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Nutritional and rheological properties of starter-produced *Lafun*, an African fermented cassava product

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Abstract

Lafun is a product of cassava fermentation that is a good source of carbohydrate but has low protein content. The present work is aimed at obtaining an optimum biomass of *Saccharomyces cerevisiae* that will ferment cassava to produce nutrient-enriched *lafun*without changing its standard rheological properties. A typed variety of cassava (TMA 419, 30572) was collected from International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. Samples of the cassava were inoculated with varying inoculum sizes of *S. cerevisiae* and fermented for 72 hours to produce *lafun*. Proximate, functional and rheological properties, and the sensory attributes of the products were determined after fermentation. Results obtained indicated that the protein content increases as the level of biomass increases. No significant difference was observed in the solubility index, breakdown viscosity and setback viscosity of sample B fermented with 1.18×10^6cfu/ml of *S. cerevisiae* and the control. Sensory evaluation of the samples revealed no significant difference in colour and texture with slight differences in the odour and flavour. This study suggests the use of about 1.18×10^6cfu/ml of *S. cerevisiae* biomass in the fermentation of cassava for *lafun* production to enhance the protein quality as well as to have a product with close rheology to the naturally fermented product.

Keywords: *Lafun*, *Saccharomyces cerevisiae*, cassava, fermentation, nutritional composition, rheological properties

Introduction

Cassava (*Manihotesculenta* Crantz)is a chief source of dietetic energy forlow-income consumers and an industrial crop in many parts of tropical and subtropical Africa, Asia and Latin American [1]. It is a leading food crop in Nigeria, supplying about 70% of the daily calorie of over 50 million people [2]. In term of production, Nigeria ranked the world's largest producer with about 45 million tonnes in 2009 which accounted for approximately 19% of the total globaloutput [3,4].

A wide range of product hasbeen developed through the fermentation of cassava. It is called *tapioca* when dried to a powdery or pearly extract while its fermented flaky form is named garri [5]. Another fermented product of cassava is *lafun* obtained through submerged fermentation of cassava for 2-5 days, sun-dried and pounded into flour [6]. *Lafun* as a staple is popular among the people of South-Western, Nigeria [7]. The flour is prepared into a thick paste in boiling water with no further heating. The stiff porridge obtained is eaten with vegetable soup or stew [6].

Various factors have been identified as responsible for variation in quality of *lafun* which include quality variation from the processor to processor and differences in processing methods [8,9]. Another important factor is the initiation of fermentation through chance inoculation by microorganisms from the environment [5]. These factors usually affect the nutritional quality and sensory attributes regarding colour, odour, and overall acceptability of *lafun*.

One of the criteria that determine the end of fermentation of cassava for *lafun* production is the degree of softening of the chopped tubers. This softening enhanced by the action of pectinolytic

enzymes produced by microorganisms associated with the fermentation of the cassava [10-12]. In the study by Padanou *et al*. [12], predominant micro-organisms associated with spontaneous fermentation of cassava for *lafun* production were identified to include yeasts (*Saccharomycescerevisiae*, *Pichiascutulata* and *Kluyveromycesmarxianus*) and lactic acid bacteria (LAB) such as *Lactobacillus-fermentum* and *L. plantarum*. To assess the role of these organisms in root softening and overall organoleptic quality of *lafun*, Padanou*et al*. [12] developed starter cultures for the standardised production of *lafun*. Results obtained by these researchers showed that the involvement of *Saccharomyces cerevisiae* contributed to the softening of the cassava during the submerged fermentation [13]. Also, there have been earlier reports of pectin-degrading ability by *S. cerevisiae* [14-16].

Saccharomyces cerevisiae has an extensive history of use in food processing. It is one of the microorganisms that is appreciated by humans based on its usefulness in food and beverages including winemaking, baking, and brewing since ancient times [17]. The organism also has many similar functional proteins to that of higher eukaryotic organisms including man [18].

Cassava root has one of the lowest protein content of all the excellent crops. The protein content of dry weight is usually between 0.7% and 3% [19]. Studies have shown that there is little or no protein in cassava after fermentation. As a result, consuming *lafun* produced traditionally without a good source of protein could lead to various manifestations of malnutrition in children and adults [20,21].

Rheological properties describe the consistency and flow of food under tightly specified conditions. It is important in quality control during food manufacture and processing, and it influences the acceptability of food products by consumers [22]. Therefore, this study investigates the level of *Saccharomyces cerevisiae* biomass that would sufficiently ferment cassava for *lafun* production without affecting its standard rheological properties.

Materials and methods

Collection of Samples: About 10 kg of a typed variety of freshly harvested cassava tubers (TMA 41, 30572) were collected from International Institute for Tropical Agriculture (IITA), Moniya, Ibadan, in clear sterile polyethene bags to avoid contamination and brought to the laboratory for immediately processing. Stock culture of *Saccharomyces cerevisiae* kept at freezing temperature of -4^0C was collected from Microbiology Department, Federal University of Technology, Akure (FUTA), Nigeria.

Sample and Inoculum Preparation: The cassava samples were washed with sterile distilled water, peeled and cut into smaller pieces to reduce the surface area. 1000 g of the cassava pieces was soaked in 5% disodium bisulphate solution for 24 hours to eliminate microbial contaminants. The cassava pieces were then transferred into 2000 ml Erlenmeyer flask containing 1000 ml of sterile water. The preparation was made for three different inoculum concentrations and in three replicates. Preparation of the inoculum and determination of inoculum size was done as described by Arana *et al*. [23].

Controlled Fermentation of Cassava for Lafun Production: This was done using the method described by Adebayo-Oyetoro *et al*.[24] with modification. The 3 inoculum preparations estimated as 5.90×10^4 cfu/ml,1.18×10^6 cfu/ml and 1.77×10^7 cfu/ml were used to inoculate the cassava in the Erlenmeyer flasks respectively. The inoculated cassava preparations were incubated at 35^oC for 3days and dried at 50^oC overnight. Samples were taking at the end of fermentation for analyses. Spontaneously fermented sample was used as control.

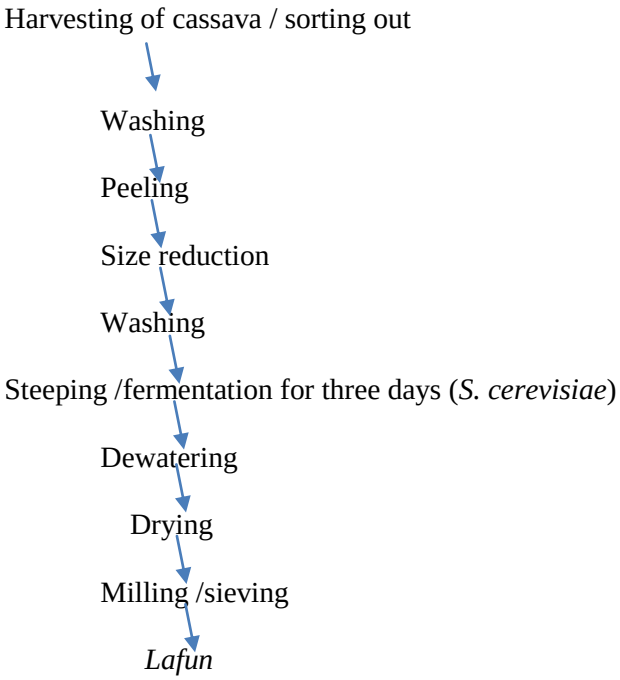


Figure 1. *Lafun* production using *Saccharomyces cerevisiae* [24]

Determination of pH and Total Titratable Acidity (TTA): The pH of the fermenting cassava was determined at the start of fermentation and 24-hourly up to 72 hours using pH meter (Jenway 3510). Similarly, the total titratable acidity was determined as described by AOAC [25].

Proximate Analyses: The ash and moisture contents, crude protein, crude fat was determined using the method described by AOAC [25] while the carbohydrate content was obtained by difference.

Determination of Functional Properties:

Water Absorption Capacity (WAC): This was determined according to the method described by Adebayo-Oyetoro *et al.* [26]. 4g each of the *lafun* samples was introduced into a 50ml centrifuge tube contain 20ml of distilled water. The mixture was occasionally stirred for up to 30 minutes after which it was centrifuged at 15000 rpm for 15 minutes. The change between the initial water added and the amount decanted after centrifugation was taken as the amount of water retained [27].

Swelling Power: The swelling power of the *lafun* samples was determined using the Leach method as described by Kusumayanti *et al.*[28]. 0.1g of each sample was heated in 10ml distilled water in a water bath at 60°C for 30 minutes with constant mixing. The samples were then centrifuged at 1600 rpm for 15 minutes. The precipitate was weighed and the swelling power calculated thus:

$$\text{Swelling power} = \frac{\text{Weight of sediment (g)}}{\text{Weight of the dry sample (g)]}} \text{ [29]}$$

Solubility: This was done using Kainuma method as described by Kusumayanti *et al.*[28]. The percentage solubility was then evaluated thus:

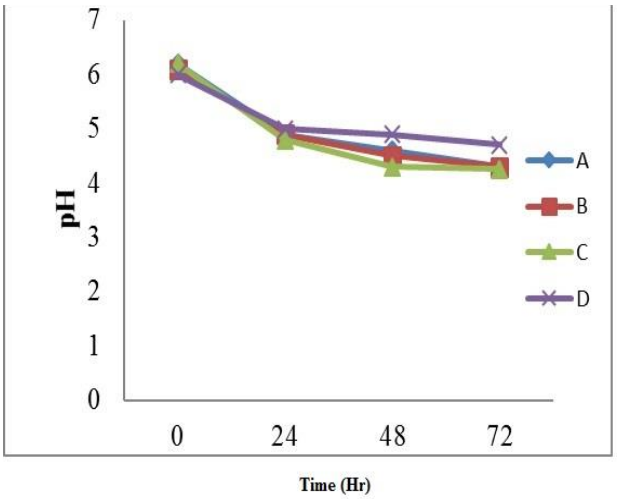
$$\%\text{Solubility} = \frac{\text{Weight of the soluble starch (g)} \times 2 \times 100\%}{\text{Weight of the dry sample (g)}} \text{ [30]}$$

Determination of Rheological (Pasting) Properties: The pasting properties were determined as described by Babajide *et al.* [31] using Rapid Visco Analyzer (RVA-4; Newport Scientific Pty. Ltd, Australia).

Sensory Evaluation: Organoleptic properties of the fermented products from controlled and spontaneous fermentation were determined. Flavour, appearance, texture, odour, and overall acceptability were assessed by 20 individuals who are regular consumers of *Lafun* using a score range of 1 (poor) to 5 (excellent). Data obtained were analysed using ANOVA for separation of means, and significance differences were accepted at 5% degree of freedom (IBM SPSS 2013).

Results

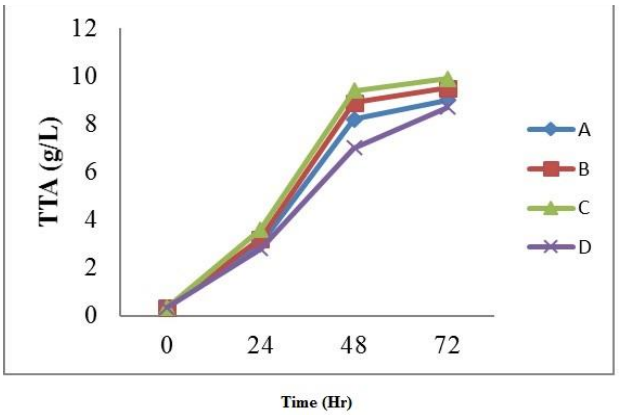
The changes in pH during the fermentation of cassava for *lafun* production is as shown in Figure 2. The pH of cassava fermented with 5.90 × 10^4cfu/ml of *S. cerevisiae* for *lafun* production (sample A) was 6.2 at the start of the fermentation. At the end of 72 hours of fermentation, the pH had dropped to 4.3. Sample B which was fermented with 1.18 × 10^6cfu/ml of *S. cerevisiae* had initial pH of 6.1 and 4.3 at the end of fermentation. The pH of 6.2 was observed for sample C fermented with 1.77 × 10^7cfu/ml of *S. cerevisiae* at the onset of fermentation with a final pH of 4.2 at the end of fermentation. The spontaneously fermented cassava (sample D – control) had a pH of 6.0 at the beginning of fermentation and 4.7 at the end of 72 hours (Figure 2).



A: Sample fermented with 5.90 × 10^4cfu/ml of *Saccharomyces cerevisiae*
B: Sample fermented with 1.18 × 10^6cfu/ml of *Saccharomyces cerevisiae*
C: Sample fermented with 1.77 × 10^7cfu/ml of *Saccharomyces cerevisiae*
D: Spontaneously fermented sample

Figure 2. pH Changes during fermentation of cassava for *lafun* production

The changes in total titratable acidity (TTA) observed in samples A-D is shown in Figure 3. Initial TTA of 0.33 g/l was observed in all the samples except sample B which had a value of 0.34 g/l. At the end of the 72 hours of fermentation, sample A had a value of 9.0 g/l TTA while 9.5 g/l was observed in sample B, 9.9 g/l in sample C and 8.7 g/l in sample D.



A: Sample fermented with 5.90 ×10^4cfu/ml of *Saccharomyces cerevisiae*
B: Sample fermented with 1.18 ×10^6cfu/ml of *Saccharomyces cerevisiae*
C: Sample fermented with 1.77 ×10^7cfu/ml of *Saccharomyces cerevisiae*
D: Spontaneously fermented sample

Figure 3. Changes in total titratable acidity (TTA) during fermentation of cassava for *lafun* production

The proximate analysis of starter fermented and spontaneously fermented *lafun* described as percentage composition is shown in Table1. The highest protein content of 10.33% was observed in sample C while the least value of 1.58% was seen in the spontaneously fermented cassava(sample D). Sample C had the highest ash content of 1.39% while the lowest value of 1.24% was observed in sample D. The value of 2.10% which was the highest for crude fat was seen in sample B. Sample D has the lowest fat content of 0.66%. The highest moisture content was found in sample D with the value of 11.83% and lowest in sample B (10.09%). The highest carbohydrate content was observed in Sample D with a value of 84.69% and lowest in sample C with a value of 76.03%.

Table 1. Proximate analyses of starter fermented and spontaneously fermented cassava for *lafun* production (% composition)

Parameter/ Samples	A	B	C	D
Crude Protein	4.14	6.57	10.33	1.58
Ash	1.30	1.31	1.39	1.24
Crude Fat	1.09	1.66	2.10	0.66
Moisture	10.95	10.09	10.15	11.83
Carbohydrate	82.52	80.37	76.03	84.69

A: Sample fermented with 5.90 $\times 10^4$cfu/ml of *Saccharomyces cerevisiae*
B: Sample fermented with 1.18 $\times 10^6$cfu/ml of *Saccharomyces cerevisiae*
C: Sample fermented with 1.77 $\times 10^7$cfu/ml of *Saccharomyces cerevisiae*
D: Spontaneously fermented sample

Table 2 describes the functional properties of starter fermented and spontaneously fermented cassava. Spontaneously fermented cassava(Sample D) had the highest swelling power of 9.20% while Sample C has the least of 8.61%. The maximum solubility index was observed in sample D with a value of 9.92% and the lowest in sample A (8.42%). The greatest water absorption capacity (99.63%) was shown in sample A while the lowest value of 86.02% was observed in sample D. There was a significant difference in the swelling power and water absorption capacity for all the samples. However, no significant difference was observed for samples B and D regarding solubility.

Table 2: Functional properties of starter fermented and spontaneously fermented cassava for *lafun* production

Sample/ Properties	Swelling power	Solubility index (%)	Water absorption capacity (%)
A	7.50^c	8.42^c	99.63^a
B	8.90^b	9.84^a	88.62^c
C	8.61^b	8.90^b	91.63^b
D	9.20^a	9.92^a	86.02^d

Means in each column with different superscripts represent significant difference ($P \leq 0.05$)
A: Sample fermented with 5.90 $\times 10^4$cfu/ml of *Saccharomyces cerevisiae*
B: Sample fermented with 1.18 $\times 10^6$cfu/ml of *Saccharomyces cerevisiae*
C: Sample fermented with 1.77 $\times 10^7$cfu/ml of *Saccharomyces cerevisiae*
D: Spontaneously fermented sample

The rheological properties of the starter fermented and spontaneously fermented *lafun* samples as described by the pasting properties is shown in Table3. The highest pasting temperature of 87.11^0C was observed in sample A while the lowest temperature of 84.00^0C was seen in sample C. Sample D has the highest peak time of 9.35 minutes. The minimum temperature of 6.05 minutes was observed in sample C. Sample A had the highest values for peak, through and final viscosities while the least values wereshown in sample C. The maximum breakdown viscosity of 39.38 RVU was observed in sample B while the lowest (31.98 RVU) was observed in sample A. There was significant difference in the rheological properties of all the samples except in the breakdown viscosity where there was no significant difference observed between sample B and D.

Table 3. Pasting properties of starter fermented and spontaneously fermented cassava for *lafun* production

Sample /Properties	Pasting temperature (^{0}C)	Peak time (Min)	Peak Viscosity (RVU)	Through Viscosity (RVU)	Final Viscosity (RVU)	Breakdown Viscosity (RVU)	Setback Viscosity (RVU)
A	87.11^a	7.50^c	312.27^a	272.90^a	440.48^a	31.98^c	67.61^b
B	84.90^c	8.45^b	211.52^c	187.21^c	289.79^c	39.38^a	77.20^a
C	84.00^d	6.05^d	195.88^d	163.89^d	235.08^d	33.94^b	60.60^c
D	85.91^b	9.35^a	219.26^b	197.35^b	291.05^b	39.20^a	77.40^a

Means in each column with different superscripts represent significant difference ($P \leq 0.05$)
A: Sample fermented with 5.90 $\times 10^4$cfu/ml of *Saccharomyces cerevisiae*
B: Sample fermented with 1.18 $\times 10^6$cfu/ml of *Saccharomyces cerevisiae*
C: Sample fermented with 1.77 $\times 10^7$cfu/ml of *Saccharomyces cerevisiae*
D: Spontaneously fermented sample

Table 4 describes the sensory evaluation of the starter fermented and spontaneously fermented *lafun*. Sample C with the highest biomass of *S. cerevisiae* has the highest acceptability regarding colour, sample B regarding odour and flavour while sample A with the lowest biomass was least accepted regarding colour and texture. Acceptability regardingodour was lowest in the starter fermented and spontaneously fermented (sample D) while sample B was least acceptedin terms of texture. However, no significant difference was observed in term of colour and texture of both the starter fermented and spontaneously fermented samples.

Table 4. Sensory evaluation of starter fermented and spontaneously fermented cassava for *lafun* production

Sample	Colour	Odour	Flavour	Texture
A	3.80^{a}	3.80^{ab}	3.80^{ab}	2.80^{a}
B	4.00^{a}	4.60^{a}	4.50^{a}	3.00^{a}
C	4.20^{a}	4.20^{ab}	4.40^{a}	3.40^{a}
D	4.00^{a}	3.40^{b}	3.40^{b}	3.20^{a}

Means in each column with different superscripts represent significant difference ($P \leq 0.05$)

A: Sample fermented with 5.90×10^{4}cfu/ml of *Saccharomyces cerevisiae*

B: Sample fermented with 1.18×10^{6}cfu/ml of *Saccharomyces cerevisiae*

C: Sample fermented with 1.77×10^{7}cfu/ml of *Saccharomyces cerevisiae*

D: Spontaneously fermented sample

Discussion

There was a progressive decrease in pH during the fermentation of cassava for *lafun* production with a corresponding rise in total titratable acidity (TTA). These observations are similar to previous reports by Wakil and Benjamin [32]who reported a decrease in pH from6.70 to 3.37, and an increase in TTA ranging from 0.027 mg/ml at 0 hr to 0.722 mg/ml at the end of 120-hour fermentation of cassava for *pupuru* production. The report also agrees with that of Rebouças*et al*.[33] during the fermentation of cassava starch. The pH conveyed by these researchers are lower than that in this present study. The higher pH obtained could be as a result of the use of single starter which might not be able to produce as much acid as compared to fermentation involving several organisms including lactic acid bacteria.

The result from this study revealed a progressive increase in protein content of the starter fermented samples as the *S. cerevisiae* biomass increases. Fermentation of cassava with *Treculia africana* (Africa breadfruit) and cocoyam has been reported to increase the protein content of the products [34,35]. There is dearth of information on the use of *S. cerevisiae*as starter in the fermentation of cassava for *lafun* production. Therefore, results from this study indicate the prospect of the ability of *S. cerevisiae* in the fermentation of cassava for *lafun* production.The amount of protein observed in the starter fermented samples exceeds the previous report in which protein-rich plant materials were utilized [34-37].

Water absorption capacity of starch granules expresses the degree of exposure of the internal structure of the starch granules to water. Thislevel of exposure relates to the viscosity, bulking and consistency of the product [38-40]. Results from the present study show significant differences in the water absorption capacity of the starter fermented and spontaneously fermented samples. These results are in apact with the observation of Bamidele *et al*. [37] who also reported a significant difference inthe water absorption capacity of*analog fufu* flour produced from cassava and cocoyam. The highest swelling power in this study was observed in the spontaneously fermented *lafun*. However, higher values were reported by Moorthy [41] and Kusumayanti *et al*. [28] for cassava starch. This contrast may be because of the differences in the degree of intermolecular association and amylose content of cassava used in the different studies [28].A significant difference was observed in the swelling power and solubility index of the starter fermented and spontaneously fermented of *lafun* in this study. Ogunnaike *et al*. [42] also noted that even though the *lafun* sample studied had same water absorption capacity, they differed significantly in their swelling power.

Pasting temperature is directly proportional to water binding capacity, gelatinization and inversely proportional to the swelling property of starch due to the association between starch granules [25,43]. The pasting temperature observed in this study were in the same range as that observed by Adebayo-Oyetoro *et al*.[25].However, observations from this study are higher than that observed by Ogunnaike *et al*. [42] who reported a temperature range of 76.75^{0}C to 76.85^{0}C in their study of *lafun* using submerged and anaerobic fermentation. Peak viscosity is often linked with the quality of the final product as well as providing an indication of the viscous loads which is likely to be encountered during mixing [44]. Pasting properties observed in this study varied considerably in pasting temperature, peak, trough and final viscosities. Though, no significant difference was noted in the breakdown and setback viscosities of sample B fermented with 1.18×10^{6}cfu/ml of *Saccharomyces cerevisiae* and the spontaneously fermented sample D (control).

The quality of a good *lafun* is described by a characteristic white colour, non-stickiness and little

or no odour [25]. Although no significant difference was observed in the colour and texture of all the samples, sample B has the highest score regarding odour and flavour. Therefore, this study suggests the use of about 1.18×10^6cfu/ml of *Saccharomyces cerevisiae* for the enrichment of *lafun*to give a product with adjacent rheological properties to the naturally fermented product.

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