



(RESEARCH ARTICLE)



Enhancing the nutritional quality of fufu with a starter culture

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GSC Biological and Pharmaceutical Sciences, 2024, 26(03), 092–102

Publication history: Received on 26 January 2024; revised on 11 March 2024; accepted on 14 March 2024

Article DOI: <https://doi.org/10.30574/gscbps.2024.26.3.0082>

Abstract

This study investigates the nutritional enrichment of fufu, a staple African food, by controlling the fermentation of cassava root tuber using a starter culture. Lactic acid bacteria (LAB) were isolated from fermented cassava and analyzed for their technological properties. The physicochemical parameters, proximate and antinutrient content of the fufu samples were determined by standard analytical methods. Twelve LAB were identified as *Lactobacilli plantarum* (42%), *L. acidophilus* (25%), *L. fermentum* (17%), *L. brevis* (8%), and *L. mesenteroides* (8%). The LAB isolates produced lactic acid, diacetyl, and hydrogen peroxide ranging from 1.90-2.90, 1.30-2.10, and 1.10 -2.90 mg/mL respectively. *Lactobacillus plantarum* (FF8) was selected as a starter culture due to its exceptional ability to produce antimicrobial substances, leading to higher yields of lactic acid, diacetyl, and hydrogen peroxide, reducing the fermenting medium's pH. The pH changes in starter-induced fermented fufu (SIFF) and spontaneous fermented fufu (SFF) samples from 0 to 96 hours were 7.10 - 2.60 and 7.10 - 3.30, respectively, while the Total Titratable Acidity (TTA) increased from 0.71-1.79 and 0.28-0.51, respectively. Starter-induced fermented fufu (SIFF) has higher protein, fat, sodium, potassium, iron, zinc, phosphorus, and Vit. C, B1, and A content of 2.93, 0.23 (%), 596.4, 270.9, 8.93, 1.67, 296.67, 5.28, 0.24, and 0.31 (mg/100g) respectively, compared to spontaneous fermented fufu and a significant decrease in antinutrient content, such as cyanide, saponin, and phytates of 0.05, 0.16, and 0.06 (mg/100g), respectively. The study found that *L. plantarum* FF8 used as a starter culture, improves the nutritional value of fufu and reduces anti-nutrients, suggesting potential health benefits for consumers.

Keywords: Cassava; Fermentation; Fufu; Lactic acid Bacteria; Starter culture

1. Introduction

Cassava (*Manihot esculenta crantz*), a staple food crop in Nigeria, a starch-rich root that provides carbohydrates and energy for sub-Saharan Africa's population [1, 2,3]. It makes up 85% of the total weight and is highly carbohydrate-rich, and deficient in protein, riboflavin, thiamin, and niacin concentrations [4, 5]. Cassava is processed before consumption to detoxify, avoid post-harvest deterioration, reduce toxicity, improve product palatability, preserve and modify [6,7,8,9]. Fermentation is a suitable method for processing cassava roots. It enhances the safety, organoleptic, and nutritional quality of cassava-derived foods [10, 11].

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Cassava root is processed into various food items such as fufu, gari, and lafun. Fufu, a fermented product, provides 70% of Nigeria's daily calories [12]. Traditional processing involves peeling, washing, cutting, and steeping in water for 4-5 days. The softened root is then pulverized, sieved, and allowed to settle for decantation, resulting in raw fufu [13].

The fermentation of cassava for fufu production involves a dominant microbiota, including yeast species such as *Saccharomyces cerevisiae*, *Pichia scutulata*, and *Kluyveromyces marxianus*, as well as lactic acid bacteria (LAB) such as *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and spore formers such as *Bacillus cereus* [14, 15]. Lactic acid fermentation is the most effective technique for processing cassava into different products due to its ability to generate antimicrobials, decrease antinutritional components, and enhance density [16, 17, 18, 19].

The controlled fermentation of cassava products is attributed to the use of microbial starter cultures, which contain a large number of viable microorganisms, primarily LAB, which often yields consistent products [20, 21, 22, 23]. Starters accelerate the fermentation process, antagonize undesirable microorganisms, and improve the final product's organoleptic and sensory attributes [24, 25, 26, 27, 28].

The development of a simple processing method for fufu can enhance its nutritional content and quality, thereby increasing consumer acceptability and ensuring its survival in the food chain. Therefore, this study aims to explore the potential nutritional enrichment of fufu using a starter culture isolated from the current study.

2. Materials and methods

2.1. Collections of samples

Fresh cassava roots were collected from Ajala village, Oluyole Local Government, Ibadan, Oyo state, and transported to the Microbiology and Biotechnology Laboratory of First Technical University, Ibadan for further processing.

2.2. Isolation of lactic acid bacteria (LAB)

Five kilograms (5 kg) of cassava root was washed, peeled, sliced into pieces, and steeped in distilled water for 72 hours at ambient temperature (28 ± 35 °C). The sample was then analyzed for microbiological analysis, added to sterile 0.1% peptone water (Oxoid, UK), and homogenized using a vortex machine (CM-101 Remi Cyclo Mixture, 1000 RPM) for 10 seconds. The homogenate was diluted to ten-fold serial dilution and then plated in sterile De Man Rogosa Sharpe (MRS) agar plates (Oxoid, UK). The colonies were incubated anaerobically in Gas Pak jars at 30°C for 48 hours. Representative colonies were randomly picked and purified, and pure cultures were grown on MRS agar slants and stored at 4°C for further use.

2.3. 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 tests by using standard procedure [29, 30, 31, 32], growth at different pH, 4% NaCl, starch hydrolysis casein hydrolysis and gelatin hydrolysis [33, 34]. Probable lactic acid bacteria were identified and confirmed using Bergey's Manual of Systematic Bacteriology [35] and Automated Biometric Identification System (ABIS online).

2.4. Technological properties of lactic acid bacteria isolates

2.6.1 Lactic acid production

The Association of Official Analytical Chemists (AOAC), [36] method was used to determine lactic acid produced by lactic acid bacteria isolates by titrating 25 mL of 24 h broth cultures with 0.1N NaOH until a pink color appeared, with phenolphthalein as an indicator. Each ml of 0.1N NaOH is equivalent to 90.08mg of lactic acid.

$$\text{Lactic acid} = \text{Volume of NaOH (mL)} \times \text{Lactic acid equivalent (mg)} \div \text{Volume of the samples used (mL)} \dots \dots$$

Equation 1

2.6.2 Diacetyl production

The Sanni [37] method was used to estimate the amount of diacetyl produced by lactic acid bacteria isolates. Twenty-five milliliters (25 mL) of MRS broth test isolates were aliquoted in 250 ml conical flasks, and residual titration was performed with 7.5 ml hydroxyl amine solution. The titration resulted in a green-yellow endpoint using bromophenol blue as an indicator. The equivalent factor of HCl to diacetyl is 21.52mg.

$$AK = (B - S)(100 - E) \div W \dots \dots \dots \text{Equation 2}$$

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.

2.6.3 Hydrogen peroxide production

Twenty milliliters (20 mL) of 0.1 M H₂SO₄ were added to 25 mL of the MRS broth cultures of the test isolates (24 h). Titration was carried out with 0.1 N potassium permanganate. Each mL of 0.1 M H₂SO₄ is equivalent to 1.70 mg of Hydrogen peroxide and decolorization of the sample was regarded as an endpoint [36].

$$H2O2 = KMnO4 (mL) \times NKMnO4 \times ME \times 100 \div H2SO4 (mL) \times Volume of sample used \dots \dots \dots \text{Equation 3}$$

2.5. Production of starter-induced fermented fufu (SIFF) and spontaneous fermentation fufu (SFF)

2.5.1 Inoculum preparation

The selected lactic acid bacteria as potential starter culture was inoculated in MRS broth and incubated anaerobically in Gas Pak jars (Gas Pak 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.

2.5.2. Production of fufu samples

The study involved two fermentation methods for the production of fufu samples. The production of the SIFF involved the inoculation of 5 mL of the inoculum into 10 kg of blanched cassava, steeped in 15 liters of distilled water, and allowed to ferment anaerobically at a temperature of 37 °C ± 2 °C for 96 hours. In the production of SFF, involved washing, peeling, slicing, and steeping 10 kg of cassava roots in 15 liters distilled water for 72 hours without adding starter culture. The fermented cassava samples were pulverized, sieved, and sedimented for 12 hours, and the resulting water was then decanted. The fufu mash, obtained through two processing methods, were drained, dried in an oven at 55°C (Thermo Oven Lab-line Vacuum) for 72 hours, milled into powder using a VTCL Excella Grinder-1000W, packed in zip-lock bag, and stored at 4°C for proximate and antinutritional analyses.

2.7 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 methods [36]. 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 [36].

$$TTA = Volume (mL) of NaOH \times Normality of NaOH \times Lactic acid equivalent \div Volume of samples uses \dots \dots \dots \text{Equation 4}$$

2.8 Determination of the proximate composition of the lafun samples

2.8.1 Determination of moisture content

The moisture content of the fufu samples were determined by drying clean crucibles in a hot air oven at 100°C for 1 hour, then cooling in a desiccator. Two grams of each sample were then weighed and dried at 100°C until a constant weight was obtained [38].

$$\% \text{ moisture content} = W2 - W3 \times 100 \div W2 - W1 \dots \dots \dots \text{Equation 5}$$

W1 = Initial weight of the empty crucible; W2 = weight of dish + sample before drying; W3 = weight of dish + sample after drying

2.8.2 Determination of fat

The Soxhlet extraction method was used to determine fat content of the fufu samples, following AOAC's guidelines [38]. 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, refluxed for 6 hours, and collected. Petroleum ether was dried at 105 °C for 1 hour and oven-cooled before weighing.

$$\% \text{ Fat} = \text{Weight of fat} \times 100 \div \text{Weight of sample} \dots \text{Equation 6}$$

2.8.3 Determination of crude protein

The micro-Kjeldahl method was used to determine protein percentage in the fufu samples. A gram of each sample was weighed into a Kjeldahl flask, and 2.5 g of anhydrous Na₂SO₄, 0.5 g of CuSO₄, and 5 mL concentrated H₂SO₄ were added. The flask was heated in a flame chamber, then transferred to a volumetric flask. The digest was distilled, mixed with 5 mL boric acid indicator and 3 drops methyl red, and titrated against 0.01 N HCl, resulting in a purple-colored endpoint. [38]. The percentage protein was calculated using the following expression.

$$\% \text{ Nitrogen} = T \times 14.01 \times 0.01 \times 20 \times 100 \div 1.0 \times 100 \dots \text{Equation 7}$$

T = Titer 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

2.8.4 Determination of total ash

The AOAC procedure [38] was used to determine ash content in well-blended fufu samples. Two grams of each sample were weighed, ignited, and cooled before being transferred to a muffle furnace at 550°C. After 8 hours, the sample was moistened, dried, and re-ashed at 550 °C for an additional hour. The percentage of ash was calculated using the following expression.

$$\% \text{ Ash} = \text{Weight of Ash} \times 100 \div \text{Weight of sample used} \dots \text{Equation 8}$$

2.8.5 Determination of crude fiber

The AOAC method [38] was used to determine crude fiber by boiling two grams of sample in 200 mL 1.25% H₂SO₄ for 30 minutes, filtering through cloth, washing with water, returning to 200 mL NaOH, washing with 1% HCl, draining, and drying. The residue was then transferred to a silica ash crucible, dried, and cooled. Percent crude fiber was calculated using this method.

$$\% \text{ Crude fiber} = \text{Loss in weight on ignition} \times 100 \div \text{Weight of the sample} \dots \text{Equation 9}$$

2.8.6 Determination of carbohydrate

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 [38].

2.8.7 Determination of vitamins and mineral

The study analyzed riboflavin, thiamine, niacin, and ascorbic acid using standard procedures and mineral analysis methods [38]. Samples were ashed (Lenton muffle furnace AF11/6) at 550 °C, boiled with 10 mL 20 % HCl, and filtered. The minerals sodium and potassium were determined using a flame emission photometer, with NaCl and KCl as standards (AOAC, 2005). Phosphorus was determined calorimetrically using the spectronic 20 with KH₂PO₄ as the standard. All values were expressed in mg/100 g.

2.8.8 Determination of antinutrients

The rapid test method of AOAC [39] was used to determine the anti-nutrients including phytates, saponin and hydrogen cyanide in the fufu samples.

2.9 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 (Free SPSS version 16.0). Significant differences between means were determined at 95% confidence limit (p< 0.05) and were compared using Duncan multiple range test.

3 Results

Twelve (12) lactic acid bacteria (LAB) were identified as *L. plantarum* (42%), *L. acidophilus* (25%), *L. fermentum* (17%), *L. brevis* (8%), and *L. mesenteroides* (8%) (Figure 1). The LAB isolates produced lactic acid, diacetyl, and hydrogen peroxide ranging from 1.90 -2.90, 1.30 -2.10, and 1.10 -2.90 mg/mL respectively (Table 1). Table 2 shows the optical density of LAB isolates at pH 3, 7, and 12, and growth at 4% NaCl at 600nm. *L. plantarum* (FF8) grew better at extreme pH values 3 and 12 and 4% NaCl of 0.39, 1.08, and 0.75 respectively.

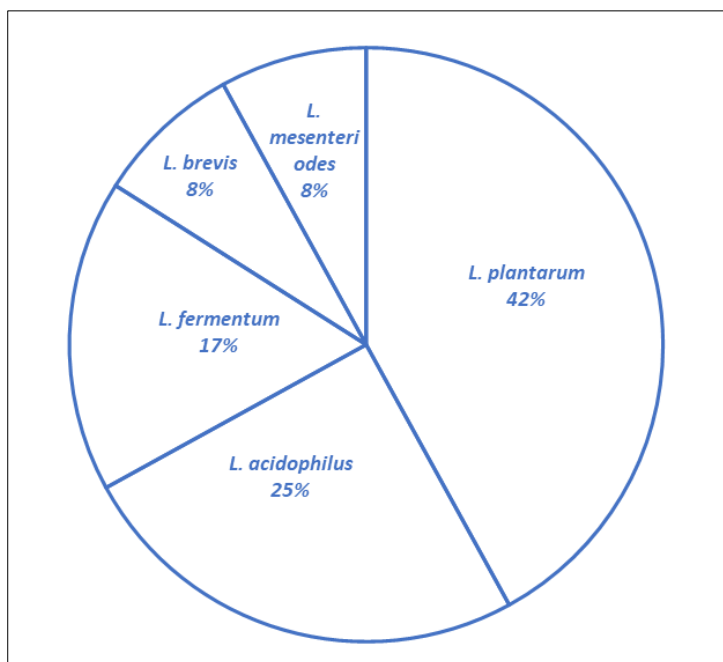


Figure 1 Percentage occurrence of the LAB isolates

Table 1 Production of antimicrobial compounds by the LAB isolates (mg/mL)

Lab isolates	Lactic acid	Diacetyl	Hydrogen peroxide
<i>L. acidophilus</i> FF1	2.60±0.20 ^b	2.10±0.20 ^b	1.50±0.20 ^a
<i>L. plantarum</i> FF2	2.30±0.20 ^b	1.70±0.20 ^a	1.30±0.20 ^a
<i>L. mesenteroides</i> FF3	2.60±0.20 ^b	1.60±0.20 ^a	1.10±0.20 ^a
<i>L. brevis</i> FF4	1.80±0.20 ^a	1.90±0.20 ^a	1.50±0.20 ^a
<i>L. acidophilus</i> FF5	2.60±0.20 ^b	1.80±0.20 ^a	1.50±0.20 ^a
<i>L. plantarum</i> FF6	2.40±0.20 ^b	1.80±0.20 ^a	1.10±0.20 ^a
<i>L. acidophilus</i> FF7	2.40±0.20 ^b	1.60±0.20 ^a	1.50±0.20 ^a
<i>L. plantarum</i> FF8	2.90±0.20 ^b	2.60±0.20 ^b	2.90±0.20 ^b
<i>L. fermentum</i> FF9	2.00±0.20 ^b	1.30±0.20 ^a	1.30±0.20 ^a
<i>L. plantarum</i> FF10	1.90±0.20 ^a	1.50±0.20 ^a	1.30±0.20 ^a
<i>L. fermentum</i> FF11	2.30±0.20 ^b	1.6±0.20 ^a	1.60±0.20 ^a
<i>L. plantarum</i> FF12	2.40±0.20 ^b	1.50±0.20 ^a	1.70±0.20 ^a

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letter within each column are significantly different (p < 0.05).

Table 2 Optical density at 600nm of the LAB isolates of different pH and 4% NaCl

Lab isolates	pH 3	pH 7	pH 12	4% NaCl
<i>L. acidophilus</i> FF1	0.30±0.0020 ^c	0.58±0.0020 ^b	0.27±0.0020 ^a	0.30±0.0020 ^b
<i>L. plantarum</i> FF2	0.32±0.0020 ^c	0.53±0.0020 ^b	0.92±0.0020 ^d	0.36±0.0020 ^b
<i>L. mesenteroides</i> FF3	0.25±0.0118 ^b	0.30±0.0020 ^a	0.67±0.0020 ^c	0.28±0.0020 ^a
<i>L. brevis</i> FF4	0.05±0.0020 ^a	0.56±0.0190 ^b	0.94±0.0020 ^d	0.53±0.0020 ^c
<i>L. acidophilus</i> FF5	0.32±0.0020 ^c	0.59±0.0230 ^b	0.68±0.0020 ^b	0.32±0.0020 ^b
<i>L. plantarum</i> FF6	0.36±0.2310 ^c	0.31±0.0014 ^a	0.80±0.0020 ^d	0.38±0.0116 ^b
<i>L. acidophilus</i> FF7	0.28±0.0020 ^b	0.58±0.0020 ^b	0.87±0.0020 ^d	0.30±0.0020 ^b
<i>L. plantarum</i> FF8	0.39±0.0020 ^c	0.72±0.0020 ^d	1.08±0.0012 ^e	0.75±0.0020 ^c
<i>L. fermentum</i> FF9	0.27±0.0020 ^b	0.63±0.0020 ^c	0.57±0.0020 ^b	0.30±0.0020 ^b
<i>L. plantarum</i> FF10	0.29±0.0020 ^b	0.59±0.0020 ^b	0.95±0.0020 ^a	0.32±0.0020 ^b
<i>L. fermentum</i> FF11	0.35±0.0020 ^c	0.59±0.0020 ^b	0.77±0.0020 ^c	0.65±0.0594 ^c
<i>L. plantarum</i> FF12	0.22±0.0020 ^b	0.61±0.0020 ^c	0.58±0.0020 ^b	0.32±0.0020 ^b

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letter within each column are significantly different (p < 0.05).

The pH changes in starter induced fermented fufu (SIFF) and spontaneous fermented fufu (SFF) samples from 0 to 96 hours were 7.10 - 2.60 and 7.10 - 3.30, respectively. The Total Titratable Acidity (TTA) increase was observed in starter induced fermented fufu (SIFF) and spontaneous fermented fufu (SFF) samples, ranging from 0.71-1.79 and 0.28-0.51, respectively (Table 3)

Table 3 pH and TTA of *lafun* samples

	SIFF		SFF	
	pH	TTA (g/L)	pH	TTA (g/L)
0 hour	7.1±0.10	0.71±0.01	7.1±0.10	0.28±0.01
24 hours	4.7±0.10	0.91±0.01	5.7±0.10	0.36±0.01
48 hours	4.1±0.10	1.25±0.01	4.5±0.10	0.41±0.01
72 hours	4.6±0.10	1.51±0.01	3.8±0.10	0.47±0.01
96 hours	2.6±0.10	1.79±0.01	3.3±0.10	0.51±0.01

Values are the Means ± Standard Deviation where n = 3; SIFF: Starter Induced Fermented Fufu; SFF: Spontaneous Fermented Fufu

The study compared the proximate compositions of starter-induced fermented fufu (SIFF) and spontaneously fermented fufu (SFF) samples. SIFF had significantly higher protein, fat, sodium, potassium, iron, zinc, phosphorus, Vit. C, B1, and A of 2.93, 0.23 (%) 596.4, 270.9, 8.93, 1.67, 296.67, 5.28, 0.24, and 0.31 (mg/100g) respectively, while SFF had higher moisture content, carbohydrate, crude fiber, and ash content of 8.80, 85.70, 3.47 and 4.8 (%) respectively (Table 4). Additionally, SIFF showed a significant decrease in antinutrient content, such as cyanide content, saponin, and phytates 0.05, 0.16 and 0.06 (mg/g), compared to SFF (Table 5).

Table 4 Proximate composition of fufu samples

Parameters	SIFF	SFF
Protein %	4.93±0.01 ^b	2.90±0.10 ^a
Moisture%	5.67±0.01 ^b	8.80±0.01 ^a
CHO %	80.36±0.01 ^a	85.70±0.10 ^b
Fiber %	2.87±0.01 ^a	3.47±0.01 ^b
Ash %	2.67±0.01 ^a	4.87±0.01 ^b
Fat %	0.23±0.01 ^a	0.12±0.01 ^b
Sodium (mg/100g)	514.64±0.10 ^b	295.94±0.10 ^a
Potassium (mg/100g)	270.90±0.10 ^b	83.92±0.01 ^a
Iron (mg/100g)	8.93±0.01 ^b	3.33±0.10 ^a
Zinc (mg/100g)	1.67±0.01 ^a	1.33±0.01 ^a
Phosphorus (mg/100g)	296.67±0.01 ^b	181.67 ±0.10 ^a
Vit. C (mg/100g)	5.28±0.01 ^b	3.62±0.01 ^a
Vit. B1 (mg/100g)	0.24±0.01 ^b	0.16±0.01 ^a
Vit. A (mg/100g)	0.31±0.01 ^b	0.25±0.01 ^a

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letter within each column are significantly different (p < 0.05). SIFF: Starter Induced Fermented Fufu; SFF: Spontaneous Fermented Fufu.

Table 5 Antinutrient content of the lafun samples

Samples	Cyanide (mg/100g)	Saponin (mg/100g)	Phytates (mg/100g)
SIFF	0.05±0.01 ^a	0.16±0.01 ^a	0.06±0.01 ^a
SFF	0.15±0.01 ^b	0.22±0.01 ^b	0.22±0.01 ^b
RC	0.98±0.10 ^c	-	-

Values are presented as Means ± Standard Deviation where n = 3. Values with different superscript letter within each column are significantly different (p < 0.05). SIFF: Starter Induced Fermented Fufu; SFF: Spontaneous Fermented Fufu. RC: Raw cassava

4 Discussion

The study found that *L. plantarum* isolates are the most prevalent possibly due to their simpler nutritional needs, potentially offering a metabolic advantage over other *Lactobacillus* spp. [40, 41, 42]. Studies by McDonald *et al.* [43] and Makimattila *et al.* [41] have identified *L. plantarum* as the predominant bacteria in natural and spontaneous lactic acid fermentation of cassava roots which aligns with the findings of Padonou *et al.* [41]. The lactic acid bacteria species identified in this study have been previously documented in cassava fermentation across a range of fermented food items [44, 45, 6,46].

Lactobacillus plantarum (FF8) exhibited the higher yield of lactic acid, diacetyl, and hydrogen peroxide, which resulted in a reduction of the fermenting medium's pH and the formation of inhibitory bioactive compounds possessing antimicrobial properties. These compounds hinder the proliferation and development of pathogenic organisms in fermented foods, which can be generated at different concentrations by distinct LAB strains [47,48]. The growth of LAB isolates at pH 3 aligns with Cotter and Hill's work [49], which suggests that this growth is due to the relative ATPase activities of microorganisms at different pH levels. This ability enhances the probiotic benefits of LAB, allowing them to survive in stomachs with low pH levels as low as 1.5. Additionally, LAB inhibits pathogenic organism growth in fermented foods [48]. *Lactobacillus plantarum* (FF8) was selected as a starter for the fermentation of starter-induced

fufu, due to its exceptional ability to produce antimicrobial properties in higher amount and outstanding performance in various tests.

The pH of cassava roots decreases during fermentation due to the production of organic acids by lactic acid bacteria. This reduction is consistent with previous studies, with a decrease observed with increasing fermentation time [46]. The production of lactic acid reduces the pH of the fermenting medium and produces inhibitory bioactive compounds such as diacetyl and hydrogen peroxide, responsible for most antimicrobial activity [50].

The increased crude protein content in the starter-induced fermented fufu (SIFF) may be attributed to the higher production of organic acids by *L. plantarum* FF8. These organic acids facilitate the growth and multiplication of single-cell proteins [51]. Additionally, the secretion of extracellular enzymes by *L. plantarum* FF8 may also contribute to the higher crude protein content [52]. The cassava root flour process, as described by Udoro *et al.* [53], effectively reduces moisture through drying, thereby enhancing resistance to microbial infestation, thereby extending the shelf life of the flour during storage. The crude fibre content in fufu samples, as determined by SIFF (2.87%) and SFF (3.47%), aligns with Afoakwa *et al.* [54] and Gil and Buitrago [55] recommendations. Consuming adequate dietary fibre can reduce the risk of diseases such as constipation, obesity, coronary heart diseases, and colon cancer, as noted by Dahl and Stewart [56]. The study found that SIFF has a reduced carbohydrate content compared to SFF of 80.6% and 85.5% respectively. The carbohydrate contents of both samples are suitable for starch production, and these values align with the reports of Alamu *et al.* [57]. The study found that starter-induced fermented fufu (SIFF) had significantly higher vitamin C, B1, and A content compared to spontaneous fermented fufu (SFF) due to the inoculation of *L. plantarum* FF8 that enhances nutrient production during fermentation. Vitamins play a role as antioxidants, helping to combat free radicals [58]. The mineral content in SIFF may increase due to the decrease in phytates during fermentation, possibly due to the loss of dry matter [59]. Additionally, fermentation increases the bioavailability of calcium, phosphorus, and iron due to the degradation of oxalates and phytates, which complex with minerals [60], indicating that *L. plantarum* FF8 has a unique capacity to enrich fufu samples.

Fermentation can decrease the cyanide content of cassava, as demonstrated by Niguse *et al.* [61]. *Lactobacillus plantarum* FF8 significantly reduced the free cyanide level from 0.98 to 0.05mg/g after 96 hours of fermentation. This decrease is due to the linamarase enzyme produced by inoculated microorganisms, which catalyzes the hydrolysis of linamarin and conversion of cyanogenic glycosides to HCN. This process prevents acute toxicity and promotes cyanide detoxification [62].

5. Conclusion

The study found that *L. plantarum* FF8 used as a starter culture, improves the nutritional value of fufu and reduces anti-nutrients, suggesting potential health benefits for consumers. Further research is needed to explore the long-term effects on gut health and overall well-being of fufu made with *L. plantarum* FF8.

Compliance with ethical standards

Acknowledgement

The authors acknowledged the invaluable support of the Department of Microbiology and Biotechnology of First Technical University, Ibadan where this research was carried out

Disclosure of conflict of interest

The authors declare no conflict of interest

References

- [1] Asante-Pok A. Analysis of incentives and disincentives for cassava in Nigeria. *Gates Open Res.* 2019; 3(915): 915.
- [2] Ferraro V, Piccirillo C, Tomlins K, Pintado ME. Cassava (*Manihot esculenta Crantz*) and yam (*Dioscorea* spp.) crops and their derived foodstuffs: safety, security and nutritional value. *Critical reviews in food science and nutrition.* 2016; 56(16): 2714-2727.
- [3] Sánchez AS, Silva YL, Kalid RA, Cohim E, Torres EA. Waste bio-refineries for the cassava starch industry: New trends and review of alternatives. *Renewable and Sustainable Energy Reviews.* 2017; 73(1): 1265-1275.

- [4] Morgan NK, Choct M. Cassava: Nutrient composition and nutritive value in poultry diets. *Animal Nutrition*. 2016; 2(4): 253-261.
- [5] France O, Chinyère N. The amelioration of cyanide induced liver toxicity with bentonite using Wistar rat as experimental model. *Journal of Advances in Biology & Biotechnology*. 2017; 14(1): 1-9.
- [6] Oyewole OB, Odunfa SA. Effects of processing variables on cassava fermentation for fufu'production. *Tropical science*. 1992; 32(3): 231-240.
- [7] Amoa-Awua WK, Appoh, FE, Jakobsen M. Lactic acid fermentation of cassava dough into agbelima. *International Journal of Food Microbiology*. 1996; 31(1-3): 87-98.
- [8] Westby A. Cassava utilization, storage and small-scale processing. In *Cassava: Biology, production and utilization*. 2001; Wallingford UK: Cabi. pp. 281-300.
- [9] Oyewole MF, Eforuoku F. Value addition on cassava wastes among processors in Oyo State, Nigeria. *Journal of Agricultural Extension*. 2019; 23(3): 135-146.
- [10] Caplice E, Fitzgerald GF. Food fermentations: role of microorganisms in food production and preservation. *International journal of food microbiology*. 1999; 50(1-2): 131-149.
- [11] Kimaryo VM, Massawe GA, Olasupo NA, Holzapfel WH. The use of a starter culture in the fermentation of cassava for the production of “kivunde”, a traditional Tanzanian food product. *International Journal of Food Microbiology*. 2000; 56(2-3): 179-190.
- [12] Oluwole OB, Olatunji OO, Odunfa SA. A process technology for conversion of dried cassava chips into “Gari”. *Nigerian Food Journal*. 2004; 22(1): 65-77.
- [13] Iwuoha CI, Eke OS. Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. *Food Research International*. 1996; 29(5-6): 527-540.
- [14] Soni SK, Sandhu DK, Vilku KS, Kamra N. Microbiological studies on dosa fermentation. *Food Microbiology*. 1986; 3(1): 45-53.
- [15] Ewanfo IJ, James IM, Ugueri U. Microbiological Quality of Commercially Ready-to-Eat Fufu Sold in Benin City, Nigeria. *American Journal of Food*. 2017; 2(5): 26-30.
- [16] Oyewole OB. Cassava processing in Africa. Application of biotechnology to traditional fermented foods. Report of an adhoc panel of the board on science and technology for international development, USA, National Research Council. 1992; 89-92.
- [17] Nweke F, Dunstan S, Spencer D, Lyman J. *The cassava transformation*. Michigan State: University press. 2002; 8992.
- [18] Ogunremi OR, Sanni AI, Agrawal RJOAM. Probiotic potentials of yeasts isolated from some cereal-based Nigerian traditional fermented food products. *Journal of Applied Microbiology*. 2015; 119(3), 797-808.
- [19] Kolapo AL, Salami RO, Onipede GO. Molecular identification and technological properties of yeasts isolated from spontaneously fermented cassava waste pulp. *Nova Biotechnology Chim* 2021; 20(2): 898
- [20] Holzapfel W. Use of starter cultures in fermentation on a household scale. *Food control*. 1997; 8(5-6): 241-258.
- [21] Holzapfel WH. Appropriate starter culture technologies for small-scale fermentation in developing countries. *International journal of food microbiology*. 2002; 75(3): 197-212.
- [22] Lacerda IC, Miranda RL, Borelli BM, Nunes ÁC, Nardi RM, Lachance MA, Rosa CA. Lactic acid bacteria and yeasts associated with spontaneous fermentations during the production of sour cassava starch in Brazil. *International journal of food microbiology*. 2005; 105(2): 213-219.
- [23] Kostinek M, Specht I, Edward VA, Pinto C, Egounlety M, Sossa C, Holzapfel WH. Characterization and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. *International journal of food microbiology*. 2007; 114(3); 342-351.
- [24] Medrano EG, Esquivel JF, Bell AA. Transmission of cotton seed and boll rotting bacteria by the southern green stink bug (*Nezara viridula* L.). *Journal of applied microbiology*. 2007; 103(2): 436-444.
- [25] Okolie NP, Ibeh IN, Ugochukwu EN. Production of improved cassava fufu, ‘akpu’, through controlled fermentation. *Food chemistry*. 1992; 44(2), 137-139.

- [26] Oguntoyinbo FA, Cho GS, Trierweiler B, Kabisch J, Rösch N, Neve H, Franz CM. Fermentation of African kale (*Brassica carinata*) using *L. plantarum* BFE 5092 and *L. fermentum* BFE 6620 starter strains. *International journal of food microbiology*. 2016; 238(1): 103-112.
- [27] Ogunremi OR, Banwo K, Sanni AI. Starter-culture to improve the quality of cereal-based fermented foods: trends in selection and application. *Current Opinion in Food Science*. 2017; 13(1): 38-43.
- [28] Laranjo M, Potes ME, Elias M. Role of starter cultures on the safety of fermented meat products. *Frontiers in microbiology*. 2019; 10(1): 853.
- [29] Harrigan WF, Mccance ME. *Laboratory Methods in Food and Dairy Microbiology*. Academic Press. London, New York. 1998; 8(8): 66-71
- [30] Olutiola PO, Famurewa O, Sonntag HG. An introduction to general microbiology: a practical approach 1991. *Germany: Heidelberg Verlaganstalt und Druckerei GmbH Heidelberg.*
- [31] Harrigan WF. *Laboratory methods in food microbiology* (1998). Gulf professional publishing.
- [32] Axelsson L. Lactic acid bacteria: classification and physiology. *Food Science and Technology-New York-Marcel Dekker*, 2004; 139(1): 1-66.
- [33] Edward VA, Egonlety M, Huch M, Van Zyl PJ, Singh S, Nesengani ND, Franz, CM. Isolation and screening of microorganisms from a gari fermentation process for starter culture development. *African Journal of Biotechnology*. 2012; 11(65), 12865-12877.
- [34] García-Cano I, Rocha-Mendoza D, Ortega-Anaya J, Wang K, Kosmerl E, Jiménez-Flores R. Lactic acid bacteria isolated from dairy products as potential producers of lipolytic, proteolytic and antibacterial proteins. *Applied microbiology and biotechnology*. 2019; 103(1): 5243-5257.
- [35] Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. *Bergey's Manual of determinate bacteriology*. 1994.
- [36] Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis, Association of official analytical chemist 19th edition 2000*; Washington D.C., USA.
- [37] Sanni A I, Onilude AA, Fatungase MO. Production of sour maize bread using starter-cultures. *World Journal of Microbiology and Biotechnology*. 1997; 14(1): 101-106.
- [38] Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis, Association of official analytical chemist 19th edition 2012*; Washington D.C., USA.
- [39] Association of Official Analytical Chemists (AOAC). *Official methods of analysis of the Association of Analytical Chemists International, 18th edition 2012*; AOAC, Gaithersburg, MD
- [40] Sanni AI, Onilude, AA, Fatungase MO. Production of sour maize bread using starter-cultures. *World Journal of Microbiology and Biotechnology*. 1997; 14(1): 101-106.
- [41] Mäkimattila E, Kahala M, Joutsjoki V. Characterization and electrotransformation of *Lactobacillus plantarum* and *Lactobacillus paraplantarum* isolated from fermented vegetables. *World Journal of Microbiology and Biotechnology*. 2011; 27(1); 371-379.
- [42] Ben Omar N, Gálvez A, Abriouel H, López RL. Bacteriocin based strategies for food bio preservation. *International Journal of food microbiology*. 2017;120 (1), 51-70
- [43] McDonald LC, Fleming HP, Hassan H. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and environmental microbiology*. 1990; 56(7): 2120-2124.
- [44] Abe MO, Lindsay RC. Evidence for a *Lactic streptococcal* role in Nigerian acidic cassava (*Manihot esculenta Crantz*) fermentations. *Journal of Food Protection*. 1978; 41(10): 781-784.
- [45] Ngaba PR, Lee JS. Fermentation of Casava (*Manihot esculenta Crantz*). *Journal of Food Science*. 1979; 44(5): 1570-1571.
- [46] Kobawila SC, Louembe D, Keleke S, Hounhouigan J, Gamba C. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *African Journal of Biotechnology*. 2005; 4(7): 689-696.
- [47] Tannock GW. A special fondness for lactobacilli. *Applied and environmental microbiology*. 2004; 70(6), 3189-3194.

- [48] Omafuvbe BO, Adigun AR, Ogunsuyi JL, Asunmo AM. Microbial diversity in ready-to-eat fufu and lafun-fermented cassava products sold in Ile-Ife, Nigeria. *Research Journal of Microbiology*. 2007; 2(11): 831-837
- [49] Cotter PD, Ross RP, Hill C. Bacteriocins—a viable alternative to antibiotics? *Nature Reviews Microbiology*. 2013; 11(2): 95-105.
- [50] Coulin P, Farah Z, Assanvo J, Spillmann H, Puhan Z. Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small-scale preparation. *International journal of food microbiology*. 2006; 106(2): 131-136.
- [51] Boonnop K, Wanapat M, Nontaso N, Wanapat S. Enriching nutritive value of cassava root by yeast fermentation. *Scientia Agricola*. 2009; 66, 629-633.
- [52] Bala EC, Ijah UJ J, Abioye OP, Emele LC. Protein enrichment of cassava with yeast for gari production. *BTALJ*. 2012. 4(1): 120-126.
- [53] Oduro I, Ellis WO, Aryeetey SK, Ahenkora K, Otoo JA. Pasting characteristics of starch from new varieties of sweet potato. *Tropical science*. 2000; 40(1): 25-28.
- [54] Polycarp D; Afoakwa EO, Budu AS, Otoo E. Characterization of chemical composition and anti-nutritional factors in seven species within the Ghanaian yam (*Dioscorea*) germplasm. *International food research journal*. 2012; 19(1): 985-992.
- [55] Gil LL, Buitrago Arbeláez, JA. La yuca en la alimentación animal. *Sistemas Modernos De Producción, Procesamiento, Utilización Y Comercialización*, 2002; p. 527–569.
- [56] Dahl WJ, Stewart ML. Position of the Academy of Nutrition and Dietetics: health implications of dietary fiber. *Journal of the Academy of Nutrition and Dietetics*. 2015; 115(11), 1861-1870.
- [57] Alamu EO, Maziya-Dixon B, Dixon AG. Evaluation of proximate composition and pasting properties of high-quality cassava flour (HQCF) from cassava genotypes (*Manihot esculenta Crantz*) of β -carotene-enriched roots. *LWT*. 2017; 86(1) 501-506.
- [58] Eleazu CO, Amajor JU, Ikpeama AI, Awa E. Studies on the nutrient composition, antioxidant activities, functional properties and microbial load of the flours of 10 elite cassava (*Manihot esculenta*) varieties. *Asian Journal of Clinical Nutrition*. 2011; 3(1): 33-39.
- [59] Day CN, Morawicki RO. Effects of fermentation by yeast and amyolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Journal of food quality*. 2018. 2018(1):1- 8.
- [60] Sripriya G, Antony U, Chandra TS. Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food chemistry*. 1997; 58(4): 345-350.
- [61] Halake NH, Chinthapalli B, Chitra DV. Role of Selected Fermentative Microorganisms on Cyanide Reduction, Protein Enhancement and Palatability of Cassava Based Food. *International Journal of Research in Agriculture and Forestry*. 2019; 6(1):1-12.
- [62] Tefera T, Ameha K, Biruhtesfa A. Cassava based foods: microbial fermentation by single starter culture towards cyanide reduction, protein enhancement and palatability. *International food research Journal*. 2014; 21(5): 1751.