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Application of a bioreactor system for the development of probiotic starter cultures for fufu fermentation

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ABSTRACT

This study aimed to develop starter cultures for fufu production to enhance fermentation efficiency, product quality, and nutritional value. Healthy cassava tubers were sourced from Umuanyagu and Abua farmlands in Rivers State, Nigeria, and fermented in sterile water for 3–5 days. Microbial isolates were obtained, and the resulting organisms were cultured in a bioreactor for 14 days. Molecular characterization identified the isolates as *Lactobacillus plantarum*, *Kluyveromyces marxianus*, and *Bacillus pumilus*. The fermentation process was monitored by measuring physicochemical parameters such as pH and temperature, which ranged from 4.25 to 6.00 and 26.2°C to 28.1°C, respectively. Proximate composition analysis revealed that moisture content ranged from 57% to 75%, crude protein from 0.025% to 1.75%, ash content from 1.1% to 2.8%, crude fiber from 1.25% to 1.75%, fat content from 0.1% to 1.3%, and carbohydrates from 30% to 39%. The bacterial population ranged from 1.07×10^9 to 2.10×10^9 CFU/ml, while fungal populations ranged from 0.00 to 3.00×10^8 CFU/ml. The study successfully demonstrated that the combination of *Lactobacillus plantarum*, *Kluyveromyces marxianus*, and *Bacillus pumilus* is effective in cassava fermentation, improving the quality of fufu. It is recommended to incorporate these starter cultures in fufu production to enhance safety, consistency, and nutritional quality.

Keywords: Starter cultures, bioreactor system, fermentation, fufu production.

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INTRODUCTION

Cassava (*Manihot esculenta*) is a tropical root crop that serves as a crucial carbohydrate source and nutritional staple for millions in Africa and Asia, providing energy to approximately 500 million people globally (Achi and Akomas, 2006). Recognized for its resilience in harsh climates, cassava is vital for food security and income generation in developing countries (Scott and Rose, 2002). Nigeria, the world's largest producer, has significantly increased its cassava production, primarily driven by small-scale farmers (Forsythe et al., 2016). With rising urbanization, cassava has become a primary food source for over 70 million Nigerians (FAO, 2005). However, it is low in protein, which can lead to malnutrition if consumed exclusively.

The demand for cassava and its derivatives, including processed foods like fufu, has surged due to its expanding applications in both the food and industrial sectors (Azogu, 2010). Fufu, a traditional fermented food, is a major cassava product, particularly in southern, western, and eastern Nigeria, and is sometimes considered more profitable than garri (Rosales-Soto et al., 2016). However, fufu's short shelf life and labor-intensive processing have led to shifting consumption patterns, with garri becoming more popular.

The preparation of fufu involves fermentation, which detoxifies cassava by breaking down harmful cyanogenic glycosides while enhancing its flavor and preservation (Flibert et al., 2016). The process typically begins with peeling and cutting cassava roots, followed by various soaking and fermentation methods, which can last from 48 hours to several days (Mokemiabeka et al., 2011; Omodamiro et al., 2012). Fermentation, an ancient food preservation technique, is essential but often presents challenges in developing countries, including extended fermentation times, inconsistent quality, and potential contamination, as it relies on spontaneous microbial processes (Holzapfel, 2002).

Cassava's significance as a staple crop continues to

grow in Nigeria, driven by its versatility and increasing market demand. However, traditional processing methods, particularly fermentation for products like fufu, present challenges that could be addressed through improved techniques to enhance food safety and consistency. Fufu undergoes lactic acid fermentation by various microorganisms, producing metabolites that contribute to preservation, flavor development, cyanide reduction, and alterations in the final product's functional properties. The use of starter cultures has the potential to standardize production, ensuring consistent quality and reducing processing time.

In Nigeria, particularly in southwestern regions, fermented cassava products are widely consumed and benefit from improved fermentation techniques. The fufu production process typically involves soaking cassava tubers for 3-5 days to allow fermentation, after which the water is drained, and the cassava paste is extracted. During fermentation, biochemical changes occur, including the breakdown of cyanogenic compounds and the formation of flavor molecules (Adebayo-Ojetaro et al., 2013).

Research into efficient starter cultures for fufu production, based on the microbial aspects of fermentation, could provide a solution by eliminating the need for toxic additives. This study aims to develop starter cultures for fufu production using bioreactor systems.

MATERIALS AND METHODS

Area and duration of study

The study was conducted over four months (February–July) in the Microbiology Laboratory of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. Sample collection was carried out at Umuanyagu farmland, Etche Local Government Area (LGA), at Latitude 4.99080°N, 7.05440°E, and Abua farmland in Abua LGA at Latitude 4.95640°N, 6.63440°E, in Rivers State, Nigeria. Within 24 hours of collection, the samples were transported to the Microbiology Laboratory, preserved in a sterile ten-liter rubber bucket, and properly covered.

Sample preparation and fermentation

Cassava tubers were peeled, and 1,900 g of the peeled tubers were washed and cut into long pieces. The pieces were soaked in 5 L of sterile water in plastic jars and covered with aluminum foil. The setup was allowed to ferment for four days. After this period, samples were taken for the isolation of microorganisms.

Enumeration and isolation of microorganisms

An aliquot (1 mL) of the fermented sample was added to 9 mL of sterile peptone water. The resulting suspension underwent a ten-fold serial dilution until a 10^{-3} dilution was obtained. Aliquots (0.1 mL) of the 10^{-3} dilution were inoculated onto Potato Dextrose Agar (PDA), De Man Rogosa Sharpe (MRS) Agar, and Nutrient Agar (NA).
Potato Dextrose Agar (PDA) was used for the isolation of *Saccharomyces* species, with inoculation performed using the spread plate method.

Nutrient Agar (NA) was used for the isolation of *Bacillus* species, while De Man Rogosa Sharpe (MRS) Agar was used for the isolation of *Lactobacillus* species (Prescott and Hotchkiss, 2011).

Characterization and identification of isolates

Cultural characteristics such as shape, size, pigmentation, opacity, surface texture, and elevation were observed and recorded. This was followed by a microscopic examination of cell types, arrangement, Gram's reaction, and motility. The biochemical characteristics of the isolates, including sugar and other chemical utilization, were then assessed.

Multiplication of microorganisms involved in the fermentation of cassava using a bioreactor

Thirteen grams (13 g) of nutrient broth powder was added to 1 L of distilled water and autoclaved at 121°C, 15 psi, for 15 minutes. The broth was allowed to cool before being added to the bioreactor vessels. The isolates were inoculated into the bioreactor vessels and observed for seven days to monitor their multiplication (Edward et al., 2012).

Packaging of the microorganisms

Fresh cow bones were collected from Mile 3 Market in Port Harcourt, Rivers State. The bones were dried in a hot air oven for four days and then ground into powder using a sterile grinder. The powdered bone, in the form of calcium carbonate, was poured into a 500 mL conical flask and sterilized in a hot air oven. Fifteen grams (15 g) of calcium carbonate was added to a beaker containing 5 mL and 10 mL of each broth culture and mixed thoroughly. The mixtures were then transferred into small sterile waterproof nylon bags and sealed using a sealing machine.

Proximate analyses

The fufu samples were analyzed for moisture content using the drying method, protein content using the semi-Kjeldahl method, fat content via Soxhlet extraction, ash content by ashing in a muffle furnace at 550°C, and

carbohydrate content by difference. These procedures were conducted in the Food Science and Technology laboratory at Rivers State University, following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2010).

Experimental design

The cultured broth and bone meal were used in preparing the experimental setups, which consisted of eight treatments, including the control. The experimental setups are presented in Table 1.

Evaluation of the packaged microorganisms

After a storage period of five days, cassava-fermenting microorganisms with calcium carbonate were evaluated. One gram of calcium carbonate containing each isolate was serially diluted and cultured using a tenfold serial dilution. The bacterial isolates were cultured on nutrient agar media plates, while the yeast isolate was cultured on potato dextrose agar plates. After incubation, the original fermenting organisms initially isolated from the cassava water reappeared on the agar plates based on their morphological characteristics.

Use of the packaged microorganisms for fufu production

Fresh cassava tubers were peeled and washed thoroughly. The cassava was cut into pieces and placed in 400 mL of sterile water in plates. Starter cultures were prepared in their respective growth media and added to the cassava tubers. The cassava tubers were monitored, and fermentation was observed within 48 hours. During this period, the microorganisms metabolized the substrates, producing acids, gases, and other fermentative products (Buba et al., 2023).

Sensory evaluation

The acceptability of the cassava products was determined through sensory evaluation using a Hedonic scale test. The products were presented in small plates and coded to avoid bias from panelists. A modified method of Iwe (2002) was used, in which a ten-member trained panel, familiar with the food product, was set up to determine general acceptability. Panelists were provided with drinking water to rinse their mouths after tasting each sample. Each sample was labeled and evaluated for taste, color, texture, odor, sourness, and overall acceptability using a 9-point Hedonic scale.

Statistical analysis

Data obtained were subjected to statistical analysis. Duncan's Multiple Range Test was used to separate mean values, and analysis of variance (ANOVA) at a 5% level of significance was used to determine differences. Data in tables were presented as mean ± standard deviation.

Table 1. Experimental setup.

Treatments	Proportion of bone mill added	Name of organism(s) added
UCBM; Control	Fifteen grams (15g)	Nil
BM+BAC;	Fifteen grams (15g)	*Bacillus sp.*
BM+BAC+LAC	Fifteen grams (15g)	*Bacillus sp.* and *Lactobacillus sp.*
BM+YEA	Fifteen grams (15g)	*Yeast sp.*
BM+LAC	Fifteen grams (15g)	*Lactobacillus sp.*
BM+YEA+LAC	Fifteen grams (15g)	*Yeast sp.* and *Lactobacillus sp.*
BM+YEA+BAC	Fifteen grams (15g)	*Yeast sp.* and *Bacillus sp.*
BM+YEA+LAC+BAC	Fifteen grams (15g)	*Yeast sp., Lactobacillus sp.* and *Bacillus sp.*

RESULTS

Microbial population in fermented cassava

The investigated microbial population in fermented cassava is presented in Table 2. As shown in the table, the total heterotrophic bacterial population ranges from 1.07±0.6 to 2.10±0.24 × 10^9 CFU/ml. The total Lactobacillus population ranges from 4.85±1.18 × 10^8 to 1.62±0.28 × 10^9 CFU/ml. The heterotrophic fungal population ranges from 0.00 to 3.00±0.45 × 10^8 CFU/ml.

Morphological and biochemical characteristics of the isolates

The morphological and biochemical characteristics of the bacterial isolates from Abua samples are presented in Table 3a. As shown in the table, all the isolates tested positive for catalase and oxidase and were negative for

Table 2. Mean population of microorganisms in cassava samples.

Sample Location	THB (CFU/ml)	TLC (CFU/ml)	THF (CFU/ml)
Abua	$1.07\pm0.6 \times 10^{9}$	$4.85\pm1.18\times 10^{8}$	$3.00\pm0.45\times 10^{8}$
Etche	$2.10\pm0.24 \times 10^{9}$	$1.62\pm0.28\times 10^{9}$	0.00

Key: THB = Total Heteortrophic Bacteria; **TLC** = Total Lactobacillus Count; **THF** = Fungi.

Table 3a. Morphological and biochemical characteristics of the bacterial isolates of cassava from Abua.

Isolate Code	Shape	Elevation	Opacity	Edge	Colour	Gram reaction	Shape	Motility	Catalase	Oxidase	Indole	Citrate	Methyl Red	Voges Proskauer	Glucose	Lactose	Starch	Sucrose	Mannitol	Probable Identity
NA 1	Round	Flat	Opaque	Rough	White	+ve	Rod	+	+	+	-	-	-	-	AG	AG	-	A	-	*Bacillus sp*
NA 2	Round	Flat	Opaque	Rough	White	+ve	Rod	-	+	+	-	+	+	-	AG	AG	-	AG	-	*Bacillus sp*
MRS 1	Round	Raised	Opaque	Rough	White	+ve	Rod	+	+	+	-	-	-	-	AG	AG	-	AG	A	*Lactobacillus sp*
MRS 2	Round	Raised	Opaque	Rough	White	+ve	Rod	+	+	+	-	+	-	-	AG	AG	-	A	AG	*Lactobacillus sp*

Key: + = positive, **-** = negative; **NA 1** = Nutrient Agar 1; **NA 2** = Nutrient Agar 2; **MRS 1** = De Man Rogosa Sharpe 1; **MRS 2** = De Man Rogosa Sharpe 2.

Voges-Proskauer. Based on comparisons with literature (Oyinlola et al., 2016; Lee et al., 2021), NA1 and NA2 from Abua LGA are suspected to be *Bacillus* sp., while MRS1 and MRS2 are likely *Lactobacillus* sp.

The morphological and biochemical characteristics of the bacterial isolates from Etche samples are presented in Table 3b. As shown in the table, all isolates tested positive for catalase and oxidase. When compared with information in the literature (Martinez et al., 2020; Taylor et al., 2018), NA1 and NA2 from Etche samples are likely *Bacillus* sp., while NA3 is suspected to be *Klebsiella* sp.

The morphological and biochemical characteristics of the fungal isolate from Etche samples are presented in Table 4a and 4b. As shown in the table, the isolate belongs to the genus *Saccharomyces* sp. In contrast to previously reported data (Miller and Ali, 2018), PDA1 from the Etche samples is suspected to be *Saccharomyces* sp. The overlayed plate and microscopy analysis suggest the presence of *Kluyveromyces* sp.

Table 3b. Morphological and biochemical characteristics of the bacterial isolates of cassava from Etche.

Isolate Code	Shape	Elevation	Opacity	Edge	Colour	Gram reaction	Shape	Motility	Catalase	Oxidase	Indole	Citrate	Methyl Red	Voges Proskauer	Glucose	Lactose	Starch	Sucrose	Mannitol	Probable Identity
NA 1	Round	Flat	Opaque	Rough	Cream	+ve	Rod	+	+	+	-	-	-	+	AG	AG	-	AG	A	Bacillus sp
NA 2	Round	Flat	Opaque	Rough	Milky	+ve	Rod	+	+	+	-	-	-	-	AG	AG	-	AG	A	*Bacillus sp*
NA 3	Round	Raised	Opaque	Smooth	Cream	-ve	Rod	+	+	+	-	-	-	-	AG	AG	-	AG	A	*Klebsiella sp*

Key: + = positive, - = negative; **NA 1** = Nutrient Agar 1; **NA 2** = Nutrient Agar 2; **NA 3** = Nutrient Agar 3.

Table 4a. Morphological characteristics of the yeast isolate of cassava from Etche.

Isolate code	Margin	Color	Shape	Elevation	Texture	Surface	Cell morphology	Sucrose	Maltose	Lactose	Mannitol	Xylose	Fructose	Glucose	Suspected organism
PDA 1	Entire	Milk	Round	Convex	Moist	Rough	Oval	AG	AG	AG	AG	AG	AG	AG	*Saccharomyces sp*

Key: PDA = Potato Dextrose Agar 1.

Table 4b. Microscopic identification of the isolated fungi.

Isolates Code	Macroscopy	Microscopy	Probable Identity
PDA 1	Milk color muccoid growth	Oval shaped cell, unicellular	*Saccharomyces sp.*

Key: PDA = Potato Dextrose Agar 1.

Proximate Composition of Fermented Cassava

The investigated proximate composition of the fermented products is presented in Figure 1. The treatment (Cassava + Bone mill) UCBM had the highest moisture content at 75%, while (Cassava + Bone mill + *Lactobacillus plantarum*) BM+LAC had the lowest at 58%.

Results from Figure 2 show that (Cassava + Bone meal + *Bacillus pumilus* + *Lactobacillus plantarum*) BM+BAC+LAC had the highest crude protein content of 1.75, while (Cassava + Bone mill + *Kluyveromyces marxianus*) BM+YEA had the lowest at 0.77.

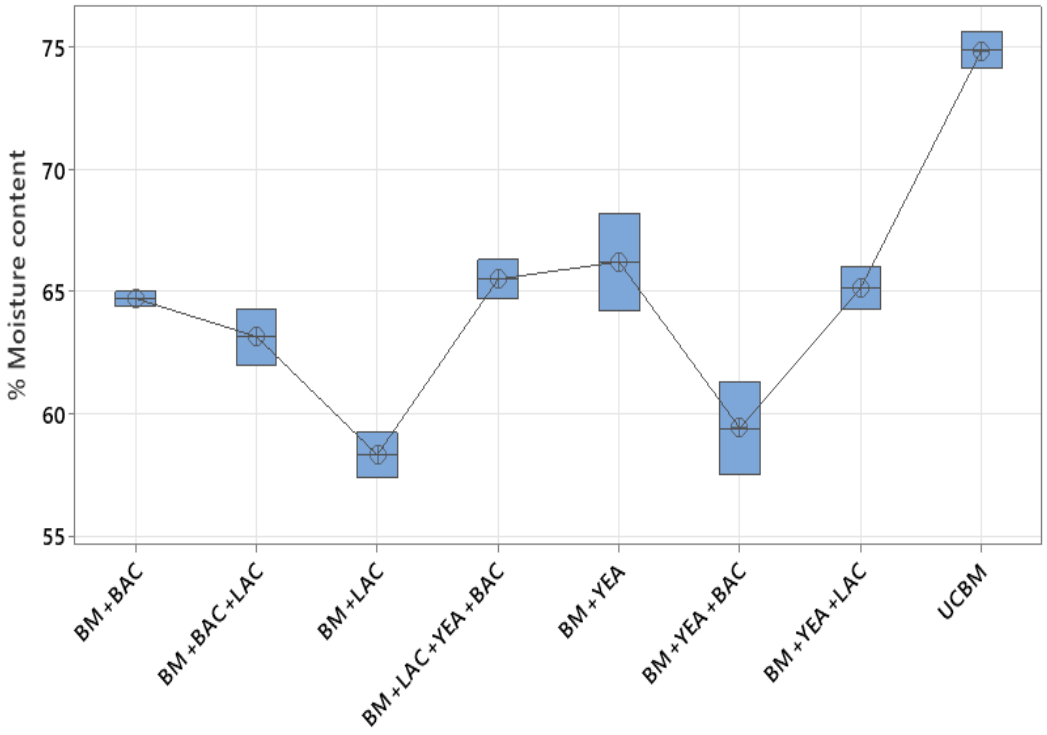

Figure 1. Percentage (%) moisture content of setups.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC** = Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA** = Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC** = Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC** = Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

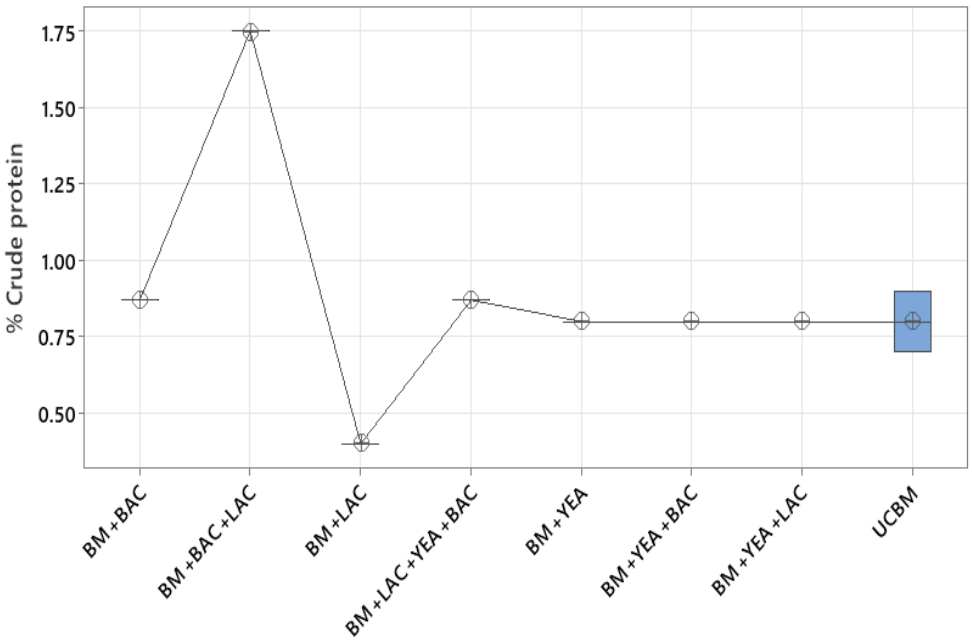

Figure 2. Percentage (%) crude protein content of setups.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC =** Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA =** Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

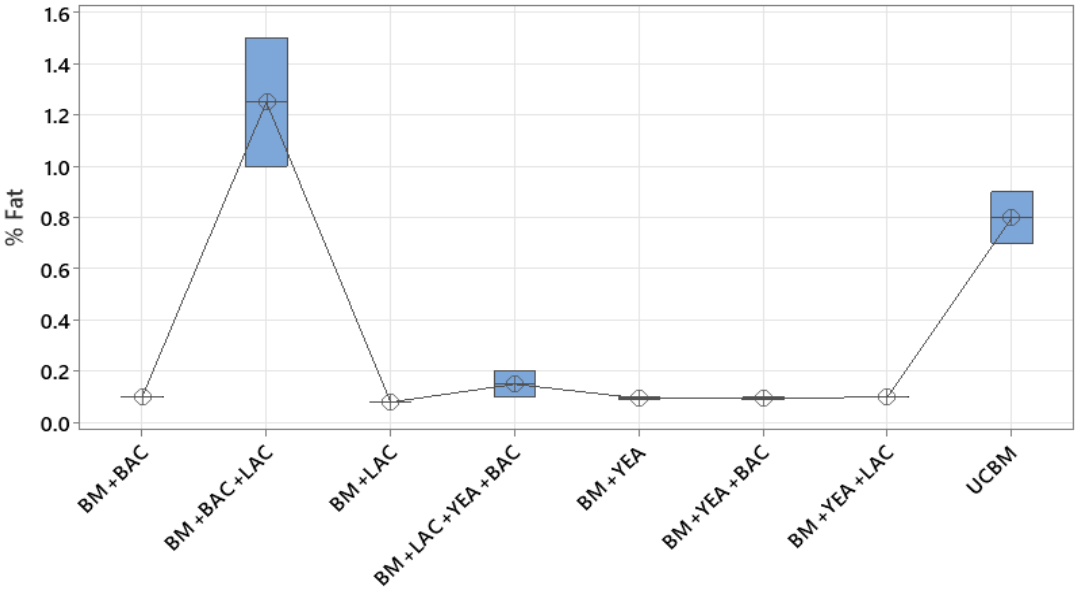

Figure 3. Percentage (%) fat content of setups.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC =** Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA =** Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

Results from Figure 3 indicate that (Cassava + Bone meal + *Lactobacillus plantarum*) BM+LAC and (Cassava + Bone mill + *Bacillus pumilus*) BM+BAC showed a slight increase in percentage fat compared to other treatments.

Results from Figure 4 show that (Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum*) BM+BAC+LAC had the highest ash content, while (Cassava + Bone mill + *Lactobacillus plantarum*) BM+LAC had the lowest.

Results from Figure 5 indicate that (Cassava + Bone mill) UCBM had the highest crude fiber content, while (Cassava + Bone mill + *Lactobacillus plantarum*) BM+LAC had the lowest.

Results from Figure 6 show that (Cassava + Bone mill + *Lactobacillus plantarum*) BM+LAC had the highest carbohydrate content, while (Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum*) BM+BAC+LAC had the lowest.

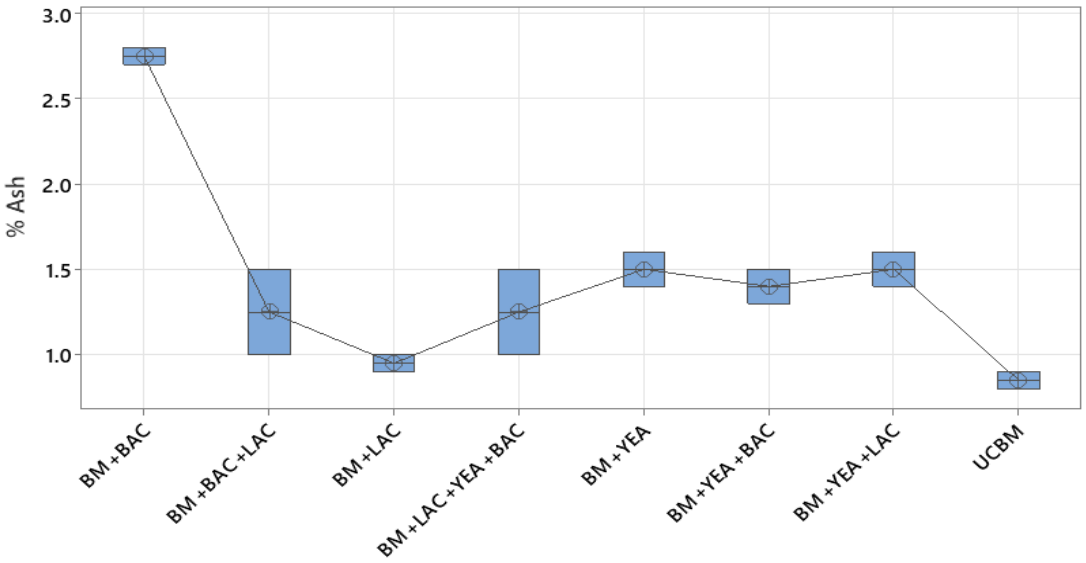

Figure 4. Percentage (%) ash content of setups.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC =** Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA =** Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

DISCUSSION

The microbial population in fermented cassava plays a critical role in determining the quality, safety, and nutritional value of the final product. The diversity and dynamics of these microbial communities are influenced by the type of starter cultures used, fermentation conditions, and the inherent properties of cassava. The notable variations in microbial population estimates recorded during cassava fermentation in this study align with recent findings by Akingbala et al. (2005), who also reported significant variations. Additionally, recent research by El-Tanash et al. (2017) reported similar variations in microbial populations during cassava fermentation for garri production. The high counts of total heterotrophic bacteria indicate that bacterial fermentation is the dominant process. The presence of a fungal population suggests that fungi also play a significant role in texture and flavor development. Kambou et al. (2019) reported microbial population dynamics and nutritional quality changes in cassava during fermentation, with potentially similar findings.

The isolation and molecular characterization of *Bacillus pumilus, Lactobacillus plantarum,* and *Kluyveromyces marxianus* in the current study indicate the microbial diversity and their roles in cassava fermentation. This process typically involves a complex consortium of microorganisms, including bacteria, yeasts, and molds. During the isolation of *Lactobacillus plantarum* and *Kluyveromyces marxianus* from cassava fermentation samples collected from Abua and Etche, *Lactobacillus plantarum* was present in all samples, with higher counts than *Kluyveromyces marxianus.* Several authors have also reported the prevalence of *Lactobacillus* in cassava fermentations (Kostinek et al., 2007; Obilie et al., 2004; Oguntoyinbo and Dodd, 2010). These high counts of

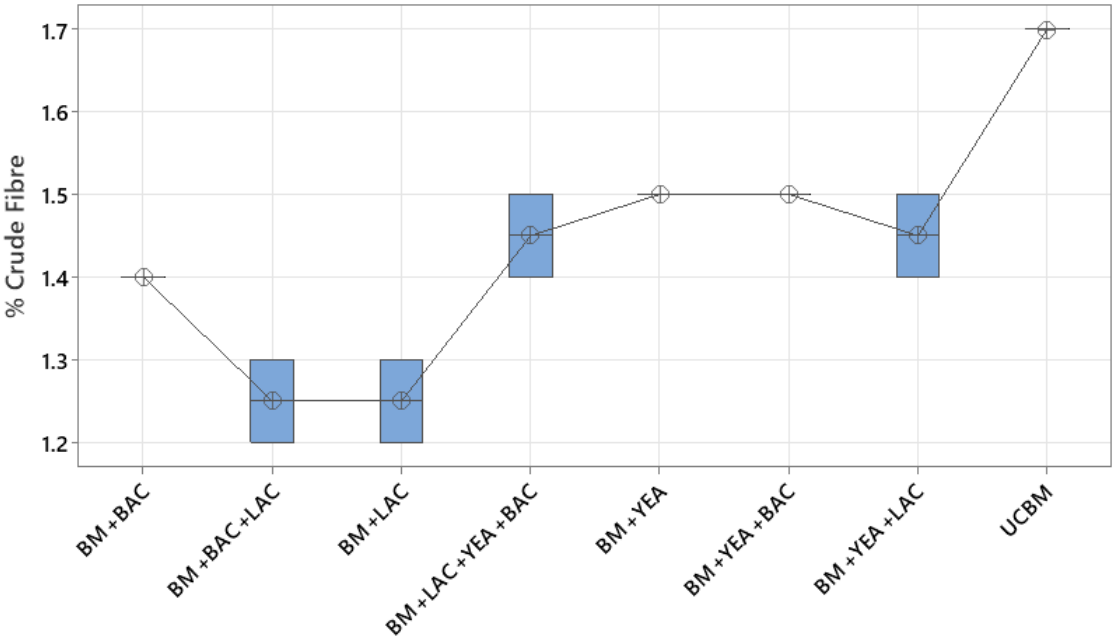

Figure 5. Percentage (%) crude fibre of setups.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC =** Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA =** Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

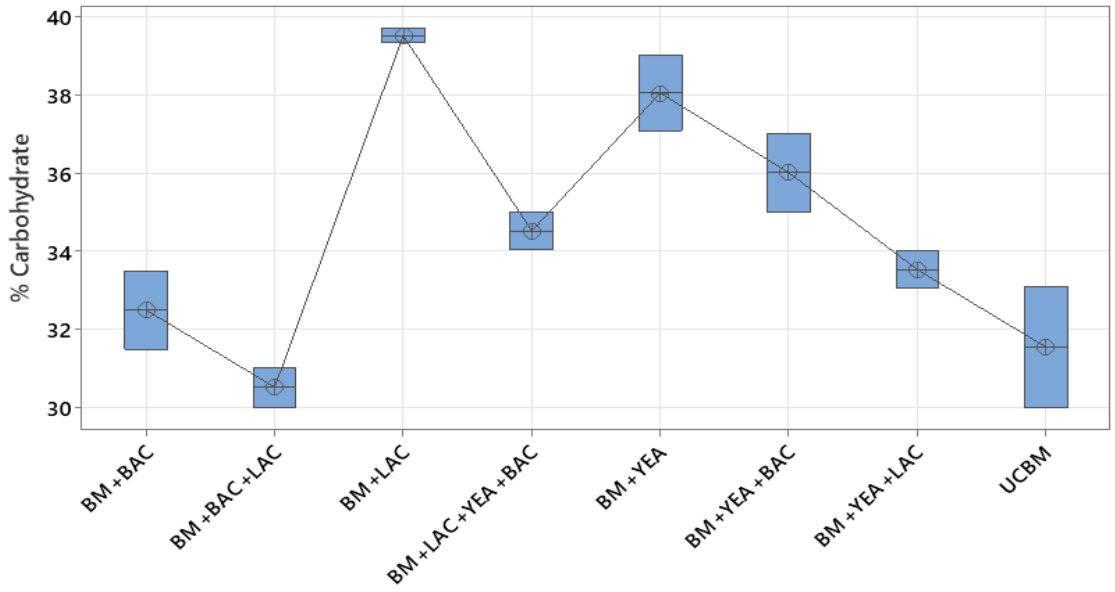

Figure 6. Percentage (%) carbohydrates of setup.

Key: BM+BAC = Cassava + Bone mill + *Bacillus pumilus;* **BM+BAC+LAC =** Cassava + Bone mill + *Bacillus pumilus* + *Lactobacillus plantarum;* **BM+LAC =** Cassava + Bone mill + *Lactobacillus plantarum;* **BM+LAC+YEA+BAC** = Cassava + Bone mill + *Lactobacillus plantarum* + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA =** Cassava + Bone mill + *Kluyveromyces marxianus;* **BM+YEA+BAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Bacillus pumilus;* **BM+YEA+LAC =** Cassava + Bone mill + *Kluyveromyces marxianus* + *Lactobacillus plantarum;* **CONTROL =** Cassava + Bone mill.

lactic acid bacteria (LAB) underscore their importance in cassava fermentation. The nutritional composition of fermented cassava has gained increasing attention due to its potential health benefits and improved nutritional profile compared to raw cassava.

Organic acids produced during fermentation facilitate the growth and multiplication of single-cell proteins (Boonnop et al., 2009). Hu et al. (2012) reported that fermentation enables microorganisms to convert substrates containing carbon and nitrogen into protein. Longer fermentation times result in higher protein content (Gelinas et al., 2007). According to Erukainure et al. (2010), this increase may be attributed to the secretion of extracellular protease by microorganisms during fermentation. Additionally, ash content was reduced when fermentation was conducted with most of the starter cultures. Ash content reflects mineral availability in fermented food, and its decrease may be due to the leaching of soluble minerals into the fermenting medium or enzymatic hydrolysis of food components into absorbable forms. A similar decrease in ash content was reported by Atti (2000) during millet fermentation, whereas Sefa-Dedeh and Kluvitse (2004) observed an increase in ash content in fermented maize-cowpea blends.

A general decrease in crude fiber was observed after fermentation. This finding contrasts with Oyewole and Ogundele (2001), who reported an increase in crude fiber content in fufu as fermentation progressed. However, Adeyemi et al. (2012) suggested that an increase in crude fiber may interfere with nutrient availability. Igbabul et al. (2014) observed a decrease in crude fiber during cocoyam fermentation, attributing it to the softening of fibrous tissues and microbial bioconversion of carbohydrates and lignocelluloses into protein. Similar findings were reported by Hwei-Ming et al. (2004) and Balagopalan (2006). A general decrease in total carbohydrate content was also observed, likely due to the breakdown of carbohydrates into simple sugars and organic acids.

The use of starter cultures in cassava fermentation plays a pivotal role in controlling the fermentation process, influencing the quality, safety, and nutritional value of the final product. The physicochemical properties of these starter cultures are crucial for their effectiveness and overall performance. The selection of *Lactobacillus plantarum, Bacillus pumilus,* and *Kluyveromyces marxianus* for cassava fermentation in this study was based on their ability to produce desirable metabolic products and adapt to the fermentation environment. This aligns with findings by Ibrahim et al. (2024), who reported that the choice of starter cultures significantly influences microbial populations in fermented cassava. The combination of *Lactobacillus plantarum, Bacillus pumilus,* and *Kluyveromyces marxianus* resulted in a more controlled fermentation process, yielding more consistent product quality. For instance, the combination of *Lactobacillus* spp. with *Bacillus* spp. has been shown to enhance fermentation by providing complementary functions such as acid production and flavor development (Adesulu and Awojobi, 2014). When coupled with appropriate temperature and pH conditions, this microbial consortium can improve fermentation efficiency by promoting beneficial microbial activity and enhancing the nutritional profile of fermented cassava (Osei et al., 2023).

CONCLUSION

This study investigated the potential for developing starter cultures from fermented cassava tubers for fufu production. The fermentation process began effectively on the second day, highlighting the prompt activity of the introduced microorganisms. The combination of *Lactobacillus plantarum*, *Kluyveromyces marxianus*, and *Bacillus pumilus* proved to be efficient in achieving successful fermentation, with *Lactobacillus plantarum* emerging as the most dominant microorganism. This dominance contributed to a significant reduction in pH values over the fermentation period, indicating efficient acid production and a favorable fermentation environment.

The bioreactor system played a crucial role in facilitating optimal growth conditions for the microorganisms, ensuring a controlled and contamination-free environment. This setup not only enhanced the proliferation of beneficial microbes but also maintained the integrity and quality of broth cultures for starter culture production throughout the process.

The fufu produced using all the starter cultures had a desirable taste and flavor and was noticeably brighter in color. The pH values indicated acidity, and it was observed that the fufu was slightly acidic due to fermentation by *Lactobacillus plantarum*. The slight acidity of the fufu makes it an unfavorable environment for the growth of pathogenic microorganisms.

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