Identification of spoilage bacteria from fermented food products in Edo state, Nigeria.

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Abstract

This study assessed the bacteriological quality of fermented food products by the enumeration of the bacteria that cause their spoilage. Three different samples of fermented food ("Palm wine", Ogi" and "Fufu") were purchased from two selling points in Ogwa and Ebelle, Esan-West and Igueben local government areas of Edo state, Nigeria respectively. de Man Rogosa Sharpe, Maconkey and nutrient agars were used for the isolation and determination of bacterial load. Standard morphological and biochemical tests were carried out for the identification and characterization of isolates. A total of twelve (12) bacteria species were isolated. The Total Heterotrophic Bacterial Count (THBC) and Lactic Acid Bacterial (LAB) count which ranged from 1.57×10^4 cfu/ml to 2.47×10^4 cfu/ml and 0.79×10^4 cfu/ml to 2.38×10^4 cfu/ml before spoilage respectively while total heterotrophic bacterial count and lactic acid bacterial count ranged from 3.47×10^4 cfu/ml to 6.15×10^4 cfu/ml and 1.91×10^4 cfu/ml to 4.05×10^4 cfu/ml after spoilage respectively. The bacteria identified include; *Salmonella enteritica, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus* and *Esherichia coli* while lactic acid bacteria were *Lactobacillus, Pedococcus, Leuconostoc, Lactococcus* and *Lactobacillis* species. Locally fermented food should be given proper handling so as to prevent food spoilage and food borne infections.

Keywords: Fermented foods, Spoilage bacteria, Lactic acid bacteria, Fermentation

Introduction

The deliberate fermentation of foods by man predates written history and is possibly the ancient method of preserving consumable foods. Evidence suggests that fermented foods were used up 7,000 years ago in Babylon. Scientist speculates that our ancestors possibly discovered fermentation by accident and continued to use the process out of first choice or necessity. Preservation through fermentation did not only make foods available for future use, but also add flavour to the food making it more nutritious [1]. According to Chelule, foods are fermented to increase shelve life or for preservation, to improve flavour, texture, add aroma, increased solubility and digestibility. Fermented food products are more palatable and do not easily get spoilt because the by-products of fermentation which also include organic acids, hydrogen peroxide, diacetyl and bacteriocin serve as preservatives against spoilage organisms apart from imparting their desired flavours and aroma. Spoilage of food may be seen as any change that occurs in a food, making it undesirable for human consumption. Food spoilage is caused by microorganisms and this is attained with the help of extracellular enzymes they manufactured, which convert the food product into new substance with the production of gas and change in organoleptic properties. Most food spoilage are due to the activities of bacteria, molds and yeasts, which alter the smell, taste, colour, texture or chemical composition of the food significantly and render it inedible. The chemical properties of the food determine how satisfactory it will be as a culture medium for microbial growth. Carbohydrate rich food is often spoilt by fungi, especially species of *Rhizopus, Mucor* and *Penicillium* which grow on starchy foods if kept in humid condition. Fermented foods are always from plant or animal origins which formed great part of an average Nigerian diet contributing main nutrients such as proteins, calories, minerals and some vitamins, and they include indigenous foods like "Nunu," "Cheese," "Youghurt," "Dawadawa," "Palm wine," "Ogi" "Fufu" etc. Local fermentation of food is a method of food refinement, where microbes, for example, Lactic Acid Bacteria (LAB) are applied in food production through the method acknowledged as fermentation. Fermentation in food processing involves the conversion of carbohydrates to alcohol and carbon dioxide or organic acids using yeast and/or bacteria, under anaerobic conditions. Fermentation is mainly one of the standard methods of food preservation with Lactic Acid Bacteria (LAB) and yeasts playing the major role in this fermentation [2]. The load of the microorganisms in the food is commonly small, but their consequence on the nature of the food, mainly in terms of flavour, and other organoleptic properties, is profound. Fermented food products are more palatable and do not easily get spoilt because the by-products of fermentation which also

include organic acids, hydrogen peroxide, diacetyl and bacteriocin serve as preservatives against spoilage organisms apart from imparting their desired flavours and aroma. Spoilage of food may be seen as any change that occurs in a food, making it undesirable for human consumption. Most food spoilage are due to the activities of bacteria, molds and yeasts, which alter the smell, taste, colour, texture or chemical composition of the food significantly and render it inedible [3]. Lactic acid bacteria like *Lactobacillus, Pediococcus, Streptococcus, Oenococcus*, etc. are the most essential bacteria in fermented foods, followed by *Acetobacter* species, which oxidize alcohol to acetic acid. The acetic acid fermentation has been used broadly to produce fruit vinegars including cider vinegar. Lactic Acid Bacteria (LAB) isolated from fermented foods demonstrated probiotic properties such as hypo-lipid emic, hepato-protective and antibacterial and had been set up to be effective in treating gastroenteritis in man and animals. Moulds are as well important organisms in food processing both as spoilers and preservers of foods [4]. Many moulds have ability to produce enzymes of economic importance such as pectinase by *Aspergillus niger*. Species of *Aspergillus* are intricate in the production of citric acid from excess like apple pomace. The *Aspergillus* species are often responsible for unwanted changes in foods triggering spoilage. In the other way, *Penicillium* species are associated with the ripening and flavour development in cheeses. While the species of ceratocystis are involved in fruit flavour production, at the same time, *Penicillium* is the causative agent for manufacture of toxin like patulin. Like bacteria and molds [5], yeasts have beneficial and non-beneficial effects in food fermentations. The most beneficial yeast in terms of desirable food fermentations are from the *Saccharomyces* family, especially *Saccharomyces cerevisiae* involved in bread production and alcohol in wine fermentations. *Saccharomyces cerevisiae* var. ellipsoideus is employed extensively in wine production. *Schizosaccharomyces pombe* and *S. boulderi* are the dominant yeasts in the production of traditional fermented beverages, mainly those obtained from maize and millet. *Saccharomyces cerevisiae* var. carlbergenisis is the yeast intricate in beer production. *Schizosaccharomyces pombe* has [6] capacity to degrade malic acid into ethanol and carbon dioxide and has been used positively to reduce the acidity in the grape and plum musts. A number of yeasts like *Rhodotorula* and *Cryptococcus* have ability to produce pigment to be used as biocolur [7].

The chemical properties of the food determine how satisfactory it will be as a culture medium for microbial growth. Carbohydrate rich food is often spoilt by fungi, especially species of *Rhizopus, Mucor* and *Penicillium* which [8] grow on starchy foods if kept in humid condition. The fermentation procedures for these foods are a local knowledge picked up by observations and practice, and move on from age to age [9].

Nigeria is a multilingual and multicultural nation which most likely reflects in their dressings, cultural practices and modes of nutrition. Each settlements and tribes has its own indigenous preferred food that is defined by customs, tradition and religion [10]. The fermentation practices are often in a small scale on domestic basis, characterized by the usage of simple non-sterile equipment, chance or natural inoculums, uncontrollable conditions, sensory variations, poor stability and unattractive package of the refined products resulting in food of unstable quality. With increasing industrialization and urbanization, efforts are presently geared toward the direction of developing a large-scale factory processing facilities [11] for these foods where the quality of the refined product will be guaranteed.

Today, there is a high consumption rate of fermented food products such as palm wine, ogi and fufu by residents in Ogwa. The consumption of these spoilt fermented food products results in illness such as diarrhea etc. The probable cause of these problems sometimes lies in the unhygienic and poor preservation methods employed by fermented foods dealers as a result of scanty information on the storage status and the organisms involved in the decay of fermented foods commonly used up. This research was carried out to study the quality of palm wine, ogi and fufu sold in Ogwa market and enumerate bacterial associated with them [12].

Materials and Methods

Collection of sample

Three different samples of fermented food ("Palm wine," Ogi" and "Fufu") were purchased from two selling points in Ogwa and Ebelle and were collected in sterile container. They were left at room temperature for 2-3 days to undergo spoilage [13].

Chemical analysis

pH determination: pH of samples was determined using a calibrated pH meter Jenway 3510, three steps calibration was done using automatic buffer of pH 4, 7 and 10 before taking the pH of sample solutions.

Microbiological analysis

Media used and their preparations: Nutrient Agar (NA) and MacConkey Agar (MCA) were used for the enumeration of total heterotrophic bacteria and coliform bacteria respectively using pour plate method. Heterotrophic bacteria and coliform were determined by incubation at 37°C for 24 hrs. Incubation was at 28°C for 72 hrs. Each medium was prepared according to the manufacturer's instructions.

Isolation of the associated bacteria

Serial dilution of sample: Ten millilitres of each sample was homogenized into 90 ml distilled water. Tenfold serial dilution (10^{-1} to 10^{-9}) was made using sterile test tubes. Nine millimetres of normal saline was pipette into the test tube and 1 ml from the initial mixture was aseptically transferred to the first test tube, 1 ml of same diluents was transferred to the second test tube [14]. This procedure was repeated until the ten-fold dilution (10^{-10}) was achieved. 1 ml from dilution was pour plated in duplicates on various media for enumeration of isolates. Bacteria were enumerated on pour plates of MacConkey Agar (MCA), Nutrient Agar (NA). The pour plate

method of inoculation after serial dilution of the samples by 10^{-4} dilution factor was conducted for viable count, after sterilizing media at 121°C for 15 mins using autoclave. All plates were incubated for 48 hours at 37°C as described by Manga and Oyeleke [15].

Inoculation and enumeration: Nutrient agar (NA) and MacConkey Agar (MCA) was used for the enumeration of bacteria coliform respectively [16]. The inoculated plates were incubated at 37°C 24 hrs. for the enumeration of total heterotrophic bacteria and total coliform bacteria. The agars were prepared according to the manufacturer's procedure. Aliquot 1 ml of appropriate ten-fold serial dilution (10^{-3}, 10^{-6}, 10^{-9}) of the food sample was inoculated into the nutrient agar plates 1 ml from dilution was pour pated in duplicates on various media for enumeration of isolates. Bacteria were on pour plates of nutrient agar. The pour plate method of inoculation after serial dilution of the sample by 10^{-4} dilution factor was conducted for viable count, after sterilizing media at 121°C for 15 minutes using autoclave. All plates were incubated for 48 hours at 37°C as described by Manga and Oyeleke, [17].

Characterization and identification of bacterial isolates: Bacteria cultures were characterized and identified using various morphological and biochemical tests including: Gram stain, motility, catalase, coagulase, indole, citrate, oxidase and sugar utilization test. The motility test was done according to the technique described by Cheesbrough to distinguish motile bacteria from the non-motile one. Pure cultures of each isolate were [18] obtained by streaking the specific colonies on nutrient agar slants and incubated overnight theses were maintained in an agar slant in bijoux bottles. The identification of the microbial isolates was established on classification scheme offered by Harrigan and McCance, Buchanan and Gibbson and Collin and Lyne. The identification was based essentially on morphological and biochemical reactions [19].

Results and Discussion

The results were presented as mean of replicate readings (Tables 1-5.

Table 1. *The averages of THBC (x 10^4 cfu/ml) counts and LAB (x 10^4 cfu/ml) counts are summarized in the table below.*

Sample ID	Before		After	
	THBC (x	LAB count (x	THBC	LAB count (x
	10^4 cfu/ml)	10^4 cfu/ml)	(x10^4 cfu/ml)	10^4 cfu/ml)
Ebele fufu 1	2.5 ± 0.146	1.38 ± 0.081	5.61 ± 0.325	3.08 ± 0.182
Ebele fufu 2	1.69 ± 0.096	0.93 ± 0.056	3.79 ± 0.202	2.09 ± 0.122
Ebele ogi 1	1.60 ± 0.096	0.88 ± 0.051	3.62 ± 0.213	1.99 ± 0.116
Ebele ogi 2	2.09 ± 0.121	1.15 ± 0.080	4.68 ± 0.269	2.58 ± 0.147
Ebele palm wine 1	1.59 ± 0.092	0.87 ± 0.050	3.51 ± 0.203	1.93 ± 0.117
Ebele palm wine 2	1.88 ± 0.112	1.03 ± 0.060	4.24 ± 0.243	2.33 ± 0.137
Ogwa fufu 1	1.84 ± 0.107	1.01 ± 0.061	4.16 ± 0.190	2.29 ± 0.132
Ogwa fufu 2	1.84 ± 0.107	1.01 ± 0.061	3.97 ± 0.223	2.19 ± 0.128
Ogwa ogi 1	2.14 ± 0.093	1.26 ± 0.071	5.79 ± 0.416	3.19 ± 0.494
Ogwa ogi 2	2.62 ± 0.153	1.44 ± 0.086	6.22 ± 0.359	3.42 ± 1.314
Ogwa palm wine 1	1.62 ± 0.092	0.89 ± 0.056	3.67 ± 0.214	2.02 ± 0.106
Ogwa palm wine 2	1.52 ±1.308	0.80 ± 0.046	3.38 ± 0.380	1.79 ± 1.684
Legend: THBC: Total Heterotrophic Bacteria Count; LAB: Lactic Acid Bacteria Count.				

Table 2. *Bacteria isolate from nutrient agar.*

Parameters	A	B	C	D	E	F	G
Cultural characteristics							
Shape	Round	Round	Round	Round	Round	Round	Round
Colour	Milky	Pale green	Milky	Milky	Milky	light green	Milky
Size	Large	Large	Large	Large	Small	Small	Large
Elevation	Flat	Flat	Raised	Raised	Flat	Flat	Flat

Transparency	Transparent	Opaque	Transparent	Opaue	Opaue	Opaque	Opaque
Morphology							
Gram stain	Negative	Negative	Positive	Negative	Negative	Positive	Negative
Cell type	Rod	Rod	Rod	Rod	Rod	Cocci	Rod
Cell arrangement	Single	Single	Pair	Pair	Single	Clusters	Pair
Biochemical test							
Citrate utilization	-	+	+	+	+	+	-
Spore forming	-	-	+	-	-	-	-
Catalase production	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	+
Motility	+	+	+	+	-	-	+
Methyl red	+	-	-	+	-	+	+
Vogues prauskeur	-	-	+	-	+	+	+
Coagulase test	-	-	-	-	-	+	-
Oxidase test	-	+	-	+	-	-	-
Fermentation test							
Lactose	-	-	+	+	+	+	-
Glucose	+	-	+	+	+	+	+
Galactose	+	+	+	+	-	+	+
Maltose	+	-	+	+	+	+	+
Raffinose	-	-	-	-	+	-	+
Manitol	+	+	-	+	+	+	-
Probable identity	*Salmonella S*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Citrobacter freundii*	*Klebsiella oxytoca*	*Staphylococcus aureus*	*Escherichia coli*
A) *Salmonella enteritica,* B) *Pseudomonas aeruginosa,* C) *Bacillus subtilis,* D) *Citrobacter freundii,* E) *Klebsiella oxytoca,* F) *Styphalococcus aureus,* G) *Escherichia coli,*							

Table 3. *Lactic acid bacteria from De Man Rogosa Sharpe (Mrs) agar***.**

Parameters	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**
Cultural characteristics					
Shape	Round	Round	Round	Round	Round
Colour	Milky	Whitish	Milky	Whitish	Whitish
Size	Large	Large	Large	Large	Large
Elevation	Raised	Raised	Raised	Raised	Raised
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque
Morphology					
Gram stain	Positive	Positive	Positive	Positive	Positive
Cell type	Rod	Rod	Rod	Cocci	Rod
Cell arrangement	Pair	Short chain	Chains	Single	Pair

Biochemical test					
Citrate utilization	+	+	+	-	+
Spore forming	-	-	-	-	-
Catalase production	-	-	-	-	-
Indole	+	-	+	+	-
Motility	-	-	-	-	-
Methyl red	+	-	+	-	+
Vogues prauskeur	+	-	+	-	-
Coagulase test	-	-	-	-	-
Oxidase test	-	+	-	+	-
Fermentation test					
Lactose	+	+	+	+	+
Glucose	+	+	-	-	-
Galactose	+	-	+	+	+
Maltose	+	+	-	-	+
Raffinose	+	-	+	-	-
Manitol	+	+	-	+	+
Probable identity	**Lactobacillus sp**	**Pedococcus sp**	**Leuconostoc sp**	**Lactococcus sp**	**Lactobacillis sp**
L_1-*Lactobacillus* Sp., L_2-*Pedococcus* Sp., L_3-*Leuconostoc* Sp., L_4-*Lactococcus* Sp., L_5-*Lactobacillis* Sp.					

Table 4. *Distribution of Isolate before spoilage***.**

		A	**B**	**C**	**D**	**E**	**F**	**G**	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**
Batch 1	Ebele fufu 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 1	Ebele fufu 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 1	Ebele ogi 1	-	+	+	+	+	+	-	+	-	-	-	+
Batch 1	Ebele ogi 2	-	-	-	+	+	+	+	+	-	-	-	+
Batch 1	Ebele palm wine 1	-	+	+	+	+	+	-	+	-	-	-	+
Batch 1	Ebele palm wine 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa fufu 1	-	+	+	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa fufu 2	-	+	+	+	+	+	+	+	-	-	-	+
Batch 1	Ogwa ogi 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa ogi 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa palm wine 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa palm wine 2	-	-	+	+	+	+	-	+	-	-	-	+

Batch 2	Ebele fufu 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 2	Ebele fufu 2	-	-	-	+	+	+	+	+	-	-	-	+
Batch 2	Ebele ogi 1	-	+	-	+	+	+	+	+	-	-	-	+
Batch 2	Ebele ogi 2	-	-	+	+	+	+	-	+	-	-	-	+
Batch 2	Ebele palm wine 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 2	Ebele palm wine 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa fufu 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa fufu 2	-	-	-	+	+	+	+	+	-	-	-	+
Batch 2	Ogwa ogi 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa ogi 2	-	-	+	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa palm wine 1	-	+	+	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa palm wine 2	-	-	+	+	+	+	-	+	-	-	-	+
Batch 3	Ebele fufu 1	-	+	+	+	+	+	-	+	-	-	-	+
Batch 3	Ebele fufu 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 3	Ebele ogi 1	-	+	-	+	+	+	+	+	-	-	-	+
Batch 3	Ebele ogi 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 3	Ebele palm wine 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 3	Ebele palm wine 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 3	Ogwa fufu 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 3	Ogwa fufu 2	-	-	-	+	+	+	-	+	-	-	-	+
Batch 3	Ogwa ogi 1	-	+	+	+	+	+	+	+	-	-	-	+
Batch 3	Ogwa ogi 2	-	-	+	+	+	+	+	+	-	-	-	+
Batch 3	Ogwa palm wine 1	-	+	-	+	+	+	+	+	-	-	-	+
Batch 3	Ogwa palm wine 2	-	-	-	+	+	+	+	+	-	-	-	+

A) *Salmonella enteritica,* B) *Pseudomonas aeruginosa,* C) *Bacillus subtilis,* D) *Citrobacter freundii,* E) *Klebsiella oxytoca,* F) *Styphalococcus aureus,* G) *Escherichia coli,* **L_1**-*Lactobacillus* Sp., L_2-*Pedococcus* Sp., L_3-*Leuconostoc* Sp., L_4-*Lactococcus* Sp., L_5-*Lactobacillis* Sp.

Table 5. *Distribution of isolates after spoilage.*

		A	**B**	**C**	**D**	**E**	**F**	**G**	**L_1**	**L_2**	**L_3**	**L_4**	**L_5**
Batch 1	Ebele fufu 1	-	+	-	+	+	+	-	+	-	-	+	+

Batch 1	Ebele fufu 2	+	-	-	+	+	+	-	+	+	-	+	+
Batch 1	Ebele ogi 1	-	+	+	+	+	+	-	+	+	+	-	+
Batch 1	Ebele ogi 2	+	-	-	+	+	+	+	+	+	+	-	+
Batch 1	Ebele palm wine 1	+	+	+	+	+	+	-	+	+	-	+	+
Batch 1	Ebele palm wine 2	+	-	-	+	+	+	-	+	+	-	-	+
Batch 1	Ogwa fufu 1	+	+	+	+	+	+	-	+	-	-	+	+
Batch 1	Ogwa fufu 2	+	+	+	+	+	+	+	+	-	-	+	+
Batch 1	Ogwa ogi 1	+	+	-	+	+	+	-	+	+	+	-	+
Batch 1	Ogwa ogi 2	-	-	-	+	+	+	-	+	-	+	-	+
Batch 1	Ogwa palm wine 1	-	+	-	+	+	+	-	+	-	-	-	+
Batch 1	Ogwa palm wine 2	-	-	+	+	+	+	-	+	+	-	-	+
Batch 2	Ebele fufu 1	+	+	-	+	+	+	-	+	-	-	-	+
Batch 2	Ebele fufu 2	-	-	-	+	+	+	+	+	-	+	+	+
Batch 2	Ebele ogi 1	-	+	-	+	+	+	+	+	-	-	-	+
Batch 2	Ebele ogi 2	-	-	+	+	+	+	-	+	+	-	-	+
Batch 2	Ebele palm wine 1	+	+	-	+	+	+	-	+	+	-	+	+
Batch 2	Ebele palm wine 2	+	-	-	+	+	+	-	+	+	-	+	+
Batch 2	Ogwa fufu 1	-	+	-	+	+	+	-	+	-	+	+	+
Batch 2	Ogwa fufu 2	+	-	-	+	+	+	+	+	-	+	+	+
Batch 2	Ogwa ogi 1	-	+	-	+	+	+	-	+	-	+	-	+
Batch 2	Ogwa ogi 2	-	-	+	+	+	+	-	+	-	-	-	+
Batch 2	Ogwa palm wine 1	-	+	+	+	+	+	-	+	+	-	-	+
Batch 2	Ogwa palm wine 2	+	-	+	+	+	+	-	+	+	+	-	+
Batch 3	Ebele fufu 1	+	+	+	+	+	+	-	+	-	-	+	+
Batch 3	Ebele fufu 2	+	-	-	+	+	+	-	+	+	+	+	+
Batch 3	Ebele ogi 1	+	+	-	+	+	+	+	+	-	+	-	+
Batch 3	Ebele ogi 2	+	-	-	+	+	+	-	+	-	+	-	+
Batch 3	Ebele palm wine 1	-	+	-	+	+	+	-	+	+	-	-	+

Batch 3	Ebele palm wine 2	-	-	-	+	+	+	-	+	-	-	+	+
Batch 3	Ogwa fufu 1	+	+	-	+	+	+	-	+	-	+	-	+
Batch 3	Ogwa fufu 2	+	-	-	+	+	+	-	+	+	-	+	+
Batch 3	Ogwa ogi 1	+	+	+	+	+	+	+	+	-	-	+	+
Batch 3	Ogwa ogi 2	+	-	+	+	+	+	+	+	-	+	-	+
Batch 3	Ogwa palm wine 1	+	+	-	+	+	+	+	+	+	+	-	+
Batch 3	Ogwa palm wine 2	+	-	-	+	+	+	+	+	+	-	-	+

A) *Salmonella enteritica,* B) *Pseudomonas aeruginosa,* C) *Bacillus subtilis,* D) *Citrobacter freundii,* E) *Klebsiella oxytoca,* F) *Styphalococcus aureus,* G) *Escherichia coli,* **L**$_1$-*Lactobacillus* Sp., L$_2$-*Pedococcus* Sp., L$_3$-*Leuconostoc* Sp., L$_4$-*Lactococcus* Sp., L$_5$-*Lactobacillis* Sp

It was recorded in the (Table 1) that among Ebelle fufu and Ogwa fufu, Ebelle fufu 1 had the highest Total Heterotrophic Bacterial Count (THBC) of 2.47×10^4 cfu/ml before spoilage and also had the highest count of 5.54×10^4 cfu/ml after spoilage [20]. For Lactic Acid Bacterial count (LAB), Ebelle fufu 1 had the highest count of 1.36×10^4 cfu/ml before spoilage and 3.05×10^4 cfu/ml after spoilage. The least among the Ebelle fufu in THBC is Ebelle Fufu 2 which had 1.67×10^4 cfu/ml before spoilage and the least after spoilage is Ebelle fufu 2 had 3.75×10^4 cfu/ml. for LAB count Ebelle fufu 2 had the least of 0.92×10^4 cfu/ml before spoilage and 2.06×10^4 cfu/ml after spoilage [21].

The results in Table 1 also recorded that among Ebelle ogi and Ogwa ogi, the highest THBC Ogwa ogi 2 had 2.44×10^4 cfu/ml before spoilage and 6.15×10^4 cfu/ml after spoilage. For LAB count before spoilage, Ogwa ogi 2 recorded as the highest count of 1.42×10^4 cfu/ml and 4.05×10^4 cfu/ml after spoilage. The least among Ogwa ogi and Ebelle ogi before spoilage for THBC recorded Ebelle ogi 1, had 1.58×10^4 cfu/ml and 3.58×10^4 cfu/ml after spoilage [22]. For LAB count Ebelle ogi 1 had the least of 0.86×10^4 cfu/ml before spoilage and 1.97×10^4 cfu/ml after spoilage [23].

The results in the table recorded that among Ebelle palm wine and Ogwa Palm wine, the highest THBC before spoilage was Ogwa palm wine 2, with 2.15×10^4 cfu/ml and Ebelle palm wine 2 with 4.19×10^4 cfu/ml after spoilage [24]. For LAB count the highest count is Ebelle palm wine 2 gave 2.38×10^4 cfu/ml before spoilage and Ogwa palm wine 2 with 2.69×10^4 cfu/ml after spoilage. The least among Ebelle palm wine and Ogwa palm wine for THBC recorded that, [25] Ebelle palm wine 2 had the least of 1.57×10^4 cfu/ml before spoilage and Ebelle palm wine 1 had the least of 3.47×10^4 cfu/ml after spoilage. For LAB count, Ogwa palm wine 2 had the least of 0.79×10^4 cfu/ml before spoilage while Ebelle palm wine 1 had the least of 1.91×10^4 cfu/ml after spoilage [26].

Table 2 revealed the presence of *Salmonella enteritica, Pseudomonas aeruginosa, Bacillus subtitles, Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus* and *Escheichia coli* from Nutrient agar. There was dominance of gram negative isolates over the gram positive [27].

Table 3 recorded that *Lactobacillus* sp, *Pedococcus* sp, *Leuconostoc* sp, *Lactococcus* sp and *Lactobacillus* sp, were isolated from MRS agar from [28] Ogi, fufu, and Palm wine samples from Ogwa and Ebelle communities [29].

Table 4 results show that, in *Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus* and Lactobacillus sp, the organisms [30] were present in all the samples and batches while *Salmonella enteritica*, *Pedococcus* sp, *Leuconostoc* sp and *Lactococcus* sp were not present in all samples and batches [31].

The results show that in *Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus*, Lactobacillus sp and Lactobacillis sp, the organisms were present in all samples and batches (Table 5) [32].

In this study, the results of the experiment indicated that the fermented foods (Palm wine, Ogi and Fufu) [33] had a Total Heterotrophic Bacterial Count (THBC) and Lactic acid Bacterial (LAB) count which ranged from 1.57×10^4 cfu/ml-2.47×10^4 cfu/ml and 0.79×10^4 cfu/ml-2.38×10^4 cfu/ml before spoilage respectively while Total heterotrophic bacterial count and Lactic acid bacterial count ranged from 3.47×10^4 cfu/ml-6.15×10^4 cfu/ml and 1.91×10^4 cfu/ml-4.05×10^4 cfu/ml after spoilage respectively [34]. This may be due to the known fact that fermentation which usually occurs during fermented food processing usually involves many bacteria species especially the lactic acid bacteria [35].

The bacteria associated with spoilage of fermented food [36] (palm wine, Ogi and Fufu) included *Salmonella enteritica, Pseudomonas aeruginosa, Bacillus subtilis, Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus* and *Esherichia coli* while lactic acid bacteria were *Lactobacillus, Pedococcus, Leuconostoc, Lactococcus* and *Lactobacillis* species [37].

The presence of some microorganisms such as *Lactobacillis, Leuconostoc, Pedococcus, Lactobacillus, Lactococcus* species

may be beneficial. Several of these have been found to have probiotic properties and immunomodulatory functions [38]. Several *Lactobacillus* sp. [39] have been reported to display stimulatory properties on cells of innate immunity *in vitro* and *in vivo* in both animal models and in humans given fermented food products containing probiotics. In humans, *Lactobacilli* are normally present in the vagina [39], gastrointestinal tract and are together with *Bifidobacterium* one of the first bacteria to colonize the infant gut after delivery. The spoilage fermented drinks has been associated with the presence of both *Bacillus subtilis* and *Pseudomonas aeruginosa*, the findings of the work agrees with the submissions of Nwachukwu [40].

Most of the bacteria identified in the study have been implicated in spoilage of ferment foods and other related infections [40]. When fermented foods are left in ambient temperature the contaminating organisms proliferates and this results in the spoilage of the fermented foods. The results were similar to the *Citrobacter freundii, Klebsiella oxytoca, Staphylococcus aureus* and *Lactobacillus* sp, were present in all the samples and batches due to their ubiquitous nature in the environment.

Conclusion

In conclusion, the study revealed that, the total Heterotrophic bacteria count and lactic acid bacteria counts in Ebele fufu 1 was very. In the three different cases reported. The after counts were more than twice the before counts in both total Heterotrophic bacteria count and lactic acid bacteria count after spoilage. This reveals high concentration of bacteria in ebele fufu 1 after spoilage. Similar findings were reported of Ebele fufu 2. Over 100% of the previous counts of bacteria were reported after food spoilage. in other food type, the results obtained were not different as the counts for bacteria increased by more than 100% after food spoilage. Ogwa Fufu is recommended for eating because it has the least total heterotrophic count and lactic acid bacterial count.

Comparing results obtained for Ebele and Ogwa, it can be seen that there was no much difference between bacteria counts before and after food spoilage.

Recommendations

Locally fermented food should be given proper handling so as to prevent food borne infections. Advanced techniques for the production of locally fermented food should be encouraged.

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