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Effects of submerged and anaerobic fermentations on cassava flour (Lafun)

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Cassava tubers for processing into cassava flour, Lafun a Nigerian locally fermented product was subjected to two different types of fermentations: submerged and anaerobic fermentation for 72 h. Physicochemical changes that occurred during fermentation and their influence on the functional, rheological and sensory properties of the resulting flour were investigated. There was no significant difference in rate of decrease of pH and hydrocyanide under both fermentation conditions but titratable acidity differed significantly (p<0.05). Crude fibre, crude protein, ash, swelling index and final viscosity were significantly higher (p<0.05) in flour from submerged fermentation. Lafun from submerged fermentation had greater sensory quality and higher consumer preference than that of anaerobic fermentation.

Key words: Cassava fermentation, physicochemical, functional, pasting properties.

INTRODUCTION

Cassava, manihot esculenta crantz, is a food crop of great importance for the nutrition of over 500 million people in the tropic (Cock, 1982). Its starchy tuberous roots are a valuable source of cheap calories (Cock, 1985; Bokanga, 1999). Additionally in many parts of Africa its leaves and tender shoots are consumed as highly prized vegetables (Bokanga, 1995). Cassava is a highly perishable crop and is usually processed as soon as it is harvested, otherwise spoilage sets in within 48 to 72 h. Cassava has been utilized in several ways and its mode of utilization in Africa shows that nearly three out of four cassava based food are fermented products (Westby, 1994). Natural fermentation of plant materials is widely used in underdeveloped countries to transform and preserve food crops because of its low technology and energy require-Ment and the unique organoleptic properties of the final products (Daeschel et al., 1987; Aro, 2008).

Fermentation has been viewed as a dynamic process during which several catabolic and anabolic reactions proceeds simultaneously depending on several factors including substrate, micro flora and environmental factors (Eleazu et al., 2011). During the fermentation of cassava the tissues are softened and disintegration of the tissues by microorganisms results in contact of cyanogenic glucosides, linamarin and lotaustralin with the enzyme linamarase located in the cell wall (Mkpong et al., 1990) leading to the formation of glucose and acetone cyano-hydrins which is spontaneously broken down to hydrocynic acid (HCN) and acetone (Cooke, 1978; Bokanga, 1999; Aworh, 2008). HCN once produced dissipates in the air since its boiling temperature is25.7°C(Bokanga, 1999). Cassava fermentation consists of two distinct
methods, aerobic fermentation (heap fermentation) and anaerobic (Hahn, 1992). Heap fermentation is the most widely used processing techniques for reducing cyanogens in Mozambique and Uganda (Essers et al., 1995; Tivana et al., 2007). It involves surface-drying the roots, 1 to 2 h after which they are heaped together, covered with leaves or straws and left to ferment 3 to 4 days until the pieces become moldy, after which they are scraped, sun-dried and milled to flour. The second method is anaerobic fermentation in which grated cassava tubers is placed in sacks and pressed with stones or a jack between two platforms (Hahn et al., 1987).

Fermentation of cassava has given rise to a wide array of fermented products. In the south-western part of Nigeria, cassava is consumed in the form of hot water flour paste called **Lafun**. The flour is made by allowing peeled tuberous roots of cassava steeped in water to ferment naturally. **Lafun** is produced by submerged fermentation of peeled sliced cassava roots in water for 3 to 5 days (Oyewole and Odunfa, 1988) or by immersing peeled or unpeeled cassava in a stream or stationary water or in an earthenware vessel and fermented until roots become soft (Hahn, 1992) after which the fermented cassava was subjected to sun-drying and milling to powder/flour. The flour is usually turned in freshly boiled water, with no further heating into a stiff porridge consumed with soup (Oyewole and Afolami, 2004). *Corynebacterium manihot, Lactobacillus spp.* and *Leuconostoc spp.* are some of the organisms that are involved in fermentation of cassava to Lafun (Odunfa, 1985).

Many constraints have been identified in the commercialization of **Lafun** processing. This includes product quality variation from one processor to the other (Oyewole and Sanni, 1995) and differences in processing methods (Akingbala et al., 1991). Idowu and Akindele (1994) enumerated the influence of age and variety of cassava roots as another constraint. Oyewole and Afolami (2004) investigated the influence of use of some new cassava varieties in comparison with local varieties in quality of **Lafun**. Another factor responsible for product variation in **Lafun** is that fermentation process is initiated by chance inoculation by microorganisms from the environment (Achi and Akomas, 2006). This study therefore aimed at investigating some of the biochemical changes that occurs when cassava is subjected to submerged fermentation and anaerobic fermentations in the processing of cassava flour, **Lafun** and the influence of these changes on the chemical, functional and pasting characteristics of the products developed from the two fermentation processes.

**MATERIALS AND METHODS**

Freshly harvested cassava tubers (*Manihot esculenta* crantz) of 12 months old TMS50395 cultivar were obtained from the International Institute of Tropical Agriculture, IITA farm in Ibadan Oyo State, Nigeria.

**Processing of cassava tubers**

The cassava tubers were sorted, peeled, washed with potable water and drained. 10 kg of the washed tubers were separated into two equal portions. A portion (5 kg) was then cut into slices of 5 to 6 cm length and soaked in a big bowl containing water at ratio of 1:3 w/v. The pieces were completely submerged in water and the bowl uncovered to allow for exposure to air (Kahn, 1992). The second portion were cut into slices, soaked with same quantity of water as in the first process and wrapped in jute sacks placed in a large bowl which was then covered with a polyethylene bag to create anaerobic conditions (Tetchi et al., 2012). Both were left for three (3) days to ferment at ambient conditions (27 to 32°C). Both fermentation conditions are similar but the only difference is the reduced availability and access of surface air to the fermenting tubers caused by the polyethylene covering resulting in anaerobic conditions. The reasoning behind creating this sort of anaerobic condition was to investigate the possible effects this will have on the actions of microorganisms on the fermenting cassava and the resulting product: **Lafun**. During the fermentation, samples of the fermenting tubers and steep water from each fermentation process were aseptically taken out and subjected to different analyses. After fermentation, the pulp was removed, water squeezed out and spread on a tray and sundried for 3 days.

The dried **Lafun** (from submerged and anaerobic fermentations) and a control sample that is traditionally produced sample purchased from a local market were separately milled, sieved and packaged in a polythene bag for analyses.

**Chemical analyses**

Moisture, ash, crude fibre, crude protein, tritratable acidity were determined by the methods of AOAC, 1990. The pH was measured using pH meter (Unicam 9450 model).

**Hydrogen cyanide determination**

This was determined by the methods of Oyewole (1990).

**Carbohydrate content**

Carbohydrate content was calculated by the difference method.

**Physical characteristics**

**Water absorption capacity**

Water absorption capacity of the flour samples were determined by the methods of Beuchat (1977).

**Swelling index**

This was determined according to the method of Ukpabi and Ndimele (1990).

**Bulk density (Loose and packed)**

These were determined by the methods of Okaka and Potter (1979).

**Rheological characteristics**

The rheological characteristic of the **Lafun** produced was
determined using Brabender Amylograph. The effects of fermentation on the pasting properties of the flours during heating, cooling and shearing were studied as described by Numfor et al. (1995).

Sensory evaluation

The fermented cassava flour was cooked by briskly turning the flour in freshly boiled water in a pot using wooden turning stick at flour/water ratio 1:4 as described by Oyewole and Afolami (2001) until a consistently smooth paste is achieved. They were served to 10-man sensory panelists who are familiar with the product to evaluate on a 9-point hedonic scale where 9 represents like extremely, 5 represents neither like nor do dislike and 1 represents dislike extremely. The means of sensory scores after being subjected to analysis of variance and separation of means by Duncan’s multiple range tests are shown in Table 3.

Statistical analysis

The analysis were performed in replicates and data obtained were subjected to analysis of variance to determine the differences with the aid of the statistical package SPSS (version 15.0) and Duncan’s multiple range tests to separate the means at 5% level of significant difference. Microsoft Works spreadsheet was used to plot graphs for presentation of figures.

RESULTS

Changes in pH, HCN and titratable acidity of cassava steep water

The changes in the pH of steep water obtained from fermenting cassava tubers over the period of 72 h are shown in Figure 1. There was no significant difference (p>0.05) in pH within 48 h of fermentation, even though there was a general decrease in pH under both anaerobic and submerged fermentation. A greater decrease in pH was observed under anaerobic fermentation. The pH decreased significantly (p<0.05) after 48 h of fermentation. pH values were much lower under anaerobic fermentation than in submerged fermentation. Figure 2 shows the changes in titratable acidity of steep water of cassava tubers fermented over a period of 72 h. There were significant differences in titratable acidity (p<0.05) under the different fermentation conditions and over 24 h interval up to the end of fermentation.

Figure 3 shows changes in HCN content of cassava steep water. There were significant differences (p<0.05) in HCN under both submerged and anaerobic fermentation. HCN content differed significantly at 24 h interval till the end of fermentation at 72 h. Under submerged fermentation, HCN reduced from 3.20 ± 0.01 mg/100 g within 24 h of fermentation to 0.98 ± 0.01 mg/100 g at 72 h while a greater reduction to 0.80 ± 0.01 mg/100 g was observed under anaerobic fermentation.

Changes in pH, titratable acidity and HCN of fermenting cassava tubers

Changes observed in pH of cassava tubers fermented between 24 and 72 h are presented in Figure 4. There was no significant differences (p>0.05) between pH under submerged and anaerobic fermentations but between 24 h fermentation interval under both conditions, pH reduced significantly.

Titratable acidity increased significantly as fermentation period increased under both submerged and anaerobic conditions. There were also significant differences (p<0.05) in the values of acidity observed under these conditions. Greater increase was observed for titratable acidity under anaerobic fermentation. HCN content of cassava tubers fermented under submerged condition differed significantly (p<0.05) from values observed under anaerobic
Figure 2. TTA of Cassava steep water.

Figure 3. HCN content of cassava steep water (mg/100g).

Figure 4. pH of cassava tubers.
fermentation. Within 24 h interval of observations, the HCN content reduced significantly under both fermentation conditions and a higher rate of reduction was observed under anaerobic fermentation as shown in Figure 6.

Changes in physicochemical properties of cassava tuber and flour fermented under different conditions

The chemical and physicochemical characteristics of peeled raw cassava tubers used for this study as well as those of the cassava flour obtained from drying and milling cassava tubers fermented under the two conditions in comparison with those of a market sample (used as control) are shown in Table 1. There was no significant difference (p>0.05) in the pH of the experimental samples and the market sample but the samples differed significantly in titratable acidity. Highest value was observed for the market sample while the anaerobic fermentation sample had the least titratable acidity (0.13 ± 0.00%). There was no significant difference (p<0.05) in the HCN acid content of all the flour samples. Moisture content, ash, crude fibre, protein and carbohydrate all differed significantly (p<0.05) among the flour samples. Moisture content was lowest in the anaerobic samples and highest in the submerged samples. Crude fibre was lowest in the anaerobic samples while the market sample had the highest. Ash content was higher in the submerged samples and highest in the market sample. Protein content was least in the anaerobic samples while the market samples had the highest protein content (1.94 ± 0.01 g/100 g). On the other hand, carbohydrate content of the anaerobic sample was higher than that of the submerged fermentation sample and the market sample.
The functional properties and pasting characteristics of the fermented cassava flour in comparison with the market samples are shown in Table 2. There was no significant difference (p>0.05) in the water absorption of all the samples. Swelling index differed significantly (p<0.05) among the samples. Submerged samples had the highest swelling index of 4.5±0.01, while the anaerobic samples had the lowest (2.00±0.01). There was no significant difference in packed bulk density between the anaerobic sample and the market sample, whereas these two samples differed significantly (p<0.05) from the submerged sample. There was no significant difference in loose bulk density between the three samples.

There was no significant difference (p<0.05) in the peak viscosity among the samples. Highest peak viscosity was observed in the market sample (424.84 ± 0.01RVU) and least in the submerged sample (400.45 ± 0.01RVU). From Table 3, peak viscosity, trough, breakdown, setback, peak time, final viscosity and pasting temperature all differed significantly (p<0.05) among the samples.

Peak viscosity was highest in the market sample, followed by the anaerobic sample. There were no significant differences (p>0.05) in the water absorption of all the samples. Swelling index differed significantly (p<0.05) among the samples. Submerged samples had the highest swelling index of 4.5±0.01, while the anaerobic samples had the lowest (2.00±0.01). There was no significant difference in packed bulk density between the anaerobic sample and the market sample, whereas these two samples differed significantly (p<0.05) from the submerged sample. There was no significant difference in loose bulk density between the three samples.

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anaerobic fermentation process. Similarly, no significant difference was observed between market sample Lafun and the submerged fermentation Lafun in colour. Lafun from anaerobic fermentation was poorly rated by panelists in colour. Interestingly, aroma of Lafun of submerged fermentation process differed significantly from and was preferred over the other flour samples. Finally, in overall acceptability there was no significant difference in overall acceptability between the Lafun from submerged fermentation and the market sample though a slightly higher value was observed for the former. Lafun from anaerobic fermentation was least rated in overall acceptability.

**DISCUSSION**

The pH and titratable acidity values of steep water and cassava tubers under submerged fermentation and anaerobic fermentation decreased and increased respectively, but a greater decrease in pH and increase in titratable acidity was observed in the steep water from both fermentation conditions signifying that a greater part of the acid produced are leached into the steep water. This has been reported to be attributed to the activities of lactic acid bacteria on carbohydrates in the fermenting cassava (Ogunsua, 1980; Oyewole and Odunfa, 1990; Oyewole and Afolami, 2001). Acid production had been reported to be dependent on microflora and processing conditions (Oyewole, 1990). The microorganisms involved in the Lafun production include four yeasts: *Pichia yinchis, Candida tropicalis, Geotrichum candida*, and *Rhodotorula* sp.; two moulds: *Aspergillus niger* and *Penicillium* sp.; and two bacteria: *Leuconostoc* sp. and *Corynebacterium* sp. (Nwachukwu and Edwards, 1987). Oyewole and Odunfa (1988) observed that the mould disappeared within 36 h of fermentation while *Bacillus* sp. which was present at the beginning of fermentation decreased drastically as fermentation progressed. The yeasts appeared within 24 to 48 h of the fermentation and increased rapidly. The lactic acid bacteria were implicated throughout the duration of fermentation. *Bacillus* sp., *Corynebacterium* sp., *Candida* sp. and the lactic acid bacteria were considered to play important roles. They have been reported to be responsible for souring of cassava fermented products through lactic acid production (Amoa-Awua et al., 1996; Oyewole and Odunfa, 1988). Some *Bacillus* species were reported to show ability to breakdown cassava tissues during fermentation process (Padanou et al., 2009) while some yeasts and fungi contribute to cassava tissue breakdown by cellulase production leading to a more intimate interaction between linamarase and cyanogenic compounds of cassava, linamarin and lotaustralin resulting in the formation of glucose and acetone cyanohydrins which is spontaneously broken down to hydrocyanic acid (HCN) and acetone (Cooke, 1978; Bokanga, 1999; Aworh, 2008) and resultant detoxification of cassava. Lactic acid bacteria, yeasts and fungi also contribute to the build-up of the aroma compounds during fermentation (Oyewole, 2001).

Moisture, pH and temperature conditions are critical for the growth of these microorganisms in roots and thus for fermentation. According to Spier et al. (2006) ambient temperature of 30°C combined with high moisture content (90%) lead to highest α-amylase produced by lactic acid bacteria for cassava starch hydrolysis. pH value of below 4.0 has been reported to be optimum for cassava fermentation for *Lafun* production (Oyewole and Odunfa, 1988). An optimum value of 3 mg/kg had been recommended as a safe level for HCH in dried fermented cassava products (Achinewhu et al., 1998).

The greater reduction in TTA observed in cassava tubers under submerged fermentation may be due to the higher water content in the fermenting medium which encourages a greater breakdown of cyanogenic compounds of cassava, linamarin and lotaustralin resulting in the formation of glucose and acetone cyanohydrins which is spontaneously broken down to hydrocyanic acid (HCN) and acetone (Cooke, 1978; Bokanga, 1999; Aworh, 2008) and resultant detoxification of cassava. Lactic acid bacteria, yeasts and fungi also contribute to the build-up of the aroma compounds during fermentation (Oyewole, 2001).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Taste</th>
<th>Colour</th>
<th>Aroma</th>
<th>Overall acceptability</th>
</tr>
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<tbody>
<tr>
<td>Market sample</td>
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<td>7.0</td>
<td>6.8</td>
<td>6.5</td>
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<td>Submerged fermentation</td>
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<td>7.0</td>
<td>7.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Anaerobic fermentation</td>
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<td>4.7</td>
<td>5.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Values in the same column with the same superscript are not significantly different (p>0.05).

Table 3. Sensory evaluation of cooked Lafun fermented under different conditions.
carbohydrates, ash, and protein during fermentation had been attributed to the leaching of these nutrients into the soaking water and microbial utilization (Oyewole and Odunfa, 1989), though a slight increase in protein content during fermentation was reported by Tivana et al., (2007). Lactic acid fermentation is heterolactic, operating in association with secondary alcoholic and anaerobic fermentation to produce alcohol and organic acid (Raimbault et al., 1996).

Higher bulk densities were observed in the market and anaerobic flours over the submerged fermentation flour. Bulk density is a measure of the heaviness of a flour sample (Oladele and Aina, 2007). Therefore, the latter sample will pack more than other in containers (Azubike et al., 2011). Also higher bulk densities increase the sinkability of powdered material (Ortega-Rivas 2012). There was no significant difference among the samples for water absorption capacities. Water absorption capacity describes the flour-water association ability under limited water supply (Oladele and Aina, 2007) and is expressed as gram of water absorbed (retained) per gram of sample.

The water absorption capacity obtained from this study was much lower than those reported for unfermented cassava flour (Azubike et al., 2011). Higher water absorption capacities of 6.0 g water/g sample and 9.5 g water/g sample were observed respectively for red and white sweet potato flour (Onuh et al., 2004). Swelling index describes the volume a particular quantity of Lafun will occupy when mixed with a specific quantity of water. Swelling index was highest in the submerged fermentation flour. Swelling index illustrates the relationship between the length of fermentation and the different fermentation conditions.

From this study it was observed that even though all the Lafun samples had the same water absorption capacities they differed significantly in swelling index. The submerged fermentation flour showed potentials for higher swelling power above the other two flour samples. This signifies that the possible changes caused by fermentation in the morphology of the starch moieties in the resultant flour is the inducement of higher swelling features and fermentation beyond four days causes a reduction in swelling index of yam flour (Iwuoha, 2004). The higher swelling index in Lafun from submerged fermentation will be of economic benefit for and consumers because only a small quantity will produce very large mass of product when the product is cooked by end user/consumer. Similar peak viscosity and water absorption values were observed for the submerged fermentation and anaerobic fermentation flours, while a higher peak viscosity was observed for the market sample (424.84 ± 0.01). It has been observed that peak viscosity usually occurs at the equilibrium between granule swelling which increases viscosity and granule rupture and alignment due to mechanical shear which causes its decrease (Ayernor, 1985, Bolade 2009). Numfor et al. (1995) studied the effect of pH on the pasting properties of starch by acidification with citric acid and observed that peak viscosity was not affected by pH between 6.9 and 4.5, however on lowering to 3.5, a reduction in peak viscosity suggested that enzyme action reduces peak viscosity of native (unfermented) starch. The rheological change also reflects greater internal stability of the fermented starch granules resulting in reduced swelling and amylose leaching.

Breakdown viscosity is regarded as a measure of the degree of disintegration of starch particles or paste stability during heating (Dengate, 1984; Bolade, 2009). The submerged fermentation flour with the lowest breakdown value was more resistant to heat and shear force followed by the anaerobic flour. This would guarantee a more stable cooked paste. Highest final viscosity was in the market sample (324.08±0.28), closely followed by the submerged fermentation flour. Higher final viscosity is attributed to aggregation of amylose molecules in the paste (Miles et al., 1985). Highest set back was observed in submerged fermentation flour (64.66±0.01) and lowest in anaerobic fermentation flour (54.42±0.01) respectively. The lower value for anaerobic flour may be due to the higher rate of reduction in titratable acidity and HCN which implies faster breakdown of starch and other carbohydrate during fermentation, thereby leading to reduction in the amount of starch to be gelatinized. It has been observed that stimulated enzymes in soaked cereals grains are capable of causing partial hydrolysis of starch molecules (Akingbala et al., 1987). Similar but significantly different pasting temperatures were observed in all the flours samples. Higher temperature of gelatinization reflects greater internal granule stability (Numfor et al., 1995). The pasting temperatures observed were in the range of 76.76±0.01 to 76.85±0.01°C for all the samples. These values were higher than those observed for fermented cassava starch by Numfor et al., 1995, but they are comparable to (73.4 to 74.8°C) observed for maize flour (Bolade, 2009). Lower pasting temperature implies that the flour will gelatinize faster that is, at reduced temperatures. The observed low pasting temperatures may probably be due to enzyme activity stimulated by the fermentation process which has broken down the matrix embedding the starch granules, thus allowing the granules to swell freely and gelatinize faster. Similar pasting temperatures (74.95±0.87 to 76.85±0.44°C) were observed by Oyewole and Afolami (2001) when different cultivars of cassava were subjected to fermentation.

The higher value of acidity of Lafun sample from submerged fermentation and the market sample must have positively impacted on the taste and aroma of the products as they were significantly highly rated than the anaerobic sample. It has been observed that consumers of Lafun described good quality Lafun as one with little or no odour, having characteristic white colour and texture which is non-sticky to the hand (Oyewole and Afolami, 2001). In all the attributes, Lafun from submerged
had the better consumer acceptability than Lafun from anaerobic fermentation which is comparable to the market sample.

Conclusion

Consequently to our investigation, it could be observed that even though both types of fermentation led to a considerable decrease in pH and increase in titratable acidity in Lafun, submerged fermentation had a higher positive impact on nutritional and functional qualities of the cassava flour. The biochemical changes that occurred led to a higher ash content (which is indicative of the mineral content), a relatively higher protein and fibre content than those observed in anaerobic fermentation. Also submerged fermentation produced Lafun of higher swelling index and higher final viscosity. It would be apparent that submerged fermentation produced a better quality Lafun than anaerobic fermentation as confirmed by consumers’ preference for Lafun from submerged fermentation. Therefore we advice that for optimum quality Lafun, cassava should be subjected to aerobic fermentation.

Conflict of interests

The authors did not declare any conflict of interest.

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