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Research Paper

Evaluation of the Microorganisms Associated with Untreated and Salt-Treated Ogi (Corn Caramel) Stored at Room Temperature

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This present research is centred on evaluation of the microorganisms associated with salt-treated and untreated corn caramel (Ogi/Akamu) kept at room temperature (28 ±2) °C for two weeks using standard techniques. The purchased ogi was stored in the Microbiology laboratory at ambient temperature and treated with different concentrations of cooking salt. The identified microbial isolates were: *Lactobacillus* sp, Bacillus sp *Micrococcus* sp, *Staphylococcus* sp, *Aspergillus* sp, *Rhizopus* sp, *Fusarium* sp and *Saccharomyces* sp. The analyses showed that Ogi 1 had higher total viable counts for both bacteria and fungi than Ogi 2. The bacterial counts ranged from 3.3×10^5 - 1.75×10^7 cfu/g while the fungal counts ranged from 3.3×10^5 - 7.4×10^6 cfu/g in the untreated samples. The same bacterial and fungal genera found in Ogi 1 and Ogi 2 were identified in the salt-treated samples (Ogi 3 and Ogi 4) but there was a significant decrease in the microbial load in samples treated with 0.5 g salt and only *Bacillus* sp and *Staphylococcus* sp were isolated in the treated samples. *Bacillus subtilis* was the most frequently occurring bacterial isolate (47%) while the least occurring was *Micrococcus* sp (23%) and the most frequently occurring fungal isolate was *Saccharomyces* sp (50%) while *Fusarium* and *Aspergillus* were the least occurring fungal isolate. The results of this research have shown that cooking salt can serve as an inexpensive material for extending the shelf-life of Ogi.

Key words: Ogi, corn caramel, room temperature, supernatant, salt- treatment, preservation.

INTRODUCTION

Maize (*Zea mays*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Abdulrahaman and Kolawole, 2006). The nutritional composition of maize had been described by Martins, (2011). Ogi (akamu) also known as Corn Caramel (Nairaland, 2013) is a product of fermented maize (*Zea mays*) widely eaten in Africa (Adams and Moss, 1995; Amakoromo, 2011). The fermented foods are derived from substrates like roots, legumes, cereals, oilseeds, nuts, meat, fish, milk and palm tree sap (Adebukunola *et al.,* 2015). One of the popular indigenous fermented foods in Nigeria is ogi which is a fermented cereal porridge made from maize (*Zea mays*), sorghum (*Sorghum vulgare*) or millet (*Pennisetum typoideum*). The ogi porridge is very smooth in texture and has a sour taste reminiscent of that of yoghurt (Adebukunola *et al.,* 2015). In Nigeria, the uncooked ogi is either prepared into a smooth porridge called “pap” or a solid gel known as “eko” or “agidi similar to kenkey, a fermented shania product in Ghana also referred to as “Akana”. Ogi is often marketed as a wet cake formerly wrapped in leaves but presently in transparent polythene bags and is mainly used as a breakfast meal for adults and weaning food by low

income earners who cannot afford the more expensive imported weaning foods (Ozoh and Kuyanabana, 1995; Amakoromo, 2011). In most parts of Africa especially in Nigeria, children are fed with mashed adult foods. These foods are bulky and this therefore reduces food intake by a child, often resulting in malnutrition (Mbakwem-Aniebo and Udemgba, 2012).

The pap used as the first native food for weaning babies does not meet the required nutritional proportion based on the processing stages (Ajanaku *et al*., 2012). The development of nutritionally balanced calorie dense, low bulk and easily digestible weaning food becomes mandatory. This involves the use of simple but time consuming traditional technology called fermentation (Marrero *et al*., 1989).

The traditional fermentation method employed in Ogi production is a wild process and microorganisms are not controlled which was described by Omemu and Omeike, (2010). Microbiological analyses have shown the presence of several genera of bacteria, moulds and yeasts in the fermented maize product- Ogi (Akinrele, 1970; Odunfa, 1985). Ogi is fairly acidic (pH 4.8), which tends to inhibit the growth of some bacteria. Its spoilage however is enhanced by some extrinsic factors amongst which are storage temperatures (Mbakwem-Aniebo and Udemgba, 2012). Extension of the shelf life of Ogi is carried out using various techniques, which include refrigeration, freezing and drying (dehydration) to reduce the microbial load and consequently spoilage (Amakoromo, 2011). This study therefore, is aimed at finding a simple and inexpensive way of extending the shelf-life of Ogi.

MATERIALS AND METHODS

Source of corn caramel

The yellow ogi (prepared from yellow maize) sample used for this study was obtained from Gwagwalada market in FCT, Nigeria. It was wrapped in a clean polythene bag to avoid further contamination and taken to the laboratory within two hours and stored following the method described by Mbakwem-Aniebo and Udemgba (2012).

Treatment of Ogi

Fifty grams of the ogi were aseptically weighed into eight sterile conical flasks and 150ml of distilled water was added. The conical flasks were labelled ogi 1, ogi 2, ogi 3A, ogi 3B, ogi 3C and ogi 4A, ogi 4B and ogi 4C respectively**.** About 0.1 g of cooking salt (NaCl) was added to ogi 3A and 4A, while 0.3 g was added to ogi 3B and 4B and 0.5 g of salt was added to ogi 3C and 4C.The conical flasks were kept at room temperature for 2 weeks. The supernatant of ogi 2 and ogi 4 (A, B, and C) was changed daily throughout the experimental period while that of ogi 1 and 3 (A,B and C) were left unchanged.

Isolation of microorganisms

One gram of each ogi sample was added to 9.0 ml distilled water in 8 different test tubes. Further ten-fold dilutions were made and from each dilution 0.1 ml was aseptically transferred to plates containing nutrient Agar and Sabourad dextrose Agar in duplicates and were also spread using a sterile bent glass rod. Plates containing nutrient Agar were incubated for 24 h at 35°C while Sabourad dextrose Agar plates were incubated for 4-5 days at 37°C. Colonies from nutrient agar were subcultured into fresh nutrient Agar and mannitol salt. Agar while colonies from Sabourad dextrose Agar were subculture into fresh Sabourad dextrose Agar. The plates were incubated for 24 h at 37°C (Cheesbrough, 2010).

Microbial count

Discrete colonies appearing at the end of the incubation period (primary culture) were counted using a digital illuminated colony counter. Total microbial counts were expressed in colony forming unit per gram (cfu/g).

Identification of bacteria

The identification of bacteria was based on morphological characteristics and biochemical tests carried out on isolates. Morphological characteristics observed for each bacterial colony after 24 h of growth included colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, indole and coagulase production, starch hydrolysis, sugar fermentation and methyl red-Voges-Proskaur reaction. The tests were performed according to the methods of (Fawole and Oso, 2001; Cheesebrough, 2010; Nester *et al*., 2007; Ochei and Kolhatkar, 2008; Olutiola *et al*., 2000). Microbial identification was performed using the keys provided in the Bergey's Manual of Determinative Bacteriology (1994).

Identification of fungi

The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi. The fungal colonies were sub-cultured on Potato Dextrose Agar (PDA). The isolates were identified based on their morphological and

Table 1. Bacterial load of ogi sample.

Sample	No of colonies	Dilution factor	Cfu/g	Standard error
Ogi 1	175	10^4	1.75×10^7	±0.08
Ogi 2	148	10	1.48×10^7	±0.07
Ogi 3	69	10^3	6.9×10^5	±1.59
Ogi 4	33	10^3	3.3×10^5	±0.13

Table 2. Fungal load of ogi samples.

Sample	No of colonies	Dilution factor	Cfu/g	Standard error
Ogi 1	74	10^4	7.4×10^6	±0.55
Ogi 2	52	10^4	5.2×10^6	±0.10
Ogi 3	48	10^3	4.8×10^5	±0.25
Ogi 4	33	10^3	3.3×10^5	±0.20

microscopic features. Two drops of cotton-blue-in-lactophenol were placed on clean glass slide and small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain. The mycelium was picked out with the needles and covered with clean cover slip carefully avoiding air bubbles and observed under the microscope for vegetative and reproductive structures (Hunter and Bamett, 2000; Efiuvwevwere, 2002).

Statistical analysis

All experiments were carried out in duplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 21.0 version). Differences were considered significant at $p<0.05$.

RESULTS

The bacterial load of the salt-treated and untreated Ogi as shown in (Tables 1 and 2) revealed that Ogi 1 had the highest bacterial and fungal count (1.75×10^7 and 7.4×10^6) respectively while Ogi 4 had the lowest bacterial and fungal count (3.3×10^5). The colonial, and morphological and biochemical characteristics of the microorganisms isolated from the Ogi samples were shown in (Tables 3 and 4) while the identification of the pure isolates are shown in (Tables 5 and 6) for the bacteria and fungi respectively. The results of the biochemical tests are shown in (Table 7). Microorganisms tentatively identified are: *Lactobacillus* sp, *Micrococcus* sp, *Staphyloccocus,* sp, *Bacillus* sp, *Aspergillus* sp*, Rhizopus* sp*, Fusarium* sp and *Saccharomyces* sp. The same bacteria and fungi present in Ogi 1 and Ogi 2 were also identified in the salt - treated Ogi samples. But there was a great decrease in the microbial load especially with the samples containing 0.5g salt where only *Bacillus* sp and *Staphylococcus* sp were isolated (Table 8 and 9). *Bacillus subtilis* was the most frequently occurring bacterial isolate (47%) while the least occurring was *Micrococcus* sp (23%) and the most frequently occurring fungal isolate was *Saccharomyces* sp (50%) while *Fusarium* and *Aspergillus* were the least occurring fungal isolate as represented in (Table 10 and 11).

DISCUSSION

The outbreak of infectious and communicable diseases in tropical parts of the world is primarily as results of food poisoning due to microbial contamination (Jay, 2005).They are often responsible for acute gastroenteritis, abdominal discomfort and pain and diarrhoea in infants and young adults (WHO, 2010). Several means have been employed in preservation and increasing the shelf life of foods such as fermentation of Ogi.

The bacterial and fungal counts recorded for the untreated samples were found to be higher in Ogi 1 than in Ogi 2 (1.75×10^7 and 1.48×10^7) cfu/g respectively. These values are lower than what was reported by Mbakwem-Aniebo and Udemgba, (2012) (2.65×10^7 and 1.65×10^7) for Ogi 1 and Ogi 2 respectively. The higher microbial load in Ogi 1 may be due to accumulation of microorganisms in the water containing the Ogi which was retained throughout the experimental period. The bacteria identified from Ogi samples were *Lactobacillus* sp, *Bacillus* sp*, Micrococcus* sp*,* and *Staphylococcus* sp*.* This observation corroborates the reports of earlier researchers (Mbakwem-Aniebo and Udemgba, (2012). *Lactobacillus* sp which was one of the organisms found in the Ogi samples has been reported by several researchers as the most important and predominant microorganism involved in the fermentation of maize during Ogi production (Odunfa, 1985; Ozoh and Kuyanbana, 1995, Amusa *et al*., 2005). Akinrele, (1970) reported that lactic acid bacteria *Lactobacillus* sp, was among the major

Table 3. Colonial and morphological characteristics of bacteria isolated from Ogi samples

Media	Sample (Ogi)	Morphology of bacteria colonies
NA	Ogi 1	Off-white circular colonies, whitish colonies with irregular shape, bright yellow, golden yellow. Whitish colonies.
MSA	Ogi 1	Golden yellow colour with circular shape.
NA	Ogi 2	Golden yellow, bright yellow, off-white with circular shape, whitish colonies with irregular shape.
MSA	Ogi 2	Golden yellow colour with circular shape.
NA	Ogi 3	Whitish colonies with irregular shape, golden yellow, white circular colonies, bright yellow circular shape.
MSA	Ogi 3	Golden yellow with circular shape.
NA	Ogi 4	Golden yellow with circular shape, whitish colonies with irregular shape.

Key: NA= nutrient agar, MSA= mannitol salt agar.

Table 4. colonial and morphological characteristics of fungi isolated from the ogi samples.

Media	Sample	Morphology
SDA	Ogi 1	Circular off-white, wooly white, black creamy, white sharp.
SDA	Ogi 2	White sharp, wooly white, black creamy, circular off-white.
SDA	Ogi 3	Circular off-white, wooly white, black creamy.
SDA	Ogi 4	Whitish circular.

Key: SDA= sabourad dextrose agar.

Table 5. morphological characteristics and identification of fungi from pure culture.

Isolates	Pigmentation	Microscopy	Organism
Isolate 1	Flat, smooth, moist, and whitish in colour	Spherical buds reproducing in irregular budding	*Saccharomyces* sp
Isolate 2	Whitish grey on the surface of the media	Sporangiosphores are smooth and ovoid. Hyphae are aseptate, simple or branched, it arise from the stolon opposite the rhizoid which is usually in group. Sporangia are round containing many spores	*Rhizopus* sp
Isolate 3	Mycelium is black covering the whole agar surface.	Conidiosphores are hyaline, smooth becoming darker at the apex. Hyphae are septate and hyaline. Conidia are ovoid.	*Aspergillus* sp
Isolate 4	Woolly white, covering the whole agar	Conidiosphores are slender and simple. Hyphae are in clusters, spectate, and hyaline. They have macro and microconidia. The macroconidia are several celled and the microconidia are one celled in chains	*Fusarium* sp

organisms responsible for the fermentation and nutritional improvement of Ogi. Studies have shown that the microorganisms involved in the fermentation of Ogi results in in the improvement of its nutritive value (Barbra, 2013). The microorganisms such as *Bacillus* sp, *Micrococcus* sp and *Staphylococcus* sp isolated in this study are of public health importance which may be due to contamination resulting from handling and processing environment. Poor handling had been reported to contaminate the ogi with enteric bacteria and possible

Table 6. Morphological characteristics of pure culture of bacteria isolate.

Isolates	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Colour	White	Golden yellow	White	Bright yellow
Configuration	Circular	Circular	Circular	Circular
Margin	Irregular	Entire	Entire	Entire
Elevation	Flat	Convex	Raised	Convex
Gram stain	Positive	Positive	Positive	Positive
Shape	Rods in chain	Cocci in clusters	Rods	Cocci in tetrads

Table 7. Biochemical test and identification of bacterial isolate.

Test	Results			
	Bacillus	**Staphylococcus**	**Lactobacillus**	**Micrococcus**
Gram stain	+	+	+	+
Endospore	+	-	-	-
Manitol	-	+	-	+
Catalase	+	+	-	+
Coagulase	-	+	-	-
Starch	+	+	-	-
Indole	-	-	-	+
Methyl red	+	-	-	+
Citrate	+	+	-	-
Glucose	A	A	A	A
Lactose	-	A	A	A
Sucrose	A	A	-	-
H_2S	-	-	+	-
Motility	+	-	-	-

Species: Bacillus, Staphylococcus, Lactobacillus, Micrococcus
Organism *B. Subtilis, S. aureus*
Key: + = Positive, - =Negative, A = Acid.

Table 8. Bacteria Isolated from salt-treated ogi samples.

Fungi	NaCl conc/ml					
	ogi 3			ogi 4		
	0.1g	**0.3 g**	**0.5 g**	**0.1 g**	**0.3 g**	**0.5 g**
Bacillus sp	+	+	+	+	+	+
Staphylococcus sp	+	+	+	_	_	+
Lactobacillus sp	+	_	_	+	+	_
Micrococcus sp	+	+	_	**–**	**–**	**–**

Key: + = present, **–** = absent

Table 9. Fungi Isolated from Salt-treated Ogi Sample.

Fungi	NaCl conc/ml					
	Ogi 3			Ogi 4		
	0.1 g	**0.3 g**	**0.5 g**	**0.1 g**	**0.3 g**	**0.5 g**
Aspergillus sp	+	+	_	_	_	_
Rhizopus sp	+	+	_	+	_	_
Fusarium sp	+	+	_	_	_	_
Saccharomyces sp	+	+	+	+	_	+

Key: + = present, **–** = absent

contamination sites like water, medium for soaking, the grinding mill and contamination that results during storage and transportation (Ijabadeniyi, 2007). The fungal counts were also higher in Ogi 1 than in Ogi 2 which also may be due to the accumulation of microorganisms in the water the Ogi was stored in and retained throughout the storage period. Fungi identified were *Aspergillus* sp*, Fusarium* sp*, Rhizopus* sp and *Saccharomyces* sp. These microorganisms were also identified by Akinrele (1970), Odunfa, (1985) and Mbakwem-Aniebo and

Table 10. Percentage frequency of occurrence of bacterial isolates in the ogi sample.

Bacterial isolates	Percentage (%) frequency
Bacillus subtilis	47
Staphylococcus aureus	35
Lactobacillus sp	29
Micrococcus sp	23

Table 11. Percentage frequency of occurrence of fungal isolates in the ogi sample.

Fungal isolates	Percentage (%) frequency
Aspergillus sp	28
Rhizopus sp	38
Fusarium sp	28
Saccharomyces sp	50

Udemgba, (2012). *Aspergillus* sp, *Fusarium* sp and *Saccharomyces*, sp were also said to be responsible for the fermentation and nutritional improvement of Ogi. *Aspergillus* sp and *Rhizopus* sp are said to produce organic acids, while *Saccharomyces* sp contributed to flavour development (Banigo and Muller, 1972). In Ogi 3 and 4 treated with different salt concentrations (0.1 g, 0.3 g 0.5 g), the bacterial and fungal counts were higher in Ogi 3 than Ogi 4. This could possibly be as a result of the not changing the water for the stored Ogi. However, there was a great decrease in the bacterial and fungal counts in Ogi 3 and 4 containing 0.1g, 0.3g and 0.5g salt in separate flasks. 0.5 g salt-treated sample recorded the least microbial count. This could be as a result of the effect of salt on the non-salt tolerant organisms. There was a significant difference in the microbial load of the two groups. The total microbial counts were more in non-salt-treated Ogi than in salt-treated Ogi.

Conclusion

From the results obtained in this study, Ogi can be kept at room temperature and the supernatant water changed daily. The presence of the microorganisms did not change the quality of the Ogi, since most of the organisms present were organisms associated with its fermentation. It is undesirable, however, to have foods with high microbial load. The use of 0.3 g/100g cooking salt in preserving Ogi should be encouraged, since it reduced the number and type of bacteria and fungi found in the product thereby extending its shelf-life. This level or amount of salt addition is so minimal and will not have any adverse health implications and did not lower the gelatinization of the Ogi when it was made into pap.

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