Production of Fermented Cassava Flour (*Lafun*) using Lactic Acid Bacteria as Starter Culture

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Abstract: *Lafun*, a fermented cassava product, confronts challenges in terms of product quality and safety as a result of spontaneous fermentation systems. To resolve these issues, a production method utilizing starter cultures was developed. Lactic acid bacteria were isolated from fermented cassava and screened for their technological properties. The physicochemical parameters, proximate and antinutrient content of the control fermented *Lafun* samples were determined by standard procedures. A total of twelve (12) lactic acid bacteria were obtained and identified as *Lactobacillus plantarum* (41.67 %), *Lactobacillus. salivarius* (25.00 %), *Lactobacillus kalixensis* (25.03 %) and *Lactobacillus pentosus* (8.3 %). Lactic acid, diacetyl and hydrogen peroxide produced by lactic acid bacteria isolates ranged from 0.06 – 11.70 1.40 – 2.50, and 0.30 - 2.00 (mg/mL) respectively. The pH of the controlled and spontaneous fermentation of cassava ranged from pH 7.20 -3.60 and pH 7.10 – 4.30 and total titratable acidity (TTA) ranged from 1.27 -1.9 and 0.61- 1.55 respectively. Controlled fermented lafun with *L. plantarum* (LF 7) as starter culture had significantly higher ($p < 0.05$) protein, fat, sodium, potassium, iron, zinc, phosphorus, vitamins C, B1, and A while containing lower cyanide, saponin, and phytates 0.10, 0.20, 0.01 mg/g respectively. The study concluded that *L. plantarum* has the potential to improve the nutritional value and degradation of antinutrients in fermented *Lafun* hence, improving food safety and quality.
Keywords: *Cassava, Fermentation, Lactic acid Bacteria, Lafun, Starter culture*

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a perennial shrub grown in the lowland tropics (Food and Agriculture Organization Corporate Statistical Database FAOSTAT, 2020). It is a popular tuber crop for food and industrial products in tropical countries (Reincke *et al.,* 2018). It is processed for detoxification (Westby, 2014) and post-harvest preservation, improving the palatability of derived products (Oyewole *et al.,* 2019). Cassava fermentation is a major method for producing traditional products such as *Abacha, Fufu*, *Gari,* and *Lafun* (Dike *et al.,* 2021) improving the safety, organoleptic, and nutritional qualities of cassava-derived foods. (Smid and Hugenholtz, 2010).

Lafun is Nigerian fermented cassava flour made by peeling and cutting fresh cassava tubers, soaking them in water for 2-5 days at room temperature (28–32^{o} C), sun-drying, and milling. The flour is used to make a stiff porridge, which is often served with stews (Abass *et al.,* 2018). Submerged fermentation of cassava leads to biochemical changes such as cyanogenic degradation, flavor formation, and softening of chunks (Eleazu *et al.,* 2011). However, spontaneous fermentation for Lafun production is dominated by contaminating microorganisms, potentially causing fermentative, pathogenic, toxigenic, or spoilage issues (Huch *et al.,* 2008). High fermentation failure risks produce unsafe, unstable, and unwholesome food products (Kostinek *et al.,* 2008).

The use of starter culture is essential for the controlled fermentation of food substrates, resulting in products with desirable, predictable, and reproducible attributes (Oguntoyinbo *et al.,* 2016; Ogunremi *et al.,* 2017; Laranjo *et al.,* 2019). Lactic acid bacteria are the predominant microorganisms involved in cassava fermentation, possessing strain-specific functional potentials such as producing antimicrobial metabolites, reducing antinutritional factors, and increasing energy density (Ogunremi *et al.,* 2015; Onipede *et al.,* 2021).

Starter cultures have been developed to improve the quality attributes of other fermented products, such as fufu and gari, however, there is limited information on starter cultures for lafun production.

Therefore, this study aims to improve the nutritional value and safety of *lafun* by controlled fermentation of cassava tuber, using indigenous LAB strains.

MATERIALS AND METHODS

Collection of samples

Fresh cassava roots were harvested and obtained from Ajala village, Oluyole Local Government, Ibadan, and collected in a sac bag. They were transported to the Microbiology and Biotechnology Laboratory of First Technical University, Ibadan.

Spontaneous fermentation of cassava

Approximately 20 kg of the cassava roots were washed, peeled, sliced into pieces (about 5 cm x 2 cm), and steeped into 10 L of distilled water for fermentation at ambient temperature (28 ± 35 ^{o}C) for 72 hours.

Isolation of lactic acid bacteria isolates

Ten grams of the steeped cassava sample was analyzed for microbiological analysis and added to 90 ml of sterile 0.1% peptone water (Oxoid, UK) and homogenized by using a vortex machine (CM-101 Remi Cyclo Mixture, 1000 RPM) for 10 seconds. The homogenate was further diluted to ten-fold serial dilution. Dilution 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5,}$ and 10^{-6} were pour-plated in sterile De Man Rogosa Sharpe (MRS) agar plates (Oxoid, UK) in duplicates and incubated anaerobically in Gas Pak jars (GasPak System, BBL) at 30°C for 48 h. Representative colonies were randomly picked and purified by repeated streaking on MRS agar plates. Pure cultures were grown on MRS agar slants and kept at 4^{o}C for further use.

Physiological and biochemical characterization of lactic acid bacteria isolates

The isolates were tested for physiological and biochemical characteristics, including Gram staining, citrate, indole, oxidase, methyl red, Voges-Proskauer, and sugar fermentation test by using standard procedure (Harrigan and McCance, (1978); Olutiola *et al.,* (1991); Harrigan, (1998); Klein *et al.,* 2003); Axelsson *et al.,* 2004), growth at different pH and 4% NaCl, starch hydrolysis (Edward *et al.,* 2012), casein hydrolysis (Garcia-Cano *et al.,* 2019), and gelatin hydrolysis (Axelsson, 2004). Probable lactic acid bacteria were identified and confirmed using Bergey's Manual of Systematic Bacteriology and Automated Biometric Identification System (ABIS online)

Technological properties of lactic acid bacteria isolates

Lactic acid production

The AOAC (2000) method was used to determine the lactic acid produced by lactic acid bacteria isolates. It involved titrating 25 mL of 24h-old broth cultures with 0.1N NaOH until a pink color appeared, with phenolphthalein (1 % w/v as an indicator. Each ml of 0.1N NaOH is equivalent to 90.08mg of lactic acid.

$$\text{Lactic acid} = \frac{\text{Volume of NaOH (mL)} \times \text{Normality of NaOH} \times \text{Lactic acid equivalent(mg)}}{\text{Volume of the sampe used (mL)}}$$

***Equation* 1**

Diacetyl production

The amount of diacetyl produced by the lactic acid bacteria isolates was estimated using Sanni, (1995) method. Twenty-five milliliters (25 ml) of MRS broth of the test isolates were aliquoted in 250 ml conical flasks, and 7.5 ml hydroxyl amine solution was used for residual titration. The flasks were titrated with 0.1N HCl to a green-yellow endpoint using bromophenol blue an as indicator. The equivalent factor of HCl to diacetyl is 21.52mg.

$$\mathbf{AK} = \frac{(\mathbf{B} - \mathbf{S})(\mathbf{100} - \mathbf{E})}{\mathbf{W}} \quad \boldsymbol{Equation\ 2}$$

AK = Percentage of diacetyl, B = ml of 0.1N HCl consumed in the titration of the sample, E =Equivalent factor of 1 mL of 0.1N HCl to diacetyl=21.52mg; W=Volume

of sample used, S= Volume of ml 0.1N HCl consumed in the titration of 7.5 mL Hydroxyl amine.

Hydrogen peroxide production

Twenty milliliters (20 ml) of 0.1M H_2SO_4 was added to 25 mL of the MRS broth cultures of the test isolates (24h). Titration was carried out with 0.1N potassium permanganate. Each mL of 0.1M H_2SO_4 is equivalent to 1.70 mg of Hydrogen peroxide and decolorization of the sample was regarded as endpoint (A.O.A.C. 2000).

$$\text{Lactic acid} = \frac{\text{KMnO4(mL)} \times \text{NKMnO4} \times \text{M.E} \times 100}{\text{H2SO4(mL)} \times \text{Volume of sample used}} \quad \textit{Equation 3}$$

Controlled fermentation of cassava for the production of *lafun* using selected lactic acid bacteria as a starter culture

Inoculum preparation and application

The selected lactic acid bacteria as potential starter culture was inoculated in MRS broth and incubated anaerobically in Gas Pak jars (GasPak System, BBL) at 30°C for 24 h. The culture was centrifuged at 3,000 rpm for 4 minutes, and the supernatant was discarded. The cell pellets were washed and resuspended in 0.9% normal saline solution, standardized to 0.1 absorbances at 600 nm for starter culture application. Five milliliters (5 ml) of the Inoculum were inoculated in 10 kg blanched cassava, steeped in 5 L of distilled water, and allowed to ferment for 96 hours at ambient temperature. An uninoculated batch was used as a control.

Determination of pH and total titratable acidity

Fermented cassava samples were aseptically taken at 24-hour intervals for pH and TTA evaluation. A digital pH meter (HANNA INSTRUMENT 8021) was used to measure pH, while total titratable acidity (TTA) was assessed using AOAC (2000) methods. Ten grams of the sample was titrated against 0.1M sodium hydroxide solution, resulting in a faint pink color endpoint (pH 8.3). One milliliter of 0.1M NaOH was equivalent to 9.008 mg of lactic acid (A.O.A.C. 2000).

TTA = Volume (ml) of NaOH × Normality of NaOH× Lactic acid equivalent ÷ Volume of sample used (Equation 4)

Production of lafun

The fermented cassava was drained, oven dried at 55 ^{C} for 72 h (3606 Thermo Oven Lab-line Vacuum), dried milled into *Lafun* flour (VTCL Excella Grinder-1000W), sieved fine mesh of 0.5mm pore size, stored at room temperature in air-tight zip-lock bags and kept at 4^{O}C until proximate, nutritional and antinutritional analyses.

Determination of the proximate composition of the *lafun* samples

Determination of moisture content

Moisture content was determined by drying clean crucibles in a hot air oven at 100 °C for 1 h to obtain a constant weight and then cooled in a desiccator. Two grams of each of the samples were then weighed into the different crucibles and dried at 100 °C until a constant weight is obtained (AOAC, 2012).

% moisture content=$W_2 - W_3$ x100 ÷ W2−W1 (Equation 5)

Where, W_1 = Initial weight of the empty crucible; W_2 = weight of dish + sample before drying; W_3 = weight of dish + sample after drying.

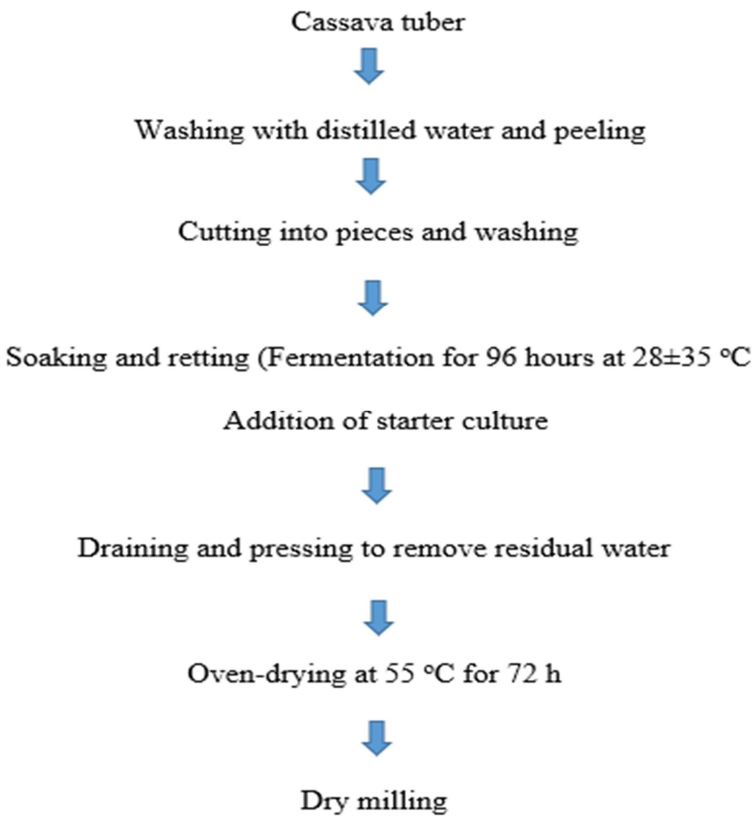

Figure 1: Production flowchart of *lafun* (Abass *et al.*, 2018 with modification)

Determination of fat

The Soxhlet extraction method was used to determine fat content as described by AOAC (2012). A Soxhlet extractor with a reflux condenser and a 500 ml round bottom flask was used. Two grams of sample were weighed into a labeled thimble, and 300 ml of petroleum ether was filled into the flask. The extractor thimble was sealed with cotton wool. The Soxhlet apparatus refluxed for 6 hours, and the thimble was removed. Petroleum ether was collected, dried at 105°C for 1 hour, and oven-cooled in a desiccator before being weighed.

% Fat =Weight of fat x 100 ÷ Weight of sample (Equation 6)

Where, F = Percent fat content; W_1 = Initial weight of flask and sample; W_2 = Final weight of flask and sample after extraction

Determination of crude protein

The micro-Kjeldahl method (AOAC, 2012) was used to determine the percentage of protein in flour samples. One gram of each lafun flour sample was weighed into a Kjeldahl flask, and 2.5 g of anhydrous Na_2S0_4, 0.5 g of $CUSO_4$, and 5 ml of concentrated H_2S0_4 were added. The flask was heated in a flame chamber, and the content was transferred to a volumetric flask. The digest was then distilled using a 5 ml volume of each sample digest mixed with 5 ml of Boric acid indicator and 3 drops of methyl red in a conical flask. The distillation was done for 5 minutes until the color changed from purple to green.

Five milliliters of the distillate were as collected and titrated against 0.01 N HC1 to obtain a purple-colored endpoint. The percentage protein was calculated using the following expression:

% Nitrogen= T x 14.01 x 0.01 x 20 x 100 ÷ 1.0 x 100 (Equation 7)

Where, *T* = Titre value; 1.0 *g* = Weight of the sample 20 = Dilution factor (i.e. from 10,015) 0.01 = Normality of HCl 14.01 = Atomic mass of nitrogen

Determination of total ash

The AOAC (2012) procedure was used to determine ash content in a mixture of well-blended samples. Two grams of samples were weighed into a shallow ashing dish, ignited, and cooled. The crucibles and their contents were then transferred to a muffle furnace at 550°C. After 8 hours of ashing, the crucible and sample were removed, moistened with water, dried in an oven at 100°C, and re-ashed at 550°C for an additional hour. The percentage of ash was calculated using the following expression:

% Ash = Weight of ash X 100 ÷ Weight of sample used (Equation 8)

Determination of crude fiber

The AOAC (2012) method was used to determine crude fiber. Two grams of the sample were weighed, boiled in 200 ml 1.25% H_2SO_4 for 30 minutes, filtered through linen or muslin cloth fixed to a funnel, washed with boiling water, and then returned to 200 ml of boiling NaOH. The residue was then washed with 1% HCl and drained. The final residue was transferred to a silica ash crucible, dried, and cooled. Percent crude fiber was calculated using the expression

% Crude fiber = Loss in weight on ignition x 100 ÷ Weight of the sample (Equation 8)

Determination of carbohydrate content (By difference)

The total carbohydrate content was estimated as the difference between 100 and the total sum of moisture, fat, protein, crude fiber, and ash as described by AOAC (2012).

Determination of vitamins and mineral

Riboflavin, thiamine, niacin, and ascorbic acid were determined using standard procedures as described by A.O.A.C (2012). The mineral including sodium, potassium, iron, zin,c, and phosphorus content of the *lafun* samples was carried out using atomic absorption spectrophotometer according to the method of Hernandez, *et al.* (2004).

Determination of antinutrients of lafun samples

Phytates content in *lafun* samples was determined using the bipyrimidine colorimeter method as described by Onwuka, (2005). Saponin content was analyzed by the double solvent extraction gravimetric method AOAC (2005), while hydrogen cyanide (HCN) was determined by the alkaline picrate colorimetric method as described by Onwuka, (2005).

Statistical Analysis

Results were presented as means with a standard deviation of triplicate values and were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0). Significant differences between means were determined at t 95 confidence limit ($p< 0.05$) and were compared using Duncan multiple range test.

RESULTS

Identification and technological properties of the lactic acid bacteria isolates

A total of twelve (12) lactic acid bacteria were obtained and identified as *Lactobacillus plantarum* (41.67 %), *Lactobacillus*. *salivarius* (25.00 %), *Lactobacillus kalixensis* (25.03 %) and *Lactobacillus pentosus* (8.3 %) (Figure 2).

Lactic acid, diacetyl, and hydrogen peroxide produced by the LAB isolates ranged from 0.06 – 11.70 (mg/mL), 1.40 – 2.50 (mg/mL), 0.30 - 2.00 (mg/mL) respectively (Table 1). The growth at different pH and 4% NaCl were shown in Table 2. *L. plantarum* LF7 had the highest absorbance at pH 3 (0.46_{600nm}) and pH 12 (1.15_{600nm}). The absorbance of LAB isolates in 4% of NaCl ranged from 0.24 - 0.65.

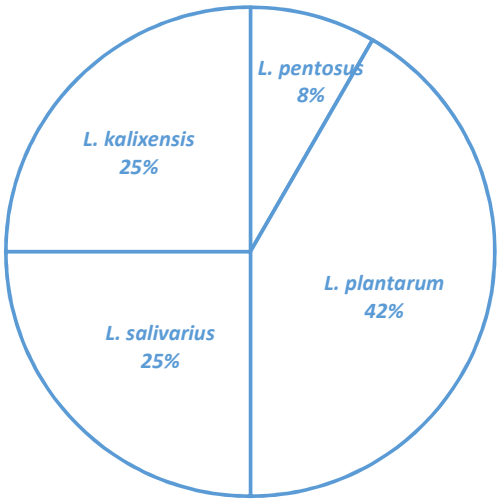

Figure 2: Percentage occurrence of the LAB isolates from fermenting cassava

Table 1: Production of Lactic acid, Diacetyl, and Hydrogen peroxide produced by the Lactic acid bacteria isolates

LAB isolate	Lactic acid (mg/mL)	Diacetyl (mg/mL)	Hydrogen peroxide (mg/mL)
L. pentosus LF1	9.50±0.20^{e}	1.80±0.20^{a}	1.70±0.20^{b}
L. plantarum LF2	4.10±0.20^{d}	2.1±0.20^{a}	1.50±0.20^{b}
L. plantarum LF3	2.00±0.20^{c}	2.30±0.20b	1.30±0.20^{b}
L. plantarum LF4	4.50±0.20^{d}	1.08±0.20^{a}	1.10±0.20^{b}
L. salivarius LF5	1.00±0.20^{b}	1.73±1.10^{a}	1.60±0.20^{b}
L. kalixensis LF6	1.40±0.20^{b}	1.80±0.20^{a}	1.10±0.20^{b}
L. plantarum LF7	11.70±0.20^{f}	2.50±0.20^{b}	2.00±0.20^{c}
L. kalixensis LF8	0.60±0.20^{a}	2.10±0.20^{b}	1.80±0.20^{b}
L. plantarum LF9	0.70±0.20^{a}	1.50±0.20^{a}	1.10±0.20^{b}
L. plantarum LF10	1.30±0.20^{a}	1.40±0.20^{a}	1.20±0.20^{b}
L. kalixensis LF11	2.30±0.20^{c}	2.20±0.20^{b}	1.10±0.20^{b}
L salivarius LF12	1.10±0.20^{b}	1.50±0.20^{a}	0.30±0.20^{a}

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letters within each column are significantly different ($p< 0.05$) using Duncan multiple range test

Table 2: Optical density$_{600nm}$ of the LAB isolates of different pH and 4% NaCl

LAB isolate	pH 3	pH 7	pH 12	4% NaCl
L. pentosus LF1	0.28±0.0020^{a}	0.55±0.0023^{c}	0.72±0.0020^{d}	0.40±0.0008^{c}
L. plantarum LF2	0.34±0.0020^{b}	0.58±0.0020^{c}	1.01±0.0020^{e}	0.62±0.0008^{c}
L. plantarum LF3	0.28±0.0020^{a}	0.22±0.0020^{a}	0.65±0.0020^{c}	0.35±0.0008^{b}
L. plantarum LF4	0.35±0.0020^{b}	0.53±0.0020^{c}	0.83±0.0020^{d}	0.39±0.0008^{b}
L. salivarius LF5	0.25±0.0020^{a}	0.23±0.0020^{a}	0.86±0.0020^{d}	0.24±0.0008^{a}
L. kalixensis LF6	0.22±0.0020^{a}	0.56±0.0020^{c}	0.62±0.0020^{c}	0.55±0.0008^{c}
L. plantarum LF7	0.46±0.0020^{c}	0.54±0.0020^{c}	1.16±0.0020^{e}	0.25±0.0008^{a}
L. kalixensis LF8	0.24±0.0020^{a}	0.58±0.0020^{c}	0.72±0.0020^{d}	0.25±0.0008^{a}
L. plantarum LF9	0.29±0.0020^{a}	0.72±0.0020^{d}	0.87±0.0020^{d}	0.57±0.0008^{c}
L. plantarum LF10	0.23±0.0031^{a}	0.80±0.0020^{d}	0.89±0.0020^{d}	0.31±0.0008^{b}
L. kalixensis LF11	0.30±0.0020^{b}	0.62±0.0020^{c}	0.82±0.0020^{d}	0.55±0.0008^{c}
L salivarius LF12	0.33±0.0020^{b}	0.59±0.0200^{c}	0.70±0.0020^{d}	0.26±0.0008^{a}

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letters within each column are significantly different (p< 0.05)) using Duncan multiple range test

pH and Total Titratable acidity of the controlled and spontaneous fermented cassava samples

The pH of the controlled and spontaneous fermentation of cassava ranged from pH 7.20 -3.60 and pH 7.10 – 4.30 and total titratable acidity (TTA) ranged from 1.27 - 1.9 and 0.61- 1.55 respectively (Table 3)

Proximate composition and antinutrient of controlled and spontaneous Lafun samples

Lafun produced by controlled fermentation using *L. plantarum* LF7 had significantly higher ($p < 0.05$) contents of protein (5.24 %), fat (0.25 %), sodium, potassium, iron, zinc, phosphorus (596.1, 294.2, 8.5, 2.4, and 191.7 mg/100g) respectively and vitamins C, B1, and A, (4.3, 0.23, 0.20 mg/100g) respectively, while spontaneous fermented *lafun* samples had significantly higher ($p < 0.05$) contents of carbohydrate, crude fiber and ash content at 83.50, 3.41and 4.90 (%) respectively. (Table 4a and 4b). Significant lower values ($p < 0.05$) of cyanide (0.10 mg/g), saponin (0.20 mg/g,) and phytates (0.01 mg/g) content were observed in controlled fermented *lafun,* compare to spontaneous fermented *lafun* (Table 4c).

Table 3: Physicochemical properties of fermented cassava

	CFL		SFL	
	pH	TTA (g/L)	pH	TTA (g/L)
0 hour	7.2±0.10	1.27±0.01	7.1±0.10	0.61±0.01
24 hours	5.0±0.10	1.40±0.10	6.7±0.10	0.74 ±0.01
48 hours	4.5±0.10	1.65±0.01	5.5±0.10	0.91 ±0.01
72 hours	4.1±0.10	1.82±0.01	4.8±0.10	1.35 ±0.01
96 hours	3.6±0.10	1.97±0.01	4.3±0.10	1.55±0.01

Values are the Means ± Standard Deviation where n = 3 CFL: Controlled fermented *lafun*; SFL: Spontaneously fermented *lafun*

Table 4a: Proximate composition of the *lafun* samples

Sample	Protein %	Moisture%	CHO %	Fiber %	Ash %	Fat %
CFL	5.24 ±0.01[a]	4.33±0.01[a]	81.9±0.01[a]	3.30±0.01[a]	4.03±0.01[a]	0.25±0.01[a]
SFL	3.68±0.01[b]	5.90±0.10[b]	83.50±0.10[b]	3.41±0.01[b]	4.90±0.10[b]	0.12±0.01[b]

Table 4b: Mineral and vitamin composition of *lafun* samples (mg/100g)

Samples	Sodium	Potassium	Iron	Zinc	Phosphorus	Vit. C	Vit. B1	Vit. A
CFL	596.4± 0.10[a]	294.2± 0.10[a]	8.5± 0.10[a]	2.4± 0.10[a]	191.7± 0.10[a]	4.3± 0.10[a]	0.23± 0.01[a]	0.20± 0.01[a]
SFL	349.3± 0.49[b]	131.2± 0.10[b]	3.5± 0.10[b]	2.1± 0.10[b]	140.0± 0.10[b]	4.1± 0.10[b]	0.18± 0.01[b]	0.18± 0.01[b]

Table 4c: Antinutrient content of the *lafun* samples (mg/100g)

Sample	Cyanide	Saponin	Phytates
CFL	0.10±0.01[a]	0.20±0.01[a]	0.01±0.01[a]
SFL	0.40±0.10[b]	0.50±0.10[b]	0.43±0.01[b]
RC	0.98±0.01[c]	-	-

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letters within each column are significantly different ($p < 0.05$). CFL: Controlled fermented *lafun*; SFL: Spontaneous fermented *lafun*. RC: Raw cassava

DISCUSSION

Previous studies have reported *L. plantarum* being the predominant LAB species in spontaneously fermenting cassava as observed in this study. This may be due to its limited nutritional requirements, which make it advantageous for substrate metabolism compared to other *Lactobacillus* species (Padonou *et al.,* 2009b; Makimattila *et al.,* 2011; Ben Omar *et al.,* 2017).

L. plantarum LF7 produced the highest amount of lactic acid, diacetyl, and hydrogen peroxide, which lowers the pH of fermenting medium and produces inhibitory bioactive compounds like diacetyl and hydrogen peroxide. These compounds have antimicrobial activity and may be produced by different LAB strains, as Tannock (2004) linked production levels and proportions to strains, medium compounds, and physical parameters. They also inhibit the growth and multiplication of pathogenic organisms in fermented foods (Omafuvbe and Enyioha, 2011). The growth of LAB isolates at pH 3 aligns with Cotter and Hill's (2013) findings, which suggest that LAB's relative ATPase activities at low pH contribute to their prophylactic effects and enhance their probiotic potential. These organisms can survive in stomachs with low pH levels, such as 1.5. *L. plantarum* LF7 was chosen as a starter for controlled fermentation cassava to produce Lafun, based on its antimicrobial compounds and stress tolerance. The acidification level in spontaneous lafun fermentation is comparable to Padonou *et al.* (2009). This leads to a reduction in pH and the production of inhibitory bioactive compounds like diacetyl and hydrogen peroxide, which are responsible for antimicrobial activity (Assanvo *et al.,* 2016). The higher crude protein content in controlled fermented lafun may be due to the production of more organic acids by *L. plantarum* LF7, which supports the growth and proliferation of yeasts which are single-cell proteins (Boonnop *et al.,* 2019). Bala (2012) suggests that this may be due to the secretion of extracellular enzymes and microbial cell growth. The carbohydrate content of lafun samples was within the range reported by Alamu *et al.* (2017), making them suitable for starch production. The crude fiber content in controlled fermented lafun and spontaneous fermented lafun were consistent with Afoakwa *et al.*

(2012) findings and Gil and Buitrago's Consuming adequate dietary fiber reduces the risk of diseases like constipation, obesity, coronary heart disease, and colon cancer (Dahl and Stewart, 2015). The processing of cassava roots into flour allows for moisture loss through the drying process, resulting in low moisture content of cassava flour, which confers high resistance to microbial infestation, resulting in extended shelf life during storage Udoro *et al.* (2020). Controlled fermentation using *L. plantarum* produced lafun with significantly higher vitamin C, B1, and A content compared to spontaneously fermented lafun. Vitamins play a role as antioxidants, helping combat free radicals. Plant-based foods are known to be good sources of vitamins (Eleazu *et al.,* 2012). The increased mineral content in controlled fermented lafun may be due to the decrease in phytates as fermentation progresses. Day and Morawicki (2018) suggest that the increase in mineral content may be due to the loss of dry matter during fermentation, as microbes degrade carbohydrates and protein. Sripriya *et al.* (2017) suggested that fermentation increase the bioavailability of calcium, phosphorus, and iron due to the degradation of oxalates and phytates that complex with minerals, reducing their bioavailability. The loss of phytates during fermentation is caused by enzymes phytases and phosphatase, which hydrolyze phytates into inositol and (2002) recommendations. orthophosphate (Tefera *et al.,* 2014). Cassava contains cyanogenic glucosides like linamarin and lotaustralin, which are recommended to be <10mg cyanide equivalents/kg DM to prevent acute toxicity to humans. Fermentation can reduce the cyanide content of cassava, as demonstrated by Niguse *et al.* (2019). After 96 hours of fermentation with *Lactobacillus plantarum* LF7, the free cyanide level dropped from 0.98mg/g of non-fermented cassava to 0.1 mg/g. *Lactobacillus plantarum* plays a crucial role in cyanide detoxification, indicating that fermentation can significantly reduce the residual HCN content of cassava. The reduction in cyanide content can be attributed to the inoculated microorganisms producing linamarase, which hydrolyzes linamarin and converts cyanogenic glycosides into HCN, which is then converted to formamide, a nitrogen and carbon source (Niguse *et al.,* 2019).

CONCLUSION

This study found that *Lactobacillus plantarum* (LF7) as a starter culture has the potential for improving lafun nutritional value and reducing antinutrient degradation. This bio-enrichment could increase cassava root usage for valuable products. Further research should focus on improving starter culture to enhance cassava root fermentation bio-enrichment.

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