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Assessment of Biochemical Changes during Fermentation Process for Production of Traditional Fermented Cassava Meal "Mchuchume"

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Abstract

"Mchuchume" is a ready to eat fermented cassava food whose production is accomplished by a consortium of microorganisms, some of which may be probiotic in nature and others pathogens to humans and of no use in the fermenting system. This work made characterization of biochemical changes evolved at different times in local (open) and biotechnological (closed) fermentation methods. The study also assessed organoleptic properties of final products. Acidification was observed in the retting water as pH ranged from 6.32 to 4.25 and titratable acidity from 0.02% to 0.32%. A decrease in total reducing sugars (6.59 to 4.03 mg/g) in fermenting pulp and an increase in total soluble solids (0.4 to 3.2°B) in retting water were observed. Cyanide detoxification occurred by decreasing cyanogenic potential from 72.72 to 5.18 mg/kg, while products organoleptic characters from two fermentation methods were not statistically different except for the case of aroma. Aseptic retting of cassava tubers using closed fermentation utilizing *Lactobacillus delbrueckii* starter cultures produces "mchuchume" which is safe from cyanide, dusts and microbiological contaminants at reduced processing time.

Keywords: Cassava; mchuchume; fermentation methods; biochemical changes

Introduction

Cassava (*Manihot esculenta*) is a short-lived perennial wood shrub; 1-5 m height that belongs to the family *Euphorbiaceae*. It is cultivated worldwide, and this plant is wholly beneficial as its leaves are good sources of protein and vitamins, tubers are rich in carbohydrates and minerals, while stems are used as animal feed and fuel sources to some societies (FAO 2013, Umeh and Odibo 2014). Cassava tubers are quantitatively the third most important food in the tropics after rice and corn, and play roles as staple food in many regions of the developing countries (Oladunmoye et al. 2010). Once seen as the "food of the poor people", cassava has emerged as a multipurpose crop for the 21st century - one that responds to the priorities of developing countries, to trends in the global economy and to the challenges of climate change (FAO 2013). It was estimated that cassava tubers provide about 40% of all the calories consumed in Africa (Umeh et al. 2007). About two thirds of the total production of cassava in Africa is consumed in various forms by human. Cassava plays a vital role as food security crop in Tanzania especially among low-income consumers. Its prevalent cultivation has been attributed by its farming chain that does not involve intensive labour and its flexibility in terms of planting and harvesting strategies. This plant nourishes in marginal soil due to its tolerance on harsh environments (Shayo and Martinm 2009).

However, cassava tubers deteriorate due to presence of tannic acids that blackens the damaged parenchyma of the tuber just after two hours of harvest. It also contains cyanogenic compounds produced by the plant as defense tools to deter predators such as insects and animals. These chemical substances are toxic when consumed by human. As part of responding to these constrains, various methods have been developed to process cassava tuber into different cassava products and the residue cyanide removed out. Large amounts of cyanoglycosides released during cassava processing might lead to increase in environmental cyanide concentrations. Fortunately, hydrogen cyanide can be used in production of chelating agents, adiponitrile, cyanuric chloride and sodium cyanide and other miscellaneous uses (WHO 2004). The hydrogen cyanide is mainly used as intermediate in the production of a number of chemicals including insecticides for fumigating enclosed spaces. Therefore, there is a need to establish a strategy utilizing cyanogenic glycoside contained in cassava tuber for developing an insecticidal formulation.

Cassava has been utilized in several ways and its modes of utilization in Africa show that nearly three out of four cassava-based-foods are fermented products (Ogunnaike et al. 2015). Fermentation, among other methods, has been reported being effective in cyanide detoxification (Aworh 2008). This technology has variously been used in the production of different types of cassava foods like "gari", "topiaca", "fufu" and "lafun" of Nigeia (Umeh and Odibo 2014); "attiéké", "placali", "attoukpou", "konkondé" and "ebrié "of Côte-d'Ivoire (Kakou et al. 2010), "bikedi" of Congo (Kobawila et al. 2005) and "kivunde" and "mchuchume" of Tanzania (Kimaryo et al. 2000, Alphonce et al. 2019).

During fermentation of cassava pulps there are several catabolic and anabolic reactions taking place simultaneously under the influence of various factors including substrates, microorganisms and environmental factors (Eleazu et al. 2011). Metabolic effects of micro flora generate biochemical changes including tissue degradation and softening associated with out fluxing of soluble solids and cyanogenesis process that releases a volatile toxic chemical substance called hydrocyanic acid (HCN). Concurrently, cassava carbohydrates are metabolized into energy and organic compounds primarily lactic and acetic acid (Giraud et al. 1994, Achi and Akomas 2006). Cassava fermentation is often of two main sorts which are heap and submerged fermentation (Oyewole 1990). Heap fermentation is carried out in dry condition and it largely depends on exogenous microorganisms entering the system in uncontrolled patterns (spontaneous); a process also known as *open fermentation*. On the contrary, submerged fermentation is carried out in a wet condition. Microorganisms to facilitate this fermentation can be allowed to enter into a system in two ways, either in uncontrolled pattern (spontaneous) for open fermentation or controlled pattern (induced) for closed fermentation (Kimaryo et al. 2000).

"Mchuchume" is an example of cassava meal produced in Western parts of Tanzania mainly in Kigoma region using submerged fermentation in which the inoculation is spontaneous (*open fermentation*). It is a ready to eat whitish paste with sour taste consumed as a snack or a daily meal by the inhabitants and refugees residing in the camps in Kigoma region. The traditional preparations of this food involve steeping cooked bitter cassava tubers in cold water contained in an open or uncovered pot to allow open fermentation. This type of fermentation relies on chance dependent inoculation and it is accomplished by consortium microorganisms, some of which are probiotic in nature and others of no use in the fermenting system but might be pathogens to humans. Subsequently fermentation time and qualities of the products vary from one producer to other or from one production batch to other. To mitigate challenges posed by open

fermentation, Okolie et al. (1992) proposed applications of biotechnology employing a processing technique known as *controlled or closed fermentation* through the use of starter culture. However, in practice "mchuchume" preparations have not yet received this much attention. Therefore, this study aimed at carrying out comparative assessments of different biochemical parameters evolved in two fermentation conditions, that is open and closed fermentation for improving "mchuchume" processing techniques.

Materials and Methods

Materials collection

Samples of cassava tubers aged 8-10 months were harvested on 19th February 2018 from a farm belonging to Mikocheni Agricultural Research Institute at Chambezi Centre in Coast region, Tanzania. The tubers chosen comprised mild bitter improved African cultivar (KBH 2006/482-Kizimbani) as they correspond to cultivar used in "mchuchume" traditional processing and they also contain quintessential amounts of cyanide for experimentation. Harvesting was done manually by digging around the standing stem using a hand hoe to facilitate the pulling of the tubers from the soil. The harvested tubers were piled into a plastic pail filled with wet soil and transported to University of Dar es Salaam for experimental studies.

Fermenting cassava tubers

Preparation of samples

500 g of bitter cassava tubers were washed using potable water to remove soil particles. Thereafter, they were peeled and sliced into small pieces of about 2 x 2 cm using a knife followed by defibring. They were then placed into a saucepan containing 250 mL portable water and then boiled on hot plate until cooked at 50 °C for 45 minutes.

Experiment design

A complete randomized block design of experiment was used. Experimentation involved submerged fermentation comprising system volume (50% w/v) that was treated with three fermentation conditions (treatments). The treatment conditions adopted two fermentation methods, open (spontaneous) and controlled/closed (the use of 2 mL and 4 mL inoculums of *Lactobacillus delbrueckii* WLP677). The fermentation processes lasted for 72 hours up to completion and the biochemical changes evolved were assessed at an interval of 12 hours.

Preparation of starter culture solution

About 250 mL of MRS agar solution was prepared according to the manufacturer's instruction and was autoclaved at 121 °C for 15 minutes. Then 20 mL of sterile agar solution was poured in three sterilized Petri dish plates and allowed to cool and solidify for 15 minutes. Each plate was inoculated with pure stains of *Lactobacillus delbrueckii* (WLP677) using inoculation loop consecutively sterilized on Bunsen burner flame. Inoculated plates were then incubated for 24 hours at 37 °C. 10 mL of 1% peptone water were added to 24 hours held plates of *Lactobacillus delbrueckii* followed by aseptic agar scrapping. The resulted suspension was used as starter culture for fermentation of cooked bitter cassava tubers in closed fermentation system.

Fermentation process

Three sets of flasks containing 50 g of boiled cassava tubers were filled with cold deionized water in the arrangement making fermentation volumes of 50% (w/v) and then autoclaved at 121 °C for 15 minutes. Two sets of flasks cooled at room temperature were inoculated with 2 mL and 4 mL of prepared *Lactobacillus delbrueckii* inoculums (Figure 1 B and C) and allowed to ferment at 30 °C while the third set of flasks was left to ferment spontaneously (open fermentation) at room temperature (Figure 1 A). Each experiment set up was conducted in triplicate and considered traditional method of "mchuchume" preparation (Figure 2(a)). Samples of fermenting cassava pulps and water for

analyses from each flask were taken aseptically at time interval of 12 hours until 72 hours for completed fermentation.

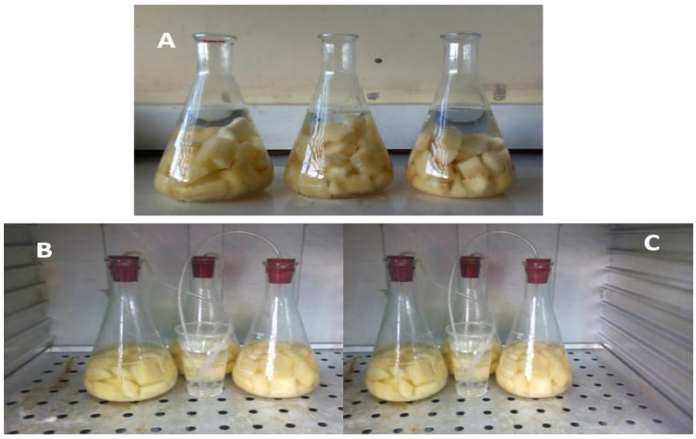
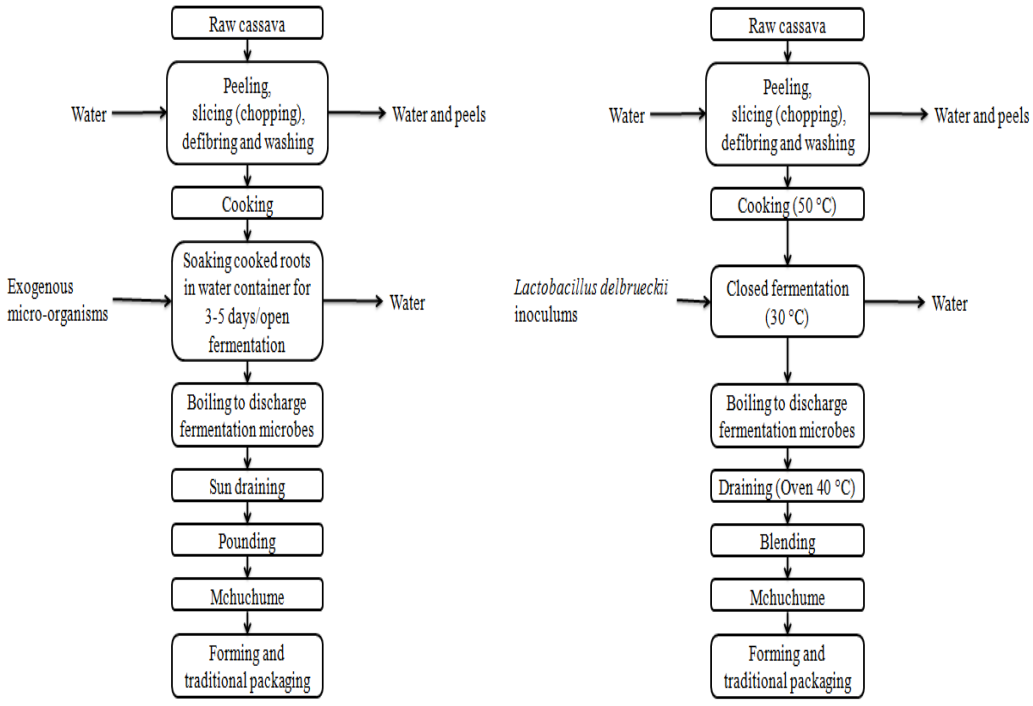


Figure 1: Illustration of wet fermentation processes for, (A) open fermentation, (B) closed fermentation using 2 mL inoculums and (C) closed fermentation using 4 mL inoculums.



(a) Process flow diagram for traditional preparation of "Mchuchume".

(b) Process flow diagram for improved preparation of "Mchuchume".

Figure 2: Diagrammatic presentation of "mchuchume" production process.

Determination of pH and titratable acidity

A method described by Kobawila et al. (2005) was used to measure the pH values and titratable acidity. About 15 mL of retting water was filtered on Whatman No. 4 filter paper and then 10 mL of filtrate was collected in the 100 mL flask. Using the digital pH meter (HANNA HI 98129 USA), pH values were measured in triplicate; meanwhile, the meter was washed thoroughly and calibrated to pH of 7 using deionized water after each measurement. Titratable acidity was determined by titrating 10 mL of filtered aliquot of retting water in the 100 mL flask with 0.1 N NaOH using three drops of 1% phenolphthalein indicator. The titratable acidity was evaluated using equation (1) on basis of lactic acid as predominated product of fermentation.

$$\text{Titratable acidity} = \frac{V_b \times N_b \times 0.09}{V_s} \times 100\% \qquad (1)$$

Where: V_b = volume of the base used; 0.09 = acid milliequivalent factor for lactic acid; N_b = Normality of the base used, and V_s = Sample volume.

Determination of total reducing sugars

Total reducing sugars present in fermenting cassava pulps at different intervals of the fermentation processes were determined using dinitrosalicylic acid (DNSA) method as described by Miller (1959) and used by Kimaryo et al. (2000) and Kakou et al. (2016).

Determination of total soluble solids (TSS)

The total amount of soluble solids in the sample of retting water was determined using a Brix, digital Refractometer (MT - 032ATC TAIWAN) with respect to a method used by Makebe et al. (2017). For each sample, a drop of retting water from fermentation of cassava pulp was placed onto the refractometer prism plate. A reading was generated on the reading scale of the refractometer and recorded in triplicate as degree of total soluble solids (°Brix). After each test, the prism plate was cleaned with deionized water and wiped dry with a soft tissue. Calibration to zero reading was done using a drop of deionized water after each cleaning.

Determination of cyanide concentration

The amount of cyanide reduced during fermentation was determined by measuring the residue cyanigenic potential in fermenting cassava pulps based on slightly modified procedures of Essers et al. (1993). Samples for assays were drawn from the fermentation system at an interval of 12 hours.

Sensory evaluation

Sensory attributes (colour, aroma, flavour, sourness, taste and appearance) of two samples of "mchuchume", produced by open and closed fermentation systems, respectively were evaluated by a panel of 11 male and female aged between 23 and 53 years who were semi trained before the exercise. The assessments were done using nine point hedonic scales 1 – 9 whereby 1 represented dislike extremely and 9 represented like extremely (Uyoh et al. 2009). Each sample was coded with two numbers and served to panellists at random to eliminate any bias.

Statistical analysis

Statistical Package for Social Statistics (IBM SPSS statistics 21) was used for data analysis. All data were reported as means ± standard deviation of replicate determinations. Except for sensory properties that used Students' t-test, two ways ANOVA was used to compare means of all collected data. The means were separately compared by Tukey's HSD test. All tests were at the $p \leq 0.05$ confidence interval.

Results and Discussion

pH and titratable acidity

Acidification was observed in the retting water as the pH values decreased from 6.32 to 4.25 and increase in titratable acidity from 0.02% to 0.32% (Figure 3 and Figure 4). Submerged fermentation of boiled cassava tuber was accompanied by production of organic acids mainly "lactic acid" by *Lactobacillus* bacteria, meaning that the

retting was done in acidic medium (Celah et al. 2016). Lactic acid produced resulted from microbiological metabolization of simple sugar obtained as the outcome of hydrolyse complex sugar. There were significant differences ($p = 0.000$) in pH recorded across the three fermentation conditions. Titratable acidity of open and 2 mL inoculums fermentation conditions were not statistically different ($p = 0.998$) but they all showed significant differences ($p = 0.002$) to that of 4 mL inoculums. According to Tefera et al. (2014), the rate of fermentation is well determined by considering changes in titratable acidity rather than pH changes. Therefore, the rate of fermentation was high in a system with 4 mL inoculums and lower in open and 2 mL inoculums fermentation systems (Table 1).

Table 1: Changes in pH, titratable acidity and total reducing sugar during the fermentation of boiled cassava

Fermentation period (hours)	pH			Titratable acidity			Total reducing sugars (mg/g)		
	OP[a]	CL. 2 mL[b]	CL. 4 mL[c]	OP[a]	CL. 2 mL[b]	CL. 4 mL[c]	OP[a]	CL. 2 mL[b]	CL. 4 mL[c]
Fresh cassava	6.27 ± 0.03			0.02 ± 0.00			5.29 ± 0.13		
0	6.32 ± 0.08	6.27 ± 0.08	6.25 ± 0.05	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	6.58 ± 0.14	6.58 ± 0.16	6.59 ± 0.12
12	5.75 ± 0.15	5.56 ± 0.09	5.39 ± 0.04	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	6.10 ± 0.06	6.07 ± 0.06	5.92 ± 0.04
24	5.16 ± 0.01	5.03 ± 0.02	4.79 ± 0.04	0.14 ± 0.00	0.14 ± 0.01	0.17 ± 0.01	5.53 ± 0.03	5.52 ± 0.16	5.14 ± 0.05
36	4.94 ± 0.03	4.84 ± 0.02	4.36 ± 0.03	0.16 ± 0.04	0.18 ± 0.01	0.2 ± 0.02	4.89 ± 0.03	4.83 ± 0.03	4.53 ± 0. 55
48	4.68 ± 0.01	4.49 ± 0.02	4.28 ± 0.01	0.21 ± 0.05	0.22 ± 0.02	0.25 ± 0.01	4.87 ± 0.02	4.80 ± 0.03	4.58 ± 0.03
60	4.54 ± 0.07	4.36 ± 0.01	4.26 ± 0.02	0.29 ± 0.03	0.29 ± 0.01	0.32 ± 0.01	4.74 ± 0.03	4.72 ± 0.02	4.38 ± 0.02
72	4.34 ± 0.02	4.25 ± 0.02	4.26 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	4.46 ± 0.03	4.58 ± 0.03	4.03 ± 0.06

OP[a] = Open (spontaneous) fermentation
CL. 2 mL[b] = Closed (induced) fermentation using 2 mL inoculums.
CL. 4 mL[c] = Closed (induced) fermentation using 4 mL inoculums.

This was caused by variations in microbial populations responsible for lactic acid production in each method. Acid production was highest in 4 mL inoculums fermentation and lowest in open fermentation. The implication for these variations entails differences in effectiveness among the methods involved in "mchuchume" production. The pH observed in 4 mL of inoculums fermentation at 36 hours (4.36) was comparable ($p = 0.07$) to that observed in 2 mL inoculums fermentation at 60 hours (4.36) and that of open fermentation at 72 hours (4.34) (Figure 3). This justified that boiled cassava fermentation by the action of high concentrations of inoculums results in a significant reduction in pH and increase of titratable acidity for shorter time (Kakou et al. 2016, Tefera et al. 2014).

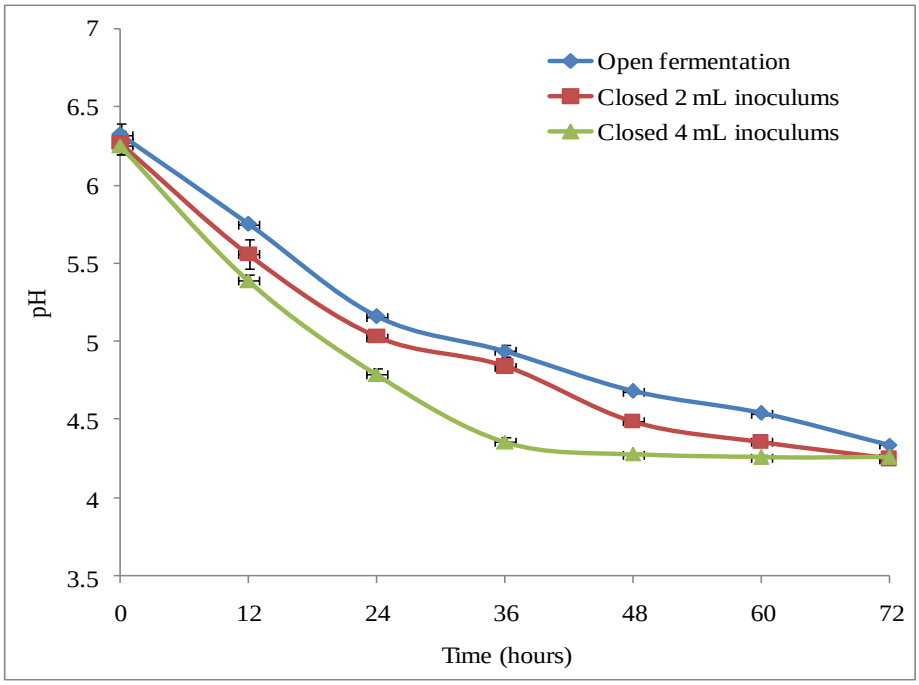


Figure 3: pH changes of retting water during the fermentation period.

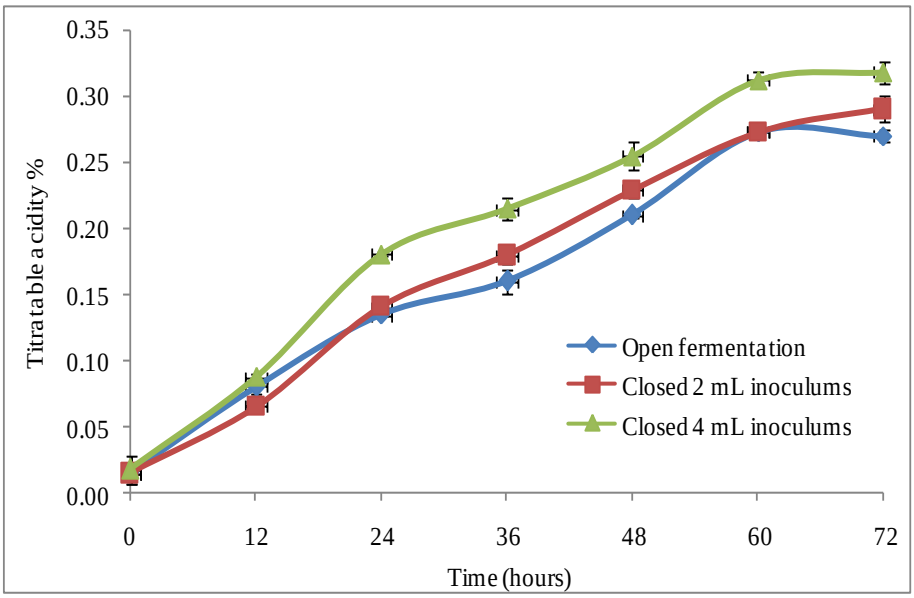


Figure 4: Change in titratable acidity in retting water during the fermentation period.

Total soluble solids (°Brix)

There were significant differences (p = 0.000) in changes of total soluble solids observed from the three fermentation conditions. The amount of total soluble solids in all fermentation conditions showed a general increasing trend along the entire period of fermentation except between 12th and 24th hours that demonstrated uninterrupted values (Figure 5). This increasing trend was due to soluble solids that underwent a movement from cassava tubers to retting water. Meanwhile, fermentative microorganisms fed on solutes dissolved in solution (Zhang et al. 2000, Tatdao et al. 2014), but the rates of consumption were smaller than that of dissolution. This pattern led to argumentation of total soluble solids (°Brix) in retting water that was from 0.4 to 3.2 °B at 0 and 72 hours, respectively for open fermentation, 0.4 to 2.9 °B at 0 and 72 hours, respectively for 2 mL inoculums closed fermentation and 0.4 to 2.5 °B at 0 and 72 hours respectively for the 4 mL inoculums closed fermentation (Figure 5).

The pattern observed between 12th and 24th hours was due to maximum consumption of soluble solutes caused by massive growth of fermentation cultures such that the rate of soluble solids consumption was higher than that of production. The findings further indicated that consumption of soluble solids during fermentation period was highly observed in system inoculated with 4 mL of inoculums and lowest in the system with open fermentation. This was due to presence of large population of fermentative microorganisms in 4 mL inoculums fermentation as opposed to that in the open fermentation. The results also depict the consumption of total soluble solids in the system inoculated with 2 mL inoculums being intermediate of the two previous methods. The °Brix consumption patterns corresponded to trends for results on growth patterns of microorganisms observed during fermentation of cassava pulp in the report of Daouda et al. (2012) and Tefera et al. (2014) as well as to the conclusion that *Lactobacillus* bacteria resist better to acid pH. Therefore, open fermentation which is occupied by mixture of species of microorganism was poorly effected as evident of its lower tendency of decreasing pH and lower °Brix consumption.

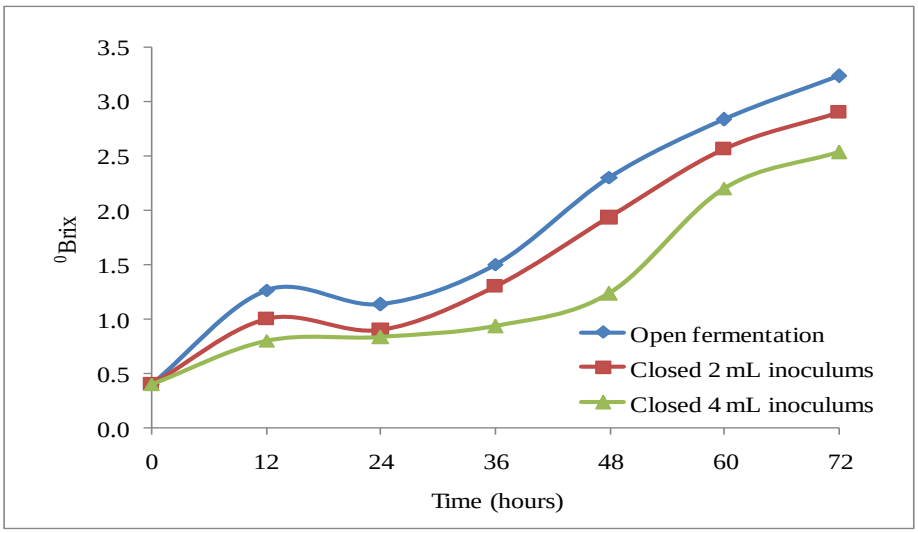


Figure 5: Change in °Brix of retting water during the fermentation period.

Total reducing sugars consumption

The change in the total reducing sugars showed a general trend of decrease within 72 hours of fermentation for all three fermentation conditions (Table 1). The decrease in total reducing sugar in open fermentation and 2 mL inoculums closed fermentation did not show significant differences ($p = 0.086$), but differed significantly ($p = 0.043$) to that of 4 mL inoculums fermentation system (Table 1). During the fermentation period, monosaccharides (glucose and fructose) coming from the breakdown of sucrose were metabolized into organic acids by different facultative anaerobic microorganisms in open fermentation set up and by only *Lactobacillus delbrueckii* in closed fermentation system (2 mL and 4 mL inoculations). According to Kimaryo et al. (2000), the decrease of total reducing sugars was due to the amylolytic activities of the microbiota which converted part of starch in boiled cassava tubers into sugars, and consequently into lactic acids. Panda et al. (2008) demonstrated that *Lactobacillus* bacteria (e.g. *L. delbrueckii,* and *L. planturum)* produce α-amylase that catalyses the hydrolysis of starch into maltose. The decrease of reducing sugars concentrations during fermentation of boiled cassava tubers could be explained by the decrease in pH and increase in titratable acidity produced during metabolization and conversion of hydrolyzed maltose into energy for the growth of fermentative micro-flora (Figure 3 and Figure 4).

Cyanide reduction during fermentation

Levels of residue cyanogenic potentials present in the fermenting boiled cassava pulps were assayed throughout the fermentation period. The degradation of cyanogenic glycosides in the cassava pulp for the open fermentation method were from 72.72 mg/kg to 5.88 mg/kg during the 72 hours of the fermentation processes, which is equivalent to 91.91% reduction. For closed fermentation using 2 mL inoculums, the corresponding changes were from 72.24 to 5.50 mg/kg (92.39% reduction), while in 4 mL inoculums fermentation, the changes were from 72.55 to 5.18 mg/kg, which is equivalent to 92.86% reduction (Figure 6). Reduction of cyanide in open fermentation and closed fermentation methods using 2 mL of inoculums were not significantly different ($p = 0.089$), but the two conditions differed significantly ($p = 0.001$) to closed fermentation using 4 mL of inoculums. Generally, during fermentation there was decreasing cyanogenic potentials, thus the processes detoxified the cyanogens in cassava pulps. The degradation of cyanogenic glycoside might have resulted from tissue disintegration brought about by water molecule dissolution and/or cyanophilic microorganisms (e.g. *Lactobacillus delbruekii* in closed fermentation) that in addition produced the enzymes linamarase, hydroxynitrilelyase and cyanide hydratase to catalyse degradation of cyanogenic glycosides into HCN. The HCN acid produced is subsequently converted into formamide and being used as both a nitrogen and carbon source (Tefera et al. 2014) by microflora or released out of fermentation system as by product.

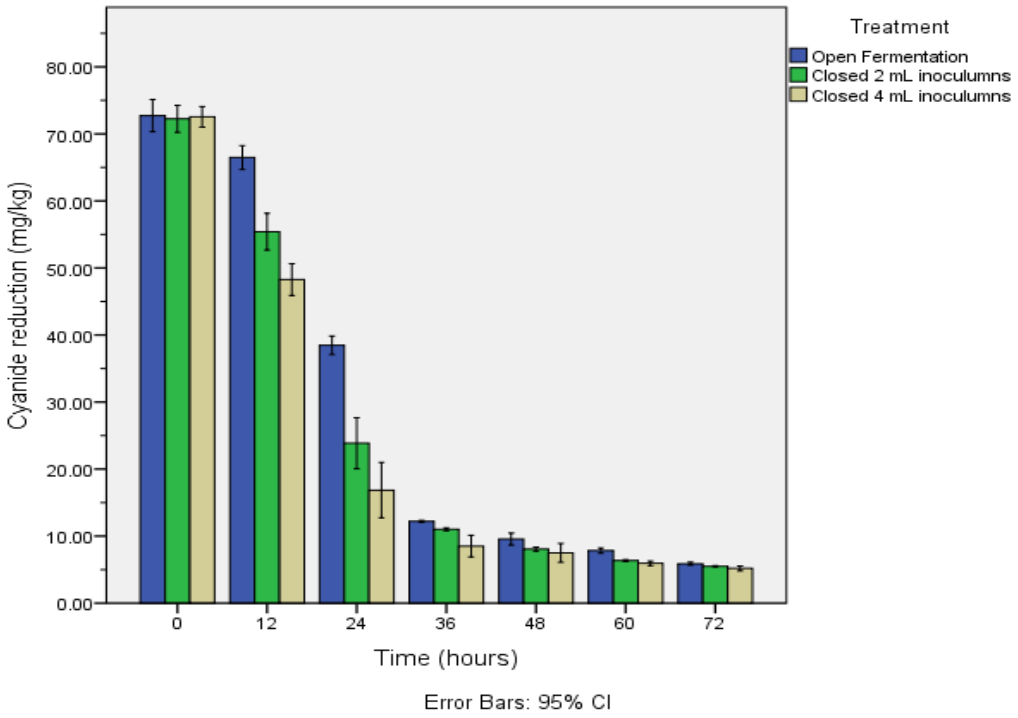


Figure 6: Cyanogenic potential content (mg/kg) in cassava pulps during fermentation process.

In all the three fermentation conditions used, cyanogenic potentials decreased with increase in time of fermentation. During the 12, 24, 36 and 48 hours of fermentation, the cyanogenic potentials of fermenting cassava pulp from the three fermentation conditions were statistically different ($p = 0.034$). This observation can be availed to the fact that the fermentation conditions have varying efficiency to detoxify cassava cyanogens. The cyanogenic potentials observed in 4 mL of inoculums fermentation at 36 hours (8.50 mg/kg), 2 mL inoculums fermentation at 60 hours (8.07 mg/kg) and that of open fermentation at 72 hours (7.84 mg/kg) were not statistically different ($p = 0.089$). This also emphasizes that closed fermentation using 4 mL of inoculums has highest rate of cyanide detoxification, while open fermentation has the lowest rate.

The values of cyanogenic potentials in the three conditions at the end of fermentation (open = 5.58 mg/kg, 2 mL inoculums = 5. 50 mg/kg and 4 mL inoculums = 5. 18 mg/kg) were not statistically different ($p = 0.080$). All these values of cyanogenic potentials recorded at 72 hours (the end of fermentation) were lower than 10 mg/kg which is the safe recommended level of cyanide in cassava based foods (FAO/WHO 1991). These findings suggest that *L. delbrueckii* has endogenous enzymes that detoxify cassava cyanide. The findings are also supported by the results of Nwokoro and Anya (2011) that showed ability of *L. delbrueckii* to release linamarase enzyme that catalyses the degradation of cyanogenic glycosides to free cyanide. Therefore, cyanide detoxification in cassava tubers through fermentation using starter cultures of *Lactobacillus delbrueckii* is possible.

Sensory analysis

The results of the sensory evaluation carried out on the fermented cassava meal "mchuchume" are given in Table 2. The general preference of the panellists for the characteristic colour, appearance, flavour, sourness and taste was more on "mchuchume" fermented with open fermentation method as evident, even though these values were not statistically different ($p > 0.05$) from those obtained with the closed fermentation method. The difference ($p < 0.05$) was only for sensory evaluation carried out on aroma for which the mean score for sample from open fermentation was 7.91 ± 0.83 (like moderately) and the mean score for sample from closed fermentation was 3.64 ± 1.75 (dislike moderately).

Table 2: Mean sensory scores of cassava "mchuchume" samples prepared by two fermentation methods

Sensory parameters	Fermentation method	
	Open	Closed
Colour	7.73 ± 1.00	7.18 ± 1.40
Aroma	7.91 ± 0.83	3.64 ± 1.75
Appearance	7.82 ± 0.75	6.73 ± 1.56
Flavour	6.64 ± 1.69	6.09 ± 1.81
Sourness	7.00 ± 1.26	6.00 ± 2.32
Taste	6.82 ± 1.54	6.55 ± 1.57

Modification of traditional processes through the use starter culture in the production of "mchuchume" by closed fermentation is acceptable as attested to by the responses of the panellists judged sensory acceptability of this food.

Implications of biochemical parameters on the cyanide detoxification

The biochemical parameters analyzed in this study provide necessary information on their roles as predicting factors toward the position of cyanogenic potential reduction and also the end of fermentation. They also provide information for comparative assessments of cyanogenic potential reduction between open and closed fermentation methods.

Correlation test indicates presence of significant correlations between cyanide reduction and either decrease in pH ($p = 0.004$), increase in titratable acidity ($p = 0.003$), increase in total dissolved solid ($p = 0.000$) or decrease in reducing sugar ($p = 0.007$) during fermentation. The values of cyanogenic potentials in cassava pulps from three fermentation conditions at 12, 24, 36 and 48 hours were significantly different. Likewise, this happened on the values of pH, titratable acidity, total dissolved solids and the reducing sugar. The values of cyanogenic potentials observed for 4 mL of inoculums fermentation at 36 hours, 2 mL inoculums fermentation at 60 hours and that of open fermentation at 72 hours were not statistically different. These observations were also noted for pH, titratable acidity, total dissolved solids and the reducing sugar. Based on the above justifications, in absence of cyanide test, the biochemical changes studied can be used to predict the position of cyanogenic potential reduction in fermenting cassava for "mchuchume" production. Both open and closed fermentation methods are capable of detoxifying cassava cyanogens. However, it is better to rely on closed fermentation method, especially through the use of *Lactobacillus delbrueckii* because it gives improved safety on a processed "mchuchume". It is a hygienic process since only selected microorganisms are allowed to carry out fermentation and it can be used to manage the rate of cyanide reduction by increasing or decreasing the inoculums levels.

Conclusion

Biochemical changes depicted are capable of supplying information on the progress of the fermentation processes in course of time. This study also has revealed that the biochemical changes can be used to predict trends in reduction of cyanide levels. The results have manifested the differences in open and closed fermentation methods. It was observed that

local technology utilizing open fermentation had least influence on the rate of cyanide detoxification compared to biotechnological closed fermentation that was more effective in the use of high concentrations of *Lactobacillus delbrueckii*. Despite of unacceptable aroma, "mchuchume" produced by closed fermentation ensure consumers profitable food safety in terms of cyanide toxicity, pathogens, dusts and other contaminations.

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