

Starter developed pupuru, a traditional Africa fermented food from cassava (*Manihot esculenta*)

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Article history

Received: 22 May 2014

Received in revised form:
20 May 2015

Accepted: 16 June 2015

Abstract

“Pupuru” is a traditional fermented, smoked-dried cassava food, commonly consumed in South western, Nigeria and other West Africa countries. Fresh cassava variety, TMS 30572 was peeled, washed, submerged in sterile distilled water and inoculated aseptically with 1ml of inoculums starter microorganisms. Fermentation was allowed for 5 days at room temperature (30±2°C) and sampling carried out 24 hourly. A sharp decrease in pH from 6.40 to 4.07 was observed while titratable acidity increases with increase in fermentation period. Starter fermented mash followed similar trend with that of spontaneous fermentation except for a rapid drop in pH within the first 24 hours and least pH of 3.37 was recorded by LPF starter fermented mash at day 5. Pupuru produced with mixed culture of *Lactobacillus plantarum* and *Candida famata* (LPC) had the highest protein content (2.82%) while the least content of 1.24 % was recorded in LCH produced pupuru. The anti-nutrient contents of all the produced pupuru were significantly different ($p < 0.05$) from each other with LPC produced pupuru having the least Tannin, cyanide, phytate and oxalate (0.05 mg/100g, 0.24 mg/100g, 102.4 mg/100g and 2.94 mg/100g respectively) contents when compared with spontaneously fermented pupuru (0.49 mg/100g tannin, 1.35 mg/100g cyanide, 201.6 mg/100g phytate and 5.21 mg/100g oxalate) and unfermented pupuru with 0.60 mg/100g tannin, 12.56 mg/100g cyanide, 215.3 mg/100g phytate and 34.44 mg/100g oxalate. Pupuru fermented with LPC mixed cultures had the highest (2.40) general acceptability, increased nutritional contents and reduced anti-nutrient contents.

Keywords

Cassava fermentation

Starter- culture

Nutritional composition

Pupuru production

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Introduction

“Pupuru” is commonly consumed by the people living in the riverine areas of the western, southern, eastern and the middle belts of Nigeria, where it is also known as “Ikwurikwu” (Aboaba *et al.*, 1988; Shittu *et al.*, 2003). It is a traditional fermented, smoked-dried cassava food consumed in South-western Nigeria. The technology of “pupuru” processing originated from the Ilaje people of the Riverine area of Ondo State, Nigeria (Shittu *et al.*, 2005).

The traditional processing of cassava (*Manihot esculenta*) into “pupuru” involves peeling of tubers, steeping of peeled tubers in stream water and fermentation of the tubers for 4-6 days. The fermented cassava mash is removed from water into a jute bag to drain-off the water. It is then ground between the two palms of the hand, moulded into balls and smoked ball upon a platform popularly called “Aka”. The smoked cassava ball has a brownish to black outer coating.

According to Shittu *et al.* (2005), after drying, the dirty outer crust of the balls are scraped off and the inner portion is pounded lightly to form large crumbs or powder which may be soaked in cold water for about five minutes before boiling with constant stirring till it forms a viscous dough that is eaten with vegetable soup. The cooked dough produced has its unique characteristic flavours and aroma that distinguishes it from other fermented cassava products like “Fufu” and “Lafun”. Smoke heat is believed to impact some characteristic flavour and aroma to this product (Aboaba *et al.*, 1988; Shittu *et al.*, 2003).

Generally, traditional carbohydrate foods such as cassava play an important role in African diet. However, cassava as a major source of carbohydrate diet is limited by low protein and antinutrients contents (Hahn, 1992; Oboh *et al.*, 2002). Opeke *et al.* (1986), reported that processing of cassava to “Pupuru” is a major income earning venture and plays a significant role in ensuring food security for some

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people in Nigeria. At least as many as 4-6 million people in Nigeria and more in other African countries eat "Pupuru" (Odetokun *et al.*, 1998). Pupuru is different from other fermented cassava products like gari, fufu, lafun and akpu based on the processing method (Faramade *et al.*, 2005). Some researchers also reported that processing method affect chemical composition of cassava products (Ogunsua and Adedeji, 1979; Ayankunbi *et al.*, 1991). In view of the above, this research work aimed at developing a wholesome and nutritious starter-produced 'Pupuru' from fermented cassava.

Materials and Methods

Sample collection and processing

Fresh cassava variety *Manihot esculenta* TMS 30572 used for the work was obtained from the Teaching and Research Farm of University of Ibadan, Ibadan, Oyo State. The cassava samples were washed with distilled water to remove adhered surface soil particles. The cassava were then peeled, chipped and thoroughly washed with sterile distilled water.

Starter fermentation procedure

One kilogram (1 kg) of peeled, chipped and washed cassava was submerged in one litre of sterile distilled water in a sterile 2 L Erlenmeyer flask and then inoculated aseptically with 1ml of the starter organisms made of different combinations of *L. plantarum*, *L. fermentum*, *C. humicola*, *C. famata* and *G. capitatum* as shown in Table 1. The inoculation was accompanied by stirring using a sterile glass rod. Fermentation was allowed for 120 hours and sampling carried out 24 hourly.

Source of starter

Pure cultures of identified *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Cryptococcus humicola*, *Candida famata* and *Geotrichum capitatum* previously isolated from fermenting cassava were obtained from the Industrial and Biotechnology laboratory of the Department of Microbiology, University of Ibadan, Ibadan. Nigeria.

Chemical analysis

Ten grams (10 g) of the fermenting samples were collected 24 hourly for 120 hours into sterile bottles, mixed with 100 ml of distilled water and pH was determined using the pin electrode of pH meter (HANNA Instrument, model HI 8424). Titratable acidity of fermenting cassava was determined by allowing the mixture to stand for 15 minutes, with shaking at 5 minutes intervals and filtered with

Table 1. The code and combinations of mixed and single starter organisms used for the fermentation of cassava

Sample Code	Combinations
LP	<i>Lactobacillus plantarum</i>
LPF	<i>Lactobacillus plantarum</i> and <i>Lactobacillus fermentum</i>
LPG	<i>Lactobacillus plantarum</i> and <i>Geotrichum capitatum</i>
LPFG	<i>Lactobacillus plantarum</i> , <i>Lactobacillus fermentum</i> and <i>Geotrichum capitatum</i>
LPC	<i>Lactobacillus plantarum</i> and <i>Candida famata</i>
LCH	<i>Lactobacillus plantarum</i> and <i>Cryptococcus humicola</i>
SF	Spontaneous Fermentation
FCT	Fresh Cassava Tuber

Whatman No. 4 filter paper. Ten milliliter aliquots (triplicates) were pipette from the filtrate into conical flask and then titrated against 0.1N NaOH using 1% phenolphthalein as the indicator in order to determine the amount of acid (as lactic acid) in the sample. The percentage titratable acidity was calculated by multiplying the titre value by 0.09 (Vasconcelos *et al.*, 1990).

Nutritional analysis

The moisture content, crude protein, crude fat, ash content, crude fibre and total carbohydrate were determined by the method of the Association of Official Analytical Chemists (A.O.A.C, 1990) while antinutrient contents (cyanide, phytates, oxalates and tannins) were determined using the methods of Oke (1969) and Markkar *et al.* (1993).

Pupuru production

The starter fermented cassava mash is removed from water into a clean sterile muslin cloth bag to drain-off the water. It is then grounded between the two palms of the hand, moulded into balls and smoked in an oven (AKA was used for traditional production, so we used oven for the laboratory production) at 80°C. The smoked cassava ball has a brownish to black outer coating and was then made into "Pupuru" meal using the method of Shittu *et al.* (2005).

Sensory evaluation

Sensory characteristics of the starter- developed fermented cassava meal/foods were assessed by 10 untrained panelists (students and staff) in University of Ibadan, Ibadan, Oyo State. The panelists were in good health and are familiar with the taste, flavour and other attributes of the food. The porridge were prepared and served in sensory evaluation plates. The samples were assessed for colour, taste, flavour, aroma, texture and over all acceptability. The judges were instructed to sip water before and after

Table 2. Proximate composition of raw cassava, spontaneously- and starter- fermented “pupuru”

Parameters (%)	LP	LPF	LPG	LPFG	LPC	LCH	SF	FCT
M.C	11.20±0.12 ^{ab}	10.80±0.17 ^a	11.80±0.06 ^c	11.22±0.06 ^{ab}	10.98±0.09 ^a	11.50±0.12 ^{bc}	12.90±0.40 ^d	54.60±0.13 ^e
C.P	1.46±0.05 ^e	1.32±0.01 ^{de}	1.36±0.06 ^{cd}	1.87±0.02 ^b	2.82±0.02 ^a	1.24±0.02 ^{ef}	1.31±0.02 ^{def}	1.20±0.06 ^f
C.Fat	3.96±0.02 ^e	4.48±0.06 ^b	3.34±0.06 ^d	3.84±0.08 ^c	4.78±0.40 ^a	3.15±0.06 ^d	1.74±0.08 ^e	0.06±0.01 ^f
C.Fibre	2.23±0.01 ^b	2.36±0.03 ^b	2.36±0.13 ^b	2.67±0.05 ^a	2.73±0.18 ^a	2.09±0.04 ^b	1.69±0.14 ^c	1.29±0.04 ^d
Ash	2.20±0.12 ^e	2.84±0.04 ^a	2.37±0.18 ^c	2.64±0.02 ^{ab}	2.42±0.12 ^{cd}	2.88±0.05 ^a	1.67±0.05 ^d	0.98±0.03 ^e
CHO	78.95±0.04 ^b	78.20±0.12 ^e	77.33±0.02 ^d	76.81±0.52 ^d	78.66±0.34 ^b	79.14±0.05 ^b	80.74±0.02 ^a	41.87±0.17 ^e

Mean Value ± Standard Error. Mean values with the same superscript along the row are not significantly different ($P < 0.05$) from each other. Starter code as described in table 1.

Table 3. Antinutrients contents of raw cassava, spontaneously- and starter- fermented pupuru

Antinutrients (mg/100g)	LP	LPF	LPG	LPFG	LPC	LCH	SF	FCT
Cyanides	0.51±0.02 ^d	0.42±0.01 ^c	0.38±0.02 ^{cd}	0.36±0.02 ^b	0.24±0.01 ^a	0.49±0.01 ^d	1.35±0.01 ^e	12.56±0.02 ^f
Phytates	192.4±0.32 ^a	184.3±0.35 ^d	181.9±0.64 ^c	130.6±0.35 ^b	102.4±0.29 ^a	185.4±0.78 ^d	201.6±0.35 ^e	215.3±0.32 ^f
Oxalates	4.50±0.17 ^b	5.20±0.06 ^e	5.02±0.09 ^d	3.10±0.05 ^a	2.94±0.03 ^a	4.81±0.01 ^{cd}	5.21±0.21	34.44±0.30 ^d
Tannins	0.22±0.01 ^d	0.33±0.02 ^f	0.16±0.01 ^a	0.12±0.01 ^b	0.05±0.01 ^a	0.25±0.01 ^c	0.49±0.01 ^e	0.60±0.02 ^b

Mean Value ± Standard Error. Mean values with the same superscript along the row are not significantly different ($P < 0.05$) from each other. Starter code as described in table 1

assessing each product. The samples were assessed using an 8 points hedonic scale ranging between 1 (like extremely) to 8 (dislike extremely).

Statistical analysis

All the data obtained were subjected to statistical analysis using analysis of variance (ANOVA) to calculate significant differences in the treatment means, and the mean separations were achieved by Duncan's Multiple Range Test ($p \leq 0.05$) using the SAS software.

Results

The pH of the samples decreased throughout the fermentation period. The initial pH of the starter fermented cassava samples was 6.70; this was followed by a rapid drop in the first 24 hr and subsequent decrease depending on the starter combination. Cassava sample fermented with mixed starter culture of LPC had the least value of pH 3.37 while the highest value (pH 3.69) was observed for sample fermented with mixed culture of LPF by day 5

(120 hr). However, increase in Total Titratable acidity (TTA) was observed throughout the fermentation period. The starter fermented cassava samples produced lactic acid that ranged from 0.027 mg/ml at 0 hr (LP) to 0.722 mg/ml by LPF at 120 hr compared to 0.80 mg/ml lactic recorded in spontaneously fermented (control) mash.

The proximate compositions for starter-developed pupuru are shown in table 2. The highest content of protein (2.82%) was observed in LPC, followed by PFG (1.87%) and the least protein content of 1.20% was recorded in unfermented fresh tuber (FCT) produced pupuru. As expected, the protein contents were relatively low (1.20 - 2.82%) while the total carbohydrate content was high. Combination of *Lactobacillus plantarum* and *Candida famata* (LPC) was found to be the mixed culture that yielded the product with highest crude protein (2.82%), crude fat (4.78%), and crude fibre (2.73%) which is significantly different ($p < 0.05$) from other starter-developed and spontaneously fermented pupuru (SF) while unfermented produced pupuru (FCT) had the least contents of all the proximate constituents.

Table 4. Sensory characteristics and acceptability of starter-produced “pupuru”

Sensory characteristics	LP	LPF	LPG	LPFG	LPC	LCH	SF
Taste	3.80±0.25 ^a	3.90±0.40 ^f	3.80±0.30 ^a	3.50±0.45 ^a	3.00±0.45 ^a	4.20±0.51 ^{ab}	5.30±0.52 ^b
Flavour	3.30±0.25 ^a	3.70±0.37 ^{ab}	3.90±0.38 ^{ab}	3.00±0.33 ^a	3.30±0.37 ^a	3.90±0.46 ^{ab}	4.80±0.55 ^b
Texture	3.00±0.30 ^a	3.40±0.34 ^{ab}	3.70±0.42 ^{ab}	3.20±0.39 ^a	3.10±0.38 ^a	3.90±0.46 ^{ab}	4.50±0.52 ^b
Colour	3.80±0.47 ^a	3.80±0.16 ^f	3.70±0.42 ^a	2.80±0.29 ^a	2.60±0.48 ^a	3.60±0.31 ^a	4.90±0.46 ^b
General acceptability	3.60±0.34 ^b	3.70±0.21 ^{bc}	3.90±0.23 ^{cd}	3.10±0.41 ^a	2.40±0.22 ^a	4.30±0.42 ^{cd}	4.80±0.47 ^d

Mean value ± Standard error. Mean values with the same superscript are not significantly different ($P < 0.05$) along rows. Using 8 points hedonic scale, 1-Like very extremely, 2-Extremely, 3-Very good, 4-Good, 5-Fair, 6-Neither good nor fair, 7-Poor, 8- Dislike extremely.

The use of both fermentation and combination of different starters in the production of pupuru was found to drastically reduce the anti-nutrient contents when compared to spontaneously fermented (SF) and unfermented pupuru (FCT) (Table 3). Fermentation of cut and submerged cassava tubers with LPC combined starter significantly reduced ($p < 0.05$) the cyanide content of the pupuru produced (0.24 mg/100 g) when compared with spontaneously fermented (SF) pupuru (1.35 mg/100 g) and the fresh unfermented (FCT) produced pupuru (12.56 mg/100 g). The use of mixed-starter combination of LPFG (*Lactobacillus plantarum*, *Lactobacillus fermentum* and *Geotrichum capitatum*) was next in reduction of anti-nutrient to LPC produced pupuru while the highest antinutrient was recorded in pupuru produced from unfermented cassava (0.60 mg/100 g tannin, 12.56 mg/100 g cyanide, 215.2 mg/100 g phytate and 34.44 mg/100 g oxalate).

Analysis of the sensory characteristics of the produced pupuru revealed no significant difference between colour of all starter-developed pupuru, except for spontaneously fermented (SF) pupuru (Table 4). In most cases, no significant difference ($p < 0.05$) were observed between the naturally / spontaneously fermented (SF) and starter produced pupuru. Overall assessment of general acceptability shows that the spontaneously fermented pupuru was the least preferred.

Discussion

A sharp decrease in pH of fermenting cassava from 6.40 to 4.07 was observed in the first 24 hours of fermentation followed by a further slight decrease in pH while Titratable acidity was seen to increase

from 0 hour to 120 hours of the fermentation. Such a decrease in pH and increase in Titratable acidity has been well documented (Aliya and Geervani, 1981; Achi, 1990). The pH values is also similar to those reported by Assanvo *et al.* (2000) on the production of attieke, and Kameni *et al.* (2006) on corn dackere production.

The result of the proximate composition showed increase in protein and fat contents of the samples when compared with the raw cassava and spontaneously fermented cassava. The protein content of the cassava fermented with combination of *Lactobacillus plantarum* and *Candida famata* (LPC) was the highest (2.82%) and significantly different ($P \geq 0.05$) from the other samples. The increase in protein content of cassava fermented products could be attributed to the possible secretion of some extracellular enzymes into cassava mash in an attempt to utilize cassava starch as a source of carbon (Akindahunsi *et al.*, 1999). Oyetayo (2006) suggested that increase in the microbial mass may also account for the increase in the protein content of the ‘pupuru’ produced from fermenting cassava.

The antinutrient (Cyanides, Phytates, Oxalate and Tannins) composition of the cassava fermented into “pupuru” was lower and significantly different from the raw sample (FCT). The Antinutrients level of the “pupuru” sample obtained from cassava fermented with *Lactobacillus plantarum* and *Candida famata* (LPC) was however the lowest except for oxalates and it is not significantly different from that of *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Geotrichum capitatum* (LPFG). Fermentation of cassava had been reported to significantly reduce the Antinutrients level (Aboua, 1995; Obboh *et al.*, 2002). The reduction in cyanide content could be

attributed to synergistic effect of loss by hydrolysis of cyanogenic glycoside (by linamarase and pH >5) (Padmaja, 1995) and evaporation of Hydrogen cyanide during drying (Okpokiri *et al.*, 1995).

The cassava fermented into pupuru with these starters could be considered safe in terms of tannin and cyanide poisoning. This is because the level of the anti-nutrients is far below the reported deleterious level of 0.76% and 30 mgkg⁻¹ for tannin and cyanide as reported by Aletor (1993) and Akinrele *et al.* (1962), respectively. The reduction of the phytic acid will also make nutritionally essential minerals available. Phytic acid had been reported to interfere with Ca, Fe, Mg and Zn absorption as a result of its ability to chelate divalent cationic minerals (Nelson *et al.*, 1968).

The sensory characteristics and acceptability shows that the product fermented with starters are significantly different from that of spontaneous fermentation but not significantly different from one another. Most members of the panel preferred pupuru fermented with *L. plantarum*, *L. fermentum* and *Geotrichum capitatum* (PFG) and that of *Lactobacillus plantarum* and *Candida famata* (LPC) in all the parameters (taste, texture, flavour and colour). On general acceptability, the panellist prefers 'pupuru' fermented with *Lactobacillus plantarum* and *Candida famata* (LPC).

Conclusion

Locally, pupuru is produced by steeping in stream water and left to ferment spontaneously. The present study reveal an increase in the protein and fat contents with a reduction in the antinutrient contents of cassava fermented into "pupuru" using different starters. *Lactobacillus plantarum* and *Candida famata* (LPC) produced 'pupuru' have the best characteristics. Pupuru can therefore be produced by the use of mixed starter of known organism since the pupuru samples obtained from these starter have considerably high protein and low anti nutrient contents.

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