



Research Paper

INFLUENCE OF *Lacto bacilli* STARTER CULTURES ON THE NUTRITIONAL CONTENT AND ANTI-NUTRITIONAL FACTORS OF FERMENTED CASSAVA FOR *usi* (EDIBLE STARCH) PRODUCTION IN NIGERIA

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Abstract

Usi, an indigenous food in Delta State, Nigeria, produced from fermented cassava has no documented information about the nutritional and anti-nutritional content when fermented with starter cultures even though fermentation is known to engender nutritional improvement on food products. This study therefore aimed at investigating the effect of starter culture fermentation on the nutritional quality of cassava for *usi* production. Cassava tubers (TME 30572), fermented both spontaneously (control experiment) and with *Lactobacilli* starter cultures (singly and in-combination) for 72 hours were analyzed for proximate and anti-nutritional factors using standard methods. Data were statistically analyzed at 5% level of probability. Spontaneously-fermented sample had lower moisture content (5.68%), crude fat (0.23%), crude fiber (1.91%) and higher carbohydrate content (90.30%) than the starter-fermented samples which had (6.79-7.35%), (0.25-0.39%), (1.93-2.24%) and (88.31-89.63%) respectively. However, higher protein content ranging from 1.02% to 1.24% was observed in the starter-fermented mashes. The least tannin content (33.4 mg/g) was evident in the spontaneously-fermented sample while cyanide was not detected in both the spontaneously-fermented sample and when the starters were combined. Although, spontaneously-fermented sample exhibited better moisture content, crude fiber content, tannin and cyanide content, utilization of starters brought about improved protein content. Furthermore, the consortium of the three starters showed better nutritional results than when utilized singly.

Key words: Cassava fermentation; Lactic Acid Bacteria; Proximate; Starter culture; *Usi*.

INTRODUCTION

Cassava, rated as the 4th most important root crop in developing countries, is one of the most useful tropical crops with a world output increase of 4.6% between 2013 and 2014, having Nigeria as one of the major producers (1). It is widely exploited as a cheap energy source despite major limitations like the presence of toxic cyanogenic glucosides, low protein content and short postharvest shelf life.

Cassava roots were reported to be rich in carbohydrates with most of it being present as starch hence, its utilization by most people in the Tropics as sources of carbohydrate (2). They are deficient in protein with about 0.7% to 1.3% fresh weight (3). Even though, cassava tuber has been criticized for its low and poor protein content, it produces more weight of carbohydrate per unit area than other staple food crops under comparable agro-climatic conditions thus, being an energy-dense food and therefore ranked high for its energy value of 250×10^3 cal/ha/day as compared to rice, wheat, maize and sorghum (4). The edible starchy flesh comprises some 60% to 70% total weight of the root as water (2). This forms the major component and it is between the range 60.3% and 87.1% according to (5). The tubers are usually processed, mostly through fermentation to overcome the aforementioned problems into various products such as *usi* (edible starch), which is a major staple diet of the Itsekiri and Urhobo in Delta, Nigeria.

Usi is an energy-rich food containing about 84% starch. Since the food is a product of fermentation, it is expected that fermentation process will confer some beneficial effect on both the improvement of its proximate composition and anti-nutritional factors. However, there is paucity of information to confirm this. Hence, this study was conducted to investigate the effect of both spontaneous fermentation and the use of different starter cultures on the nutritional qualities of *usi*.

MATERIALS AND METHODS

Cassava samples

Healthy cassava variety TME 30572 was obtained from the International Institute of Tropical Agriculture, Ibadan. Oyo State, Nigeria.

Starter culture

Lactobacillus pentosus F2A (Accession number KJ778115), *L. plantarum* subsp. *argentinae* F2B (KJ778116) and *L. plantarum* F2C (KJ77117) previously isolated from a spontaneously-fermented cassava and identified (6) using the NCBI Basic Local Alignment Search Tools (BLAST) were used as starter cultures.

Preparation of cassava for *usi* fermentation

Cassava tubers were visually assessed, peeled, washed with clean tap water, cut into pieces and sterilized using 0.1% HgCl in 70% ethanol followed by rinse with sterile distilled water (7). One hundred gram was grated and the pulp were fermented both spontaneously (control experiment) and with *Lactobacilli* starter cultures, under sterile conditions in 2-litre sterile distilled water in a 5-litre capacity fermenter for 72 hours.

Inoculum size determination

The starters were inoculated into sterile de Man Rogosa and Sharpe (MRS) broth and incubated at 30°C for 48 hours. Aliquot (1 ml) of broth cultures were introduced into another batch of sterile broth and incubated at 30°C for 24 hours. At the end of incubation period, the broth cultures were centrifuged (Himac CR21GII, Japan) at $5000 \times g$ for 10 minutes. The supernatant was decanted while the pellet was washed with sterile distilled water and re-centrifuged before being suspended in sterile normal saline.

The inoculum size was determined using McFarland standard. Dilutions were made with sterile normal saline to McFarland standard (No 4) using a spectrophotometer (Cecil CE 1011, Cambridge, England) to give 0.669 optical density at 600 nm, resulting in an approximate cell density of 1.2×10^9 CFU/ml. Aliquot (5 ml) of resultant diluent was used as inoculum singly and in-combination to inoculate 100 g of the steeped cassava and fermentation was allowed for 72 hours at room temperature. Spontaneously-fermented cassava served as the control experiment. Samples for analysis were taken at the end of the fermentation period.

Nutritional analysis

Moisture content, crude protein, fat, fiber, ash and total carbohydrate were estimated according to Association of Official Analytical Chemists (AOAC) methods (8).

Anti-nutritional factor analysis

Determination of cyanide content

The cyanide content of the fermented cassava mash was determined using the method of (9). Twenty gram (20 g) of sample was homogenized in 200 ml distilled water for 10 minutes. The homogenate was incubated for 18 hours at room temperature after which 100 ml of 5% NaHCO_3 was added to it before distillation. After distillation, the filtrate was collected and titrated against 0.2% iodine solution using 1% starch as indicator. Percentage cyanide content was calculated using the titre value.

Estimation of phytic acid

The phytic acid was determined using the procedure described by (10). Two gram (2 g) of sample was weighed into 250 ml conical flask. A hundred millilitre concentrated HCl acid (2% v/v) was used to soak the sample in the conical flask for 3 hours and then filtered through a double layer of Whatman filter papers. The filtrate (50 ml) was placed in 250 ml beaker and 100 ml of distilled water was added. Ten millilitres (10 ml) of 0.3% (w/v) ammonium thiocyanate solution was added to the solution as indicator. The solution was titrated with standard iron chloride solution, which contained 1.95 mg iron per millilitre. The end point colour was slightly brownish-yellow which persisted for 5 minutes. The percentage phytic acid was calculated using the titre value.

Estimation of tannin content

The method described by (10) was adopted. Four hundred milligram (400 mg) of sample was placed in 500 ml conical flask and 40 ml diethyl ether containing 1% acetic acid (v/v) was added, this was properly mixed to remove the pigment materials. The supernatant was carefully discarded after 5 minutes and 20 ml of 70% aqueous acetone was added. The flask was sealed with cotton plug covered with aluminum foil, then kept in shaker for 2 hours for extraction. The content of the flask was filtered through Whatman filter paper (No. 1) and the filtrate was used for analysis. Aliquot (50 ml) of tannin extract from the sample was introduced into test tube and the volume was made up to 100 ml with distilled water. Folin-Ciocalteu reagent (0.5 ml) was added, mixed properly after which 2.5 ml of 20% (w/v) sodium carbonate solution was added and further mixed. The mixture was kept for 40 minutes at room temperature, and absorbance was read at 725 nm using spectrophotometer (Cecil CE 1011, Cambridge, England). Tannin concentration was estimated from tannic acid standard curve.

STATISTICAL ANALYSIS

Data obtained were subjected to Analysis of variance (ANOVA) using SPSS software at 5% level of significance to determine differences and were presented as mean \pm standard deviation of three replicates.

RESULTS AND DISCUSSION

The proximate composition and anti-nutritional factor of the fresh and spontaneously fermented cassava was presented in Table I. The cassava variety used for this study had 87.90% total carbohydrate and protein content as low as 1.02% which makes it a predominantly starchy food with a moisture content of 7.28% per 5 g fresh cassava. It had 1.57% ash, 1.75% crude fiber and 0.48% crude fat. Phytate, tannin and cyanide content of the fresh cassava root were 0.3 mg/g, 35.4 mg/g and 0.1 mg/g respectively. The above moisture content was within the range of 7.31% and 8.40% that was reported during the analysis of the nutritional quality of *fufu* from different cassava cultivars (11). The authors also put the range of ash content and crude fiber to be between 0.15% – 1.5% and 0.12% – 0.65% respectively, among the different cultivars used. Lower moisture content of 5.43%, ash content 1.05% and higher crude fiber 1.06% was however, observed in fresh cassava (12). A much higher (8.98%) moisture content, 1.87% ash and 3.23% crude fiber had earlier been observed in fresh cassava during the study on effect of duration of fermentation on *gari* quality (13). These values were higher than what was observed in the present study. All these findings were indications that different cassava cultivars have varied nutritional content.

After 72-hour fermentation with starter cultures, the moisture content of the cassava mash ranged between 6.79% and 7.35%. Samples fermented with consortium of the starters had the most reduced (6.79%) moisture content. This was closely followed by those fermented with *L. pentosus* F2A starter, having 6.88% moisture content (Table II). Decrease in moisture content after fermentation is an indicator for stable shelf life and this could be due to uptake of water by the fermentation substrate which resulted in their soft and porous texture after fermentation.

Table I: Proximate composition and anti-nutritional factor of fresh and spontaneously-fermented cassava

		Fresh cassava	Spontaneously-fermented cassava
Proximate composition (%)	Moisture content	7.28 \pm 0.03	5.68 \pm 0.42
	Crude protein	1.02 \pm 0.12	0.95 \pm 0.12
	Crude fat	0.48 \pm 0.01	0.23 \pm 0.02
	Crude fiber	1.75 \pm 0.03	1.91 \pm 0.03
	Ash	1.57 \pm 0.04	0.93 \pm 0.06
	Total carbohydrate	87.9 \pm 0.11	90.3 \pm 0.52
Anti-nutritional factor (mg/g)	Phytate	0.30 \pm 0.004	0.29 \pm 0.001
	Tannin	35.4 \pm 0.03	33.4 \pm 0.01
	Cyanide	0.10 \pm 0.01	0.00

Values are means \pm Standard Deviation of three replicates

Table II: Proximate composition and anti-nutritional factor of starter-fermented cassava

		<i>Lactobacillus pentosus F2A</i>	<i>Lactobacillus plantarum subsp. argentolarensis F2B</i>	<i>Lactobacillus plantarum F2C</i>	Combination of the three starters
Proximate composition (%)	Moisture content	6.88 ± 2.25 ^{c*}	7.32 ± 0.06 ^b	7.35 ± 0.02 ^a	6.79 ± 0.03 ^d
	Crude protein	1.02 ± 0.12 ^b	1.02 ± 0.12 ^b	1.24 ± 0.13 ^a	1.24 ± 0.13 ^a
	Crude fat	0.31 ± 0.02 ^b	0.25 ± 0.01 ^d	0.29 ± 0.02 ^c	0.39 ± 0.02 ^a
	Crude fiber	1.93 ± 0.05 ^c	1.94 ± 0.05 ^c	1.96 ± 0.02 ^b	2.24 ± 0.02 ^a
	Ash	0.93 ± 0.02 ^b	0.89 ± 0.02 ^c	0.78 ± 0.03 ^d	1.03 ± 0.01 ^a
	Total carbohydrate	89.63 ± 0.18 ^a	88.63 ± 0.16 ^b	88.37 ± 0.13 ^c	88.31 ± 1.28 ^d
	Antinutritional factor (mg/g)	Phytate	0.32 ± 0.003 ^a	0.32 ± 0.003 ^a	0.28 ± 0.001 ^b
Tannin		46.4 ± 0.4 ^a	35.5 ± 0.03 ^c	40.6 ± 0.02 ^b	34.6 ± 0.02 ^d
Cyanide		0.20 ± 0.01 ^b	0.10 ± 0.01 ^c	0.80 ± 0.02 ^a	0.0 ^d

Values are means ±Standard deviation of three replicates

* Means reported with the same superscript in each row indicated no significant difference (p<0.05).

However, increase in moisture after fermentation has been linked to hydrolytic activity of the fermenting organisms whereby moisture could be released as part of their metabolic products (14). As shown in the experimental results, fermentation with starter cultures brought about increased moisture content than the spontaneous fermentation (5.68%) and this could be as a result of faster utilization of the substrates by the starters which released moisture as one of their end products since spontaneous fermentation has been characterized with a longer lag phase. Even though obtained values after fermentation were lower than the moisture content (7.28%) of the fresh unfermented cassava, fermentation with starter cultures brought about overall increased moisture content when compared with the value obtained (5.68%) in the spontaneously fermented sample.

Earlier studies on protein improvement in foods involved fortification and supplementing with protein-rich legume based substrates (15), but as observed in this study, where it was indicated that starter fermented samples had increased protein content than the spontaneously fermented samples, Tilahun and colleagues in 2013 reported that fermentation with starter cultures have also proven to be an effective method of protein improvement especially in cassava fermentation (16). The spontaneously-fermented cassava mash had lower protein content (0.95%) when compared to the fresh cassava, while there was no significant difference (1.02%) in the protein content when starters *L. pentosus F2A* and *L. plantarum subsp. argentolarensis F2B* were utilized. However, *L. plantarum F2C* and combination of the three starters had

highest protein content (1.24%) after the 72-hour fermentation. Structural proteins are part of the microbial cell and this could have contributed to the protein content increase observed even though, such increase has been attributed to the ability of microbial biomass to secrete cellulolytic enzymes that break down non-starch polysaccharides to monomer sugars. These are easily metabolized to protein and further extensive hydrolysis to amino acid and other simple peptides (17).

Crude fat was generally reduced after both fermentations even though, starter fermented samples had higher fat content (between 0.25% and 0.39%) when compared with the spontaneously fermented samples (0.23%).

Samples fermented with the combined starter had the highest ash content (1.03%) while those fermented with single starters ranged between 0.78% and 0.93%, however, ash content was generally lower in fermented samples (0.78% -1.03%) than the fresh cassava (1.57%). This reduction could be ascribed to leaching of soluble mineral elements into the fermenting medium or as a result of enzymatic hydrolysis of food components into their absorbable forms. Similar decrease after fermentation was also consistent with a 2015 report during the analysis of effect of fermentation on quality and composition of cassava mash (18) as well as millet (19). However, observable increase in ash content was reported (20) (21) when fermenting *Jatropha curcas* and *Candida albidum* seeds with fungi.

Crude fiber of the starter fermented samples ranged between 1.93% and 2.24%. This was higher than the fiber content (1.91%) of the spontaneously fermented sample. However, both fermentations showed increased fiber content when compared with the fresh cassava which had 1.75%. A general increase in crude fiber observed after fermentation was similar to the findings of Oyewole and Ogundele (22) who also reported an increase in crude fiber of *fufu* as fermentation progressed even though, it was suggested that increasing crude fiber interferes with other nutrients thereby, making such nutrients unavailable for use (21). However, the findings of this study indicated that the use of starter cultures and fermentation generally, could lead to increased crude fiber of cassava. On the contrary, during the fermentation of cocoyam, decrease in crude fiber which was reported as an indication of softening of the fibrous tissues during fermentation and the decrease was attributed to microbial bio-conversion of carbohydrates and lignocelluloses into protein (23).

Total carbohydrate of all samples increased after the 72-hour fermentation in both starter- and spontaneously-fermented mashes with ranges from an initial 87.9% in the fresh cassava to 90.6%. Such increase, as fermentation time progressed, could be a factor of decrease in moisture content (23) or the proportionate increase in protein content (24).

The samples fermented with single starters had higher carbohydrate content (88.4% - 89.6%) than that of the combined starter (88.3%) However, the spontaneously-fermented sample had the highest total carbohydrate (90.3%) after fermentation.

Since fermentation has been known to denature anti-nutritional factors and increase nutritional values of food products (25), decrease in anti-nutrients would be expected and such was attributed to leaching and microbial activities (26). The fresh cassava sample had 0.30% phytate. Some samples fermented with single had increased phytate content (0.32%) while others showed significant reduction. It was however noted that samples of the combined starter had the least phytate content (0.27%). Phytate reduction could be as a result of the effect of phytase activity, an endogenous enzyme from the substrate or inherent in the organisms. The enzyme hydrolyzes the phytic acid in the fermented food preparations into inositol and orthophosphate (27).

However, contrary to expectation, the increased phytate (0.32%) observed when starters *L. pentosus* F2A and *L. plantarum subsp. argentolarensis* F2B were utilized could not be established, but might be as a result of some plant metabolites being converted to phytate-like products (28). The least value being observed in the starter consortium could be due to the effect of combined microbial action.

The spontaneously-fermented sample had the most reduced tannin content (33.4%). Samples fermented with single starters exhibited increased tannin content (35.5% - 46.4%) while the combination of the three starters showed reduced tannin (34.6%) when compared to the initial 35.4% tannin content of the fresh cassava. Cyanide content of samples fermented with *L. pentosus* F2A, *L. plantarum subsp. argentolarensis* F2B and *L. plantarum* F2C had 0.2%, 0.1% and 0.8% respectively whereas there was no detectable cyanide when the combined starter was utilized and this could be said to be due to combined effect of the mixed starter or from the effect of other cyanide-degrading organisms in the spontaneous fermentation as no cyanide was also detected in the spontaneously-fermented cassava. This reduction in cyanide had mostly been linked to the fermentation involving water since it has been established to be the simplest method to reduce cyanide content as the water will swell the cells and allow linamarase to come into contact with linamarin, thus, initiating cyanide hydrolysis (29). Samples fermented with the single starters did not satisfy the Standards Organization of Nigeria regulation which puts the minimum tolerant cyanide level at 0.05 mg/g (30) even though, the deleterious level was put at 0.03 mg/g (31). In spite of both, observed values deviated from the findings of Oboh and Akindahunsi, who put the usual cyanide content of major cassava products in Nigeria as 0.019 mg/g (32).

Conclusively, despite the fact that fermentation process had significantly improved effect on the nutritional content of the cassava as observed in the spontaneous fermentation, utilization of starter cultures, most especially the combined starter elicited a more desired effect. Furthermore, the starters, even though belonged to the same genus, exhibited significantly different nutritional qualities.

Conflict of interest statement

Authors declared no conflict of interest.

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