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Development of Starter Culture for the Improvement in the Quality of *Ogiri*, a Food Condiment

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Abstract

The objective of the present study was to isolate Lactic Acid Bacteria (LAB) from *ogiri*, a Nigerian fermented vegetable product, with the primary focus of selecting suitable isolates as candidates for starter cultures for use in possible improvements in the quality of the product. LAB was isolated from *ogiri* using phenotypic methods and then subjected to technological tests to evaluate its suitability as a starter culture. Based on their considerable technological properties, two isolates of LAB were selected as candidates for starter cultures. The starter cultures were inoculated at 10^3 CFU/g during the production of *ogiri*, while un-inoculated samples served as a control. The *ogiri* samples were stored for nine days, within which samples were taken for microbial and proximate analyses. Four LAB isolates were isolated and identified phenotypically from *ogiri* procured from a commercial market, including *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Enterococcus* sp. and *Lactobacillus plantarum*. The species of *Lactobacillus* displayed the usual cell shapes of rods when examined under the microscope, which is typical of most members of the genus. The cells of the *Enterococcus* sp. were, however, cocci in shape, and this is also typical of members of the genus. The basis of the identification of the LAB isolates was their ability to utilize a wide range of carbon sources in their physiological and biochemical activities. Among the LAB isolates, *L. acidophilus*, *L. fermentum* produced less than 0.35 and 0.024 mg/l of acetic acid and hydrogen peroxide, respectively, and were therefore chosen as starter cultures for the production of *ogiri*. Inoculated *ogiri* samples showed reduced counts of coliforms, yeast, and moulds in comparison with their un-inoculated counterparts during storage. Coliform counts increased beyond 10^5 CFU/g in the un-inoculated control samples, whereas counts were lower in samples inoculated with *L. acidophilus* and *L. fermentum*. Yeast and mould count of 8.1 10^6 CFU/g was recorded as the highest value in the un-inoculated control samples, but the count was generally below 10^6 CFU/g in the starter culture inoculated samples. Inoculation with LAB did not have significant difference ($p > 0.05$) in the proximate compositions of the fermented product. The LAB cultures *L. acidophilus* and *L. fermentum* demonstrated considerable control of coliforms and fungi in *ogiri*. Storage of the fermented product should not exceed 5 days for safety concerns, as an increase in counts of coliforms was recorded beyond this period. No significant difference ($p > 0.05$) was recorded in the proximate compositions of starter culture inoculated *ogiri* and un-inoculated samples.

Keywords: Technological Properties; *Ogiri*; Lactic Acid Bacteria; *Lactobacillus*; *Enterococcus*; Phenotypically, Food Technology.

1. Introduction

Ogiri is a fermented condiment, which gives a pleasant aroma to soups and sauces in many countries, especially in Africa and India, where protein calorie malnutrition is a major problem. Fermented condiments have great potential as

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key protein and fatty acid sources, and are good sources of gross energy. Therefore, condiments are basic ingredients for food supplementation, and their socio-economic importance cannot be over emphasized [1]. They constitute a significant proportion of the protein content of diets in rural populations across West Africa, and they are usually introduced in fairly small quantities during cooking because of their strong flavour and aroma characteristics [2]. The production of *ogiri* involves fermentation during which microorganisms utilize biochemical constituents of the substrate, changing them from one form to another with the aid of microbial enzymes [3]. This process enhances the palatability and increases the protein value, vitamin content, and mineral levels of such foods. It also improves food preservation, food safety, enhances flavour and acceptability, it increases variety in the diet, improves nutritional value, reduces anti-nutritional compounds and, in some cases, improves functional properties [4].

Lactic acid bacteria (LAB) have a GRAS (generally regarded as safe) status, and have been widely used as starters in the production of fermented foods [5]. The ability of LAB to inhibit the growth of undesirable bacteria has been reported, and inhibition may be due to the production of organic acids, hydrogen peroxide, carbon dioxide, acetaldehyde, diacetyl or bacteriocins [6, 7]. Occurrence of LAB such as *Lactobacillus fermentum* during the production of *ogiri* has been reported [8]. The authors also noted that *L. fermentum* was among the principal participants in the fermentation process of the product. Besides *L. fermentum*, other species of LAB belonging to the genera *Streptococcus*, *Pediococcus,* and *Leuconostoc* have been identified to be associated with the production of *ogiri* [4, 9].

The association of microorganisms of public health significance has been reported in *ogiri*. Such microorganisms include species of *Staphylococcus*, *Pseudomonas*, *Proteus*, *Escherichia*, and *Enterobacter* [1, 9], some of which belong to the group of coliforms and may be pathogenic in nature. There is therefore a need to contain many of these indicator microorganisms which may pose health risks to consumers of the product, especially through the use of lactic acid bacteria as biological bio-preservatives by exploiting their technological properties. The present study, therefore, is aimed at using LAB isolates as co-cultures in the fermentation process during the production of *ogiri* from different vegetables, with the primary focus on controlling coliforms in the product.

2. Materials and Methods

2.1. Collection of Samples

Seeds of castor beans (*Ricinus communis*), fluted pumkin (*Telfairia occidentalis*), and melon (*Cucumis melo)* were purchased from Orieugba market in Umuahia North local government area of Umuahia, Abia State, Nigeria (Figure 1). The seeds were transported in clean plastic containers to the laboratory for processing. Commercial *ogiri* samples were also obtained from the same source, for the purpose of isolating lactic acid bacteria to be used as starter cultures (after subjecting them to technological assessments) during laboratory preparations of the product.

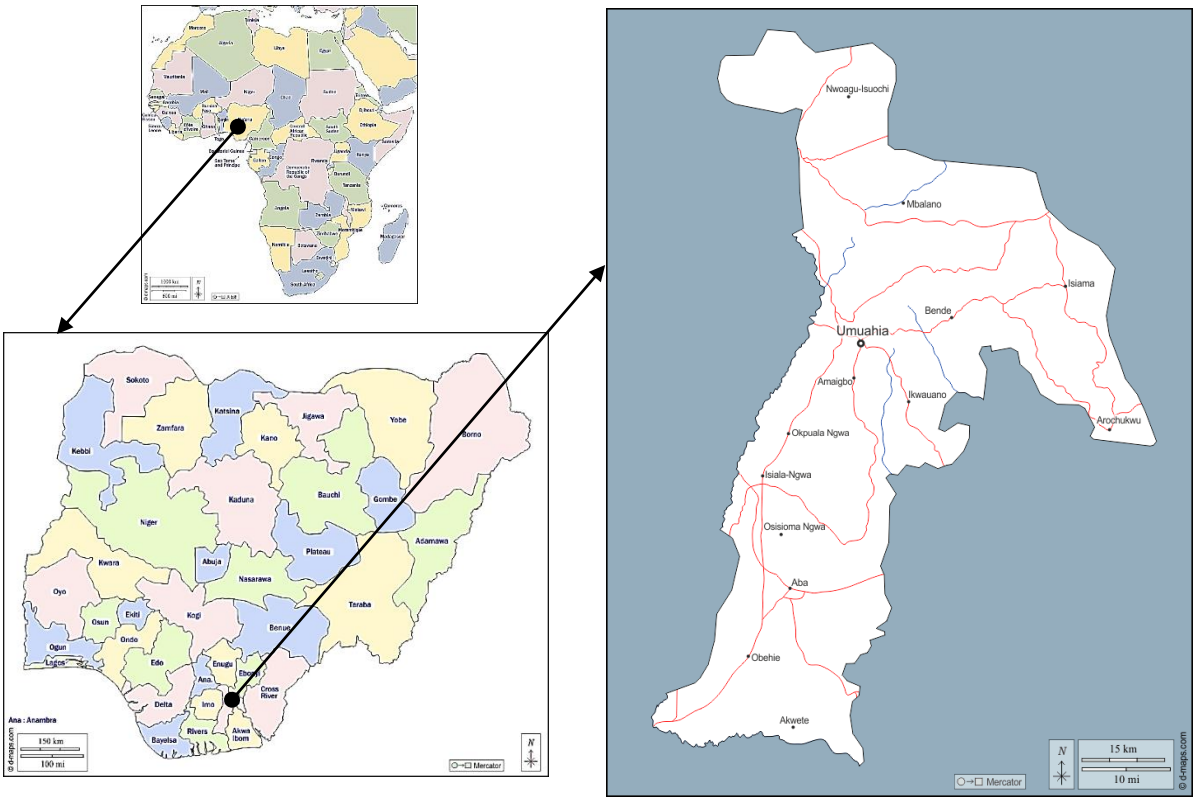

Figure 1. The Location of data collection in Umuahia North, local government area of Umuahia, Abia State, Nigeria

2.2. Isolation and Phenotypic Properties of Lactic Acid Bacteria from *Ogiri*

Lactic acid bacteria (LAB) were isolated from commercial *ogiri* using the modified method of Olaoye. Ten grams (10 g) of *ogiri* were macerated in 90 ml sterile saline solution (1% w/v). The resulting macerate was plated by spreading 1 ml of it in sterile petri dishes containing deMan Rogossa Sharpe (MRS, Oxoid UK) agar, with the aid of sterile spreaders. The plates were then incubated at 30ºC for 24 h inside anaerobic jars. Plates were examined for growth of visible colonies after incubation; colonies that tested negative to catalase test were presumed to be lactic acid bacteria, and picked and sub-cultured repeatedly to obtain pure cultures. Cultures were subjected to Gram staining and microscopic examinations to ascertain purity and cell morphologies. The morphological and biochemical characteristics of the colonies were determined using the method of Stiles and Holzapfel [10] to aid in their identifications; only Gram positive isolates were picked as presumptive LAB isolates and stored on MRS agar slants in the refrigerator (approximately 4ºC) for further use.

2.3. Screening of Lactic Acid Bacteria for Technological Properties

The lactic acid bacteria isolates were screened for production of organic acids, diacetyl and hydrogen peroxide using the modified methods of Olaoye and Onilude [11]. Evaluations of organic acids (lactic and acetic acids) and diacetyl produced by the LAB isolates were carried out using high performance liquid chromatography and gas chromatography respectively. For the determination of acidification abilities of the strains, the isolates were initially grown in brain heart infusion (BHI) broth and then in sterile reconstituted skim milk supplemented with yeast extract (3 g/l) and glucose (2 g/l) for two successive sub culturing. Sterile reconstituted skim milk (100 ml) was inoculated with 1% (v/v) of a 24 h activated culture and pH changes were determined using pH meters (glass electrode, HANNA instruments, Padova, Italy).

2.4. Preparation of *Ogiri* from Seeds of Melon, Fluted Pumpkin and Castor Seeds

Ogiri samples were prepared separately from the seeds of melon, fluted pumpkin and castor seeds, using traditional methods with some modifications [4, 12]; the flow chart is represented in Figure 2.

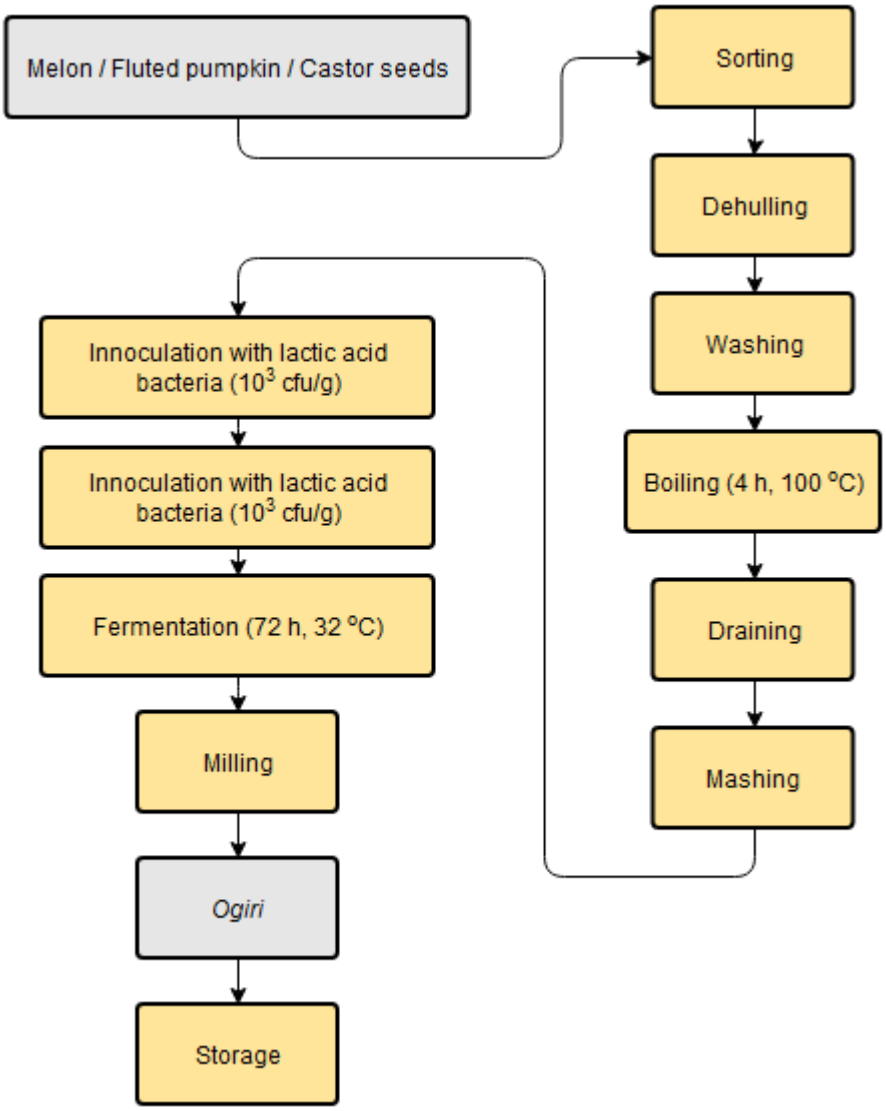

Figure 2. Flow chart for preparation of *Ogiri* from different vegetable sources

After preparing the seeds (by removing coats, and through washing with clean water), they were transferred into separate pots and covered completely with water and then boiled. The seeds were subjected to boiling (approximately 4 h) to soften and probably reduce anti nutrients in the seeds. The seeds were then drained, mashed and then wrapped in plantain leaves for 72 h at ambient temperature (~32^{o}C) for fermentation to take place. For the inoculation of lactic acid bacteria, the mashed seeds were inoculated with isolates of *Lactobacillus fermentum* and *L. acidophilus* (inoculum size of 10^{3}CFU/g) prior to fermentation.

2.5. Microbial Enumerations of *Ogiri* Samples

The *ogiri* samples were subjected to microbial enumerations, including yeasts and moulds, and coliforms, using the methods of Olaoye and Onilude [7]. Yeasts and moulds (fungi) were enumerated using Rose Bengal Chloramphenicol Agar (Oxoid, UK), incubated at 25^{o}C for 72 h while MacConkey Agar (Oxoid, UK) was used for coliforms at 37^{o}C for 24 h.

2.6. Proximate Composition of *Ogiri* Samples

The proximate composition, including moisture, ash, fat, and protein contents of the *ogiri* samples were determined using the methods of Association of Official Analytical Chemists [13]. Carbohydrate was determined by difference.

2.7. Statistical Analysis

The data obtained, which depended on *ogiri* samples produced with inoculation of lactic acid bacteria and uninoculated (control) samples were analyzed using the means of three replicates of each sample. Means of data were separated and analyzed using the *t*-test in data analysis functionality of Microsoft Excel 2010 SP2 (version 14.0.7015.1000) to determine differences. Significant differences among samples were determined at $p < 0.05$.

3. Results and Discussion

In the present report, four lactic acid bacteria (LAB) were isolated and identified through their morphological and biochemical characteristics (Table 1) from *ogiri*. The identified LAB include; *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum* and *Enterococcus* sp. The species of *Lactobacillus* displayed usual cell shapes of rods when examined under the microscope, which is typical of most members of the genus. The cells of the *Enterococcus* sp. were, however cocci in shapes, and this is also typical of members of the genus. The basis of identification of the LAB isolates was on their ability to utilize wide range carbon sources in their physiological and biochemical activities [14]. Other authors have reported isolation and identification of LAB from traditional fermented foods. For example, Ukaoma *et al.* [4] isolated and identified strains of *Lactobacillus* from *ogiri*. David and Aderibigbe [9] also reported the isolation and identification of species belonging to various genera of LAB, including *Leuconostoc*, *Streptococcus*, *Pediococcus* and *Lactobacillus*.

Research findings have suggested that there is increasing attention on the use of naturally occurring metabolites produced by selected lactic acid bacteria (LAB) to inhibit the growth of undesirable microorganisms [5, 15]. LAB growing naturally in foods produce antimicrobial substances such as lactic and acetic acids, diacetyl, hydrogen peroxide and bacteriocins, which could serve vital roles in bio-preservation of foods [16, 17]. While species of the genus *Bacillus* have been noted as dominant organisms in the production of *ogiri* by Adebayo and Obiekezie [8] and Ademola et al. [18], same authors observed that certain species of LAB contribute positively to the quality attributes of the fermented product, especially the aroma and flavor.

Table 1. Morphological and biochemical characteristics of the presumptive lactic acid bacteria (LAB) isolates

Isolates	Gram Reaction	Cell Shape	Indole	Citrate	MR	VP	SH	Mannitol	Lactose	Sucrose	Maltose	Catalase	Probable Identity
LAB1	+	Long Rods	-	-	+	-	-	+	+	+	+	-	*Lactobacillus acidophilus*
LAB2	+	Rods	-	-	+	-	-	-	+	+	+	-	*Lactobacillus fermentum*
LAB3	+	Cocci	-	-	-	-	-	-	-	-	-	-	*Enterococcus* sp.
LAB4	+	Short Rods	-	-	-	-	-	+	+	+	+	-	*Lactobacillus plantarum*

MR: Methyl red, VP: Voges Prokauer, SH: Starch hydrolysis

In the present study, the four LAB isolates identified from *ogiri* were subjected to technological assessment in terms of their abilities to produce organic acids (mainly lactic and acetic acids), diacetyl and hydrogen peroxide. From the result of the technological properties (Table 2), *Lactobacillus acidophilus* and *L. fermentum* produced reduced

quantities of hydrogen peroxide and acetic acid; hydrogen peroxide values (mg/l) of 0.02 and 0.006 were recorded for the former while 0.34 and 0.41 were obtained in terms of acetic acid (mg/l). The least production of hydrogen peroxide and acetic acid concentrations by the two LAB isolates could make them suitable candidates of starter cultures for production of fermented foods such as *ogiri*, as high production of these metabolites by LAB has been reported to be disadvantageous in fermented foods. Production of high concentrations of hydrogen peroxide, even though has antimicrobial properties, could lead to loss in food qualities, as it can interfere with the organoleptic properties of fermented food products, through undesirable promotion of rancidity and discoloration [19]. The lower concentrations of acetic acids by *L. acidophilus* and *L. fermentum* may be attributed to their homo-fermentative nature, as homofermenters have been noted to produce more lactic acids than acetic acids [20]. The author also reported that acetic acid may impart unpleasant taste on food products when compared to lactic acid, thus making the two LAB isolates better candidates of start cultures for production of *ogiri*, than their other two counterparts.

Table 2. Technological properties of the phenotypically identified lactic acid bacteria isolates

Isolates	H_2O_2 (mg/l)			Lactic acid (mg/l)			Diacetyl (mg/l)			Acetic acid (mg/l)			pH		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Lactobacillus acidophilus	0.02	0.04	0.04	0.72	0.99	1.22	0.43	0.57	0.74	0.34	0.69	1.02	4.0	4.1	3.8
Lactobacillus fermentum	0.006	0.012	0.03	0.84	1.22	1.38	0.38	0.47	0.64	0.41	0.72	1.21	3.7	3.5	3.2
Enterococcus sp.	0.024	0.031	0.03	0.42	1.04	1.26	0.57	0.62	0.78	0.66	0.85	1.18	4.8	4.3	3.8
Lactobacillus plantarum	0.06	0.062	0.064	0.62	0.68	1.18	0.46	0.58	0.61	0.62	0.90	1.26	4.4	4.0	4.0

After careful consideration of the technological properties of the LAB isolates, *L. acidophilus* and *L. fermentum* were selected as starter cultures for use during production of *ogiri*. Some of the key considerations before the choice of the two isolates include reduced productions of acetic acid and hydrogen peroxide and moderate production of lactic acid and diacetyl, which are very vital to competitive exclusion of unwanted organism in foods [11]. Table 3 shows the results of coliform counts in the *ogiri* samples inoculated with LAB as starter cultures, and uninoculated control samples, during storage. Results indicate that coliforms were generally lower in starter culture inoculated samples than in their uninoculated counterparts. There were slightly higher counts of coliforms as storage progressed, although counts were generally not significant ($p > 0.05$) in the starter culture inoculated samples, except in few cases; however significant differences ($p < 0.05$) were recorded for their uninoculated control counterparts. During storage, the highest coliform count of 5.8×10^6 CFU/g was observed on day 9 for the uninoculated control sample. The decrease in counts of coliforms in the starter inoculated samples suggests the protective ability of LAB cultures against coliforms. In the report by Olaoye and Onilude [7] on meat inoculated with LAB cultures in Nigeria, a reduction in coliform counts was noted during storage. The researchers concluded that the LAB used as protective cultures may have produced antimicrobial agent possibly responsible for reduction in coliform counts. Reports of the presence of coliforms and other pathogenic organisms in *ogiri* produced from melon seeds have been made by other researchers. David and Aderibigbe [9] reported the occurrence of pathogenic organisms, including *Pseudomonas aeruginosa*, *Klebsiella* sp., *Escherichia coli* and *Staphylococcus aureus*. In a related study, Ogunshe and Olasugba [1] also reported the occurrence of coliforms in some *ogiri* samples randomly selected from middle-belt and South Western Nigeria. In the present study, the use of *L. acidiphilus* and *L. fermentum* may therefore be of public health significance towards combating the occurrence of coliforms, and other organisms in *ogiri* that may be pathogenic in nature.

Table 3. Coliform counts (log CFU/g) in the *ogiri* samples during storage

SD	FPL1	FPL2	FPL3	CSL1	CSL2	CSL3	MSL1	MSL2	MSL3	Control
1	$2.4^{d}\pm0.2\times10^2$	$4.4^{b}\pm1.2\times10^2$	$3.1^{b}\pm0.7\times10^2$	$3.8^{c}\pm0.6\times10^2$	$4.2^{c}\pm0.5\times10^2$	$4.7^{c}\pm2.1\times10^2$	$3.8^{d}\pm0.3\times10^2$	$5.4^{b}\pm1.1\times10^2$	$5.8^{c}\pm1.5\times10^2$	$1.3^{e}\pm0.1\times10^2$
3	$2.7^{bc}\pm0.3\times10^3$	$4.2^{b}\pm1.0\times10^2$	$3.8^{a}\pm1.5\times10^3$	$3.5^{b}\pm1.1\times10^3$	$3.7^{b}\pm1.1\times10^3$	$8.2^{c}\pm1.6\times10^2$	$3.8^{c}\pm0.5\times10^3$	$5.2^{b}\pm1.3\times10^2$	$4.7^{b}\pm0.8\times10^3$	$7.9^{d}\pm2.3\times10^3$
5	$6.7^{a}\pm0.5\times10^3$	$3.2^{a}\pm0.4\times10^3$	$3.3^{a}\pm0.3\times10^3$	$3.2^{b}\pm0.5\times10^3$	$3.4^{b}\pm0.6\times10^3$	$4.0^{b}\pm1.9\times10^3$	$5.1^{b}\pm1.1\times10^3$	$3.4^{a}\pm0.7\times10^3$	$7.7^{a}\pm2.3\times10^3$	$1.8^{c}\pm0.7\times10^4$
7	$3.2^{b}\pm0.8\times10^2$	$3.5^{a}\pm0.5\times10^3$	$3.6^{a}\pm1.3\times10^3$	$3.1^{a}\pm0.7\times10^4$	$3.0^{a}\pm07\times10^4$	$3.4^{b}\pm0.4\times10^3$	$3.7^{a}\pm0.7\times10^4$	$4.4^{a}\pm1.2\times10^3$	$9.2^{a}\pm1.4\times10^3$	$8.8^{b}\pm2.1\times10^5$
9	$4.9^{b}\pm1.2\times10^3$	$3.8^{a}\pm1.3\times10^3$	$3.7^{a}\pm0.9\times10^3$	$4.2^{a}\pm1.4\times10^4$	$2.4^{a}\pm0.9\times10^4$	$2.6^{a}\pm0.5\times10^4$	$5.7^{a}\pm1.9\times10^4$	$3.8^{a}\pm1.6\times10^3$	$8.1^{a}\pm0.8\times10^3$	$5.8^{a}\pm0.9\times10^6$

Values are means of replicate determinations. Means with different superscripts across columns are significantly different ($p < 0.05$); CFU, colony forming units; SD, storage days; FPL1, *ogiri* from fluted pumpkin seeds inoculated with *Lactobacillus acidophilus*; FPL2, *ogiri* from fluted pumpkin seeds inoculated with *L. fermentum*; FPL3, *ogiri* from fluted pumpkin seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; CSL1, *ogiri* from castor seeds inoculated with *L. acidophilus*; CSL2, *ogiri* from castor seeds inoculated with *L. fermentum*; CSL3, *ogiri* from castor seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; MSL1, *ogiri* from melon seeds inoculated with *L. acidophilus*; MSL2, *ogiri* from melon seeds inoculated with *L. fermentum*; MSL3, *ogiri* from melon seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*

The yeasts and moulds (Y & M) count of the *ogiri* samples are presented in Table 4. From the results, it was observed that Y & M counts (CFU/g) ranged between 5.5×10^2 and 7.9×10^3 at the beginning of storage. The counts

generally increased in all samples as storage period progressed. Among the samples inoculated with LAB starter cultures, the highest count of 9.7×10^5 was observed in the sample produced from fluted pumpkin seeds and inoculated with mixed LAB cultures on the last day of storage (day 9). The LAB starter cultures, especially *L. acidophilus* and *L. fermentum*, were noted to exert antagonistic activities on the Y & M, especially when used as singly, and the effect was sustained throughout the storage period. This observation was similar to the research investigations reported by Erkmen [21] and Olaoye and Onilude [7]. The former reported reduction in the Y & M counts in a Turkish sausage after inoculation with LAB strains as protective cultures in comparison with uninoculated control samples, while the latter also made similar findings in meat samples inoculated with LAB cultures. The findings recorded in the present study of the antagonistic activities of the species of *Lactobacillus* further support the work of Lipinska et al. [22] who reported the antagonistic of some strains of *Lactobacillus* against yeasts and moulds, including *Aspergillus niger*, *Fusarium latenicum*, *Geotrichum candidum*, *Mucor hiemalis* and *Candida vini.* In their research investigation, Lipinska *et al.* [22] concluded that the antifungal activities of the *Lactobacillus* strains used could be due to antimicrobial substances produced by them *in vitro* in the broths employed as growth media.

Table 4. Yeast and moulds counts (log CFU/g) in the *ogiri* samples during storage

SD	FPL1	FPL2	FPL3	CSL1	CSL2	CSL3	MSL1	MSL2	MSL3	Control
1	$4.2^{b}\pm1.0x10^3$	$4.1^{c}\pm0.6x10^3$	$4.6^{c}\pm1.6x10^3$	$5.2^{d}\pm0.9x10^2$	$4.7^{c}\pm1.3x10^3$	$5.5^{d}\pm0.9x10^2$	$4.7^{b}\pm1.7x10^3$	$4.5^{b}\pm0.8x10^3$	$5.8^{c}\pm1.7x10^3$	$7.9^{d}\pm1.3x10^3$
3	$2.6^{b}\pm0.3x10^3$	$2.7^{b}\pm0.1x10^4$	$8.9^{c}\pm0.3x10^3$	$3.9^{c}\pm1.0x10^3$	$6.4^{c}\pm0.8x10^3$	$2.7^{c}\pm0.3x10^3$	$3.8^{a}\pm0.8x10^4$	$4.4^{a}\pm1.2x10^4$	$5.1^{b}\pm1.2x10^4$	$7.9^{c}\pm1.7x10^4$
5	$2.2^{a}\pm0.2x10^4$	$2.4^{b}\pm0.7x10^4$	$2.5^{b}\pm0.5x10^4$	$3.1^{c}\pm1.2x10^3$	$2.6^{b}\pm0.6x10^4$	$7.3^{bc}\pm1.7x10^3$	$3.6^{a}\pm0.5x10^4$	$3.2^{a}\pm1.0x10^4$	$2.4^{b}\pm0.6x10^4$	$8.4^{c}\pm2.1x10^4$
7	$4.3^{a}\pm1.3x10^4$	$3.1^{b}\pm0.5x10^4$	$5.9^{a}\pm1.0x10^5$	$3.3^{b}\pm0.8x10^4$	$2.3^{b}\pm0.3x10^4$	$2.3^{b}\pm0.6x10^4$	$2.6^{b}\pm0.7x10^3$	$2.9^{b}\pm0.9x10^3$	$3.5^{b}\pm1.3x10^4$	$7.9^{b}\pm1.2x10^5$
9	$3.8^{a}\pm0.7x10^4$	$9.8^{a}\pm0.9x10^4$	$9.7^{a}\pm1.3x10^5$	$2.6^{a}\pm0.4x10^5$	$1.7^{a}\pm0.1x10^5$	$2.1^{a}\pm0.3x10^5$	$2.5^{b}\pm0.1x10^3$	$2.3^{a}\pm0.5x10^4$	$3.2^{a}\pm1.1x10^5$	$8.1^{a}\pm2.1x10^6$

Values are means of replicate determinations. Means with different superscripts across columns are significantly different ($p < 0.05$); CFU, colony forming units; SD, storage days; FPL1, *ogiri* from fluted pumpkin seeds inoculated with *Lactobacillus acidophilus*; FPL2, *ogiri* from fluted pumpkin seeds inoculated with *L. fermentum*; FPL3, *ogiri* from fluted pumpkin seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; CSL1, *ogiri* from castor seeds inoculated with *L. acidophilus*; CSL2, *ogiri* from castor seeds inoculated with *L. fermentum*; CSL3, *ogiri* from castor seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; MSL1, *ogiri* from melon seeds inoculated with *L. acidophilus*; MSL2, *ogiri* from melon seeds inoculated with *L. fermentum*; MSL3, *ogiri* from melon seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum.*

Table 5. Proximate composition of the *ogiri* samples

Samples	Dry Matter (%)	Moisture Content (%)	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Ash (%)	Carbohydrate (%)	Energy Value (%)
FPC	$65.20^{g}\pm0.14$	$34.80^{a}\pm0.26$	$22.43^{c}\pm0.01$	$5.70^{e}\pm0.02$	$2.87^{e}\pm0.01$	$2.72^{a}\pm0.01$	$9.48^{e}\pm0.16$	$238.94^{d}\pm0.64$
FPL1	$67.60^{f}\pm0.14$	$32.40^{a}\pm0.14$	$26.82^{a}\pm0.03$	$5.70^{e}\pm0.01$	$2.91^{e}\pm0.01$	$2.72^{a}\pm0.03$	$8.45^{f}\pm0.19$	$276.38^{b}\pm0.55$
FPL2	$69.25^{f}\pm0.07$	$30.75^{b}\pm0.07$	$27.98^{a}\pm0.01$	$5.66^{e}\pm0.01$	$2.91^{e}\pm0.01$	$2.75^{a}\pm0.02$	$8.96^{f}\pm0.08$	$286.68^{b}\pm0.38$
FPL3	$68.25^{f}\pm0.21$	$31.75^{b}\pm0.21$	$26.23^{b}\pm0.01$	$5.01^{f}\pm0.01$	$2.91^{e}\pm0.01$	$2.77^{a}\pm0.03$	$9.03^{e}\pm0.25$	$279.29^{b}\pm1.09$
CSC	$75.35^{d}\pm0.21$	$24.65^{d}\pm0.21$	$12.60^{f}\pm0.03$	$7.19^{d}\pm0.02$	$3.32^{a}\pm0.01$	$2.62^{b}\pm0.01$	$12.49^{c}\pm0.26$	$237.37^{cd}\pm0.74$
CSL1	$77.70^{c}\pm0.14$	$22.30^{e}\pm0.14$	$17.09^{e}\pm0.01$	$6.14^{e}\pm0.04$	$3.34^{b}\pm0.01$	$2.71^{a}\pm0.01$	$10.32^{d}\pm0.14$	$238.30^{cd}\pm0.49$
CSL2	$78.80^{b}\pm0.14$	$21.20^{e}\pm0.14$	$15.83^{e}\pm0.03$	$6.01^{e}\pm0.04$	$3.32^{b}\pm0.01$	$2.72^{a}\pm0.01$	$10.79^{d}\pm0.18$	$246.65^{c}\pm0.24$
CSL3	$77.65^{c}\pm0.14$	$22.35^{e}\pm0.21$	$16.32^{e}\pm0.01$	$6.32^{d}\pm0.03$	$3.89^{c}\pm0.01$	$2.73^{a}\pm0.01$	$10.56^{d}\pm0.14$	$233.00^{d}\pm0.99$
MSC	$76.50^{a}\pm0.00$	$23.50^{f}\pm0.28$	$16.26^{e}\pm0.01$	$10.04^{a}\pm0.02$	$2.28^{d}\pm0.01$	$2.02^{c}\pm0.01$	$17.24^{a}\pm0.29$	$303.87^{a}\pm1.03$
MSL1	$78.40^{b}\pm1.14$	$21.60^{e}\pm0.14$	$20.05^{d}\pm0.04$	$9.01^{b}\pm0.02$	$2.23^{b}\pm0.01$	$2.01^{b}\pm0.01$	$15.91^{b}\pm0.19$	$283.23^{b}\pm0.53$
MSL2	$70.85^{e}\pm0.07$	$29.15^{c}\pm0.07$	$19.40^{d}\pm0.03$	$8.01^{c}\pm0.03$	$2.55^{c}\pm0.04$	$2.12^{b}\pm0.02$	$12.27^{c}\pm0.01$	$253.29^{c}\pm0.38$
MSL3	$71.45^{e}\pm0.07$	$28.55^{c}\pm0.26$	$21.92^{c}\pm0.03$	$7.89^{d}\pm0.02$	$2.03^{b}\pm0.03$	$2.72^{a}\pm0.01$	$11.16^{d}\pm0.16$	$277.95^{b}\pm0.35$

Values are means of replicate determinations. Means with different superscripts across columns are significantly different ($p < 0.05$); FPC, *ogiri* from fluted pumpkin seeds; FPL1, *ogiri* from fluted pumpkin seeds inoculated with *Lactobacillus acidophilus*; FPL2, *ogiri* from fluted pumpkin seeds inoculated with *L. fermentum*; FPL3, *ogiri* from fluted pumpkin seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; CSL1, *ogiri* from castor seeds inoculated with *L. acidophilus*; CSL2, *ogiri* from castor seeds inoculated with *L. fermentum*; CSL3, *ogiri* from castor seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum*; MSL1, *ogiri* from melon seeds inoculated with *L. acidophilus*; MSL2, *ogiri* from melon seeds inoculated with *L. fermentum*; MSL3, *ogiri* from melon seeds inoculated with mixed cultures of *L. acidophilus* and *L. fermentum.*

Results of the proximate composition (%) of the *ogiri* samples indicate that dry matter contents ranged between 65.2 and 86.5, showing varying levels of significant differences ($p < 0.05$) among samples. Moisture content ranged from 21.20 to 34.80. The values recorded for moisture in the samples in the present study were similar to those reported by David and Aderibigbe [9]. The highest crude protein and ether extract of 27.98 and 10.04 were recorded for *ogiri* samples made from fluted pumpkin that was inoculated with *L. fermentum* and un-inoculated melon seed, respectively [23, 24]. In terms of crude fiber, *ogiri* samples produced from castor seeds had higher values than their counterparts from fluted pumpkin and melon seeds, a value of 3.89 was recorded for a sample from castor seed inoculated with mixed LAB cultures. The highest content of ash (2.75) was recorded for an *ogiri* sample obtained

from a fluted pumpkin that was fermented with *L. fermentum*, while the lowest value of 2.02 was obtained for an *ogiri sample* from an un-inoculated melon seed. The values of the crude fibers and ash in the *ogiri* samples obtained in this study show correlation to those reported by David and Aderibigbe [9] and Nnennaya et al. [25] in *ogiri* made from melon seed and sandbox seed. Generally, inoculation with LAB starter cultures did not significantly affect the proximate compositions of the *ogiri* samples, though there were variations in the values of the respective proximate parameters, depending on the type of vegetable used.

4. Conclusion

In conclusion, the use of LAB starter cultures in this study demonstrated considerable control of coliforms and fungi in *ogiri*; the effect was more noticeable in samples inoculated with *L. acidophilus* and *L. fermentum*. However, based on the results of the microbial analysis, it is suggested that when *ogiri* is produced using LAB as starter cultures, storage should be limited to a maximum of five (5) days in order not to compromise safety, as an increase in counts of coliforms was recorded beyond this period. Furthermore, the inoculation of LAB as starter cultures in the production of *ogiri* had no pronounced significant difference in the proximate compositions of the fermented product.

5. Declarations

5.1. Author Contributions

Conceptualization, O.A.O. and U.V.N.; methodology, O.A.O.; software, O.A.O.; validation, O.A.O., J.C.O., A.C.N. and U.V.N.; formal analysis, U.V.N.; investigation, O.A.O.; resources, O.A.O.; data curation, U.V.N.; writing—original draft preparation, O.A.O.; writing—review and editing, O.A.O.; visualization, O.A.O.; supervision, O.A.O.; project administration, O.A.O.; funding acquisition, U.V.N. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in article.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Institutional Review Board Statement

Not Applicable.

5.5. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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