ 10^{12}$	$3.7x10^{6}$	$2.6x10^{6}$

CB = Cellulolytic bacteria
LAB= Lactic Acid Bacteria

Table 3. Analysis of fermented fluid and fermented cassava flour

Treatment	Fermentation fluid analysis			Flour quality analysis			Yield (%)
	Acid number (ml NaOH 0.1N/100ml)	pH	Sugar content (mg/ml)	Whiteness (%)	HCN content (ppm)	Water content (%)	
A.1	26.2^{bc}	4.3	28.1^{c}	87.14^{a}	0.38^{d}	2.9	36.05^{a}
B.1	14^{a}	5.5	2.4^{a}	83.73^{a}	0.69^{l}	3.0	33.17^{a}
C.1	31.3^{cd}	4.1	1.5^{a}	86.36^{a}	0.56^{h}	2.2	33.33^{a}
D.1	35.60^{de}	4.3	3.3^{b}	86.05^{a}	0.40^{e}	2.9	32.56^{a}
E.1	25.70^{bc}	4.5	2.2^{a}	**88.23^{a}**	**0.20^{a}**	**1.1**	33.21^{a}
F.1	21.1^{b}	4.7	2.2^{a}	84.27^{a}	0.68^{i}	2.3	32.26^{a}
A.2	42.20^{e}	3.9	6.0^{b}	85.77^{a}	0.68^{i}	2.4	36.11^{a}
B.2	36.0^{de}	4	3.1^{a}	87.32^{a}	0.37^{c}	2.0	33.91^{a}
C.2	30.20^{cd}	4.2	2.8^{a}	86.18^{a}	0.27^{b}	1.7	33.72^{a}
D.2	28.90^{cd}	4.3	2.0^{a}	86.59^{a}	0.57^{h}	1.7	34.37^{a}
E.2	31.40^{cd}	4.5	2.9^{a}	87.45^{a}	0.47^{f}	2.7	32.74^{a}
F.2	27.80^{bc}	4.7	3.5^{b}	86.82^{a}	0.53^{g}	2.5	33.65^{a}

longstanding shortage due process and the growth of microorganisms could not be controlled. Today the use of a starter in the production of cassava flour also increased. Use of lactic acid bacteria as a starter in the manufacture of cassava flour conducted by Hanif (2009) showed good results. The degree of white flour was higher than the spontaneous fermented flour, besides cyanide acid content in flour also decreases. Other microorganisms that can be used in the fermentation of cassava are a group of cellulolytic bacteria capable of producing cellulase enzymes (Oyewole, 2001).

Using polysaccharide degrading enzymes in the field of industry and agriculture have been done for cost efficiency of production. One of the enzymes used to degrade cellulose is the enzyme cellulase. The enzyme consisting of endo-1 ,4-β-glucanase, exo-1 ,4-β-glucanase, and β-D-glucosidase. Endo-1 ,4-β-glucanase cut the cellulose chains in cellulose molecules is shorter, exo-1 ,4-β-glucanase cut ends of cellulose chains to produce cellobiose molecules, whereas β-D-glucosidase cut the molecule into two molecules of cellobiose glucose (Coughland and Mayer, 1991).

Bacillus pumilus C11-1 isolates has a high activity on 1% cassava medium. This isolate also has high activity on CMC media, corn cob, and rice straw (Meryandini et al. 2009). It showed that *Bacillus pumilus* C11-1 isolate has a potential cellulase enzyme, which could be used as a starter in fermented cassava. In addition to *Bacillus pumilus* C11-1 isolates we used lactic acid bacteria (LAB) isolated from brine of fermented pickle mustard. Lactobacillus is an amylolytic bacteria which are used to make pickles and curd (Ray et al. 2008). Lactic acid bacteria are also common used for cassava fermentation (Kostinek et al. 2007; Kobawila et al. 2005; Sobowale et al. 2007). Based on its growth curve, LAB entered exponential phase at the 30 hours of incubation. According to Pelczar and Chan (2007) exponential phase is an optimal bacterial growth, so its use in the fermentation process.

Total Plate Count (TPC) showed all the microorganisms that used are able to live in cassava substrate. This can be seen from the high number of colonies of each microorganism after 48 hours compared with the number of colonies which incorporated as a starter in the fermentation. Data in Table 2 showed a drastic increase in the number of microorganisms occurred on cellulolytic bacteria and yeasts. Growth of lactic acid bacteria is not very high compared to two other microorganisms. Microorganisms that grow on cassava fermentation are most amylolytic, part of them are cellulolytic and have polygalacturonase and linamarinase activities (Guyot et al. 1998). In the spontaneous fermentation the amount of microorganisms was very high after fermentation. This displayed that many kind of microorganisms could grow at cassava. Sugar content at the spontaneous fermentation was higher compared to other treatment. It could be due to the activity of the microorganisms that degraded the substrate to oligomer and monomer and then used it to growth. It can be seen from the sugar content that decreased after 48 hours of fermentation. Statistically, the sugar content from spontaneous fermentation is different with other treatment.

Table 3 showed the highest acid content obtained on spontaneous fermentation A.2. This value is positively correlated with a very low pH value of 3.9 indicating a high level of acidity. The high total acid in the treatment A.2 was due to a longer treatment time and occurs spontaneously so that the microorganisms involved could not be controlled. The lowest acid content was occurred at the B1 treatment (with addition of yeast for 24 hours). In this treatment it also showed that quantity of BAL was smaller compared to other treatment. Acid formation is a result of microorganism activities that changes simple sugars into organic acids, especially BAL. The pH range was the same as that from Achinewhu et al. (1998) using six different cassava cultivar.

Analysis on the quality of fermented flour showed the highest whiteness value produced by the treatment of E, but statistically all treatment did not showed differences.

The fermentation process lowers the value of HCN. This is because the microorganisms that are used were producing linamarinase which can hydrolyze linamarin (Guyot et al. 1998). Fermentation cassava using water is also the simplest method to reduce the cyanide content (Cumbana et al. 2007; Bradbury and Denton, 2010). The water will swell the cells of cassava and allows linamarinase to come into contact with linamarin and hydrolysis proceeds (Bradbury, 2006). Uyoh et al. (2009) also show that fermentation using unchanged water during fermentation will reduce HCN content significantly. The lowest HCN concentration was obtained from treatment E (24 hours fermentation with the addition of 30 ml cellulolytic bacteria). Concentration of HCN in Fresh cassava was 0.84 ppm, it was higher than describe by Djazuli and Bradbury (1999). The higher HCN content in the cassava roots can due to the low rainfall at that periode. The effect of low rainfall has greatly increase the total cyanide content (Cardoso et al. 1999). After fermentation the concentration of HCN was decreasing, showing fermented cassava flour was safe for consumption. The highest HCN concentration was obtained from treatment B1 (with yeast addition and 24 hours of fermentation). *Bacillus pumilus* are able to used cyanide for their nutrition (Knowles, 1976), so that they could contribute to the decrease the cyanide content. Statistically, there were differences in every treatment for HCN concentration. In generally, the concentration of HCN decreased more than 50% after fermentation. Achinewhu et al. (1998) also show that 72 hours of fermentation will reduce the HCN concentration by all cultivar by 85%.

Microscopic observations determined the effects of starch modification on the structure of fibers, granules, and birefringence properties of starch. In all treatments could be seen a change in the structure of starch granules and fibers. More and more the addition of cellulolytic bacteria, the structure of the broken fiber and starch were bigger. These changes have occurred in the treatment of 24 hours. Fiber structures of the most devastated seen at 24 hours treatment with the addition of 50 ml cellulolytic bacteria. This proves the addition of cellulolytic bacteria influenced the changes in fiber structure. With polarized light visible amorphous cellulose structure could easily be penetrated by light (Figure 3:F.1) than the cellulose fraction in fresh and spontaneous fermented cassava (Figure 3: A.1).

Fermentation of cassava is expected to improve the quality of flour in a relatively short time. After fermentation process, the cassava flour produced will have a better flavor, aroma, and texture, and the amount of HCN is lower.

From the twelve treatment of fermentation, treatment E was the best treatment that is generated by the addition of 30 ml with cellulolytic bacteria. This was evident from the resulting whiteness of 88.23% and low levels of

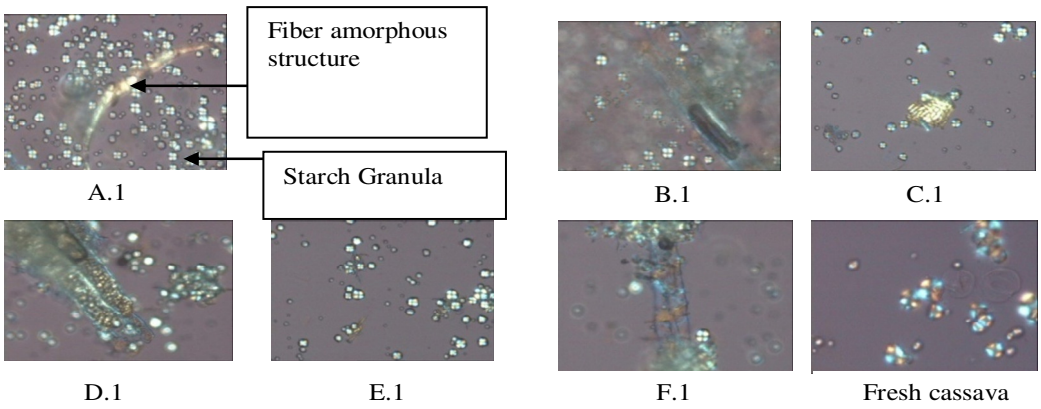

Figure 3 . The appearance of microscopic fibers and granules of cassava starch in fresh and fermented cassava flour 24-hour treatment on polarized light microscopy

HCN at 0.20 ppm.

REFERENCES

Achi OK, Akomas NS (2006). Comparative assessment of fermentation techniques in the processing of fufu, a traditional fermented cassava product. Pakistan J of Nutr 5(3):224-229.

Achinewhu SC,Barber LI, Ijeoma IO (1998). Physicochemical properties and garification (gari yield) of selected cassava cultivars in Rivers State. Plant foods f Human nutr. 52:133-140.

[AOAC] Association of Official Analytical Cemists (1995). *Official Methods of Analysis of The Association of Analytical Chemistry*. Washington DC: AOAC

Blagbrough IS, Bayoumi SAL, Rowan MG, Beeching Jr (2010). Cassava: An appraisal of its phytochemistry and its biotechnological prospects. Phytochemistry 71: 1940 – 1951

Bradbury JH (2006). Simple wetting method to reduce cyanogens content of cassava flour. J of Food Compost. Anal. 19:388 – 393

Bradbury JH, Denton IC (2010). Simple method to reduce the cyanogens content of gari made from cassava. Food. Chem. 123:840 - 845

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantitaties of protein in utilizing the principle of protein-dye binding. Ann Rev Biochem 72:248-254

Cardoso AP, Ernesto M, Cliff J, Bradbury JH (1999). High levels of total cyanogens in cassava flour related to drought in Mozambique. Roots 6: 4-6

Coughland MP, Mayer F (1991). The cellulose decomposing bacteria and their enzyme systems, In Balows A, Triper HG, Dworkin M, Harder W, Schleifer KH, editor. *The Prokaryotes ,A Handbook on Biology of Bacteria: Ecophysiology, Isolation, Identification, Application*. Vol I. New York: Springer Verlag. p:460-516.

Cumbana A, Mirione E, Cliff J, Bradbury JH (2007). Reduction of cyanide content of cassava flour in Mozambique by the wetting method. Food Chem. 101: 894 – 897

Djazuli M, Bradbury JH (1999). Cyanogen content of cassava roots and flour in Indonesia. Food Chem. 65: 523-525

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F1(956). Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350-356.

Febriyanti, T (1990). Studi karakteristik fisiko kimia dan fungsional beberapa varietas tepung singkong [skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Guyot JM *et al*. 1998. *Lactobacillus manihotivorans* sp. nov., a new starch-hydrolysing lactic acid bacterium isolated during cassava sour starch fermentation. Int J of Syst. Bacteriol 48: 1101-1109.

Hanif M (2009). Produksi dan karakterisasi tepung kasava termodifikasi [skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.Knowles CJ (1976). Microorganisms and Cyanide. Bacteriol. Rev. 40:652 – 680

Kobawila sc, Louembe D, Keleke S, Hounhouigan J, Gamba C (2005). Reduction of cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. Afr. J of biotechnol. 4: 689 – 696

Kostinek M, Specht I, Edward VA, Pinto C, Egounlety M, Sossa C, Mbugua S, Dortu C, thornart P, Taljaard L, Mengu M, Franz CMAP, Holzapfel WH (2007). Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. Int. J of Food microbiol. 114; 342 – 351

Meryandini A, Widosari W, Maranatha B, Sunarti TC, Rachmania N, Satria H (2009). Isolasi bakteri selulolitik dan karakterisasi enzimnya. MAKARA 13: 33-38

Miller GL (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 31:426-428.

Obilie EM, Debrah KT, Awua WKA (2003). Microbial modification of the texture of grated cassava during fermentation into akyeke. Int J of Food Microbiol 89:275-280.

Onwueme IC. 1978. *The Tropical Tuber Crops (Yams, Cassava, Sweet Potato, and Cocoyams)*. New York: John Wiley & Sons.

Oyewole OB.(2001). Characteristics and significance of yeast involvement in cassava fermentation for fufu production. Int J Food Microbiol. 63:213-218.

Pelczar MJ, Chan ECS (2007). Dasar-Dasar Mikrobiologi. Jakarta: UI Press.

Prasetya R (1985). Perubahan Kimia dan Mikrobiologi dalam Fermentasi Rebung [skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Ray RC, Mohapatra S, Panda S, Kar S (2008). Solid substrate fermentation of cassava fibrous residue for production of a- amylase, lactic acid and ethanol. J. of Environ. Biol. 29: 111 – 115

Sobowale AO, Olurin TO, Oyewole OB (2007). Effect of lactic acid bacteria starter culture fermentation of cassava on chemical and sensory characteristics of fufu flour. Afr. J. of Biotechnol. 6: 1954 – 1958

.Uyoh EA, Ntui VO, Udoma NN (2009). Effect of local cassava fermentation methods on some physiochemical and sensory properties of FUFU. Pakistan J. Nutri 8: 1123 - 1125