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Effect of Fermentation on the Chemical and Microbial Load of Cassava Mash

Amajor, J.U.

National Root Crops Research Institute Umudike, Abia State, Nigeria
Corresponding Author's e-mail.amajorjohn2010@yahoo.ca

Abstract
Fermentation is an economic processing method used in homes to improve the nutritional quality of plant foods. It is the most current process of cassava by- product valuation. It improves nutritional value, hygiene and sanitary qualities, food preservation, energy density and organoleptic characteristic of food. The materials for the study were procured from National Root Crops Research Institute (NRCRI), Umudike, and taken to the food laboratory for processing. The effect of fermentation on physicochemical properties and microbial population were evaluated for six days. On daily basis, changes in pH, titratable acidity, antinutrients (Alkaloid and HCN), chemical composition and microbial population were evaluated. The pH values decreased significantly ($p<0.05$) from 5.9 to 3.0 on the sixth day, while titratable acidity (TTA) increased significantly ($p<0.05$) from 0.03% to 0.34% from the first to the five day and thereafter reduced to (0.14%). The alkaloid reduced significantly from 5.12mg/100mg to 0.05mg/100mg, while the hydrocyanic acid followed the same trend ranging from 22.4mg/100mg to 3.0mg/100mg, which could be completely eliminated by roasting to flakes. The moisture content increased significantly ($p<0.05$) from 64.60% to 75.10%, while protein, ash and fiber followed the same trend and were significantly highest within the first four days of fermentation and thereafter, decreased till the end of fermentation. The total plate count increased significantly ranging from 4.5×10^2 cfu/g to 9.0×10^5 cfu/g at the end of fermentation. The result of the study revealed that fermentation for four days (96h) will provide a better attribute of nutritional and sensory quality of the final product.

Keywords: Fermentation days, cassava, physicochemical properties, anti-nutrients, microbial population

Introduction

Cassava (*Manihot esculenta crantz*) is an important root crop in Africa, South America, Asia and India, which provide energy to about 500 million people (Mroso, 2003). Cassava is cultivated in the tropical regions and may be the main valuable root crop in terms of overall output and cultivated area (Ano, 2003). It is a main food crop in Nigeria according to Ogbe *et al.* (2007). It is important for its position in food protection, alleviating poverty as well as its export market potential in Nigeria (Egesi *et al*., 2007). Cassava roots are potentially toxic due to the presence of high levels of cynaogenic glycosides, linamarin, lotaustralin anti- nutrients factor cyanide (Akinrele *et al.,* 2000). These factors interfere with digestion and absorption of nutrients (Bandna, 2012). However, cyanide occurs in two forms in cassava; cyanogenic glycosides, linamarin and lotaustrelin as reported by Ukenye and Okafor (2010). The linamarin and lotaustralin undergo a sequential enzymatic breakdown yielding toxic free cyanide. The sum of the two forms is known as cyanogenic glycoside. Cyanogenic glycoside acts as effective defense agents against generalist herbivores and humans as reported by Gleadow and Woodrow (2002). However, ingestion of varying does of cyanide from cassava products over time could cause cyanide toxicity showing symptoms of headaches, diarrhea, dizziness, goiter and may result to death (Aworh, 2008). Cassava can be processed and consumed in different forms depending on local customs and preferences which are done at home, villages and small-scale cottage industries. They are sold at rural populace who buy them for food and social ceremonies. Processing of cassava can generally reduce toxicity of cassava. Fermentation as a processing method significantly reduces cassava cyanide content in cassava fermented food to enhance flavour and aroma of fermented food and help in bio-preservation of fermented food. The cyanide reduction by fermentation is due to enzymatic activity of the microorganisms. The reduction varies according to the fermentation time. Olaoye *et al*. (2015), observed a few level of residual cyanide content of 0.24 and 126.83 mg/100g

respectively in *pupuru* and *gari.*

In processing of foods, fermentation has been identified as an economic processing method that could be used in the homes to improve the nutritional quality of plant foods (Ezeama and Amajor 2012). Fermentation of cassava by bacteria and yeast not only prefer detoxification, it also improves food quality and safety by product preservation, flavor development, and cyanide reduction (Amajor *et al.*, 2020). In Nigeria, cassava can be processed into many fermented and unfermented products in many ways. Few of the fermented products are cassava flakes (*gari*) which is produced by grating, soaking, fermenting, roasting of cassava mash; cassava flour (*Lafun*), produced by drying and milling of fermented cassava tubers and fermented cassava slurry used to produce '*fufu*'. The Bulkiness and high perishability of the harvested and stored cassava roots is a major barrier to wide utilization of the crop and there is need to diversify uses to enhance demand and increase rate of turn over or sales of the products. The length of fermentation period and the quality of the fermented cassava products varies from one processor to the other in different localities. Hence the objective is to study the effect of fermentation period on the changes in the physico-chemical composition and microbial population of the fermenting cassava mash for flakes and other products.

Materials and Methods

Cassava roots of selected cassava TMS 30572 were harvested at 12 months after planting from the farm of National Root Crops Research Institute (NRCRI), Umudike and taken to the food laboratory for processing.

Production of mash

Freshly harvested 2kg cassava roots were washed in clean water, twice and peeled manually with stainless steel kitchen knife, washed and grated into mash using stainless steel grater. The mashed cassava was divided into six (6) parts of 250g each and stored in six (6) plastic bags and allowed to ferment at ambient temperature for six (6) days and the mash were dewatered as fermentation progressed as samples were collected at 24 h intervals for analyses.

Total titratable acidity (TTA)

Total titratable acidity of the fermenting mash samples were determined by titrating 10ml of the homogenized sample against 0.1M NoaH using 2 drops of phenolphthalein indicator (0.5 in 50% alcohol). The titratable acidity was calculated as percentage lactic acid (v/v). Each milliliter of 0.1M NaoH is equivalent to 0.9008mg of tactic acid (AOAC, 1990).

pH

Ten grams of the fermenting mash samples was aseptically removed and homogenized with 100ml of sterile distilled water. The water was decanted and its pH determined in triplicates. The pH media was calibrated using buffer of pH 4.0 and 7.0. The pH was determined using kent pH meter model 7020 equipment with glass electrode.

Proximate composition of the fermenting mash

This was determined using the method of AOAC (2010). Parameters such as moisture content, crude fibre, crude protein, ash, fat and carbohydrate were determined from the samples.

Determination of anti –nutrients (hydrogen cyanide and alkaloid)

The method of Onwuka (2005) was used to determine the hydrogen cyanide content of the cassava fermenting mash samples. About 5g of each sample were dispersed into 50ml cm^3 of distilled water (1:10 w/v) and allowed overnight. The filtrate extract from the filtered samples were used for analysis. The solution developed a reddish brown colour, which its absorbance was read in US-vis spectrophotometer 752 at 490nm wave length.

Determination of Alkaloid

The Alkaloid was determined by a gravimetric method described by Onwuka (2005). Five grams (5g) of the samples were weighed into 250ml beaker and 200ml of 10ml acetic acid in ethanol added, covered and allowed to stand for 4 hours. The solution was filtered and filtrate was concentrated using water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The solution was allowed to settle and precipitates collected, and washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed which is the alkaloid.

The Alkaloid content was calculated in percentage

$$\text{Alkaloid} = \frac{\text{weight of residue x 100}}{\text{Wight of the sample}}$$

Microbiological Analysis

One gram of the mash samples were homogenized in 9ml sterile distilled water. The dilution was serially made until 10^{-7} dilution was obtained. The poor plate method as described by Ezeama (2007) was used. Samples were introduced into each sterile petri dish, then 15ml of liquefied tryptone soy agar medium at 45°c was poured into each plate and mixed by rotating the plates first in one direction and then in the opposite direction. The agar on the plates were allowed to solidify on a level surface and plates incubated aerobically at 37°c for 48h at ambient temperature (28±2°c). The colonies were observed and counted and expressed as colony forming unit per gram (cfu/g).

Statistical Analysis

Data generated were subjected to analysis of variance (ANOVA) using statistical package for social science (SPSS) 15.0 version.

Results and Discussion

The changes in pH and titratable acidity of the cassava mash are shown in Figure 1. The pH of the fermenting cassava mash decreased from 5.9 to 3.0 during six days

of fermentation. There was no significant difference ($p>0.0$) in day one and two, but between day 2 to day 4 showed significant difference ($p<0.05$) and gradually decreased to the sixth day. Similar pH values have been reported by Oduah *et al.* (2015). The range of pH obtained especially towards the end of fermentation was enough to inhibit the growth of undesirable microorganisms. All the samples fermented had a reduction in their pH values. The decrease in pH is attributed to the production of lactic acid and other organic acids in the fermenting mash, which is the characteristic of carbohydrate food fermentation, due to the amylolytic activity of the fermenting microorganisms. As the fermentation of cassava mash progressed, there was a significant decrease in pH and an increase in titratable acidity of the fermented mash ranging from 0.03% to 0.34% and decreased to 0.14% by the end fermentation (Figure1). Titrable acidity differed significantly ($p<0.05$) in the fermenting mash throughout the fermentation days. There was a rapid increase followed by a decrease at the end of fermentation. Similar decrease was obtained by Oduah *et al.* (2015) from the 4th day to the end of the fermentation. The increase in titratable acidity in relation to fall in pH has been attributed to the production of lactic acid and organic acid in the cassava mash produced by the amylolytic activity of the lactic acid bacteria, and yeast which are the dominant microorganisms found towards the ending of fermentation (Oyewole and Odunfa, 1988). According to the authors, lactic acid bacteria have been implicated throughout the period of fermentation of cassava to fermented products. However, the increase in lactic acid followed the same trend as reported for some fermented foods (Dziedzoaze *et al*., 1996).

The results of the alkaloid and hydrocyanic acid of fermenting cassava mash are shown in Figure 2. On the initial day, the alkaloid and HCN content were 5.12±0.05mg/100g and 22.4±0.10mg/100g respectively. The alkaloid content showed a pronounced significant difference from the first third days of fermentation and followed with a slight decrease on the 4th day till the end of fermentation 0.05^{f}±0.13. The HCN also followed the same trend of pronounced significant decrease in HCN content which reduced to a final value of 3.00^{c}±0.16 at the end of fermentation. The anti-nutrients of the fermenting cassava mash showed decrease in hydrogen cyanide and alkaloid during the fermentation. The low alkaloid content could be attributed to fermentation. The level of hydrocyanic acid decrease observed at the end of fermentation could be credited to the breakdown of the cyanogenic glucosides to cyanohydrins which become hydrolysed at a lower pH to form HCN during fermentation as reported by (Tetchi *et al*., 2012). The high pronounced reduction in HCN observed during this study within the first four days of fermentation, shows that large part of the process of hydrolysis of cyanide compounds to HCN took place within this period. The grating process and microbial activities attributed to the fast reduction of the cyanogenic compound and alkaloid content. The reduction of cyanide level of the mash confirm reports that processing of cassava roots helped to reduce cyanide level in the product and enhance palatability of the cassava products as reported by Nwabueze and Anoruoh (2011). Endogenous cyanide compounds in cassava roots have been reported to be eliminated by fermentation after 48h as stated by Titchi *et al*. (2012). The product of the HCN observed in the mash fell within a safe level of 10mg HCN/kg as stated by FAO/WHO (2013).

Table 1 presents the changes in the chemical composition of cassava mash during the days of fermentation. The results show the moisture content, fat, protein and crude fibre increased within the first four days of fermentation. However, as the cassava mash absorbs more moisture, the chemical components were observed to increase in moisture within the first four days. The result of the moisture content during 6th day of fermentation is shown in Table 1. The moisture content increased significantly ($p<0.05$) as the fermentation progressed, with day 5 and 6 having the highest moisture content of 75.00% and 75.10% respectively, which did not differ from each other. The increase in the percentage moisture content of the mash can be attributed to the degrading activity of the different micro-flora during fermentation (Egbebi *et al*., 2011). The protein content increased from 1.20% to 1.75% on the first four days which declined significantly towards the 6th day of fermentation. There was a slight significant ($p<0.05$) increase in fat content, with an initial value of 0.25±0.01% to 0.30±0.02% within the first four days and a gradual decrease to end of the fermentation. The lipid content of the study was low. This justifies the report that fat content of cassava roots is so low that it is seldom of any nutritional significance (Nwagbara and Iwe, 2008). The increase could be attributed to fermenting micro flora not utilizing fat from the food as energy source as reported by Reebe *et al*., (2000). Crude fibre content had the initial value of 0.7±0.01% and was observed to increase significantly to 1.40±0.01% within the first four days and later reduced to 0.60±0.02% by the end of fermentation. However, crude fibre does not contribute nutrients to the body, it adds bulk to food thus, facilitating bowel movement (peristalsis) and preventing many gastroinstesinal diseases in man and lower faecal pH (Amajor *et al*., 2014). Ash content also increased significantly ($p<0.05$) from 0.60± 0.01% to 0.80±0.01% within first four days of fermentation and thereafter reduced gradually to the end of fermentation. Carbohydrate content decreased significantly as fermentation progressed till the end of fermentation. However, cassava root is made up of carbohydrate as the main nutritional make up which contains high amount of starch and serve as energy food (Purseglove 1991).

The microbial population during fermentation of cassava mash evaluated using the total plate counts are shown in Figure 3. There was significant ($p<0.05$) increase in the population of micro-organisms from 4.5 x10^{2}cfu/ml to 9.0 x10^{5}cfu/g during the first four days of fermentation. This was followed by a slight decrease in

population to 4.0 x10^{2}cfu/g at the end of fermentation period. The counts obtained in the study were higher than the counts obtained by Oduah *et al*. (2015). The pattern of the microbial population showed the microbial growth phases. The first two days count represent the lag phase of the microbial growth followed by the log phase represented by the third and the fourth days of fermentation with a rapid increase in microbial count. The 5th day showed the stationary phase which cloud led to the death phase at the end of fermentation. However, the increase in the microbial population as observed in the first four days of fermentation may be due to ready availability of adequate nutrient for microbial growth and proliferation. The decrease in population may probably be due to depletion of available nutrient as observed in the carbohydrate content of the cassava mash which reduced with fermentation days. This indicates that carbohydrates are broken down to monosaccharaides like glucose and fructose which are metabolized into lactic acid and organic acids by lactic acid bacteria (LAB) and yeast (Otunola and Balogun 2002), and production of toxic products in the fermentation media. As the carbohydrate and nutrient level reduced after four days, the rate of microbial growth reduced which may also be attributed to increased competition for nutrient and increased level of acidity as a result of low pH in the fermenting mash. This also resulted to accumulation of waste substances which could inhibit growth of other bacteria and death of organisms. Lactic acid bacteria (LAB) are one of the species that ferment food. They are generally regarded as safe (GRAS). They play an important function in the majority of food fermentation and preservation (Elyas *et al*., 2015). The major food pathogens are killed during fermentation due to acid and bacteriocin production by lactic acid bacteria (Nout, 1994). Although, fermented food condiments has constituted a significant proportion of the diet of many people, Nigeria has exhibited an ambivalent attitude in terms of consumer's tastes and preferences for such foods (Achi, 1994).

Conclusion

The study showed that fermentation of cassava mash occurred in the plastic bag. The result revealed that a wide diversity of microbial population was involved in the fermentation process of the cassava mash for cassava flakes. The nutritional and physico-chemical properties of cassava mash for flakes revealed that all the chemical changes were achieved within three to four days of fermentation, indicating that fermentation of this variety can be done within 72h to 96h of fermentation. Therefore, it is recommended that fermentation of cassava mash for flakes and other products be done for three to four days, for better attributes of nutritional and sensory quality of the final product. During processing, the maintenance of proper hygiene standards in the environment should be of utmost importance. Fermentation beyond four days will produce a low quality product. Also recommended is the use of starter culture for cassava mash fermentation.

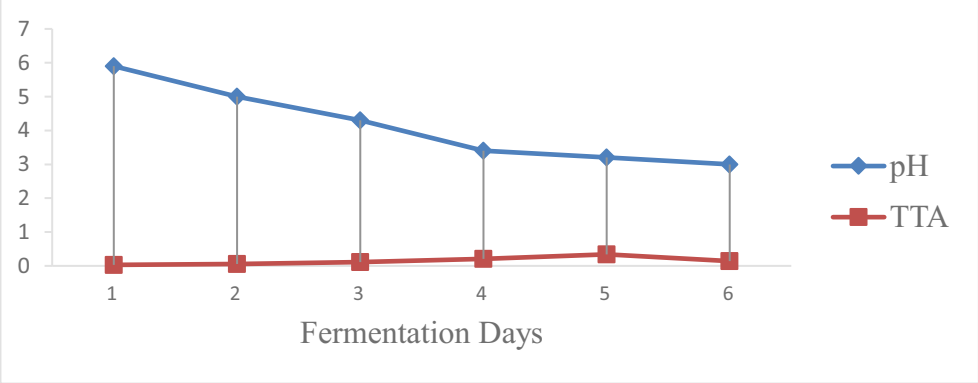

Figure 1: Changes in pH and Titratable acidity (TTA) of the cassava mash.

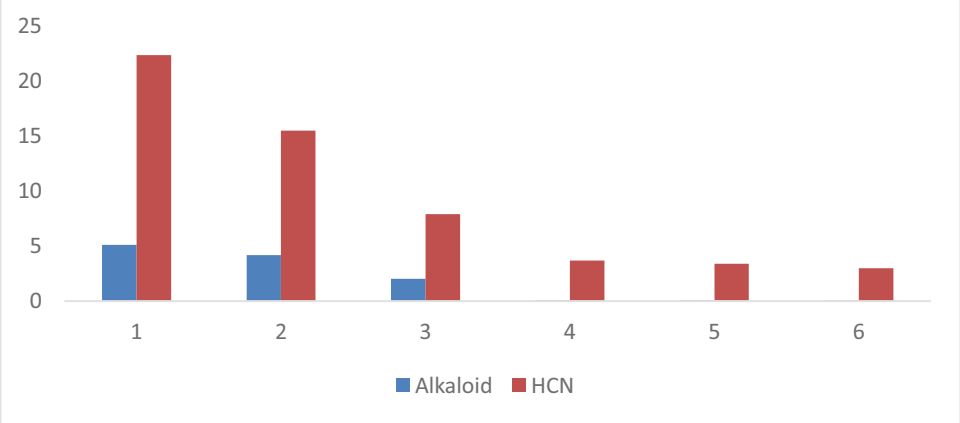

Figure 2: Changes in alkaloid and hydrocyanic acid (HCN) of cassava mash

Table 1: Chemical changes during fermentation of cassava mash

Days	1	2	3	4	5	6
Moisture	$64.60^{d}\pm0.01$	$70.60^{c}\pm0.15$	$74.00^{b}\pm0.06$	$74.15^{b}\pm0.12$	$75.01^{a}\pm0.01$	$75.03^{a}\pm0.02$
Fat	$0.25^{b}\pm0.02$	$0.30^{a}\pm0.01$	$0.30^{a}\pm0.02$	$0.30^{a}\pm0.02$	0.20b±0.01	$0.10^{c}\pm0.01$
Protein	$1.20^{e}\pm0.02$	$1.30^{d}\pm0.05$	$1.60^{b}\pm0.02$	$1.75^{a}\pm0.02$	$1.40^{c}\pm0.01$	$1.10^{f}\pm0.01$
Ash	$0.60^{f}\pm0.01$	$0.74^{c}\pm0.01$	$0.79^{b}\pm0.01$	$0.80^{a}\pm0.01$	$0.72^{d}\pm0.01$	$0.62^{e}\pm0.01$
Crude fiber	$0.7^{d}\pm0.01$	$1.30^{b}\pm0.01$	$1.40^{a}\pm0.02$	$1.40^{a}\pm0.01$	$0.90^{c}\pm0.01$	$0.06^{e}\pm0.02$
Carbohydrate	$41.06^{a}\pm0.02$	$34.30^{b}\pm0.01$	$31.10^{c}\pm0.02$	$31.02^{c}\pm0.01$	$29.97^{d}\pm0.01$	$28.40^{f}\pm0.01$

values are means and standard deviation triplicate determinations

Means in the same row with different superscripts differ significantly (p <0.05)

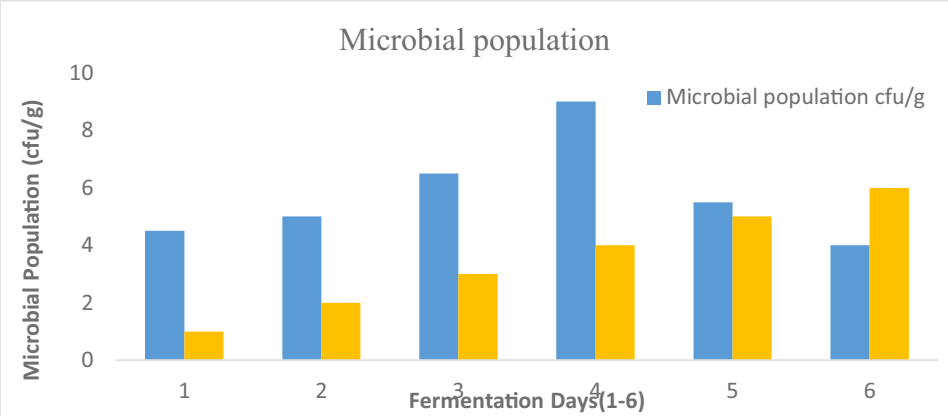

Figure 3: Microbial population during fermentation of cassava mash.

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