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## Full Length Research Paper

## Shelf life extension study of *ogi* and *fufu* using bacteriocin isolated from *Lactobacillus acidophilus* of fermented dairy products

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**Bacteriocins are antimicrobial peptides produced majorly by lactic acid bacteria (LAB), which act against food spoilage and pathogenic bacteria, thereby, extending the shelf life of food products. Bacteriocin produced by *Lactobacillus acidophilus* (isolated from yoghurt and *nono*) was extracted and incorporated in samples of *ogi* and *fufu* to evaluate its bio-preservative potential. Microbiological analysis was done at the beginning of spoilage test lasting for a period of 60 days. Inoculation of bacteriocin resulted in the extension of shelf life of *ogi* to 60 days and *fufu* beyond 60 days under refrigeration conditions as compared to the uninoculated samples of *ogi* and *fufu* which had a shelf life of 45 and 55 days, respectively.**

**Key words:** Shelf life, bacteriocin, *ogi*, *fufu*, bio-preservative.

### INTRODUCTION

Food fermentation processes generally involve the conversion of carbohydrates to alcohol and carbon dioxide or organic acids using yeast or bacteria, mostly under anaerobic conditions (William and Dennis, 2011). Various traditional fermented foods are produced in many Africa countries (Chelule et al., 2010). In Nigeria, however, the most common substrates for fermentation are cassava and cereal grains such as maize, sorghum and millet (Adesokan et al., 2010).

Majority of Nigerian fermented foods are products obtained through lactic acid fermentation (Ogunbanwo et al., 2004). The production of many indigenous African

foods is often plagued by premature spoilage due to their high moisture content (Adebayo and Famurewa, 2002). Many food preservation techniques including physical, chemical and biological methods have been explored as remedies to food spoilage.

Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatment (e.g. high temperature). Nevertheless, these types of solutions have many drawbacks such as the proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the

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organoleptic and nutritional properties of foods, as well as the recent consumer trends in purchase and consumption of food that are generally safe, minimally processed and without additives.

To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in food are being replaced by combinations of innovative technologies that include biological antimicrobial system such as LAB and/or their bacteriocins. The use of LAB and/or their bacteriocins may be an efficient way of extending shelf life and food safety through the inhibition of spoilage and pathogenic bacteria without altering the nutritional quality of raw materials and food products (Ross et al., 2002).

Fermented foods are largely consumed in Africa where they constitute a bulk of the diet among the many African traditionally fermented food stuffs like *ogi* and *fufu*. *Ogi* is popular in Nigeria and in most of West Africa. *Ogi* is a fermented semi-solid food product manufactured from cereals. Gelatinized *ogi* is commonly referred to as pap (already prepared *ogi* into porridge in the presence of water and heat). It is commonly used as weaning food for babies and for young children (Opeifa et al., 2015) and as a standard breakfast cereals in many homes. While *fufu* is a product of fermented cassava tuber, fermented cassava roots are mashed and cooked into dough (Oyinlola et al., 2016). However, these food products are plagued with the problem of reduced shelf life due to random inoculation by spoilage organisms and the humid conditions in the tropics (Adeyeye, 2016).

LAB is the main organisms associated with the fermentation of foods. They had been known to produce antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide and bacteriocin (Stellato et al., 2015). They constitute a group of bacteria that have morphological, metabolic and physiological similarities, and they are also closely related phylogenetically. They are Gram-positive, non-sporulating, non-respiring cocci or rods which ferment carbohydrates to produce lactic acid as their major end product (Pei et al., 2017).

LAB made it possible for human to increase the shelf life of food and food products by utilizing their antimicrobial activities without damaging food contents (Tamang et al., 2016). Lactic acid bacteria are usually found in food products, also known as probiotics and produce bacteriocins which are proteinaceous compound.

LAB is also able to produce small organic substances that contribute to aroma and give specific organoleptic attributes to the products (Hattingh et al., 2015). These micro-organisms are found in milk, meat and fermented products; as well as in fermented vegetables and beverages inhibiting the growth of pathogenic and deteriorating micro-organisms, maintaining the nutritive quality and improving the shelf life of foods.

Bacteriocin is the most potent of all the antimicrobial compounds produced by LAB (Aabha and Santosh,

2015). The term “bacteriocin” comprises of a large and diverse group of ribosomally synthesized extracellular antimicrobial low molecular mass proteins or peptides produced by strains of diverse bacterial species. The antimicrobial activity of this group of natural substances against food borne pathogens, as well as spoilage bacteria, has raised considerable interest for their application in food preservation (Gong et al., 2010; Ana, 2012).

In the past years, a lot of work has aimed to detect, purify and characterize bacteriocin, as well as their application in food preservation strategies. Application of bacteriocins may help reduce the use of chemical preservatives and/or the intensity of heat and other physical treatments, satisfying the demands of consumers for foods that are fresh tasting, ready to eat, and lightly preserved (Saito and Nitisinprasert, 2015).

The LAB bacteriocins have many attractive characteristics that make them suitable candidates for use as food preservatives such as protein nature, non-toxic to laboratory animals tested and generally non-immunogenic, inactive against eukaryotic cells, and generally thermo resistant (Oduro-Yeboah, 2016).

## MATERIALS AND METHODS

### Isolation of *Lactobacillus acidophilus*

*L. acidophilus* was isolated from dairy products (yoghurt and *nono*) in the laboratory of Kaduna State University (KASU), Kaduna State, Nigeria and was maintained on MRS (De Mann Rogosa Sharpe) agar.

### Isolation and identification of lactic acid bacteria (*L. acidophilus*)

LAB were isolated using De Mann Rogosa Sharpe agar (oxide MRS UK) (MRSA-35 g<sup>l</sup><sup>-1</sup>): peptone (10 g); yeast extract (5.0 g); K<sub>2</sub>HPO<sub>4</sub> (2.0 g); NaNO<sub>3</sub> (4.0 g); MnSO<sub>4</sub> (50 mg); MgSO<sub>4</sub> (2.0 g); (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> (2.0 g); Tween (0.1 ml); Lab M powder (10.0 g); and distilled water (1 L). The medium was prepared according to the manufacturer's instruction.

1 ml of each dairy product (yoghurt and *nono*) was serially diluted by six folds into sterile test tubes containing 9 ml of distilled H<sub>2</sub>O. 1 ml of the diluent 10<sup>-4</sup> was inoculated into MRS agar plates using pour plate technique, incubated anaerobically at 37°C for 24 h. Bacteria colonies were sub-cultured on MRS agar until discrete colonies were obtained. The pure culture was maintained on agar slant for further characterization and identification. The isolates were characterized based on colony morphology, cell morphology, cell arrangements, motility and biochemical test (Fawole and Oso, 1998; Oyeleke and Manga, 2008). The lactic acid bacteria strain was identified by reference to the Bergey's Manual of Systematic Bacteriology and the Genera of Lactic Acid Bacteria.

### Bacteriocin production and extraction

To determine bacteriocin production, the *L. acidophilus* was inoculated into 5 ml of MRS broth under anaerobic conditions and incubated at 37°C for 24 h. The culture extract was obtained by

centrifugation at 10,000 rpm for 15 min. The supernatant was decanted and adjusted to pH 7.0 by adding sodium hydroxide (1 M NaOH) to eliminate any effect of acidity; hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was neutralized by the addition of 5 mg/ml catalase. The mixture of supernatant of LAB culture, NaOH and catalase was filtered and sterilized with a 0.2 µm cellulose acetate filter to obtain cell free bacteriocin.

The obtained cell free supernatant was then precipitated with 40% solution of ammonium sulphate. The mixture was stirred for 90 min at 4°C and then centrifuged at 10,000 rpm for 90 min at 4°C. The precipitates were dissolved in phosphate buffered saline (PBS) and then further used in well diffusion assay to check the antimicrobial activity (Savadogo et al., 2006).

#### **Determination of antibacterial activity of bacteriocin using agar well diffusion method**

The total volume of 100 µl from the purified Bacteriocin was placed in Mueller Hinton agar wells (wells were bored using 5 mm cork borer) in Petri dishes seeded with the bio-assay strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium* spp. and *Salmonella dysenteriae*), separately and incubated overnight at 37°C. The diameters of the zone of inhibition were recorded by measuring the zone of inhibition around the well using a meter rule (Soomro et al., 2002).

#### **Characterization of bacteriocin**

The purified bacteriocin sample was characterized with respect to their heat stability, pH stability and stability during storage.

#### **Heat stability of bacteriocin**

The effects of temperature on the bacteriocin were tested by heating the bacteriocin in different test tubes at 40, 60, 100 and 121°C for 15 min and incubated for one hour at 37°C. The treated bacteriocin sample was assayed for antimicrobial activity against *S. aureus* and *E. coli*.

#### **pH stability of bacteriocin**

Aliquots of the bacteriocin were placed in test tubes and the pH value of the contents was adjusted to pH 2-12 individually using either NaOH or HCl (1 M NaOH or 1 M HCl solution) and then incubated for 1 h at 37°C. Thereafter, assay for antimicrobial activity against *S. aureus* and *E. coli* was carried out on the treated bacteriocin samples.

#### **Stability of bacteriocin during storage**

The purified bacteriocin was incubated at 37°C and refrigerated at 4, 10 and -20°C, respectively, for thirty days. The residual antimicrobial activity against *S. aureus* and *E. coli* was thereafter determined.

#### **Preparation of samples (ogi and fufu)**

For *ogi*, guinea corn (*Sorghum bicolor*) was washed and steeped in water and allowed to ferment for 3 days by the natural flora of the grains. After fermentation, the grains were drained, wet-milled into slurry in a grinding mill and sieved through a fine mesh. The chaff was discarded and the resulting starch paste at the bottom of the

container was the *ogi*.

For *fufu*, the fresh cassava (*Manihot esculenta*) tubers were peeled and cut into piece. The cassava tissues were then soaked in water to ferment for 4 days at ambient temperature. The soft fermented cassava tissues were meshed manually and later passed through a plastic sieve. The fibres were discarded and the thick paste (mash) that settled under the water was *fufu*.

#### **Shelf life study of ogi and fufu**

A volume of 5 ml of the bacteriocin obtained was added to 10 g of *ogi* and *fufu*, respectively, and the two different combinations (products) were stored under refrigerated conditions (4°C) for 60 days to determine their shelf-lives in comparison with the experimental control (*ogi* and *fufu* without bacteriocin). The food samples were then observed daily to determine when spoilage would start. The total microbial load was determined at the beginning of spoilage (the growth of microorganisms on the food samples showed sign of spoilage). Initial plate count of samples (*ogi* and *fufu*) was serially diluted at 10<sup>6</sup> and the plates were incubated at 37°C for 24 h. The colony count was recorded and compared with the control (without bacteriocin) (Narayanapillai et al., 2012).

#### **Statistical analysis**

The data generated were subjected to analysis of variance (ANOVA) using the statistical software package SPSS (Statistical package for social science version 20) and standard error of mean (SEM) for all the graphs plotted were represented with error bars.

## **RESULTS AND DISCUSSION**

### **Isolation and identification of *L. acidophilus***

The fermented milk products (yoghurt and *nono*) analyzed contained lactic acid bacteria (LAB) in varying proportions which included *L. acidophilus*. It was identified based on colony morphology and characterized based on cell morphology and biochemical test (Table 1).

It is clear that the bacteria was Gram positive, rod shaped coccobacilli occurring singly or in chains. The main task of carbohydrate fermentation test was to investigate the ability of bacteria to ferment different types of carbohydrates. Table 1 shows that the isolated bacteria could ferment maltose, lactose, sucrose and glucose, but not sorbitol and arabinose. Thus, the results obtained coincided with *L. acidophilus* strain characteristics.

### **Antimicrobial properties of bacteriocins**

The results of antagonistic effects of the bacteriocin against 5 pathogenic strains are shown in Table 2. The results revealed that the bacteriocin exhibited strong inhibition on the growth of *Pseudomonas aeruginosa*, *Escherichia coli* and *Clostridium* spp. (inhibition zone of 16, 15 and 10 mm, respectively) and good inhibition against *S. aureus* and *Salmonella dysenteriae* with inhibition zones of 8 and 7 mm, respectively.

**Table 1.** Biochemical, morphological and physiological characteristics of *L. acidophilus*.

Isolate	<i>Lactobacillus acidophilus</i>
Cell morphology	Cocci bacilli
Colony morphology	Convex, small, rough edges and white
Gram staining	Gram +
Motility	Non motile
Catalase	-
Indole	-
Arabinose	-
Sorbitol	-
Maltose	+
Lactose	+
Sucrose	+
Glucose	+

(-) Negative, (+) positive.

**Table 2.** Antimicrobial activity (mm) of crude bacteriocin against test organisms.

Test organism	Bacteriocin	Zone of inhibition (in mm) Gentamycin (control)
<i>Staphylococcus aureus</i>	8	14
<i>Escherichia coli</i>	15	12
<i>Salmonella dysenteriae</i>	7	12
<i>Clostridium</i> spp.	10	13
<i>Pseudomonas aeruginosa</i>	16	17

**Table 3.** Effects of temperature changes on antimicrobial activity of obtained bacteriocin against *E. coli* and *S. aureus*.

Temperature (°C)	Zone of inhibition (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
37	10.00±0.50 <sup>e</sup>	10.00±0.30 <sup>e</sup>
40	9.00±0.60 <sup>d</sup>	10.00±0.50 <sup>e</sup>
60	10.00±0.80 <sup>e</sup>	8.00±0.40 <sup>d</sup>
80	7.00±0.40 <sup>c</sup>	6.00±0.20 <sup>c</sup>
100	4.00±0.20 <sup>b</sup>	3.00±0.10 <sup>b</sup>
121	3.00±0.10 <sup>a</sup>	2.00±0.10 <sup>a</sup>

Values are Mean ± SEM; values with different superscript within the column are significantly different (P<0.05) by Duncan multiple range test.

The inhibitory activities shown by this bacteriocin against selected microorganisms revealed that bacteriocin was actually produced by the *Lactobacillus* strain. Earlier reports by Ogunshe et al. (2007) revealed the presence of the compound bacteriocins in the *Lactobacillus* strains and bacteriocins have inhibitory effect against several bacteria. Possession of bacteriocin in *Lactobacilli* strains indicates their probiotic potentials (Ogunshe et al., 2007).

#### Heat stability of bacteriocin

The bacteriocin produced by the *L. acidophilus* was heat

stable after heat treatment at 37, 40, 60, 80 and 100°C for 15 min.

Table 3 shows that at 37 and 60°C, the highest significant (P < 0.05) zone of inhibition in *E. coli* was 10.00 mm, respectively, while at autoclaving temperature 121°C, it had the lowest zone of inhibition of 3.00 mm, respectively. At 37 and 40°C, the highest significant (P < 0.05) zone of inhibition obtained in *S. aureus* was 10.00 mm, respectively while at temperature 121°C, it had the lowest value of inhibition (2.00 mm). Similar results were recorded for a number of bacteriocins produced by *Lactobacillus* strains which was resistant at 100°C for 15 min (Joshi et al., 2006). The phenomenon of heat stability

**Table 4.** Effects of pH changes on antimicrobial activity against *E. coli* and *S. aureus*.

pH	Zone of inhibition(mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
2	8.00±0.40 <sup>cd</sup>	8.00±0.90 <sup>d</sup>
4	9.00±0.80 <sup>d</sup>	8.00±0.70 <sup>d</sup>
6	7.00±0.60 <sup>c</sup>	9.00±0.20 <sup>d</sup>
8	5.00±0.30 <sup>b</sup>	5.00±0.40 <sup>b</sup>
10	3.00±0.70 <sup>a</sup>	4.00±0.50 <sup>b</sup>
12	3.00±0.50 <sup>a</sup>	2.00±0.30 <sup>a</sup>

Values are Mean ± SEM; values with different superscript within the column are significantly different (P<0.05) by Duncan multiple range test.

of LAB bacteriocins have been reported earlier in literatures (Moigani and Amirinia, 2007; Ogunbanwo et al., 2004). This present research is also in agreement with the above mentioned reports as it was observed that the bacteriocin used in this study still retained its antimicrobial activity after heating at 121°C for 15 min which means it could be placed within the heat stable low molecular weight group of bacteriocins. This quality of the bacteriocin makes it superior in processed foodstuffs where high heat is applied.

Thermostability is a very useful characteristic in case of using bacteriocin as food preservative, because many food processing procedures involve a heating step (Panesar and Bera, 2011). Moigani and Amirinia (2007) also stated that it is a good property of bacteriocin that it remains effective even at 121°C for 15 min. Due to this property, it remains effective during many food safety processes like pasteurization.

#### pH stability of bacteriocin

The bacteriocin obtained from *L. acidophilus* in this study was observed to be active over a pH range of 2 to 6, but its activity reduced along neutral to alkaline pH range of 7 to 12 (Table 4). This indicates strong probiotic potential because most of the bacteriocins are resistant to acidic pH more than basic pH. It was observed that at pH 4, the highest significant ( $p < 0.05$ ) zone of inhibition was 9.00 mm against *E. coli*, while at pH 10 and 12, the lowest zone of inhibition of 3.00 mm was obtained respectively. This implies that bacteriocin obtained from *L. acidophilus* will be effective against Gram negative bacteria such as *E. coli* at acidic pH ranges and not at alkaline pH range. Also, against *S. aureus*, the bacteriocin showed maximum activity at pH 6 which had the highest significant ( $p < 0.05$ ) zone of inhibition of 9.00 mm, while at pH 12, it had the lowest of 2.00 mm, respectively.

Similar results were reported by Adebayo and Famurewa (2002) who opined that the bacteriocin of

*Lactobacillus* were active over a wide range of pH 2 to 6 and is the optimum pH range for good inhibitory activity of bacteriocin from *Lactobacillus* strains against a wide range of various pathogenic organisms for example *S. aureus*, while inactivation occurred mostly at pH 12, suggesting an inhibitory effect of acidity on the growth of *S. aureus* and *E. coli*. Most of lactic acid bacteria excrete acid that has been shown to inhibit growth of pathogens. These observations are in agreement with those reported by Tatsadjieu et al. (2009) in their work with LAB bacteriocins with antimicrobial activities against Chicken *Salmonella enteric* and *E. coli*. The findings of the present study are also in agreement with those reported by Holzapfel et al. (2010) who showed that *L. plantarum* excreted other compounds such as bacteriocins that inhibited the growth of pathogens. Bacteriocins produced by *L. acidophilus* in this investigation proved to have high activity and stability at pH 2, 4 and 6, respectively against the range of pathogenic and spoilage microorganism (Table 4).

#### Storage stability of bacteriocin

The bacteriocin produced by *L. acidophilus* was stable at 4 and -20°C, slightly stable at 10°C and unstable at room temperature (24±1°C). Table 5 shows that the maximum zone of inhibition was observed at 4°C against *E. coli* (10 mm) and *S. aureus* (9 mm), respectively. The percentage of effectiveness reduced more at 10°C as compared to 4 and -20°C storage temperature. These implies that the bacteriocin can be stored at -20 and 4°C, indicating that cold temperature may be the most appropriate preservation technique for storing bacteriocins. Similar results was reported by Panesar and Bera (2011) that the high stability of bacteriocin during prolong storage makes them superior and can have a positive impact on their use as bio-preservative with a view to improving the hygiene and safety of food products, especially processed foods. Bacteriocin is pH, heat and storage temperature dependent. So, due to these qualities, bacteriocins produced by LAB constitute the best option as bio-preservative for the preservation of food at commercial level.

#### Bio-preservative efficiency of bacteriocin in *ogi*

The effectiveness of bacteriocin isolated from *L. acidophilus* to act as bio-preservative and its role in increasing shelf life of *fufu* and *ogi* was checked in the presence of bacteriocin as compared to control.

Table 6 presents the result of the shelf life study carried out on inoculated *ogi* and uninoculated *ogi* (as control) which were monitored and compared throughout the 60 days period of study. In the case of the control sample at day zero, the total microbial count in *ogi* was  $8.9 \times 10^5$

**Table 5.** Effects of storage temperature on bacteriocin activity obtained from *L. acidophilus* during storage.

Temperature (°C)	Zone of inhibition (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
-20	9.00±0.70 <sup>b</sup>	9.00±0.60 <sup>b</sup>
4	10.00±0.80 <sup>c</sup>	9.00±0.30 <sup>b</sup>
10	6.00±0.50 <sup>a</sup>	5.00±0.20 <sup>a</sup>

Values are Mean ± SEM; values with different superscript within the column are significantly different (P<0.05) by Duncan multiple range test.

**Table 6.** Effects of bacteriocin on microbial count in *ogi* during storage at 4°C.

Day	Uninoculated <i>ogi</i> (control) (CFU/ml)	Ogi Inoculated with bacteriocin (CFU/ml)	P-value
0	8.9±0.02×10 <sup>5</sup>	3.5±0.01×10 <sup>5</sup>	0.000
45	2.20±0.20×10 <sup>6</sup>	2.8±0.02×10 <sup>5</sup>	0.000
50	2.40±0.10×10 <sup>6</sup>	2.2±0.02×10 <sup>5</sup>	0.000
55	2.70±0.20×10 <sup>6</sup>	1.8±0.02×10 <sup>5</sup>	0.000
60	3.00±0.20×10 <sup>6</sup>	1.5±0.20×10 <sup>5</sup>	0.000

Values are Mean ± SEM; p-value less than 0.05 is considered significantly different (P<0.05) by independent sample t-test.

**Table 7.** Effects of bacteriocin on microbial count in *fufu* during storage at 4°C.

Day	Uninoculated <i>fufu</i> (control) (CFU/ml)	Fufu inoculated with bacteriocin (CFU/ml)	P-value
0	2.8±0.20×10 <sup>5</sup>	1.5±0.01×10 <sup>5</sup>	0.000
55	2.20±0.02×10 <sup>6</sup>	1.1±0.01×10 <sup>5</sup>	0.000
60	2.35±0.02×10 <sup>6</sup>	8±0.01×10 <sup>4</sup>	0.000

Values are Mean ± SEM; p-value less than 0.05 is considered significantly different (P<0.05) by independent sample t-test.

CFU/ml, while inoculated *ogi* after 24 h was  $3.5 \times 10^5$  CFU/ml, this could be due to bactericidal effect of bacteriocin on the microorganisms initially present in the sample (Samelis et al., 2005). During storage of the *ogi*, physical examination showed signs of spoilage after 45 days of storage which caused a sharp increase in the microbial count,  $2.2 \times 10^6$  CFU/ml which could be due to the initiation of spoilage of *ogi* at 4°C, while inoculated *ogi* after 45 days of storage was  $2.8 \times 10^5$  CFU/ml. The result depicted that the microbial load of bacteriocin treated samples and the control samples after day 45 were not comparable. The result showed that there was a decrease in the microbial count in the inoculated *ogi* throughout the period of study. After 45 days, the microbial analysis was made after every 5 days interval.

The results of this investigation have shown that the uninoculated *ogi* had a shelf life of 45 days before spoilage occurred. With bacteriocin, the shelf life of *ogi* was increased up to 15 days. Ohenhen and Ikenebomeh

(2007) monitored and compared inoculated fermented *ogi* slurry and uninoculated *ogi* slurry throughout a 60 days period of study in which the uninoculated *ogi* slurry had a mouldy flavour by 40 days of study and by the end of 60 days period of study, it was no longer acceptable nor edible in terms of colour and flavour. This observation correlated with the observation of Mensah et al. (2002) that the method of preparation, handling and environmental factors were probably responsible for the early sign of spoilage observed during the study.

#### Bio-preservative efficiency of bacteriocin in *fufu*

The effect of bacteriocin on the shelf life of *fufu* is presented in Table 7. The total microbial count in *fufu* (control) was  $2.8 \times 10^5$  CFU/ml as compared to  $8.9 \times 10^6$  CFU/ml in *ogi* at day zero. There was a significant difference (P<0.05) in the microbial count of uninoculated

*fufu* and *ogi*. The initial low microbial load in *fufu* as compared to *ogi* might be due to the heat treatment (heating process) involved in the cooking of the fermented mashed cassava root into ready to eat *fufu* (Omafuvbe et al., 2007).

Similarly, previous studies indicate that the treatment of heat during the meat-ball preparation inactivates vegetative cells, which was indicated by zero microbial count in control and treated samples at day zero (Intarapichet and Gosaarak, 2008). The data also shows the bactericidal effect of bacteriocin has drastic decrease in viable cell count in initial sample (day zero),  $1.5 \times 10^5$  CFU/ml.

Physical examination of the uninoculated *fufu* showed sign of spoilage after 55 days of storage, with microbial load of  $2.2 \times 10^6$  CFU/ml, while the bacteriocin treated sample showed  $1.1 \times 10^5$  CFU/ml viable cell counts; there was a decrease in the number of microbial load in the treated sample. The results of this investigation have shown that the shelf life of inoculated *fufu* was over 60 days; this indicates that the product can keep well beyond this period, whereas the uninoculated *fufu* had a shelf life of 55 days before spoilage started.

Previous studies (Intarapichet and Gosaarak, 2008) also reported that crude bacteriocin from *Lactococcus lactis* TISTR 1401 prevented the growth of total aerobic bacteria up to day 6 in treated meatball batter as compared to control.

## Conclusion

The *Lactobacilli* strain used in this study, *L. acidophilus* produced an appreciable quantity of bacteriocin that inhibited the pathogenic organisms associated with spoilage of *ogi* and *fufu* under study.

The use of bacteriocin also extended the shelf life of both *ogi* and *fufu* by 15 and 5 days, respectively. From the results obtained, the bacteriocin could be frozen or refrigerated for storage purposes. A maximum reduction of bacterial load was observed when the bacteriocin was introduced into fermented food products.

The production, purification and characterization of bacteriocins are imperative because bacteriocins are important in the improvement of shelf life of foods products. Bacteriocins obtained from LAB are regarded as safe (GRAS).

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

Aabha G, Santosh KT (2015). Probiotic potential of bacteriocin-producing *Enterococcus hirae* strain LD3 isolated from dosa batter, Ann. Microbiol. 3(9):223-232.

- Adebayo CO, Famurewa O (2002). Antimicrobial effectiveness of in-house-use concentration of disinfectants in a Nigerian hospital. I Technosci. 6(1):17-20.
- Adesokan AO, Abiola OP, Ogundiyamo O (2010). Influence of ginger on sensory properties and shelf-life of *ogi*, a Nigerian traditional fermented food. Afr. J. Biotechnol. 9(12):1803-1808.
- Adeyeye SAO (2016). Safety Issues in Traditional West African Foods: A Critical Review. J. Culinary Sci. Technol. 75(3):213-229.
- Ana AZ (2012). Antimicrobial activities of lactic acid bacteria strains isolated from Nile Tilapia Intestine (*Oreochromis niloticus*). J. Biol. Life Sci. 25(4):164-171.
- Chelule PK, Mbongwa HP, Carries S, Gqaleni N (2010). Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. Food Chem. 122(3):656-661.
- Gong, HS, Meng XC, Wang H (2010). Plantaricin MG active against Gram – negative bacteria produced by *Lactobacillus plantarum* KLD51.0391 isolated from Jiaoke; a traditional fermented cream from China. Food Control 21:89-96.
- Hattingh M, Alexander A, Meijering I, Van Reenan CA, Dicks LMT (2015). Amyolytic strains of *Lactobacillus plantarum* isolated from barley. Afr. J. Biotechnol. 14(4):310-318.
- Holzapfel WH, Cho G, Huch M, Franz CMP (2010). Genetic Analysis of the Plantaricin Efl. Locus of *Lactobacillus plantarum* PCS20 reveals an unusual plantaricin E gene sequence as a result of mutation. Intl. J. Food Microbiol. 141:5117-S124.
- Intarapichet K, Gosaarak S (2008). The use of crude bacteriocins from *Lactococcus lactis* TISTR 1401 as biopreservative to extend shelf life of aerobically packed pork meatballs. In. Proceedings of the 54th International Congress of Meat Science and Technology (54th ICoMST). Elsevier. pp. 1-3.
- Joshi VK, Somesh S, Neerja SR (2006). Production, purification, stability and efficacy of bacteriocin from isolates nature lactic acid fermentation of vegetables. Food J. Biotechnol. 44(3):435-439.
- Moigani N, Amirinia C (2007). Kinetics of Growth and Bacteriocin production in *Lactobacillus casei* RN 78 isolated from a dairy sample in Iran. Intl. J. Dairy Sci. 5(2):1-12.
- Narayanapillai U, Duraisamy S, Balakrishnan S, Ramasamy G (2012). Production of bacteriocin and their application in food products. Asian Pac. J. Trop. Biol. Med. 56(3):406-410.
- Oduro-Yeboah C (2016). The role of traditional food processing technologies in preservation of foods: the Ghanaian experience. Afr. J. Food Agric. Nutr. Dev. 16(2):1-3.
- Ogunbanwo ST, Sanni AI, Onilude AA (2004). Effect of bacteriocinogenic *Lactobacillus* sp. On the shelf life of *fufu* a traditional fermented cassava product. World J. Microbiol. Biotechnol. 20(2):57-63.
- Ogunshe AAO, Omotoso MA, Adeyeye JA (2007). *In vitro* antimicrobial Characteristics of Bacteriocin Producing *Lactobacillus* Strains from Nigerian Indigenous Fermented Foods. Afr. J. Biotechnol. 6(3):445-453.
- Ohenhen RE, Ikenebomeh MJ (2007). Shelf stability and enzyme activity studies of *ogi*: a corn meal fermented product. J. Am. Sci. 3(1):34-37.
- Omafuvbe BO, Adigun AR, Ogunsuyi JL, Asunmo A.M. (2007). Microbial Diversity in Ready-to-eat *fufu* and Lafun-fermented Cassava Products sold in Ile-Ife, Nigeria. Res. J. Microbiol. 48(2):831-837.
- Opeifa AO, Olatidoye OP, Adesala SO, Fayomi MJ (2015). Production and Quality Evaluation of *Ogi* Produced from Fermented Maize and Horse Eye Bean (*Mucuna urens*). Pak. J. Nutr. 14(7):417-425.
- Oyinlola KA, Onilude AA, Garuba OE (2016). Towards the development of a common starter culture for *fufu* and *usi* (edible starch): Screening for potential starters. Intl. J. Food Stud. 5(1):718-722.
- Panesar S, Bera MB (2011). Isolation, screening and evaluation of antimicrobial activity of potential bacteriocin using LAB Isolate. J. Microbiol. 1(2):113-119.
- Pei J, Yue T, Yuan Y, Dai L (2017). Activity of paracin C from lactic acid bacteria against *Alicyclobacillus* in apple juice: Application of a novelty bacteriocin. J. Food Safe. 75(3):265-267.
- Ross RP, Morgan S, Hill C (2002). Preservation and fermentation: past, present and future. Intl. J. Food Microbiol. 79(1-2):3-16.
- Saito T, Niitsinprasert S (2015). Detection and partial characterization of



- bacteriocin produced by *Leuconostoc* isolated from Thai fermented food. *J. Sci. Technol. Humanit.* 1(2):149-158.
- Samelis J, Bedie GK, Sofos K, Smith GC (2005). Combinations of Nisin with Organic Acids or Salt to Control *Listeria monocytogens* a sliced pork Bologna stored at 4°C in Vacuum Packages. *LWT Food Sci. Technol.* 38(5):21-28.
- Savadogo A, Outtara CAT, Bassole IHN, Traore SA (2006). Bacteriocins and lactic acid bacteria – a mini review. *Afr. J. Biochem.* 5(9):678-683.
- Stellato G, De Filippis F, La Storia A, Ercolini D (2015). Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy-processing environment. *Appl. Environ. Microbiol.* 55(3):225-263.
- Tamang JP, Watanabe K, Holzapfel WH (2016). Review: diversity of microorganisms in global fermented foods and beverages. *Front. Microbiol.* 5(9):678-683.
- Tatsadjieu NL, Njintang YN, Sonfack TK, Mbofung CM (2009). Characterization of Lactic acid Bacteria Producing Bacteriocins against Chicken *Salmonella enteric* and *Escherichia coli*. *Afr. J. Microbiol. Res.* 3(5):220-227.
- William CF, Dennis CW (2011). *Food Microbiology*, Fourth edition, McGraw Hill, India. 6(2):1949-1953.