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Microbial and some chemical constituent changes of high cyanide cassava during simultant spontaneous submerged and solid state fermentation of "gadungan pohung"

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Abstract

Fermentation is one ways to reduce cyanogenic compounds in cassava. Fermentation of cassava is usually performed by submerged fermentation and solid state fermentation separately. "Gadungan pohung" is one of Indonesian traditional of cassava fermentations that combines spontaneous submerged and solid state fermentation simultantly. The objective of this study was to evaluate some chemical compound changes, microbiological profile, and cyanides reduction of three high cyanide cassava varieties during 3 days submerged fermentation and 3 days solid state fermentation simultaneously, in "gadungan pohung" processing. The resuls showed that submerged and solid state fermentation decreased most of nutritonal compounds of cassava except carbohydrate. Submerged fermentation reduced pH value from 6 to 4.5, meanwhile solid state fermentation increased pH value from 4.5 to 6. All cassava varieties revealed similar changes. Bacteria, mainly lactic acid bacteria, dominated during submerged and solid state fermentation. Yeasts were also found in higher number than molds. Cassava varieties slightly affected the pattern of microbial growth during solid state fermentation. Submerged fermentation reduced cyanide compounds. Some cyanide compounds were found in fermenting medium. Significant declined was found in cyanogenic glycosides, meanwhile cyanohydrin and free HCN increased in all cassava varieties. Solid state fermentation decreased cyanogenic glycosides and cyanohydrin, meanwhile concentration of free HCN were fluctuated during 3 days fermentation. Degradation of cyanides in solid state fermentation was due to microbial activity and increasing pH to above 5. Combination of submerged and solid state fermentation effectively reduced cyanide compounds in high cyanide cassava varieties to about 89-93%.

Introduction

Cassava or manioc (*Manihot esculenta* Crantz), a perennial shrub of the New World, is the third worldfood crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (Sornyotha *et al.*, 2010; Murugan *et al.*, 2012). Cassava tuber is one of cheap sources of carbohydrate especially starch (Charoenkul *et al.*, 2011; Aprianita *et al.*, 2014). Meanwhile, cassava root easily deteriorates and contains toxic bitter cyanogen compounds in the range of 1-1500 ppm (Cardoso *et al.*, 2005) that depends on varieties. The safe level of cyanide is 10 ppm (FAO / WHO, 2012). Consumption of 50 to 100 mg of cyanide has been associated with acute poisoning which is lethal to humans (Bandna, 2012). Residual cyanogens in cassava foods may cause neurological disorders or paralysis, particularly in nutritionally compromised individuals (Siritunga and Sayre, 2004; Kambale *et al.*, 2017).

In cassava, cyanide is mainly (93%) found in the form of cyanogenic glycosides or linamarin in cell vacuole, and to some extent (7%) in the form of lotaustralin (Bandna, 2012; Morgan and Choct. 2016; Pinto-Zevallos *et al.* 2016). Most of cyanogenic glycosides have β linkage with D-glucose (FSANZ, 2005). Actually, cassava also has linamarase enzyme

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in cell wall (Sornyotha *et al.*, 2010) which is able to degrade cyanogenic glycosides into glucose and acetone cyanohydrin (Iyuke and Idibie, 2007). Cyanogenesis is initiated in cassava when the plant tissue is damaged. Rupture of the vacuole releases linamarin, which is hydrolyzed by linamarase, a cell wall-associated β-glycosidase (Pinto-Zevallos *et al*., 2016). Unstable hydroxynitrile intermediate, acetone cyanohydrin, is easily decomposes into free HCN (Montagnac *et al*., 2009) and aldehyde or keton (Sharma *et al.*, 2005; Bradbury and Denton, 2011) by hydroxynitrile liase (HNL) enzyme or spontaneously at pH >5.0 or temperature >35°C (Siritunga and Sayre, 2004; Bradbury and Denton, 2011). Cassava roots however have no HNL activity, presummably accounting for the accumulation of potentially toxic levels of acetone cyanohydrin in poorly processed cassava roots (Nambisan, 2011).

Therefore, cyanide removal needs some stages, the first is contact between cyanogenic glycosides as substrate and linamarase, the second is acceleration of degradation of acetone cyanohydrin, and finally HCN removal by vaporization (Esser *et al*., 1996). Free HCN is easily evaporates and leaches into water (Nwokoro, 2016).

The cyanogens, including linamarin and its deglycosylated product, acetone cyanohydrin, can be efficiently removed from the root by various processing procedures (Siritunga and Sayre, 2004). Many efforts have been done to reduce cyanide level of cassava such as evaporation, slicing, grinding, boiling, fermentation, drying, sieving, or combination of those process (Lambri *et al*., 2013)

Traditional fermentation of cassava in Asia and Africa is a local wisdom to process cassava into many food products and can also reduce cyanide level. Cyanide reduction occurs during spontaneous fermentation which is used to produce many cassava products such as agbelima, gari, atieke, lafun, bikedi, in Africa and wikau maombo in Indonesia (Obilie *et al*., 2003; Kobawila *et al.*, 2005; Padonou *et al*., 2009; Adepoju *et al*., 2010; Wahyuni *et al*., 2016). Traditional fermentation of fufu and pupuru by using spontaneous fermentation through water steeping, is proved to decrease cyanide level (Agbor-Egbe and Mbome, 2006; Wakil and Benjamin, 2015). Gari is produced by involving solid state fermentation meanwhile lafun is produced by submerged fermentation (Obadina *et al*., 2009), and gari has lower cyanide content compared to unfermented products (Irtwange and Achimba, 2009).

According to Esser *et al.* (1995), solid state fermentation increased pH of substrate that created suitable condition for acetone cyanohydrin degradation. According to Bradbury and Denton (2011), spontaneous acetone cyanohydrin degradation occurred at pH 6–6.5 at temperature 30–50°C. Meanwhile, traditional fermentation that simultaneously combined submerged and solid state fermentation is limited.

One of Indonesian traditional cassava fermentation is "gadungan pohung" that simultaneously ferments cassava by submerged fermentation and solid state fermentation. It is interesting to evaluate level of cyanide reduction during "gadungan pohung" fermentation, because usually this type of fermentation uses high cyanide cassava varieties. Generally, high cyanide cassava varieties have high productivity and are used for cassava starch or tapioca production.

In this study, we compared chemical, cyanide level, and microbiological changes of three varieties of high cyanide cassava during simultaneous submerged and solid state fermentation of "gadungan pohung". The effect of solid state fermentation time was also evaluated.

Materials and Method

Materials

Three varieties of high cyanide Malang 4, Malang 6 and Sembung with age of 10 months were obtained from Malang, East Java, Indonesia.

Fermentation by "gadungan pohung" method

Cassava tubers were peeled, washed, and grated. Fresh grated cassava tuber was analyzed for chemical composition, microbiological profile, and cyanide compounds. Grated cassava was soaked in water at ratio grated cassava to water 1:1 for 3 days spontaneous submerged fermentation. After submerged fermentation, grated cassava and fermenting medium were analyzed similar to fresh tuber. After draining for 1 h, drained grated cassava was put into woven bamboo containers and they were put in humid room for solid state fermentation for 1, 2, or 3 days. Everyday, solid state fermented grated cassava was analyzed similar to submerged fermented grated cassava.

Chemical analysis

Grated cassava of fresh tuber, grated cassava from submerged fermentation, and grated cassava during solid state fermentation were analyzed for water, protein, ash, crude fiber (AOAC 2011), carbohydrate by difference, amylose by spectrophotometric method (AOAC, 1990), starch by acid hydrolysis method (AOAC, 2011), titratable acidity by using molecular weight of lactic acid for calculation (Medoua, 2008),

and pH.

Microbiological profile analysis

Microbiological analysis of total microbes, total mold, total bacteria, and lactic acid bacteria (LAB) were determined according to Fayemi and Ojokoh (2012). For all determinations, 5 g of the sample were homogenized in a stomacher with 45 ml of sterile peptoned buffered water. Tenfold serial dilutions of stomacher fluid, ranging from 10^{-2} to 10^{-8}, were prepared and spread-plated for the determination of microbial counts.

Total microbial were counted by cultivation on Plate Count Agar (PC agar) after incubation at 30°C for 48 hours. Enumeration of lactic acid bacteria was carried out using plates of DeMan, Rogosa and Sharpe agar (MRS-agar), which were incubated aerobically at 30°C for 48 hours. Yeasts were enumerated on plates of PGY agar incubated at 30°C for 72 hours. Molds were enumerated on plates of potato dextrose agar (PD agar) incubated at 30°C for 5-7 days. Extraction of cyanide and analysis of cyanide (linamarin, cyanohydrin and free HCN) was by Bradbury *et al.* (1991) method.

Statistical analysis

Data was analyzed by using analysis of varance in Split Plot Designs for solid state fermetation. Duncan multiple-range test was employed to know the difference between treatments by using SPSS.

Results and Discussion

Spontaneous submerged fermentation

Microbiological profile

Some microbes grew in fermenting medium and grated cassava after 3 days submerged fermentation in "gadungan pohung" processing (Figure 1 and 2). Cassava varieties significantly affected the growth of microbes in medium and grated cassava. The highest total microbes in fermenting medium was found in Sembung variety, beside total molds, yeasts, bacteria, and lactic acid bacteria. The lowest total microbes was found in Malang 6 variety. Fresh tuber of Sembung variety had the lowest cyanide content, meanwhile cyanide content of Malang 4 was the highest. It seemed that there was no correlation between cyanide level and the growth of microbes.

In all cassava varieties, predominant microbes in fermenting medium was bacteria mainly lactic acid bacteria. Molds were found to some extent, and their amount was lower than yeasts. Most of molds are aerobic that implied on the low growth of mold in fermenting medium. Brauman *et al.* (1996) found that yeasts (mostly *Candida* spp.) did not seem to play a significant role in the process, but their increasing numbers in the last stage of the process might influence the flavor and the preservation of the end foo-foo products. Meanwhile, Kimaryo *et al.* (2000) reported that during submerged fermentation of kivunde, yeasts and molds could not be isolated throughout the period of fermentation (detection limit: 10 colony forming units/g). It appeared that the type microrganisms involved in submerged fermentation of cassava was vary and depended on several factors such as condition of fermentation and enviroment. Interestingly, in the process of "gadungan pohung", a significant number of yeasts and molds was found in fermenting medium. The ratio of water to grated cassava in submersion was 1:1 that caused some grated cassava was not covered well by water. This condition made some molds grew. Degradation of starch during submerged fermentation possibly took place due to amylolitic activity of some microbes. Kostinek *et al.* (2007) found some biochemical properties of fermenting cassava including α amylase. Sugar produced in fermenting medium was suitable substrate for yeasts that caused the number of yeasts was higher than molds.

Fermenting medium was dominated by lactic acid bacteria. This finding was in accordance to that reported by Brauman *et al* (1996) that lactic acid bacteria were largely predominant (over 99% of the total flora after 48 h) and governed the fermentation. Moreover, Oyedeji *et al.* (2013) revealed that dominant lactic acid bacteria in fermenting cassava in lafun processing by submerged fermentation was *Lactobaccillus plantarum.*

In general, microbes in fermenting medium was higher then in grated cassava. Predominant microbes during 3 days submerged fermentation was bacteria that are suitable to grow in abundant water such as "gadungan pohung" submerged fermentation. These microbes preferred to grow in fermenting medium with excess water. Similar to fermenting medium, the predominant microbes in grated cassava was bacteria, mainly lactic acid bacteria (Figure 2). Some molds also were found in grated cassava and their number was lower than yeasts. The number of molds and yeast in grated cassava was lower than their number in fermenting medium, although during "gadungan pohung" fermentation, some grated cassava was not covered by water that enabled some molds to grow in the surface. However, overall the number of molds in fermenting medium was higher than grated cassava.

As fermenting medium, cassava varieties also affected the number of microbes. The lowest total

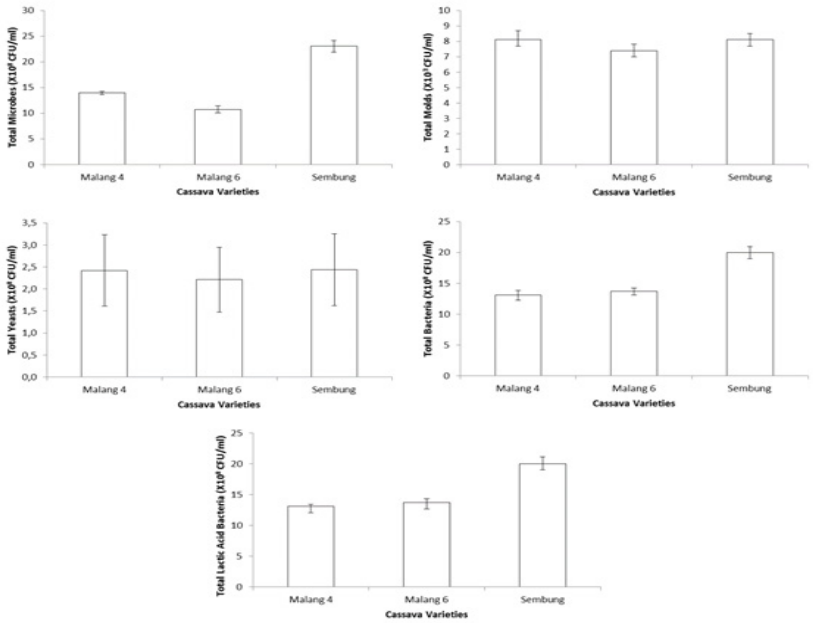

Figure 1. Microbiological profile of fermenting medium after 3 days submerged fermentation of high cyanide cassava varieties in "gadungan pohung" processing

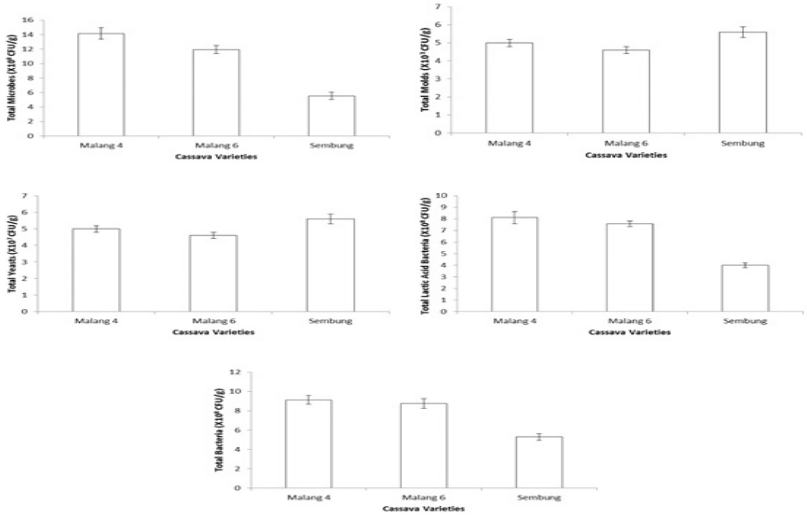

Figure 2. Microbiological profile of grated cassava after 3 days submerged fermentation of high cyanide cassava varieties in "gadungan pohung" processing

microbes in grated cassava was found in Sembung variety, meanwhile the highest was found in Malang 4 variety. Sembung varitey showed the lowest bacteria that dominated by lactic acid bacteria. This fact made total microbes of this variety was the lowest. This variety had the highest molds and yeast. It is interesting that the varieties of cassava affected the growth pattern of microbes. Intrinsic factors of cassava as a substrate such as nutrition composition might also affect the number of certain microbe. It seemed that cyanide content of cassava did not affect the growth of microbes which Sembung variety was the lowest cyanide content but had the lowest total microbes.

Chemical constituent changes

Data in Table 1 shows that there are some changes in cassava characteristics after spontaneous submerged fermentation. In all cassava varieties, all nutrient compounds tended to decrease except moisture and carbohydrate content. Similar result was reported by Ogunnaike *et al.* (2015) that submerged fermentation in lafun processing increased moisture content. Increasing water absorption occurred during soaking. Starch granules were easier to absorp water possibly because some structural changes due to microbial activity to degrade starch. Kostinek *et al.* (2007) reported that during fermentation of

Table 1. Some chemical constituent changes during fermentation in "gadungan pohung" processing

Variety	Day	Moisture (%, db)	Ash (%, db)	Crude Fat (%, db)	Crude Protein (%, db)	Crude Fiber (%, db)	Carbohydrate (%, db)	Starch (%, db)	Amlyose (%, db)	pH	Titrable acidity (%)
fresh tuber											
Malang 4		(63.65±0.28)	(2.70±0.05)	(2.60±0.10)	(1.58±0.05)	(3.25±0.10)	(81.05±0.36)	(79.92±0.10)	(20.42±0.23)	(6.28±0.02)	(0.10±0.00)
Malang 6		(61.70±0.16)	(2.61±0.11)	(2.54±0.04)	(1.60±0.07)	(3.48±0.08)	(82.37±0.47)	(79.65±0.22)	(21.97±0.33)	(6.10±0.02)	(0.09±0.00)
Sembung		(61.71±0.33)	(2.48±0.11)	(2.70±0.09)	(1.17±0.04)	(3.30±0.16)	(83.47±0.08)	(70.64±0.15)	(22.42±0.14)	(6.41±0.02)	(0.09±0.00)
Submerged fermentation											
Malang 4	3	(68.47±0.92)^{cd}	(1.80±0.08)^{f}	(2.46±0.08)^{de}	(1.55±0.07)^{f}	(2.06±0.01)^{ab}	(81.55±0.93)	(73.63±1.56)^{d}	(24,93±0,38)^{ab}	(4.54±0.11)^{b}	(0.65±0.03)^{d}
Malang 6	3	(68.51±0.78)^{cd}	(1.35±0.01)^{bc}	(2.32±0.09)^{cd}	(1.42±0.05)^{e}	(2.16±0.03)^{b}	(83.81±0.29)	(73.42±1.50)^{d}	(25,21±0,61)^{ab}	(4.59±0.13)^{bc}	(0.65±0.02)^{d}
Sembung	3	(69.23±0.86)^{d}	(1.34±0.04)^{b}	(2.51±0.01)^{e}	(0.97±0.07)^{ab}	(2.51±0.01)^{c}	(84.34±0.51)	(66.59±0.37)^{b}	(24,94±0,51)^{ab}	(4.52±0.02)^{ab}	(0.84±0,03)^{f}
Solid state fermentation											
Malang 4	1	(67.63±0.41)^{c}	(1.74±0.08)^{f}	(2.14±0.08)^{b}	(1.45±0.07)^{ef}	(2.00±0.08)^{a}	(83.52±0.34)	(70.51±2.05)^{c}	(24,99±0,09)^{ab}	(4.39±0.12)^{a}	(0.86±0.01)^{g}
	2	(67.77±0.79)^{c}	(1.81±0.07)^{f}	(2.25±0.03)^{bc}	(1.29±0.03)^{d}	(2.01±0.07)^{a}	(83.41±0.59)	(65.61±0.25)^{b}	(24,92±0,12)^{a}	(5.45±0.04)^{d}	(0.28±0.01)^{c}
	3	(67.55±0.29)^{c}	(1.80±0.02)^{f}	(1.55±0.09)^{a}	(1.01±0.06)^{b}	(2.01±0.07)^{a}	(86.44±0.53)	)65.18±0.42)^{b}	(26,01±0,81)^{b}	(6.90±0.06)^{e}	(0.09±0.00)^{a}
Malang 6	1	(67.75±0.65)^{c}	(1.24±0.04)^{a}	(2.24±0.08)^{bc}	(1.30±0.03)^{d}	(2.01±0.10)^{a}	(85.17±0.49)	(70.52±0.24)^{c}	(25,08±0,57)^{ab}	(4.73±0.07)^{c}	(0.73±0.03)^{e}
	2	(67.61±0.70)^{c}	(1.34±0.08)^{bc}	(2.16±0.07)^{b}	(1.13±0.05)^{c}	(2.10±0.09)^{ab}	(85.69±0.16)	(66.94±0.30)^{b}	(24,62±0,33)^{a}	(6.83±0.09)^{e}	(0.27±0.01)^{c}
	3	(67.76±0.22)^{c}	(1.44±0.08)^{cd}	(1.64±0.10)^{a}	(0.94±0.05)^{ab}	(2.00±0.07)a	(87.54±0.18)	(65.11±0.32)^{b}	(26,10±0,98)^{b}	(6.89±0.10)^{e}	(0.09±0.00)^{a}
Sembung	1	(66.09±0.59)^{b}	(1.47±0.06)^{de}	(2.29±0.04)^{bc}	(0.92±0.03)^{a}	(2.09±0.03)^{ab}	(86.21±0.34)	(62.95±0.27)^{a}	(24,81±0,97)^{a}	(6.94±0.08)^{e}	(0.17±0.01)^{b}
	2	(63.77±0.51)^{a}	(1.45±0.02)^{d}	(2.25±0.05)^{bc}	(0.93±0.04)^{ab}	(2.09±0.06)^{ab}	(87.25±0.06)	(61.86±1.48)^{a}	(25,68±0,190^{ab}	(6.88±0.04)^{e}	(0.10±0.00)^{a}
	3	(63.68±0.64)^{a}	(1.56±0.04)^{e}	(1.51±0.05)^{a}	(0.99±0.05)^{ab}	(2.04±0.05)^{ab}	(88.81±0.31)	(61.21±1.06)a	(26,22±1,09)^{b}	(6.96±0.08)^{e}	(0.08±0.00)^{a}

cassava, some biochemical changes were related to some enzymes produced by microflora such as production of α-amylase, β-glucosidase, and tannase. Slight increase in carbohydrate was due to decreasing of other components and carbohydrate mainly starch granule did not easily dissolve in water, although some microbes had amylolytic and cellulolytic activities. Oboh *et al.* (2005) found amylase activity from waste water from fermented cassava mash. Previously, Oyewole and Odunfa (1992) reported that amylase activity increased rapidly during the early period of the fermentation of cassava for 'fufu' production, attaining their peak activities after 12 h. Cellulase activity was lower and approximately constant for most of the fermentation period. Possibly, predominant factor that changed carbohydrate content was leaching during 3 days of spontaneous submerged fermentation in "gadungan pohung".

Significant decrease was found in ash and crude fiber, meanwhile fat and protein only declined slightly. Changes of nutrient components of grated cassava was related to leaching into soaking water, especially for ash. Grating treatment facilitated some nutrients to leach into soaking water. Slight decrease in protein and fat content highly related to utilization by microbes during fermentation. Protein was a source of nitrogen for microbial growth. Fat is water insoluble therefore its decrease might relate to utilization as nutrient source for microbes.

Significant decrease in crude fiber was also corresponded to microbial activity. During cassava fermentation, some microbes involved have enzymatic activities. Okolie and Ugochukwu (1988) reported that the cell wall degrading enzymes polygalacturonase, pectinase, cellulose, and xylanase, as well as α-amylase and phosphorylase, were monitored during fermentation of cassava with *Citrobacter freundii.*

According to Ogunnaike *et al*. (2015), some yeasts and fungi contribute to tissue cassava breakdown by cellulose production. Enzymes from lactic acid bacteria hydrolize cell wall components partially such as hemicellulose, pectin, and mucilage that destroy the firm structure of cell. Degradation of cellulose leads to fragmentation and hydrolysis of cell wall and starch granule that facilitates starch granule to leach (Adetunji *et al.*, 2016) and decreases starch content. Cellulase broke cellulose down as part of crude fiber. Moreover, Ogunnaike *et al.* (2015) indicated that tissue breakdown made more intimate interaction between linamarase and cyanogenic compounds of cassava.

Starch was decrease in all cassava varieties after submerged fermentation in the range 4-6% (db) and conversely amylose increased after fermentation (Table 1). Similar result was reported by Sobowale (2007) that starch decreased after 96 h spontaneous fermentation. There was a considerable decrease in sugar content with fermentation in all the cultivars, indicating a predominant utilization of the easily assimilable sugars by the microorganisms than starch. Amylopectin presummably was hydrolyzed more than amylose. Starch granule structure was modified during fermentation such as depolymerization (Mestres and Rouau, 1997), and this occured mainly in amylopectin. The starch granule consists of alternating semi-crystalline and amorphous layers and semi crystalline layer consisted of double-helical α-glucans (Svihus *et al.*, 2005) mainly amylose

Table 2. Cyanide compound changes during fermentation in "gadungan pohung" processing

Variety	Day	Cyanogenic glycosides (mg HCN eq./kg db)	Acetone cyanohydrin (mg HCN eq./kg db)	Free HCN (mg HCN eq./kg db)	Total cyanogens (mg HCN eq./kg db)	Reduction* (%)
fresh tuber						
Malang 4		(337.98±1.40)	(3.45±0.14)	(4.50±0.22)	(345.94±1.36)	
Malang 6		(240.15±0.08)	(4.98±0.24)	(3.83±0.08)	(248.96±0.37)	
Sembung		(145.99±4.15)	(4.83±0.16)	(4.54±0.13)	(155.35±3.87)	
fermenting medium after 3 day submerged fermentation						
Malang 4	3	(18.59±0.58)	(26.33±0.89)	(8.68±0.22)	(53.60±0.39)	
Malang 6	3	(27.50±1.28)	(14.30±0.17)	(9.15±0.36)	(50.95±0.95)	
Sembung	3	(26.74±0.56)	(2.90±0.22)	(16.38±0.38)	(46.03±0.60)	
submerged fermentation						
Malang 4	3	(43.45±0.57)^{h}	(62.94±0.95)^{h}	(9.40±0.04)^{i}	(115.79±0.44)^{i}	66.53
Malang 6	3	(40.62±1.20)^{g}	(29.18±0.25)^{e}	(6.29±0.24)^{g}	(76.81±0.93)^{f}	69.15
Sembung	3	(64.67±0.96)^{i}	(27.56±0.35)^{d}	(0.53±0.08)^{e}	(95.21±0.93)^{h}	38.71
Solid state fermentation						
Malang 4	1	(37.99±1.00)^{f}	(42.54±1.09)^{f}	(7.01±0.22)^{h}	(88.20±0.62)^{g}	74.50
	2	(23.43±0.77)^{e}	(3.49±0.17)^{a}	(2.99±0.00)^{c}	(27.76±0.63)^{e}	91.97
	3	(17.41±0.91)^{c}	(3.77±0.16)^{a}	(7.67±0.06)^{d}	(22.75±0.73)^{c}	93.42
Malang 6	1	(22.30±0.49)^{e}	(48.42±0.61)^{g}	(0.30±0.03)^{f}	(77.02±1.12)^{f}	69.06
	2	(12.33±0.47)^{a}	(12.79±0.19)^{c}	(0.84±0.03)^{c}	(25.89±0.41)^{d}	89.60
	3	(13.58±0.71)^{b}	(4.17±0.20)^{a}	(0.77±0.03)^{c}	(18.52±0.67)^{b}	92.56
Sembung	1	(20.01±0.47)^{d}	(6.37±0.19)^{b}	(1.56±0.01)^{a}	(26.68±0.31)^{d}	82.83
	2	(12.41±0.50)a	(3.31±0.12)^{a}	(0.77±0.00)^{b}	(16.25±0.60)^{a}	89.54
	3	(12.30±0.30)^{a}	(3.30±0.12)^{a}	(0.40±0.02)^{b}	(16.00±0.20)^{a}	89.70

*compared to fresh tuber

The data are presented as mean value±standard deviation of triplicate analyses. Different letters in the same row indicate statistically significant values ($p \leq 0.05$).

chain. Amorphous layer that consists of particularly amylopectin was easier to hydrolyze during submerged fermentation of cassava.

Data in Table 1 shows that after submerged fermentation pH dropped slightly from around 6 to 5. Decrease in pH correlated well with increasing titratable acidity. Similar result was reported by Ogunnaike *et al.* (2015), that pH of fermenting medium decreased to about 5 after 72 h of submerged fermentation. Adetunji *et al.* (2016) reported that more sharp decrease in pH from 6.39 to 4.09 after 7 d fermentation due to LAB activity mainly *Lactobacillus plantarum*. LAB produces lactic acid and acetic acid to some extent (Coulin *et al.*, 2006). Fermentation time greatly affected final pH. Similar pattern in some chemical constituent changes was found for all cassava varieties. It meant that cassava varieties did not affect significantly on this alteration.

Reduction of cyanide

All cassava varieties in this study are high cyanide and very toxic cassava that indicated by total cyanide content more than 100 ppm. According to Kobawila *et al*. (2005), cassava is classified into 3 catagories 1) very toxic (cyanide content more 100 ppm), 2) moderately toxic (cyanide content 50-100 ppm), and 3) non toxic with cyanide level less than 50 ppm.

Cyanogens or cyanogenic compounds in fresh tuber consisted mainly in the form of cyanogenic glycosides (Table 2), and in the form of cyanohydrin or α hydroxynitrile and free HCN to some extent. Cyanohydrin and free HCN could be formed during sample preparation due to enzymatic hydrolysis (Agbor-Egbe and Mbome, 2006). The tissues of healthy cyanogenic plants do not contain detectable HCN, suggesting that the cyanogenic glycosides are normally separated from the enzymes that catalyse HCN release (Iriti and Faoro, 2009).

Spontaneuos submerged fermentation for 3 days reduced cyanide compounds in grated cassava (Table 2). Cassava varieties showed different level of cyanide reduction. The highest degree of reduction (%) was found in Malang 6 variety and the lowest was Sembung. The highest cyanide level among varieties was Malang 4. Actually, Malang 4 had the highest level of cyanide reduction because this variety was the highest cyanide, which cyanide level reduced from 346 to 116 ppm.

During submerged fermentation for 3 days, the changes in cyanide compounds occured. Cyanogenic glycosides decreased significantly in all varieties. The highest degree of reduction was found in Malang 4 variety from 338 to 43 ppm. Meanwhile, cyanohydrin and free HCN increased, and the significant enhancement was found in cyanohydrin compounds. In general, total cyanide declined in all varieties due to the significant decrease in cyanogenic glycosides.

Decreasing cyanogenic glycosides during 3 days submerged fermentation was presummably related to dissolvation and degradation into acetone cyanohydrin. Data in Table 2 shows that some cyanogens dissolved into fermenting medium during fermentation. Malang 4 as the highest cyanide

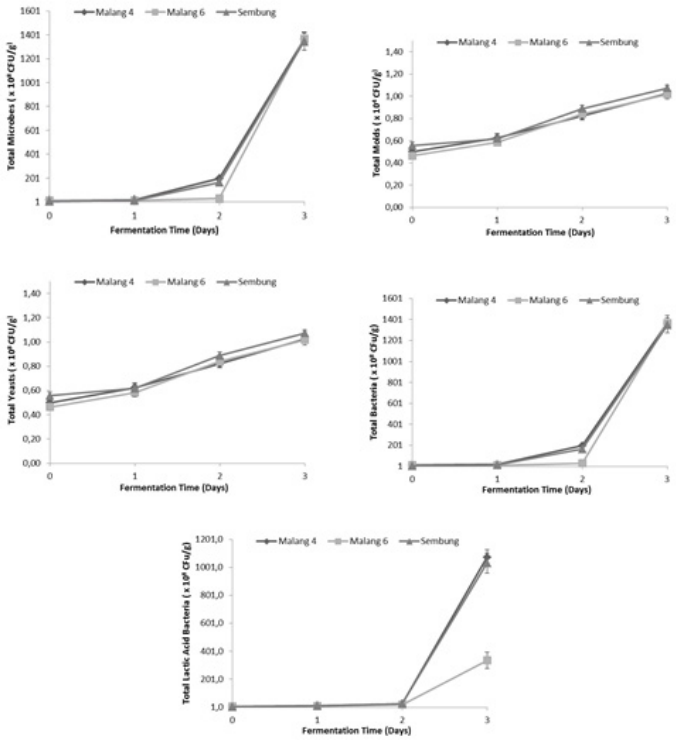

Figure 3. Microbiological profile during solid state fermentation of high cyanide cassava varieties in "gadungan pohung" processing

variety had the highest total cyanide in fermenting medium. Degree of dissolvation among cyanogenic compounds was different and it seemed that cassava varieties also affected. Cyanogenic glycosides in fermenting medium of Malang 4 variety was the lowest among varieties. However, level of glycoside cynogenic reduction of this variety was the highest. It meant, presummably there was other mechanism for cyanogenic glycoside reduction, mainly by hydrolysis due to enzymatic activity.

Hydrolysis of cyanogenic glycoside involves linamarase or β glucosidase activity (Gueguen *et al*., 1997). Cassava root also has endogeneous β-D-glucosidase linamarase (Nwokoro, 2016) that enabled to have a role in degradation of cyanogenic glycoside into acetone cyanohydrin. According to Giraud *et al*. (1983), endogenous linamarase released during the grating of the roots was sufficient to permit the complete and rapid breakdown of linamarin during cassava fermentation using *L. plantarum* starter. Previously, Maduagwu (1983) suggested that the contribution of the fermentation process in cyanide detoxification of pulped cassava roots is minimal. Grating of cassava before fermentation also facilitated liberation of linamarase from cell wall and rupture of vacuole that enabled a contact between enzyme and substrate. Endogen linamarase is not sufficient to degrade linamarin, therefore additional exogen linamarase is required such as from *Lactobacillus plantarum* (Nwokoro, 2016). The decline in linamarin was related to activity of degrading enzymes from microbes to destroy cell wall of cassava, that facilitated the contact between linamarin with exogen linamarase from microbes or endogen linamarase from cassava.

Reduction of cyanogenic glycosides was accompanied by an increase of cyanohydrin in all varieties (Table 2). Degradation of cyanogenic glycosides produced cyanohydrin as intermediate product. Interestingly, not all of fermenting medium showed increasing cyanohydrin (Table 2). Enhancement of cyanohydrin in fermenting medium was only found in Malang 4 as the highest cyanide variety. Malang 6 and Sembung showed a decrease in cyanohydrin. Probably, this required enough time for gradually degradation of linamarin in fermenting medium. Ikediobi and Onyike (1982) revealed that linamarin demonstrated unsual stability in dilute acid even at 100ºC thus making it unlikely that detoxification is due to solely to chemical hydrolysis by the weak acid by microbial fermentation. Therefore, linamarin degradation in fermenting medium might be in low rate, because linamarase is maximally active in the pH 5.5 (Nambisan, 2011). Final pH of fermenting medium after 3 day fermentation was below 5.

Cyanohydrin could degrade into free HCN and acetone. This degradation was facilitated by hydroxynitrile liase (HNL) enzyme, but this enzyme activity in parenchyma was only slight (Siritunga and

Sayre, 2004). Cyanohydrin degrades spontaneously at pH > 5 and at temperature 30–50°C (Siritunga and Sayre, 2004; Bradbury and Denton, 2011). Free HCN increased significantly in grated cassava of all varieties, except Sembung variety. Sembung variety showed the highest concentration of HCN in fermenting medium. It seemed that in this variety, HCN released more rapidly and easily dissolved into fermenting medium. Conversely, Malang 4 variety had the highest level of cyanohydrin in grated cassava but its fermenting medium showed the lowest free HCN. It appeared that degradation of cyanohydrin was accompanied by increasing free HCN in fermenting medium. Mechanism of cyanohydrin degradation was supposed to HNL activity. After 3 day submerged fermentation, pH decreased from 6 to 4.5 that passed the optimum pH for HNL activity. According to McMohan (1995), HNL activity from cassava was optimum at pH 5.5. Therefore, activity of HNL contributed to degradation of cyanohydrin into free HCN. Free HCN in grated cassava increased in comparison to fresh tuber for all varieties, as a result of cyanohydrin degradation. Sembung variety showed very low concentration of free HCN. Free HCN from cyanohydrin degradation easily disappeared by vaporization. Temperature of submerged fermentation was ambient temperature about 28±2ºC, and boiling point of HCN is 26ºC (Bradbury, 2006).

Solid state fermentation

Microbiological profile

In "gadungan pohung" processing, solid state fermentation is conducted simultaneously after submerged fermentation. Some microbes grew during solid state fermentation (Figure 3). Significant increase in total microbes was found after 2 day fermentation. Similar pattern was also found in total bacteria and total lactic acid bacteria (LAB), meanwhile the growth of yeasts and molds increased steadily since the beginning of fermentation. All varieties of cassava showed similar pattern of microbial growth.

Predominant microbes in solid state fermentation was bacteria, mainly lactic acid bacteria. Lactic acid bacteria from submerged fermentation continued to grow during solid state fermentation. Sharp increase in lactic acid bacteria occured after 2 days fermentation. Meanwhile, total bacteria started up after 1 day fermentation although lactic acid bacteria dominated at the end of fermentation. Tetchi (2012) also found that during solid state fermentation of cassava in attieke processing, lactic acid bacteria became dominant and contributed to the most acidification of the product. Condition of fermentation in solid state fermentation of "gadungan pohung" processing was microaerophilic, but lactic acid bacteria was able to grow. Tetchi (2012) also found other microbes in attieke processing including yeast and enterobacteria as aerobic mesophiles.

The number of yeasts and molds during solid state fermentation of "gadungan pohung" increased almost constantly and there was no sharp enhancement during 3 days fermentation. The growth of these microbes was as the continuation of the growth during submerged fermentation. As in the submerged fermentation, the number of yeasts was higher than molds. This finding was in accrodance to the study of Coulin *et al.* (2006) that the mean numbers of *Candida tropicalis,* the main yeast present, remained relatively constant at about 10^5 cfu/g throughout attiéké production.

Varieties of cassava affected the growth rate of microbes. The slowest growth of total microbes was found in Malang 6 variety, meanwhile Malang 4 and Sembung varieties indicated similar growth. The growth of molds and yeasts was similar for all varieties, but the growth of total bacteria had different pattern. The slowest growth of total bacteria was found in solid state fermentation of Malang 6, meanwhile Malang 4 and Sembung varieties exhibited similar rate. Interestingly, the growth of lactic acid bacteria in Malang 6 variety was much lower after 2 days fermentation than that in Malang 4 and Sembung. The cyanide level of cassava varieties in this study was Malang 4 > Malang 6 > Sembung. It seemed that the level of cyanide generally did not affect microbial growth, although high cyanogenic glycosides content which could have an inhibitory effect on fungal (Moller, 2010). Antifungal activity of cyanogens presummably resulted in slow growth of molds during solid state fermentation mainly in Malang 4 as the highest cyanide variety.

Chemical constituent changes

Data in Table 1 showed the changes of proximate composition and other chemical compounds, as well as pH and titratable acidity. Moisture content, protein, fat, fiber, and starch decreased slightly during solid state fermentation, meanwile ash and carbohydrate increased. Metabolism of microbes during fermentation needed nutrition from grated cassava such as protein, fat, and starch. Water was required for hydrolysis activity of microbes that made moisture content of grated cassava decreased. Also, some microbes mainly molds have the ablity to degrade cellulose that made fiber content also

decreased. Pothiraj *et al.* (2006) revealed that during solid state fermentation of cassava waste, some fungi produced cellulase. During "gadungan pohung" fermentation, some molds possibly produced cellulase that resulted in slight decrease of fiber. Soccol *et al.* (1994) found amylase activity of *Rhizopus* in raw cassava by solid state fermentation. The molds in solid state fermentation during "gadungan pohung" processing are still identified. However, we proposed some enzymes were produced, including amylase, resulted a decrease of some nutritional compounds of grated cassava. Interestingly, total carbohydrate increased significantly during solid state fermentation in all cassava varieties. This increase possibly was due to the decrease in moisture content and other compounds, because carbohydrate was analyzed by difference.

During 3 days solid state fermentation, pH of grated cassva increased significantly in all cassava varieties. It was on the contrary to pH value during submerged fermentation. Mahanta *et al*. (2008) reported that pH increased during solid state fermentation using *Jatropha curcas* seed. Degradation of nutrition compounds especially protein presumably made an increase of pH value. Increasing pH from 4 to 6 during solid state fermentation generated suitable condition for HNL activity that promoted degradation of cyanohydrin. Titratable acidity decreased during 3 days solid state fermentation that was in line with increasing pH value.

Reduction of cyanide compounds

Data in Table 2 showed the reduction of all of cyanide compounds during 3 days solid state fermentation in all cassava varieties, except free HCN. Cyanogenic glycosides declined during solid state fermentation, but its decrease was not as sharp as its reduction during 3 days submerged fermentation. Activity of linamarase presummably contributed to reduction of cyanogenic glycosides. However, its activity possibly was not as high as during submerged fermentation. During solid state fermentation, pH of substrate increased from 4 to 6, that was suitable for linamarase activity. According to Nambisan (2011), optimum pH of linamarase during gari fermentation was 5.5, and linamarase is a rate limiting enzyme in cyanogenesis (Mc Mahon *et al.*, 1995). However, other factors might influence linamarase activity during solid state fermentation such as temperature and water activity.

In contrast, cyanohydrin decreased sharply during solid state fermentation. The responsible enzyme for degradation of cyanohydrin is HNL, that has optimum activity at pH 5.5 (Mc Mahon *et al.*, 1995). Degradation of cyanohydrin was also facilitated by increasing pH. Suitable condition of cyanohydrin degradation during solid state fermentation resulted in great reduction of this cyanide compounds. In contrast to solid state fermentation, residual cyanohydrin was still found in significant amount and its quantity was higher than fresh tuber. It was supposed that rate of cyanohydrin degradation was higher during submerged fermentation. At Malang 4 variety, cyanogenic glycosides degraded from 344 into 43 ppm that produced about 301 ppm cyanohydrin. However, residual cyanohydrin after submerged fermentation was 63 ppm. During submerged fermentation, pH declined from 6 to 4.5 that also generated suitable condition for HNL activity. Free HCN was fluctuating during 3 days solid state fermentation. Free HCN was liberated from cyanohydrin by HNL activity and increasing pH. Free HCN could be removed by heating or dissolution into water. However, solid state fermentation did not use water as fermenting medium, therefore free HCN was probably removed by heat that generated from microbial activity.

Final cyanide compound level after solid state fermentation for all cassava varieties was 16-23 ppm. This level was not toxic (Kobawila *et al*., 2005) but was still higher than 10 ppm as the safe level of consumption (FAO/WHO, 1991). The magnitude of reduction for all cassava varieties was about 90%. The higher reduction was found in the highest cyanide cassava variety (Malang 4) and the least reduction was in the lowest cyanide variety (Sembung). For all varieties, predominat residual cyanogenic compound was cyanogenic glycosides. This residue was not easy to remove. Presumably, longer solid state fermentation time will assist to reduce residual cyanogens.

Conclusions

Combination of submerged and solid state fermentation was an effective way to reduce cyanides in high cyanide cassava varieties. Some chemical changes occured during submerged and solid state fermentation. Submerged fermentation reduced cyanide compounds. Some cyanide compounds were found in fermenting medium. Significant declined was found in cyanogenic glycosides, meanwhile cyanohydrin and free HCN increased in all cassava varieties. Cassava varieties significantly affected the growth of microbes in fermenting and grated cassava. Predominant microbes in fermenting medium was bacteria mainly lactic acid bacteria. Molds were found to some extent, and their amount was lower

than yeasts. During submerged fermentation the microbes grew were bacteria, yeasts, and molds, with predominant microbe was lactic acid bacteria. The growth pattern of yeasts and molds was similar, meanwhile mold growth was affected by varieties. Degradation of cyanides was due to microbial activity and increasing pH to above 5 in solid state fermentation.

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References

Adepoju, O. T., Adekola, Y. G., Mustapha, S. O. and Ogunola, S. I. 2010. Effect of processing methods on nutrient retention and contribution of cassava (*Manihot* spp) to nutrient intake of Nigerian consumers, African Journal of Food, Agriculture, Nutrition and Development 10 (2): 2099-2111.

Adetunji, A. I., du Clou, H., Walford, S. N. and Taylor, J. R. N. 2016. Complementary effects of cell wall degrading enzymes together withlactic acid fermentation on cassava tuber cell wall breakdown. Industrial Crops and Products 90:110–117.

Agbor-Egbe, T. and Mbome, L. L. 2006. The effects of processing techniques in reducing cyanogen levels during the production of some cameroonian cassava foods. Journal of Food Composition and Analysis 19: 354-363.

AOAC. 2011. Official methods of analysis of the AOAC, 18th ed. Rev 4. Association of official analytical chemists. Arlington, USA.

Aprianita, A., Purwandari, U., Watson, B. and Vasiljevic, T. 2009. Physico-chemical properties of flours and starches from selected commercial tubers available in Australia. International Food Research Journal 16: 507-520.

Bandna, C. 2012. Effect of processing on the cyanide content of cassava products in Fiji. Journal of Microbiology, Biotechnology and Food Sciences 2(3): 947-958..

Bradbury, J. H. 2006. Simple wetting method to reduce cyanogen content of cassava flour. Journal of Food Composition and Analysis 19: 388–393.

Bradbury, J. H. and Denton, I. C. 2011. Mild methods of processing cassava leaves to remove cyanogens and conserve key nutrients. Food Chemistry 127: 1755–1759.

Bradbury, J. H., Egan, S. V. and Lynch, M. J. 1991, Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glycosidess. Journal of the Science of Food and Agriculture 55: 277-290.

Brauman, A., Keleke, S., M. Malonga, E. Miambi, and F. Ampe. 1996. Microbiological and biochemical characterization of cassava retting, a traditional lactic acid fermentation for foo-foo (cassava flour) production. Applied and Environmental Microbiology 62: 2854–2858.

Cardoso, A.P., Mirione, E., Ernesto, M., Massaza, F., Cliffc, J., Haque, M.R. and Bradbury, J.H. 2005. Processing of cassava roots to remove cyanogens. Journal of Food Composition and Analysis 18:451–460.

Charoenkul, N., Uttapap, D., Pathipanawat, W. and Takeda, Y. 2011. Physicochemical characteristics of starches and flours from cassava varieties having different cooked root textures. Food Science and Technology 44 : 1774-1781.

Coulin, P., Farah, Z., Assanvo, J., Spillmann, H. and Puhan, Z. 2006. Characterisation of the microflora of attie´ke´, a fermented cassava product, during traditional small-scale preparation. International Journal of Food Microbiology 106: 131 – 136.

Essers, A. J. A., Bennik, M. H. J. and Phut, M. J. R. 1995. Mechanisms of increased linamarin degradation during solid-substrate fermentation of cassava. orld Journal Microbiology Biotechnology 11: 266-270.

Essers, A.J.A., Van der Grift, R. M. and Voragen, A.G. J. 1996. Cyanogen removal from cassava roots during sun-drying. Food Chemistry 55 (4): 319-325.

FAO/WHO. Joint FAO/WHO. 2012. Food Standards Programme. Codex Alimentarius Commission 35th. FAO. Rome. Italy.

Fayemi, O. E. and Ojokoh, A. O. 2012. The effect of different fermentation techniques on the nutritional quality of the cassava product (fufu), ournal of Food Processing and preservation 38: 183-192.

FSANZ. 2005. Cyanogenic glycosides in cassava and bamboo shoots. A Human Health Risk Assessment Technical Report Series No 28. Food Standards Australia New Zealand.

Giraud, E., Gosselin, L. and Raimbault, M. 1993. Production of a Lactobacillus plantarum starter with linamarase and amylase activities for cassava fermentation. Journal of the Science Food and Agriculture 62(1): 77-82.

Gueguen, Y., Chemardin, P., Labrot, P., Arnaud, A. and Galzy, P. 1997. Purification and characterization of intracellular β glucosidase from a new strain of Leuconostoc mesenteroides isolated from cassava. Journal of Applied Microbiology 82: 469-476.

Ikediobi, C.O. and Onyike, E. 1982. Linamarase activity and detoxification of cassava *(Manihot esculenta)* during fermentation for gari production. Agricultural and Biological Chemistry 46(6): 1667-1669.

Iriti, M. and Faoro, F. 2009. Chemical diversity and defence metabolism: how plants cope with pathogens and ozone pollution. International Journal of Molecular Sciences 10: 3371-3399.

Irtwange, S. V. and Achimba, O. 2009. Effect of the duration of fermentation on the quality of gari.

Current Research Journal of Biological Sciences 1(3): 150-154.

Iyuke, S. E. and Idibie, C. A. 2007. Recovery of linamarin by adsorption of cassava extract onto activated carbon. Industrial and Engineering Chemistry Research 46(14): 4974-4981.

Kambale, K. J., Ali, E. R., Sadiki, N. H., Kayembe, K. P., Mvumbi, L. G., Yandju, D.L., Boivin, M. J., Boss, G. R., Stadler, D. D., Lambert, W. E., Lasarev, M. R., Okitundu, L. A., Mumba Ngoyi, D., Banea, J. P. and Tshala-Katumbay, D. D. 2017. Lower sulfurtransferase detoxification rates of cyanide in konzo—A tropical spastic paralysis linked to cassava cyanogenic poisoning. NeuroToxicology 59: 256–262.

Kimaryo, V. M., Massawe, G. A., Olasupo, N. A. and Holzapfel ,W. H., 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.

Kobawila, S.C., Louembe, D., Keleke, S., Hounhouigan, J. and Gamb,a C. 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. African Journal of Biotechnology 4(7): 689-696.

Kostinek, M., Specht, I., Edward, V. A., Pinto, C., Egounlety, M., Sossa C., Mbugua, S., Dortu, C., Thonart, P., Taljaard, L., Mengu, M., Franz, C. M. A,. P. and Holzap, W.H. 2007. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. International Journal of Food Microbiology 114(3): 342-351.

Lambri, M., Fumi, M. D., Roda, A. and de Faveri, D. M. 2013. Improved processing methods to reduce the total cyanide content of cassava roots from Burundi. Africa Journal Biotechnology 12(19): 2685-2691.

Maduagwu, E. N. 1983. Differential effects on the cyanogenic glycoside content of fermenting cassava root pulp by β-glucosidase and microbial activities. Toxicology Letters 15(4): 335-339.

Mahanta, N., Gupta, A. and Khare, S. K. 2008. Production of protease and lipase by solvent tolerant Pseudomonas aeruginosa PseA in solid-state fermentation using Jatropha curcas seed cake as substrate. Bioresource Technology 99(6): 1729–1735.

McMahon, J. M., White, W. L. B. and Sayre, R. T. 1995. Cyanogenesis in cassava (*Manihot esculenta* Crantz). Journal of Experimental Botany 46(288): 731-741.

Medoua, G.N., Mbomea I. L., Agbor-Egbe T. and Mbofung C. M. F. 2008. Influence of fermentation on some quality characteristics of trifoliate yam *(Dioscorea dumetorum)* hardened tubers. Food Chemistry 107: 1180–1186.

Mestres, C. and Rouau, X. 1997. Influence of natural fermentation and drying conditions on the physicochemical characteristics of cassava starch. Journal the Science Food and Agriculture 74(2): 147–155.

Moller, B. L. 2010. Functional diversifications of cyanogenic glucosides. Plant Biology 13: 338–347.

Montagnac, J.A., Davis, C.R., Tanumihardjo, S.A. 2009. Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. Comprehensive Reviews in Food Science and Food Safety (8):17-27.

Morgan, N. K. and Choct, M. 2016. Cassava: nutrient composition and nutritive value in poultry diets. Animal Nutrition 2: 253-261.

Murugan, K., Yashotha, Sekar K. and Al- Sohaibani S. 2012. Detoxification of cyanides in cassava flour by linamarase of *Bacillus subtilis* KM05 isolated from cassava peel. African Journal of Biotechnology 11(28): 7232-7237.

Nambisan, B. 2011. Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. Food Chemistry Toxicology 49: 690–693.

Nwokoro, O. 2016. Linamarase production by some microbial isolates and a comparison of the rate of degradation of cassava cyanide by microbial and cassava linamarases. Hemijska industrija 70(2): 129–136.

Obadina, A. O., Oyewole, O. B. and Odusami, A. O. 2009. Microbiological safety and quality assessment of some fermented cassava products (lafun, fufu, gari). Scientific Research and Essay 4(5): 432-435.

Obilie, E. M., Tano-Debrah, K. and Amoa-Awua, W. K., 2004, Souring and breakdown of cyanogenic glycosidess during the processing of cassava into akyeke, International Journal of Food Microbiology 93 : 115– 121.

Oboh, G. 2005. Isolation and characterization of amylase from fermented cassava (*Manihot esculenta* Crantz) wastewater. African Journal of Biotechnology 4(10): 1117-1123.

Ogunnaike, A. M., Adepoju P. A., Longe A. O., Elemo G. N. and Oke O. V. 2015. Effects of submerged and anaerobic fermentations on cassava flour (Lafun). African Journal of Biotechnology 14(11): 961-970.

Okolie, P. N. and Ugochukwu, E. N. 1988. Changes in activities of cell wall degrading enzymes during fermentation of cassava (*Manihot esculenta* Crantz) with Citrobacter freundii.

Oyedeji, O., Ogunbanwo, S. T. and Onilude, A. A. 2013. Predominant lactic acid bacteria involved in the traditional fermentation of fufu and ogi, two Nigerian fermented food products. Food and Nutrition Sciences 4: 40-46.

Oyewole, O. B. and Odunfa, S. A. 1992. Extracellular enzyme activities during cassava fermentation for ‘fufu’ production. World Journal Microbiology Biotechnology 8: 71.

Padonou S. W., J. D. Hounhouigan and M. C. Nago, 2009, Physical, chemical and microbiological characteristics of lafun produced in Benin African Journal of Biotechnology 8(14) : 3320-3325.

Pinto-Zevallos D. M., Pareja M. and Ambrogi B. G. 2016. Current knowledge and future research perspectives on cassava (*Manihot esculenta* Crantz) chemical

defenses: An agroecological view. Phytochemistry 130: 10-21.

Pothiraj C., Balaji P. and Eyini M. 2006. Enhanced production of cellulases by various fungal cultures in solid state fermentation of cassava waste. African Journal of Biotechnology 5(20): 1882-1885.

Sharma M., Sharma N. N. and Bhalla T. C. 2005. Hydroxynitrile lyases: At the interface of biology and chemistry. Enzyme and Microbial Technology 37: 279–294.

Siritunga D. and Sayre R. 2004. Engineering cyanogen synthesis and turnover in cassava *(Manihot esculenta)*. Plant Molecular Biology 56: 661–669.

Sobowale, A. O., Olurin T. O. and Oyewole O. B. 2007. Effect of lactic acid bacteria starter culture fermentation of cassava on chemical and sensory characteristics of fufu flour. African Journal of Biotechnology 6(16):1954-1958.

Soccol C. R., Marin B., Raimbault M. and Lebeault J. M. 1994. Breeding and growth of *Rhizopus* in raw cassava by solid state fermentation. Applied Microbiology and Biotechnology 41(3): 330-336.

Sornyotha S., Kyu K. L. and Ratanakhanokchai K. 2010. An efficient treatment for detoxification process of cassava starch byplant cell wall-degrading enzymes. Journal of Bioscience and Bioengineering 109(1): 9–14.

Svihus B., Uhlen A. K. and Harstad O. M. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Animal Feed Science and Technology 122: 303–320.

Tetchi F. A. 2012. Effect of cassava variety and fermentation time on biochemical and microbiological characteristics of raw artisanal starter for attiéké production. Innovative Romanian Food Biotechnology 10: 40-47.

Wahyuni, S., Ansharullah, Saefuddin, Holilah and Asranudin. 2016. Physico-chemical properties of Wikau maombo flour from cassava (*Manihot esculenta* Crantz). Journal of Food Measurement and Characterization 11(1): 329–336.

Wakil, S.M. and I.B. Benjamin. 2015. Starter developed pupuru, a traditional Africa fermented food from cassava *(Manihot esculenta)*. International Food Research Journal 22: 2565-2570.