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SENSORY AND MICROBIOLOGICAL EVALUATION OF FUFU PREPARED TRADITIONALLY AND WITH LACTIC ACID BACTERIA ISOLATES

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Abstract: Lactic acid bacteria (LAB) metabolites possess notable antimicrobial properties. Laboratory fermentation of retted cassava for the production of *fufu* was carried out. Lactic acid and hydrogen peroxide were quantified. Starter cultures were developed antagonistic activities against spoilage moulds. Sensory evaluation was carried out. *Lactobacillus plantarum* was the most predominant, while *Pediococcus acidilactici* was the least among the isolates. *Lactobacillus plantarum* 1M91 had the highest lactic acid production of 18.37 mg/ml and hydrogen peroxide of 0.015 mg/ml production at 48 h. *Lactobacillus fermentum* 1M92 gave the best antagonistic activity (12.0) mm against *Aspergillus niger*. *Lactobacillus plantarum* 1M91 and *L. fermentum* 1M92 were used singly and in combination for controlled fermentation of *fufu.* The *fufu* prepared using starter cultures had a longer shelf life than the traditionally prepared *fufu*.

Keywords: Lactic acid bacteria, Fufu, Biopreservation, Metabolites, Starter cultures.

INTRODUCTION

Food fermentation involves the biotransformation of complex organic compounds into simpler products by the action of metabolites produced by microorganisms, including yeast, moulds and bacteria (Canos et al., 2007). Food processing using microorganisms is one of the oldest methods for food value addition and improvement. Many developing countries in Africa and Asia depend on various fermented foods in their diets (Sharma et al., 2006). Biopreservation is defined as a technique used to improve food quality and shelf life using natural or starter cultures and/or their metabolites (Stiles, 1996).

Lactic acid bacteria (LAB) are aerotolerant, acid tolerant, organotrophic, and are strictly fermentative rod or coccus, producing lactic acid as a major end product of carbohydrate fermentation (Holzapfel, 2014; König & Fröhlich, 2017). LAB is included in the Lactobacillales order, which comprises of 6 families, 36 genera, with over 200 species (Leyva et al., 2017). Organic acid production during metabolic activities leads to pH reduction, contributing to the aggregate effect, particularly by ensuring the successful early dominance of lactic acid bacteria (Dagnas et al., 2015). The lowered pH inhibited the survival of pathogenic and food spoilage microbes, thus prolonging the shelf life of fermented foods (Abbasiliasi et al., 2017).

Several publications in the literature report the role of LAB in food preservation in various fermented foods, for example, in millet (*Pennisetum typhoideum*) and cassava (*Manihot esculenta* Crantz) (Oyewole & Odunfa, 1990) & (Adebiyi et al., 2018). Cassava is a perennial woody shrub with an edible root grown in the tropics, but has its genetic origins in South America, from where it was introduced into Africa in the sixteenth century and subsequently into South East Asia. The foods made from cassava include *garri, fufu* and *lafun,* which are widely consumed in West Africa. Garri is prepared by grating freshly plucked cassava tubers that have been peeled to obtain pulp. The cassava pulps are then placed in jute bags, pressure is applied using a press machine and then fermented spontaneously for 2-3 days at ambient temperature (Beuchat, 2001; Obueh & Kolawole, 2016). After this, it is dried by heating and then mixed with palm oil. *Fufu* is a widely consumed staple food in several West and Central African countries and the Caribbean. *Fufu* is produced from the acid fermentation of cassava root tubers and is a principal dish in the consuming areas (Odunfa, 1985). *Fufu* is prepared by spontaneous fermentation, which modifies the cassava root and prevents rapid deterioration of the cassava after harvest (Oyedeji et al., 2013). Lactic acid bacteria have been observed isolated during the fermentation processes, where they impart flavour, texture, and aroma development (Oyedeji et al., 2013; Oyewole, 1992).

Food-fermenting LAB has been reported to inhibit spoilage bacteria, pathogens and provide immunomodulation of blood cholesterol levels, immune system stimulation, and to serve as an alternative to antibiotics (Vieco-Saiz et al., 2019). The final quality of the fermented food is mostly a reflection of the microbial diversity, their dynamics, and frequency of occurrence (Ogunbanwo et al., 2004).

Nowadays, the development of stable starter cultures for fermentation processes is mostly a product of quality planning other than random screening (Hansen, 2002). The development of starter cultures is dependent on principles derived from the knowledge of microbial physiology and biotechnology, and interaction with the food products (Rau & Zeidan, 2018). However, very few publications exist in the literature on the detailed utilisation of starter LAB cultures and their metabolites in controlling spoilage in *fufu*.

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This study was aimed at assessing the influence of single and mix starter LAB cultures on the shelf-life of a selected fermented food (*Fufu*) and to evaluate the antagonistic effect of some of the LAB metabolites on spoilage moulds from isolated *fufu* and *koko*.

MATERIALS AND METHODS

Sample collection

The millet (*Pennisetum typhoideum*) and the cassava tuber (*Manihot esculenta* Crantz) were purchased from a local food store in Bodija, Ibadan, Southwest Nigeria (7°26'06" N 3°54'51" E). The millet and cassava were collected and transported in clean polythene bags and kept in the refrigerator at 5 °C until use.

Sample processing

The millet grains were sorted out to remove stones, debris, and defective seeds. It was then weighed and steeped in sterile water in a clean container for 48 hours at room temperature. The steeping water was drained off, and the grains were grounded using a blender (Sapphire Mixer Grinder VTCL). The resultant paste was then separated as described by (Adedire et al., 2018; Sanni & Onilude, 2003) and allowed to ferment for 72 hours at ambient temperature [23,](Adedire et al., 2015).

The cassava tubers were washed with clean water, peeled with a clean knife, then cut into small portions and steeped in clean tap water for 72 hours according to the methods described by (Oyedeji et al., 2013).

Microbiological analysis

Microbial analysis of the fermenting substrate was estimated daily for 72 hours at 24 hours interval as described by (Abegaz, 2007; Chibuzor-Onyema et al., 2021; Oyedeji et al., 2013).

Physicochemical analysis

The pH changes and titratable acidity (TTA) of fermenting samples were monitored daily (24 hours) till the end (72 hours) of fermentation using the method of (AOAC, 2015) (pH meter - Surgifield Medical England Sm - 6021A).

Enumeration of lactic acid bacteria

Samples were collected at 24 hours intervals during fermentation of the millet and the retted cassava for 72 hours. The millet sample and retted cassava were agitated for 120 seconds before sampling to ensure homogeneity. The samples were serially diluted, and microbial isolation and enumeration were carried out as described by (Oyedeji et al., 2013; Petkova et al., 2021).

Isolation of spoilage moulds

The isolation of spoilage mould from spoilt *fufu* and *koko* was carried out according to the method of (Dike & Sanni, 2010). Characterisation was carried out using standard methods described by (Osho MB & Shobande OE, 2019). Identification was carried out by processing the biochemical reports using ABIS online.

Antagonistic activity of lab metabolites against spoilage moulds

The antagonistic activities of Lactic acid bacteria against spoilage moulds were carried out according to the method of (Ogunbanwo et al., 2014). The culture assay plates were incubated at 30 °C for 2 to 7 days and were observed for zones of inhibition (Adebiyi et al., 2018; Ogunbanwo et al., 2014; Schillinger & Lucke, 1989). Control samples were not inoculated.

INFLUENCE OF STARTER CULTURES ON FUFU PRODUCTION

Preparation of fufu

Approximately 1kg of cassava roots was washed and peeled. The peeled cassava tubers were washed several times in sterile water inside a clean vessel, and the water drained. The processed cassava tubers were inoculated with the starter cultures obtained from this study (individually and mixed) at about 3.72×10^5 CFU/ml and fermented for 72 hours at 26 °C ± 1.0 (Ogunbanwo et al., 2004).

Sensory evaluation/shelf-life study of fufu

Sensory evaluation of the fermented *fufu* was carried out by a 10 – untrained panel member who was quite familiar with the product (Dike & Sanni, 2010). The variables examined include appearance, colour, odour, taste, and texture. The evaluations were presented on a 9-point Hedonic scale (ranging magnitude of higher to lower acceptability of the food sample). Shelf life studies were carried out (Dike & Sanni, 2010).

RESULTS

From this study, 30 lactic acid bacteria strains were isolated during the fermentation of retted cassava and millet samples; 20 of the isolates have been reportedly isolated during the fermentation of retted cassava, while 10 LAB isolates were found in the fermenting millet sample. The total viable counts as observed on the De Man, Rogosa and Sharpe (MRS) agar plate are shown in Table 1. The findings from the cultural and morphological characteristics showed that most of the isolates were small, circular, whitish to creamy colour, raised with entire edges. All the isolates were gram-positive cocci, short to medium-long rods.

The pH change during the fermentation of the retted cassava was significant ($P \leq 0.05$) with time (Table 2).

The highest mean value for pH change of fermentation, 5.80 ± 0.28, was observed at 0 hr onset of fermentation, followed by mean values of 5.37 ± 0.14 and 3.70 ± 0.10 at 24 hrs and 48 hrs during the fermentation of retted cassava, respectively. The lowest mean value for retted cassava fermentation during pH change of 3.70 ± 0.10 was obtained at 72 hrs (Table 2). The pH change of retted cassava differed significantly during fermentation (Table 2).

The pH change during the fermentation of millet was significant ($P \leq 0.05$) over time duration (Table 2). The highest pH during the fermentation, 5.60 ± 0.25, was recorded at 0 hr fermentation, followed by pH values of 5.23 ± 0.46 and 4.82 ± 0.14 at 24hrs and 48hrs during the fermentation of millet. The lowest pH change of 3.77

± 0.23 was observed at 72 hrs during millet fermentation (Table 2).

Physiological characteristics of the LAB showed them as *Lactobacillus plantarum*1M91, *Lactobacillus fermentum*1M92, *Lactobacillus brevis, Leuconostoc mesenteroides*, *Pediococcus acidilactici* (Table 3)

The microorganisms associated with spoilage were isolated daily, and the cell counts (Logcfu/ml) increased as spoilage proceeded.

Cultural and microscopic examination of the fungi isolated from spoilt *fufu* and *koko* identified them as *Aspergillus niger, Aspergillus flavus, Penicillium* sp, *Rhizopus nigricans*. *Aspergillus* sp (65 %) had the highest occurrence, while *Rhizopus nigricans* (10 %) had the least occurrence. The percentage frequency of occurrence of mould isolated from the two samples, *fufu* and *koko,* is shown in Table 4.

The antagonistic activities of the cell-free supernatants of isolated lactic acid bacteria against spoilage moulds revealed that *Lactobacillus fermentum* 1M92 showed the highest zone of inhibition of 12 mm against *Aspergillus niger code*, while the least inhibition of 6mm was observed for *Lactobacillus plantarum* 1M91 against *Rhizopus nigricans code*. (Table 5)

Lactobacillus plantarum 1M91 had the highest lactic acid production of 30 mg/ml and hydrogen peroxide of 0.015 mg/ml production at 72 h and 48 h, respectively (Figures 1 and 2).

The influence of different starter cultures on *fufu* samples was assessed with the traditional fermented *fufu*. The findings showed that *fufu* produced using starters of *Lactobacillus plantarum* 1M91 and *Lactobacillus fermentum* 1M92 individually and mixed had a longer shelf life than the samples produced using the traditional method.

The shelf life of *fufu* produced using mixed starter cultures of both *Lactobacillus plantarum* 1M91 and *Lactobacillus fermentum* 1M92 was 10 days, while *fufu* samples produced by the traditional method was 4 days, followed by the onset of observable spoilage after the 72 hours fermentation period when kept at room temperature.

Change in viable counts of lactic acid bacteria and mould

Table 6 depicts the total lactic acid bacteria (LAB) and moulds counts from the *fufu* sample inoculated with single and combined starter cultures of *L. fermentum* 1M92 and *L.plantarum*1M91 species and the traditional fermentation of *fufu* (control). In all the cases of fermentation, the changing lactic acid bacteria population resulted in a very rapid increase in the total viable counts of the fermenting organisms.

Total viable counts of the spoilage moulds increased rapidly in the traditional *fufu*. The *fufu* that was improved with the introduction of the starter had a longer shelf life.

Sensory analysis

Organoleptic assessments showed significant acceptance of *fufu* inoculated with starter cultures of lactic acid bacteria and *fufu* produced by traditional fermentation (Table SM1). The change in colour, texture, taste, and aroma of the *fufu* produced using single starter and mixed starters differed significantly, and the samples were more acceptable to *fufu* produced by uncontrolled fermentation (Tables 6 and 7).

Table 1.

Total viable counts of the lab in retted cassava and millet samples

Sample/ Hours	24hrs	48hrs	72hrs
Retted cassava	3.8×10^7	5.0×10^7	6.9×10^7
Millet sample	3.9×10^7	4.8×10^7	5.7×10^7

Table 2.

PH change during the fermentation of retted cassava and millet

Time	Retted cassava	Millet
0hrs	5.80 ± 0.28^a	5.60 ± 0.25^a
24hrs	5.37 ± 0.14^{ab}	5.23 ± 0.46^{ab}
48hrs	4.88 ± 0.16^b	4.82 ± 0.14^b
72hrs	3.70 ± 0.10^c	3.77 ± 0.23^c

Each value is presented as Mean ± SEM (n =3). Values with different letters as superscripts across the column are considered significant

Table 3.

Percentage occurrence of lactic acid bacteria isolated from selected traditional fermented millet

Isolates	Number	Percentage (%)
Lactobacillus fermentum (1M92)	8	26.7
Lactobacillus plantarum (1RC51)	10	33.3

Isolates	Number	Percentage (%)
Pediococcus acidilactici (1M51)	3	10
Lactobacillus brevis (2M51)	4	13.3
Leuconostoc mesenteroides (1RC72)	5	16.7
Total	30	100

Table 4.

Percentage occurrence of moulds isolated from spoiled *fufu* and *koko* samples

Isolates	Samples	Number	Percentage (%)
Penicillium sp	*Fufu*	2	10
	Koko	3	15
Aspergillus flavus	*Fufu*	2	10
	Koko	1	5
Aspergillus niger	*Fufu*	4	20
	Koko	2	10
Rhizopus nigricans	*Fufu*	2	10
	Koko	0	0
Aspergillus fumigatus	*Fufu*	2	10
	Koko	2	10
	Total	20	100

Table 5.

Antagonistic activity of lactic acid bacteria metabolites against selected moulds

Fungi isolates	*Lactobacillus plantarum*1rc51 (mm)	*Lactobacillus plantarum* 1M91 (mm)	*Lactobacillus fermentum*1m92 (mm)
Aspergillus flavus	7.0	4.0	8.0
Rhizopus nigricans	4.0	6.0	7.0
Aspergillus fumigatus	2.0	3.0	6.0
Penicillium sp	3.0	6.0	8.0
Aspergillus niger	6.0	5.0	12.0

Inoculum size 1 × 10^4 spore/ml

Table 6.

Influence of starter cultures on the shelf life of *fufu* samples produced (cfu/g)

Sample code/Days	Total viable counts for Lactic acid bacteria (CFU/g)					
	3	4	5	6	7	9	10
A	NG	NG	2.5×10^3	2.7×10^5	2.0×10^4	1.8×10^8	1.5×10^8
B	NG	NG	NG	1.0×10^4	1.2×10^8	1.8×10^6	1.9×10^8
C	NG	NG	NG	1.0×10^5	1.1×10^6	1.4×10^5	1.6×10^6
D	NG	NG	NG	NG	1.8×10^4	2.0×10^6	2.4×10^8

Keys**:**
A: Traditional fermentation (control)
B: Fufu fermented with *Lactobacillus plantarum* 1M91
C: Fufu fermented with *Lactobacillus fermentum* 1M92
D: Fufu fermented with a mixed culture of *L. Plantarum* 1M91 and *L. Fermentum* 1M92
NG: No growth

Table 7.
Organoleptic assessment of the traditional prepared *fufu* and laboratory prepared *fufu* using starter culture

Sample	Taste	Texture	Colour	Aroma	Overall acceptability
Traditional *fufu* (control)	3.52[a]	2.25[a]	3.25 [a]	3.05 [a]	3.55 [b]
Fufu + L. Fermentum 1C51	4.45[b]	3.76 [a]	2.80 [a]	3.00 [a]	2.85 [a]
Fufu + L. Plantarum 1M91	3.02[a]	3.55 [a]	3.00 [a]	3.35 [a]	3.75 [b]
Fufu + L.fermentum 1C51 + *L. Plantarum* 1M91	4.87[b]	4.90 [b]	4.80 [a]	4.85 [b]	5.00 [b]

Values are presented as Mean ± SEM (n = 10). Values with different letters as superscripts across the column are considered significant (p≤ 0.05)

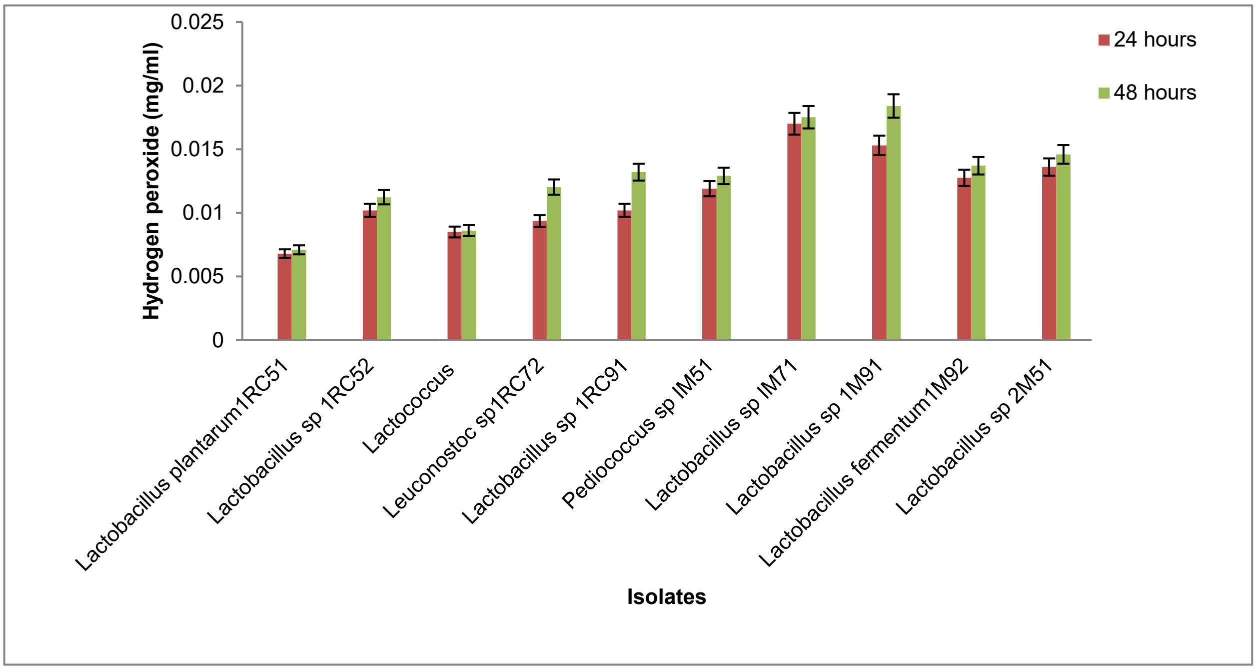

Fig. 1. Hydrogen peroxide production (mg/ml) by Lactic acid bacteria isolated from fermented Retted cassava and Millet.

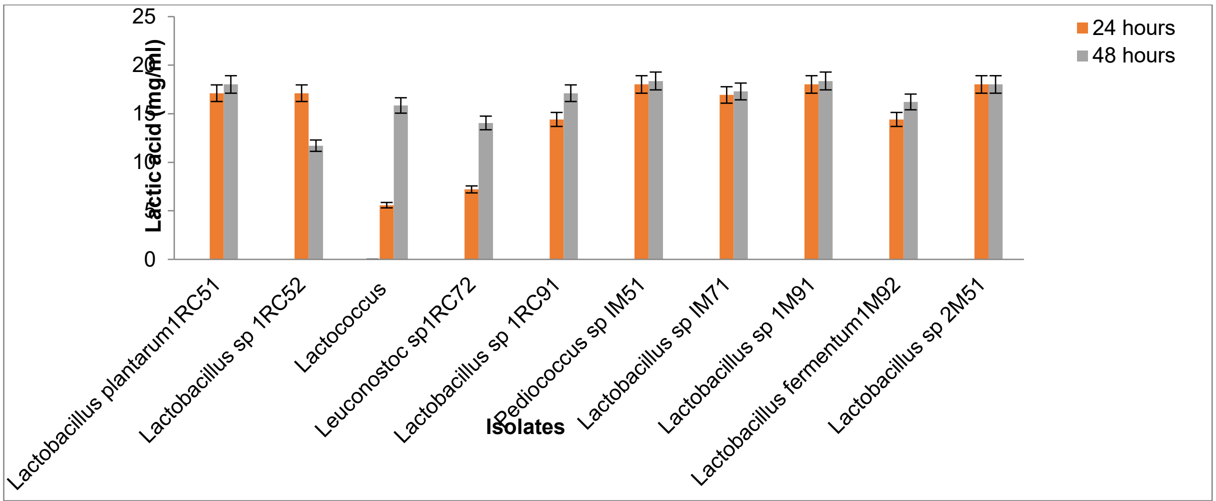

Fig. 2. Lactic acid production (mg/ml) by Lactic acid bacteria isolated from fermented Retted cassava and Millet.

DISCUSSION

From this study, the dominance of Lactic acid bacteria LAB as observed after the fermentation is in agreement with findings reported by several authors (Adedire et al., 2018; Ogunbanwo et al., 2004; Oyedeji et al., 2013; Oyewole, 1992) who reported the dominance of several LAB species (*L. plantarum, L. fermentum, L. brevis*, *Pediococcus acidilactici* and *Leuconostonoc mesenteroides*) during the fermentation of *fufu* and *ogi*. *Lactobacillus plantarum* and *L. fermentum* were more dominant in the microbial succession. The fermenting microorganisms significantly influenced the acidity of the media through the liberation of organic acids such as lactic acid during which resulted in a lowered pH of the fermenting media (Adebiyi et al., 2018; Oyedeji et al., 2013). Also, lactic acid is a major by-product of the metabolism of carbohydrates (Steinkraus, 2002). Spoilage moulds associated with the spoilage of fufu and koko in this study were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* and *Rhizopus nigricans,* these findings are in consonance with reports by (Blandino et al., 2003; Corsetti et al., 1998; Omemu et al., 2015) who reported the isolation of *Aspergillus niger, A. flavus, Penicillium* spp and *Rhizopus* spp as spoilage organisms in fermented cereals and cassava. The antagonistic effects of the metabolites of LAB against the isolated spoilage moulds showed appreciable inhibition on all the moulds with *A. niger* having the best inhibition to *Lactobacillus plantarum*1M92. This is similar to reports by (Leyva et al., 2017; Parafati et al., 2015) who reported growth inhibition of *Penicillium, Aspergillus,* and *Cladosporium* genera to some *Lactobacillus plantarum* strains. This could be as a result of the composition of MRS agar, which could stimulate the expressions of antifungal effects by LAB due to its acetate content, thus reinforcing LAB antifungal activity and a pseudo-increase in the number of active isolates (Le Lay et al., 2016).

The quantification of the hydrogen peroxide produced showed a significant increase for all the LAB isolates over 48 hours, while the lactic acid production showed a significant increase at 48 hours, and no doubt had a prolonged effect on the shelf-life extension of *fufu*.

The shelf life of the laboratory prepared *fufu* with starter cultures showed marked improvement in contrast to the traditionally fermented samples, which closely agrees with reports by (Ogunbanwo et al., 2014; Oyedeji et al., 2013) who highlighted the role of starter LAB in the retarding food spoilage. This could also be attributed to the fact that traditional fermentation, which is a product of the competitive activities of several microorganisms, resulted in the best adaptive strains dominating the fermentation process (Madoroba et al., 2009). The LAB counts showed a significant increase in the starter fermented *fufu,* as evident from their increasing counts up to Day 10, when compared to the traditional *fufu* fermented spontaneously, with the *fufu* fermented using consortia showing the highest counts.

Findings from the organoleptic assessment also showed that *fufu* fermented using the LAB consortia had higher acceptability compared to *fufu* fermented using a single LAB and the traditionally fermented *fufu*. This was observed in the significant improvement in the aroma of the starter fermented *fufu,* which shows the potential of the starter LAB to improve the quality of *fufu*. These findings agree with reports by (Achi & Akomas, 2006) who associated the acceptance of *fufu* to the effects of processing parameters but differ from (Sobowale et al., 2007) who reported no significant difference in the acceptability of starter fermented *fufu* with traditionally fermented *fufu*.

CONCLUSION

The metabolites produced by *Lactobacillus fermentum* 1M92 were effective against the spoilage moulds *Aspergillus niger* and *Rhizopus nigricans* and can therefore be used as biopreservatives upon further studies. The incorporation of starter cultures to cassava during steeping affected the pasting and aroma of *'fufu'*.

More research should be done on the use of genome and peptidome data in the characterisation of novel bacteriocins with little or no homology to known bacteriocins. Focus on the use of emerging technologies and inexpensive media is also important for the large-scale production of bacteriocin to overcome the commercial viability of bacteriocin production.

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AUTHORS CONTRIBUTION

Conceptualization, T.C.R and A.I.S; methodology, T.C.R., K.B. and A.O.O, data collection, data validation, and data processing T.C.R., K.B. and A.O.O.,; writing-original draft preparation, T.C.R., K.B. and A.O.O.; writing-review and editing, A.O.O.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with other people or organization that might be construed to influence the results or interpretation of this manuscript.

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