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Article

Effects Of Fermentation On The Quality And Composition Of Cassava Mash(*Gari*).

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Abstract: The changes that took place during the fermentation of cassava mash over a period of ten days at ambient temperature (28 – 32°C) were investigated. On daily basis, changes in pH, titratable acidity, hydrocyanic acid (HCN), ascorbic acid as well as chemical composition were determined. pH decreased significantly ($p<0.05$) from 6.4 to 3.2 while titratable acidity initially increased significantly ($p<0.05$) from 0.2% to 0.8% from the first to the fourth day and thereafter reduced to 0.5%. HCN reduced significantly from 6.5 mg/100g to 3.2 mg/100g, while ascorbic acid increased significantly from 24 mg/100g to 28 mg/100g. Moisture content increased significantly ($p<0.05$) from 65.94% to 77.1% while protein, crude fibre and ash increased significantly ($p<0.05\%$) within the first four days and thereafter decreased all through. Total plate count increased significantly from 5.2 x 10^3cfu /g to 8.4 x 10^5cfu/g within four days and thereafter reduced to 3.0 x 10^2cfu/g. Results obtained showed that fermentation for a period of four days will be adequate for optimum development of nutrients and organoleptic quality of cassava mash for *gari* processing.

Keywords: cassava, fermentation period, hydrocyanic acid, physicochemical properties.

1. Introduction

Cassava (*Manihot esculeuta Cranz*) ranks fourth on the list of the main food crops in developing regions after rice, wheat and maize (Bokanga, 1999). Cassava is an important root crop in Africa, Asia and Latin America (Achi and Akomas, 2006), providing nutrition and energy for over 800 million people (Bokanga and Oto, 1994).

Cassava roots are potentially toxic due to the presence of high levels of cyanogenic glycosides linamarin, lotaustralin and anti-nutritional factor cyanide (Akinrele *et al*, 2000). Cassava contains 10 – 500 mg/Kg of roots, the value exceeding 1000 mg/Kg in some varieties (Aworh, 2008). Cyanogenesis is initiated in cassava when the plant cell is damaged. Rupturing of the vacuoles releases linamarin which is hydrolysed by linamarinase, a cell wall-associated glycosidase (Akinpelu *et al.*, 2011). The linamarin content of cassava have been reported to be more than double during drought, leading to the outbreak of the disease called Konzo (Cardoso et al., 2005). More than 100 cases of the disease were reported during the drought of 2005 in Nampula and Zambezia provinces of Mozambique (Cliff *et al.*, 2011). Continuous ingestion of varying doses of cyanide from cassava products over time results in acute cyanide toxicity with symptoms of dizziness, headaches, diarrhoea, and sometimes death. Other symptoms include increased prevalence of goitre and cretinism in iodine deficient areas (Bokanga, 1999, Aworh, 2008). Children are more susceptible to cyanide poisoning since its lethal dose is proportional to body weight.

Many processing methods have been developed empirically for reducing cassava toxicity in most cassava producing populations (Njoku and Obi, 2010). These processing techniques consists of a combination of procedures such as peeling, boiling, steaming, pounding, slicing, grating, roasting, soaking, pressing and fermentation (Hahn and Kersey, 1985). Fermentation is one of the oldest and most important traditional food processing and preservation techniques (Aworh, 2008). Natural fermentation of plant materials is widely used in under-developed countries to transform and preserve vegetables because of its low technology and energy requirements and the unique organoleptic properties of the final products (Daeschel *et al.*, 1987). During fermentation, endogenous linamarase present in cassava roots hydrolyses linamarin and lotaustralin releasing hydrocyanide (HCN). Crushing of the tubers exposes the cyanogens located in the cell vacuoles to the enzyme which is located on the outer cell membrane, facilitating their hydrolysis (Aworh, 2008). It has been reported that most processes to which cassava is subjected in preparation of food products lead to reduction in protein, vitamin and mineral content (Lancaster *et al*, 1982). Bokanga (1999) reported that protein is reduced by 50 – 87% in the preparation of food stuffs from cassava roots in Cameroon, while vitamin C, niacin and thiamine undergo considerable losses (Favier *et al.*, 1971). However, Riboflavin levels has been

found to be higher in fermented cassava products than in fresh cassava roots, thereby suggesting that fermentation leads to synthesis of this vitamin (Watson,1976).

In Nigeria, cassava has been processed into many fermented and unfermented products in many ways. Some of the fermented products include cassava flour (*Lafun)* produced by drying and milling of fermented cassava tubers, cassava flakes (*Gari)* produced by grating, soaking, fermenting, roasting of cassava mash. Other products include fermented cassava slurry used to produce "*Fufu*".

The quality of these fermented cassava products varies from one processor to the other and the length of fermentation employed. There is therefore need to document the effects of length of fermentation on these products. This work is focused on monitoring the changes in the composition of fermenting cassava mash intended for production of roasted cassava granules (*gari*) in order to determine the optimum fermentation period at which the maximum nutritive composition is achieved and to determine the fermentation time at which the hydrocyanide content is at a safe level.

2. Materials and Methods

The cassava used for this study was those of 12 months old sweet (low cyanide) variety locally called *Odigbo* cassava. They were purchased from a farm near Ikosi Ketu in Lagos, Nigeria and transported immediately to the laboratory for sample preparation. About 5kg tubers were washed repeatedly in water peeled manually using pre sterilized stainless steel knives and grated into mash using stainless steel graters.

2.1. Sample Preparations

The mashed cassava was divided into ten parts of 250g each and stored in 10 plastic bags and allowed to ferment under ambient laboratory conditions (28-32°C) for ten days. As fermentation progressed, the mash was dewatered and samples were analysed at 24 hour intervals.

2.2. Chemical Analyses

The pH of the fermenting cassava was determined using the method of Oyewole, (1990) while titratable acidity was determined using the method described by Amoa-Awua *et al.*, (1996). Crude fibre was determined using the trichloroacetic acid method of Joslyn (1970). The moisture content, crude protein, fat and ash contents were determined by the methods of AOAC (1990).

Carbohydrate was determined by difference using the formula,

Carbohydrate content (%) =100- (M+P+F+C+A)

Where M=moisture content, F=fat content. P=protein content, C=crude fibre and A=ash

Determination of Hydrocyanic Acid (HCN)

This was determined by the method described by Oyewole, (1990).

2.3. Microbiological Analysis

The microbial growth during the fermentation of cassava mash was determined as total plate count by the method described by Kirmayo *et al.*, (2000).

2.4. Statistical Analysis

All analysis were done in triplicates and data obtained were subjected to analysis of variance with the aid of statistical software SPSS, version 15.0 and means separated using Tukey's test. Microsoft Works spreadsheet was used to plot graphs for presentation of figures.

3. Results and Discussion

3.1. Results

3.1.1. Changes in pH and titratable acidity of Cassava Mash.

The changes in the pH and titratable acidity of the cassava mash fermented over a period of ten (10) days are shown in figure 1. The initial pH and titratable acidity were 6.40 ± 0.20 and 0.20 ± 0.02% respectively. Within the first two days of fermentation there was no significant difference ($p > 0.05$) in the pH but between the second and fourth day. A rapid decrease was observed which differed significantly ($p<0.05$) from earlier observation followed by a steady decline throughout the remaining days of fermentation. Titratable acidity differed significantly ($p<0.05$) in the fermenting mash throughout the period of fermentation. There was an initial rapid increase within four days of fermentation, followed by a rapid decrease till the end of fermentation.

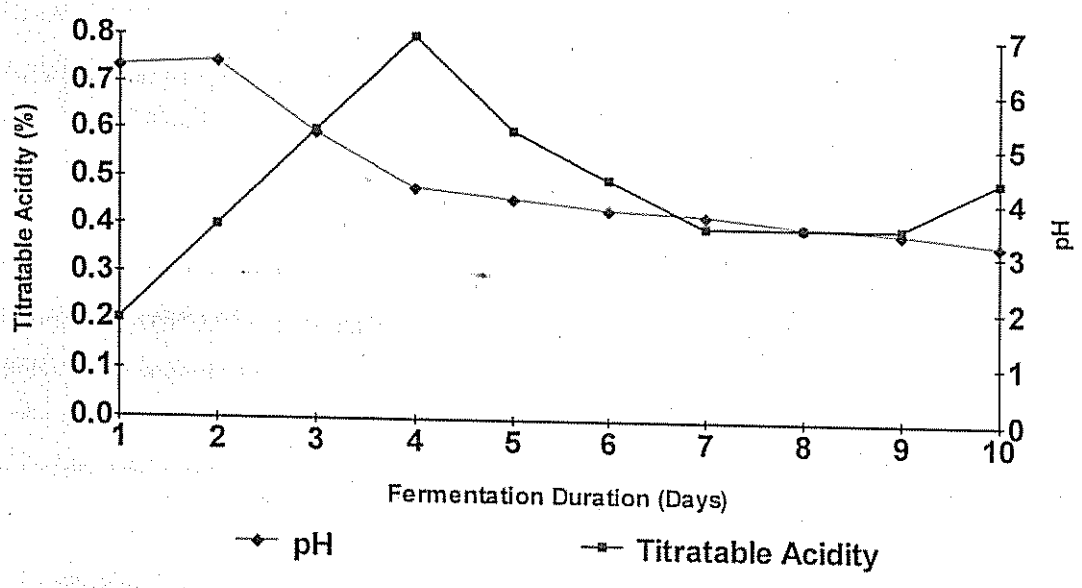

Figure 1: Changes in pH and titratable acidity of cassava mash

3.1.2. Changes in hydrocyanic acid and ascorbic acid

Results of changes in Hydrocyanic acid (HCN) and Ascorbic acid contents of fermenting cassava mash are shown in figure 2. On the first day of fermentation, the HCN and ascorbic acid were 6.5±0.2 mg/100g and 24±0.4 mg/100g respectively. During the first four days of fermentation, there was a highly pronounced significant decrease in HCN content which reduced to a final value of 3.2±0.0 mg/100g at the end of fermentation. However for ascorbic acid there was a significant difference ($p<0.05$) observed from an initial value of 24±0.4 mg/100g. There was an increase within the first three days of fermentation which was followed by a slight decrease on day five and an increase on day six which remained constant till the end of fermentation at which final value of 28± 0.00 mg/100g was observed.

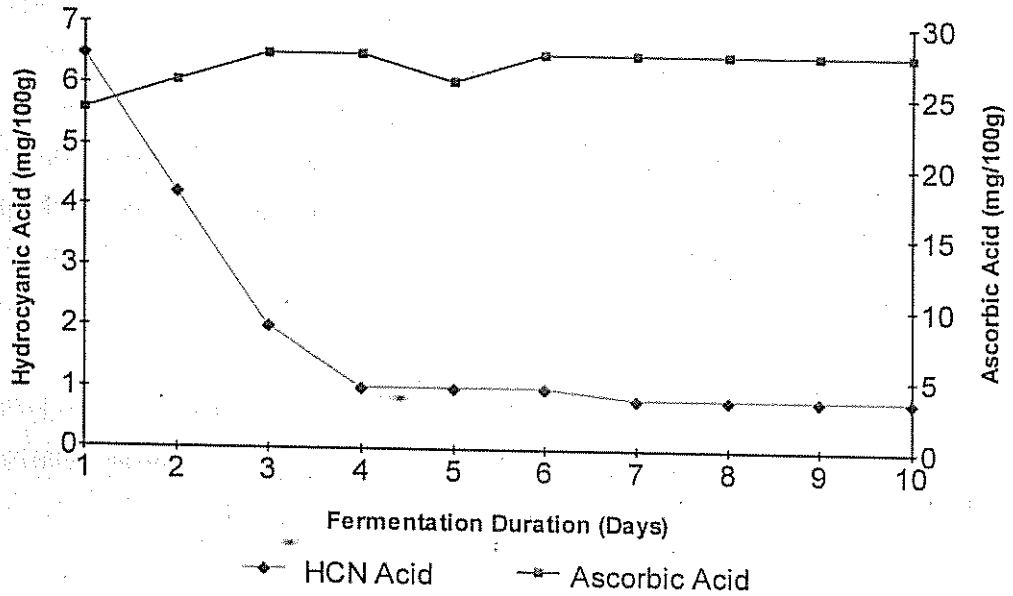

Figure 2: Changes in hydrocyanic acid and ascorbic acid of cassava mash

3.1.3. Changes in chemical composition

The changes in chemical composition of cassava mash fermented over a period of ten days are shown in table 1. Moisture content increased significantly ($p<0.05$) throughout the period of fermentation ranging from 65.94±0.12% to 77.10±0.10%.

Values are means and standard deviations of triplicate determinations. Means in the same row with different superscripts differ significantly ($p<0.05$). There was a slight but significant ($p<0.05$) increase in protein level from 1.20±0.01 day one to 1.60±0.01 on day five after which it declined significantly towards the end of fermentation period. There was a slight significant ($p<0.05$) increase in fat levels within the first four days followed by a gradual decrease till the end of fermentation.

Table 1: Chemical Changes during fermentation of cassava mash

Days	1	2	3	4	5	6	7	8	9	10
Moisture (%)	65.94 ±0.01^{e}	71.70 ±0.36^{d}	75.10 ±0.08^{c}	75.17 ±0.12^{c}	76.07 ±0.09^{b}	76.13 ±0.09^{b}	76.00 ±0.08^{b}	77.03 ±0.05^{a}	77.07 ±0..09^{a}	77.10 ±0.08^{a}
Protein (%)	1.20 ±0.01^{d}	1.30 ±0.01^{c}	1.50 ±0.01^{b}	1.60 ±0.01^{a}	1.20 ±0.01^{d}	1.10 ±0.01^{e}	1.10 ±0.01^{e}	0.90 ±0.02^{f}	0.90 ±0.01^{f}	0.90 ±0.01^{f}
Fat (%)	0.20 ±0.01^{b}	0.30 ±0.01^{a}	0.30 ±0.01^{a}	0.30 ±0.01^{a}	0.20 ±0.01^{b}	0.20 ±0.01^{b}	0.20 ±0.05^{b}	0.10 ±0.01^{c}	0.10 ±0.01^{c}	0.10 ±0.00^{c}
Crude Fibre (%)	0.9 ±0.01^{d}	1.20 ±0.01^{c}	1.30 ±0.01^{b}	1.40 ±0.01^{a}	0.90 ±0.01^{d}	0.70 ±0.01^{e}	0.50 ±0.01^{f}	0.30 ±0.01^{g}	0.20 ±0.01^{h}	0.20 ±0.00^{h}
Ash (%)	0.70 ±0.01^{e}	0.84 ±0.01^{c}	0.89 ±0.01^{b}	0.90 ±0.01^{a}	0.72 ±0.01^{d}	0.68 ±0.01^{f}	0.66 ±0.01^{g}	0.60 ±0.01^{h}	0.60 ±0.01^{h}	0.60 ±0.01^{h}
Carbohydrate (%)	31.05 ±0.05^{a}	24.36 ±0.02^{b}	21.01 ±0.02^{g}	21.10 ±0.02^{g}	21.19 ±0.01^{c}	20.98 ±0.01^{g}	21.38 ±0.13^{d}	21.54 ±0.01^{c}	21.10 ±0.01^{f}	21.10 ±0.01^{f}

For crude fibre, an initial value of 0.90±0.01% was observed which increased significantly ($p<0.05$) to 1.4±0.01% within the first four days and thereafter reduces steadily to 0.20±0.0% by the end of fermentation. Ash content also increased significantly ($p<0.05$) from 0.70±0.01% within the first four days to 0.90±0.01% and thereafter reduced gradually to the end of fermentation. Carbohydrate content decreased significantly throughout the fermentation period.

3.1.4. Microbial growth

The growth of micro organisms during the fermentation of cassava mash measured as total plate count is shown in figure 3. Within the first four days of fermentation, there was significant increase ($p < 0.05$) in population of micro organism from 5.2 x 10^3cfu/ml to 8.4x10^5cfu/g. There was a slight reduction to 2.8x 10^4 cfu/g on the seventh day and a gradual decrease to 3.0 x 10^2 cfu/g at the end of the fermentation period. The first two days represents the lag phase of the microbial growth while the third and fourth day appears to represent the log phase as evidenced by the rapid increase of the microbial population. Day five to seven represent the stationary phase while the eighth to tenth represents the death phase.

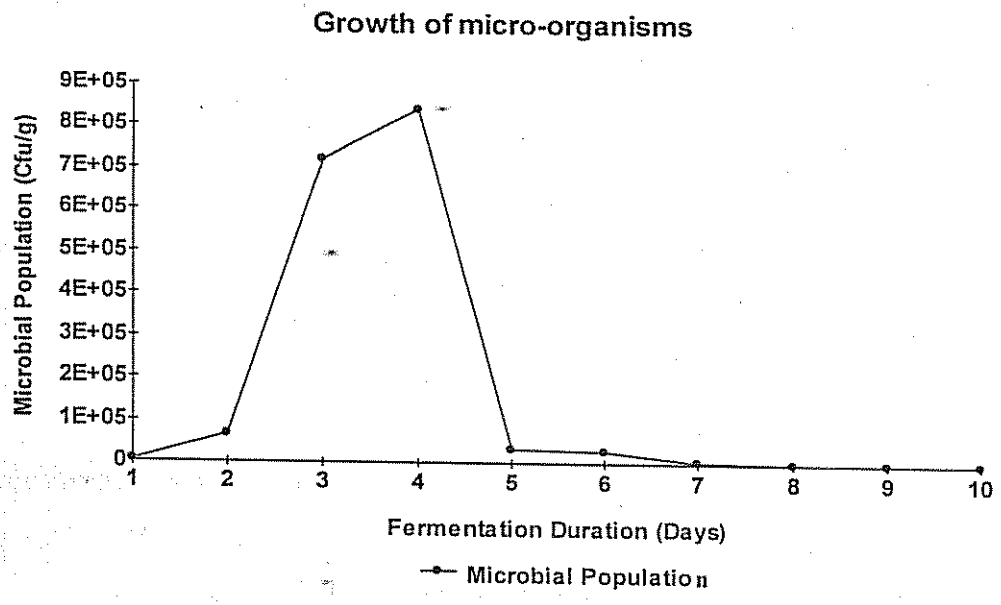

Figure 3: Microbial growth during fermentation of cassava mash

3.2. Discussion

During the fermentation of cassava mash, there was significant decrease in pH and increase in titratable acidity. Fermentation has been reported to lower pH values and lead to increase in titratable acidity (Ogunsua, 1980, Oyewole, 1990, Ocloo and Ayernor, 2008, Abdjo *et al.* 2010). The level of acidification increased with increasing period of fermentation (Oyewole and Ogundele, 2001). The lowering of pH and the concomitant increase in titratable acidity has been attributed to the accumulation of organic acids such as lactic and acetic acids in the fermenting cassava produced by activities of bacteria and yeasts which contribute the dominant specific microflora (Oyewole and Odunfa, 1988). pH is a critical factor in developing flavour and aroma characteristic of foods (Tetchi *et al*, 2012). Lactic acid bacteria have been reported to be implicated throughout the duration of fermentation of cassava into fermented products, their activity imparting the typical sour fermented taste to these products (Oyewole and Odunfa, 1988).

The decrease in the level of hydrocyanic acid observed during cassava mash fermentation has been attributed to the degradation of cyanogenic glucosides to cyanohydrins which at lower pH becomes hydrolysed to form HCN (Tetchi *et al.*, 2012). The highly pronounced reduction in HCN observed during this study within the first four days of fermentation shows that a large part of the process for hydrolysis of cyanide compounds to HCN took place within this period. Although a high proportion of the microorganisms present have the ability to hydrolyse linamarin, 95% of the initial linamarin hydrolysis have been reported to occur within three hours of grating (Westby and Choo,

1994), demonstrating that grating is the key step in cyanogenic glycoside hydrolysis bringing endogenous linamarinase in contact with linamarin. This is quite unlike cyanogenic hydrolysis in soaked cassava roots where efficient cyanogenic reduction only occurs when microbial growth takes place as the root softens. Ogunsua (1980) in a study to determine whether any cyanogenic glycoside survived processing of fermented cassava mash into *"gari"*, and "*lafun*" through thin layer chromatography of the cyanogenic glycoside observed that *"lafun"* prepared from fermented cubed cassava tubers subsequently dried slowly and ground into flour had a low cyanide content while *"gari"* a product obtained from roasting of cassava mash fermented for over four days were totally free of cyanide. Fermentation has been reported to cause elimination of endogenous cyanide compounds in cassava roots after 48 hours (Tetchi *et al.*, 2012). Shortening the fermentation period of cassava mash to about 24 hours has been implied to constitute a health hazard to consumers of *gari* (Ihedioha and Chineme, 2003). Various health disorders are associated with consumption of cassava containing residual cyanogens (Delange et al., 1994). Ingested cyanide is converted to thiocyanate, a reaction catalyzed by the enzyme rhodonase which uses up part of the pool of sulphur containing essential amino acids, methionine and cysteine (Aletor, 1993, Osuntokun, 1994, Akinpelu et al., 2011). Reduction of these sulphur containing amino acids leads to limitations in protein synthesis, resulting in stunted growth in children as evidenced by the study of Cardoso et al., (2005) in the Democratic Republic of Congo.

Cassava had been reported to contain ascorbic acid of about 120mg – 150mg/100g of fresh cassava on wet basis and this value reduced to about 25 – 30 % of the original value in 5 days and subsequently dropped to insignificant values after 8 days (Ogunsua and Adedeji, 1979). In the present study, a slight increase was observed for ascorbic acid in the fermenting mash. In their study on fermentation of cassava, Ogunsua and Adediji (1979) observed that up to about 50% of the original dehydro-ascorbic acid (DAA) was present at the end of fermentation and concluded that adequate level of ascorbic acid may be available in the fermented cassava mash after processing because a large amount of dehydro-ascorbic acid (DAA) was retained and DAA retains about 70% of vitamin C activity.

A positive correlation was observed with the changes in chemical composition of the cassava mash during fermentation. Protein, fat, crude fibre and ash content all increased within the first four days of fermentation as moisture content increased. It appeared as though as the cassava mash absorbs more moisture, the increase in chemical components become more pronounced. A concomitant increase was also observed in the microbial population of the fermenting mash within the first four days. Fibre content has been observed to increase within the first 48 hours of fermentation (Morthy *et al.*, 1993) while an increase in protein and fat content was reported in fermented cassava flour and *gari* (Oboh and Akindahunsi,2003,).

The microbial growth observed in the first four days coincides with the period in the fermentation of cassava mash in which the nutrient composition was highest. Apart from this increase in nutrient composition, carbohydrate content of the cassava mash reduced with fermentation period. During fermentation, carbohydrates to a large extent, sucrose, are broken down to monosaccharide such as glucose and fructose which are then metabolized into organic acids by facultative anaerobic micro organisms such as lactic bacteria and yeasts (Matthew *et al*., 1995, Brauman *et al*., 1996). As the level of nutrients and carbohydrates drop after four days, the rate of microbial growth reduced possibly due to the combination of increasing competition for nutrients and increasing level of acidity and low pH in the mash, leading to unfavourable environment for susceptible micro organisms and increasing metabolic wastes which could inhibit growth of other bacteria finally leading to the death of the biomass. Also the antimicrobial property of lactic acid bacteria leading to production of bacteriocins and some other antimicrobial products have been shown to have inhibitory effect on coliform growth (Abdjo *et al.*, 2010).

4. Conclusions

From this study, it is deduced that most of the biochemical changes required for the fermentation of cassava mash to produce "*gari*", a roasted cassava mash of acceptable nutritional and physicochemical properties were achieved within four to five days of fermentation. The removal of cyanogenic glycosides through hydrolysis to hydrocyanic acid and the subsequent degradation and reduction of this to a low level was much pronounced within four to five days of fermentation, after which the hydrocyanic acid can be completely eliminated during the subsequent roasting to "*gari*".

We therefore recommend that fermentation of cassava mash for *gari* processing should be carried out for four to five days because all the biochemical processes required for development of necessary organoleptic and nutritional qualities desired for the product were optimally achieved within this period. Fermentation beyond this number of days will produce a product of low nutritional and unacceptable quality.

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