

BIOPRESERVATIVE EFFECT OF LACTIC ACID BACTERIA ON MICROBIOLOGICAL, SHELF-LIFE AND SENSORY QUALITY OF UGBA, A TRADITIONAL NIGERIAN FERMENTED FOOD

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ABSTRACT

The study was conducted with the aim of evaluating the biopreservative potentials of lactic acid bacteria isolated from *ugba* and the safety nature of the product. The LAB isolates used as starter cultures in this study are *Lactobacillus plantarum*, which have been isolated from *ugba* in a previous study by same authors, based on their ability to produce considerable quantities of lactic acid under a reduced pH and good fermentative activity exhibited. The pure culture were successively screened and used (in single) to ferment African oil bean slices. The samples were subjected to shelf-life study and microbiological analysis. Sample B inoculated with LAB starter culture was able to store for more than 7 days with less microbial load showing improved keeping qualities than those products processed locally that were purchased from the market that started spoiling from day 4. The results also showed that the following bacterial isolates were involved in the fermentation: *Bacillus* spp., *Lactobacillus* spp., *Streptococcus* spp., *Micrococcus* spp., and spoilage of *ugba*: *Proteus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Escherichia coli*. Fungal contaminants: *Penicillium* spp.,

Aspergillus spp and *Rhizopus* spp., were also observed. Sample B had good sensory attributes especially in appearance and texture while Sample D was rated best in taste and aroma (flavour). It was concluded that pure cultures of *Lactobacillus plantarum* used as starter culture had good biopreservative effect on the fermented African oil bean slices as antimicrobial agents by improving the safety and keeping qualities of the product.

Keywords: *Biopreservation, lactic acid bacteria, microbiological, sensory, shelf life, starter cultures, ugba.*

INTRODUCTION

Food security, the availability of food and its accessibility to people, has been an important concern in most developing countries where food preservation techniques have been very inadequate (Olaoye and Onilude, 2010).

Biopreservation, preservation by the use of biological agents, refers to the extension of the shelf-life and improvement of the safety of foods using microorganisms and/or their metabolites (Rosset *al.*, 2002). Nowadays, the consumer pays a lot of attention to the relation

between food and health. The general public wants to reduce the use of chemical preservatives in food or feed. In other words, consumers require high quality, preservatives free, safe but mildly processed food with extended shelf-life. This is of course not an easy task to solve. In addition, present legislation has restricted the use of some currently accepted preservatives in different foods (Brul and Coote, 1999).

Lactic acid bacteria have been used as biopreservatives in food and animal feed, sauerkraut and

silage (Messens and De-Vuyst, 2002). One of the main roles of lactic acid bacteria in biopreservation is to improve food safety and enhance its shelf-life through its antimicrobial activity by inactivating pathogens and spoilage microorganisms via acid production and bacteriocins (Olaoye and Idowu, 2010). However, they also have beneficial influence on the nutritional and sensory characteristics as well as on the standardization of end products (Olaoye and Onilude, 2009). The use of starter cultures has generally been recognized as one major way of ensuring product consistency and to a reasonable extent eliminates the problem of food-borne pathogens (Eman, 2009). Preservation and safety are presently the two major challenges of the food industry especially at the local level because,

huge economic losses are sustained yearly due to food spoilage while numerous consumers have been reported to develop adverse sensitivity reactions to chemical based preservatives (Buddeet *al.*, 2003). The lactic acid bacteria (LAB) which are used widely as starter cultures for food fermentation are thought of as having the potential to bridge this gap (Delves - Broughton, 2005).

Ugba, a fermented product of African oil bean seed (*Pentaclethra macrophylla* Benth) is one of the common fermented legumes predominantly consumed by the Igbos and other smaller ethnic groups of South Eastern Nigeria as a delicacy and food flavouring (Okorie and Olasupo, 2013). Preparation of *ugba* is by mixed fermentation carried out spontaneously by a number of microorganisms.

Uncontrolled activities of fermentative organisms' after production result to the very short shelf life of *ugba* (Orji *et al.*, 2003). The short shelf-life of the product is one of the factors limiting its production on a large scale (Amachree, 1997). Under room temperature, fermented *ugba* spoils within 3 - 4 days. Spoilage is identified with increased softness (Enujiugha and Akanbi, 2008), colour change, off flavour and sliminess (Mbata and Orji, 2008) and production of pungent ammonical odour (Ogbulie *et al.*, 1993) which leads to product rejection, poor sales etc. The main objective of the research work is to use isolated LAB (*Lactobacillus plantarum*) as starter culture to ferment African oil bean seeds and investigate their biopreservative abilities on the product (*ugba*).

Significance of study

Prolonging the shelf life of *ugba* has been a case of interest to many researchers with many attempts carried out. While many scientific reports are available on both fermented and unfermented African oil bean seeds, there is presently, paucity of scientific information on the ecological contribution of the lactic acid bacteria for the safety and biopreservation of the food product (*ugba*) which will help to reduce the problem of occurrence of pathogens, enhance the product quality and improve the poor keeping quality of the product being experienced by the local processors.

MATERIALS AND METHODS

Collection of Samples

This research work was carried out in Umuahia-Abia State, Nigeria. The African oil bean seeds (*Pentaclethra*

macrophylla Benth) and the *Alchornealaxiflora* Benth leaves (*Akwukwo ugba* - the popular leaves for wrapping *ugba*) were purchased from different selling points at urban main market, Umuahia - Nigeria. The seeds were identified at the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike. The seeds were sorted, graded and washed in order to remove spoilt seeds, dust and extraneous materials from wholesome seeds prior to processing. Already produced *ugba* samples were purchased from the market while the control and LAB inoculated samples were prepared in the laboratory. The purchased *ugba* samples were collected in sterile polyethylene bags and sent immediately to the laboratory for analysis.

Lactic acid bacteria used

The LAB isolates used as starter cultures in this study are *Lactobacillus plantarum*, which have been isolated from *ugba* in a previous study (Olaoye *et al.*, 2018). The choice of the isolate from a number of LAB strains was based on their ability to produce considerable quantities of lactic acid under a reduced pH and good fermentative activity exhibited.

Laboratory preparation of *Ugba* and the application of starter culture

The traditional and experimental procedures of Olaoye *et al.*(2018) for preparing *ugba* were employed in the laboratory to ferment the product. The processing of the large brown glossy seeds of the African oil bean to obtain '*ugba*' involves the following; 4 kg of raw African oil bean seeds were boiled in an autoclave at a temperature

of 121°C and a pressure of 15 pounds per square inch (psi) for 1 h to soften the hard brown testa (shell). The heating was discontinued and the seeds were removed in batches and dehulled while hot. After dehulling, a local vegetable shredder (called *Nkwoo* (in Ibo) - this was a perforated piece of metal which when the seed was ran over it at a certain angle, the seed came out in shreds) was used to shred the seeds. Then into boiling water in a pot, the shred of the African oil bean seeds were added and boiled with stirring at 5 min intervals, for 30 min. The boiled shred were poured into sterile sieve to drain out the hot liquor. Sprays of water were sprayed on the slices to completely remove hot liquor. Then the shreds were washed 3 times, drained of wash water and steeped in distilled water in a pot and covered. The

shreds were steeped for 10 h. After the steeping period, the shreds were vigorously stirred and poured into a sterile sieve (which has been autoclaved at temperature 121°C and pressure of 16psi) to completely drain out the steep water from the shreds. Ten millilitres (10ml) of the cultures containing (8.3 - 12.5 x 10¹⁰Cfu/ml) was used to inoculate 100g sterile African oil bean shreds in singles and aseptically wrapped in sterile *Alchornealaxiflora* Benth leaves (*akwukwo ugba*) washed and sterilized over a steam and lightly tied. The wrapped samples were kept in warm environment (34°C) to initiate fermentation and further left to ferment for 3 days (72 h) at room temperature (29 - 32°C) to yield '*ugba*'. The prepared sample (*ugba*) was labelled appropriately and reserved for further analysis.

Evaluation of Microbiological quality of Ugba

Twenty five grams (25g) of the test samples (*ugba*) was crushed in a mortar and 225ml of deionised water was added to make a dilution of 10^{-1} of the *ugba* extract. From this dilution, further 10 fold serial dilutions were made. One millilitre (1.0 ml) supernatant of various dilutions were inoculated separately into the poured plates of Tryptone Soya Agar (TSA) and Nutrient Agar (NA) for general purpose detection, Potato Dextrose Agar (PDA) for fungi detection at 30°C for 72 h, de Man Rogosa Sharpe Agar (MRS) for lactic acid bacteria, Manitol Salt Agar (MSA) for *Staphylococcus* spp, and McConkey Agar (McC) for *E. coli* and other coliforms. The plates were properly labelled and anaerobically

incubated for 24-72 h at 35-37°C. At the end of the incubation, isolates on the different media were characterized and identified based on the cultural and morphological features of the isolates on the plates and biochemical tests as well as Sugar Fermentation tests.

Fungal isolates; their characterization and identification

Fungal isolates were picked with sterile spatula and spread on sterile slides. The properly spread isolates were stained with lactophenol in cotton blue and examined microscopically using various magnifications. The fungi were identified based on their macroscopic cultural characteristics (colonial morphology) and microscopic characteristics on slide culture with reference to standard identification

atlas and keys (De-Hooget *al.*, 2007; Tsuneo, 2010)

Shelf life study of the fermented *Ugba* samples

The shelf life study was carried out on the physical properties of *ugba*, which include colour, texture, smell and sliminess being the organoleptic indices of a well fermented *ugba* during storage at ambient temperature.

Sensory Attributes

The sensory tests for taste, aroma, appearance, texture and general acceptability was carried out using a 30-man taste panel who were very familiar with *ugba* (consisting of students and workers from Michael Okpara University of Agriculture, Umudike) and were briefed about the aim of the experiment. The rating test method was used and scoring was done using a 9-point Hedonic scale in a brightly lit room at room

temperature. Each panelist was provided with 2 g of the test sample and asked to freely evaluate, comment and score the samples taste, aroma, appearance, texture and general acceptability. Scale used was as follows, 9-Liked extremely and 1-Disliked Extremely. To eliminate bias, coded samples were presented to panel individually with sufficient privacy to guarantee independent judgment. The acceptability of the samples was based on the scores and remarks made by the panel. The result of the test was assessed using the Hedonic preference test (Iwe, 2010).

Statistical analysis

The experimental design used was Completely Randomized Design (CRD). All data obtained were subjected to one-way Analysis of Variance (ANOVA) using Statistical

Package for the Social Sciences (SPSS) version 22 for Windows; to determine any significant difference at 5% level (LSD) and reported as means of three replicates

RESULTS

Tables 1 shows the physiological and fermentative characteristics of the isolated organisms from *ugba* samples. Four samples of *ugba* were analyzed for

total heterotrophic bacterial count. And all the samples examined exhibited microbial contamination. Based on the results of the Gram reaction and biochemical test performed, the genera of bacteria identified were *Bacillus* spp, *Proteus* spp, *Lactobacillus* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Streptococcus* spp., and *Micrococcus* spp.

Table 1: Physiological and fermentative profile of the isolated organisms

Bacterial	Sa	Biochemical Tests/Stains					Sugar Fermentation			Probable Identification				
		Gra	in	Spo	re	Cat	ribs	Mo	Oxi		das	e	Glu	Lac
1	C,D,E	+	+	+	+	+	+	+	+	+	A	A	A	<i>Bacillus</i> spp.
2	D	-	-	+	+	-	-	-	-	-	-	A	A	<i>Proteus</i> spp.
3	B,C	+	-	-	-	-	-	-	-	-	A	A	A	<i>Lactobacillus</i> spp.
4	C,D	+	-	+	-	-	-	-	-	-	A	A	A	<i>Staphylococcus</i> spp.
5	C,D	-	ND	+	-	+	-	+	-	-	A	-	-	<i>Pseudomonas</i> spp.
6	C	-	ND	+	+	-	-	-	-	-	A	A	A	<i>Escherichia</i> spp.
7	D	+	-	-	-	-	-	-	-	-	A	A	A	<i>Streptococcus</i> spp.
8	C,D,E	+	+	+	+	+	+	+	+	+	A	-	A	<i>Micrococcus</i> spp.

+: Positive, -: Negative, A: Acid Production, AG: Acid and Gas Production, ND: Not Determined

Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

Table 2 shows the morphological properties of the fungal species isolated from *ugba* samples stored at ambient temperature. Fungal species such as *Penicillium*, *Aspergillus* and *Rhizopus* spp have been reported to have active involvement in the contamination and spoilage of many foods

(Ezeama, 2007; Chuku, 2012) and these fungal species were isolated in this work from the samples C and D purchased from the local market.

Table 2: Morphological profile of fungal isolates

Fungal Isolates	Morphological Characteristics (Moulds)	Samples				Probable organisms
		B	C	D	E	
FI-1	Septate hyphae, formed conidiophores on a blue-green, brushlike conidia head.	-	+	-	-	<i>Penicillium</i> spp
FI-2	Septate hyphae, black spores on conidia occurring in chains, presence of foot cell and conidiophores.	-	-	+	-	<i>Aspergillus</i> spp
FI-3	Non-septate hyphae, cottony mycelia, formed sporangiophores in large-black sporangium, presence of rhizoids.	-	+	-	-	<i>Rhizopus</i> spp

(+ = Isolated, - = Not Isolated)

Sample B = Fermented African Oil bean slices
(*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

Table 3 shows the shelf-life study of *ugba* samples during storage at ambient temperature for eight days. The physical properties of *ugba*, which include colour, texture, smell and sliminess being the organoleptic indices of a well fermented *ugba* were examined during the shelf life study of the product. After the storage period of the product (*ugba*) at

room temperature in plantain leaves, the results in Table 3 were obtained.

Table 3: Shelf-life study of the *ugba* samples

Samples/Parameters	Storage Days					
	0	2	4	6	8	
B	Colour:	Light-brown	Light-brown	Light-brown	Light-brown	Brown
	Texture:	Hard	Hard	Hard	Hard	Soft
	Smell:	MAG	MAG	Ammonia gas	Ammonia gas	Ammonia gas
	Sliminess:	----	----	----	----	Slimy
C	Colour:	Light-brown	Light-brown	Brown	Brown/Green	Brown/Green
	Texture:	Hard	Hard	Soft	Very soft	Very soft
	Smell:	Ammonia gas	Ammonia gas	SAG	SAG	Ammonia gas
	Sliminess:	----	----	Slimy	Very slimy	Very slimy
D	Colour:	Light-brown	Brown	Brown	Dark-brown	Dark-brown
	Texture:	Hard	Hard	Hard	Very Hard	Very hard
	Smell:	Ammonia gas	Ammonia gas	SAG	SAG	MAG
	Sliminess:	----	----	Sticky	Sticky	Very sticky
E	Colour:	Light-brown	Light-brown	Light-brown	Brown	Brown/Green
	Texture:	Hard	Hard	Soft	Soft	Very soft
	Smell:	MAG	MAG	Ammonia gas	Mildly pungent	Pungent
	Sliminess:	----	----	Ammonia gas	Slimy	Very slimy

MAG: Mild Ammonia Gas, SAG: Strong Ammonia Gas

Sample B = Fermented African Oil bean slices
(*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

Table 4 shows the sensory attributes of *ugba* samples and their respective scores after a thorough evaluation by the sensory panel. Sample B (*ugba* produced by LAB starter culture) had good sensory attributes especially in appearance and texture while Sample D (salted market *ugba*) was rated best in taste and aroma (flavour). However, sample B and C were more preferred.

Table 4: Sensory attributes of *ugba* samples

Samples	Taste	Aroma	Appearance	Texture	General Acceptability
B	7.57 ^{ab} ± 1.07	7.40 ^b ± 1.07	8.33 ^a ± 0.80	8.00 ^a ± 0.74	8.07 ^a ± 0.91
C	7.27 ^b ± 1.05	7.07 ^b ± 0.87	7.90 ^{ab} ± 0.96	7.53 ^{ab} ± 1.04	8.20 ^a ± 0.81
D	7.83 ^a ± 1.01	8.07 ^a ± 0.69	7.60 ^b ± 0.97	7.43 ^b ± 1.22	7.53 ^b ± 0.94
E	7.13 ^b ± 0.94	7.23 ^b ± 1.07	6.90 ^c ± 1.18	7.17 ^b ± 0.87	7.57 ^b ± 0.77
LSD	0.42	0.39	0.52	0.50	0.45

Values in the same column having the same superscripts are not significantly different ($P > 0.05$) and those with different superscripts are significantly different ($P < 0.05$)

Sample B = Fermented African Oil bean slices (*Ugba* inoculated with LAB starter culture)

Sample C = Market *Ugba*-01

Sample D = Market *Ugba*-02

Sample E = Control Sample

DISCUSSION

Results in Table 1 shows the potential of lactic acid bacteria to inhibit growth of several common food-spoiling microorganisms such as *Bacillus* spp, *Micrococcus* spp, *Staphylococcus* spp, *Proteus* spp, *E. coli*, and *Pseudomonas* spp. as these organisms were not isolated from Sample B being the *ugba* inoculated with starter culture. The microbial load of the market *ugba* could be as a result of cross contamination because of the air, nature of market environment as well as the human handlers. It could also be attributed to the nature of production and the unhygienic condition of the baskets and other materials employed such as water, leaves used for wrapping during the production (Nwuche, 2013). The biochemical and morphological

characteristics of the isolated microorganisms in this work are in line with the reports made by other researchers (Eze et al., 2014; Enujiugha, 2009)

Results in Table 2 have proven the potential of lactic acid bacteria to inhibit growth of several common food-spoiling fungi as these organisms were not isolated from Sample B being the *ugba* inoculated with LAB starter culture. The inhibitory activity of LAB isolates in this study confirms the prospect of LAB as a potential antifungal agent in control of fungal contamination and spoilage of food and feed products. The antifungal effect of lactic acid bacteria could not simply be assigned to the low pH, but most probably to the formation and secretion of antifungal organic metabolites or organic acids

(De-Muyncket *al.*, 2004). This result supports the observations of Oranusiet *al.* (2013) and Eze *et al.* (2014), who reported that spores of *Aspergillus*spp, *Mucors*spp and *Rhizopus*spp are widely distributed in nature.

From Table 3, shelf-life studies conducted showed that the sample inoculated with LAB starter culture (Sample B) retained their physical qualities for 7 days after production as there was no significant change on the quality attributes of the product from day 0 to 8. Slight changes in colour, smell, texture were observed on day 8 which proved that the product was able to store at room temperature for 8 days. This showed a remarkable improvement in the keeping quality of the product which does not normally last beyond 3 days. However,

the other samples started deteriorating from day 4.

Table 4 shows the scores from the sensory evaluations of the *ugba* samples. It could be observed that sample B (*ugba* produced by LAB starter culture) had a good sensory attributes especially in appearance and texture and was able to compare favourably with the conventional market *ugba* since both were more preferred.

CONCLUSION

From the results of this study, pure cultures of *Lactobacillus plantarum* used as starter culture was observed to have a good biopreservative effect on the fermented African oil bean slices as antimicrobial agents by improving the safety and keeping qualities of the product.

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