EVALUATION OF MICROORGANISMS AT DIFFERENT STAGES OF PRODUCTION OF OGI IN ALIMOSHO COMMUNITY, AREA SOUTHWEST, LAGOS, NIGERIA

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ABSTRACT

Traditional method of preparation was simulated in the laboratory fermentation of maize to produce Ogi. The maize grains were steeped for 72hr; wet milled, sieved and fermented for 48hr separately at 32±2^{0}c by the maize natural microflora. The mould and bacterial isolated were identified using standard cultural, microscopic and biochemical methods. The pH of Ogi was determine using pH meter while titratable acidity was determined by titration method. The moulds isolated were Aspergillus niger, Aspergillus flavus, Fusarium oxysporium and Penicillium species. The yeasts isolated during the fermentation period include Mucormucedo, Saccharomyces cerevisae, Rhizopus stolonifer and Candida albicans. Seven bacterial were also isolated with Lactobacillus plantarum has the highest isolation rate 10.31%, followed by Lactobacillus fermentum and Staphylococcus aureus 9.28% each respectively. Escherichia coli 6.19%, Klebsiella spp 5.15%, Pseudomonas aeruginosa 4.12% and Corynebacterium spp 2.06%. The temperature of fermenting maize

remained relatively constant between 28^0c to 32^0c throughout the fermentation.

The pH decreased and titratable acidity increased during fermentation.

Despite the unhygienic wet-milling and wet sieving processes involved in the traditional preparation of Ogi, the low pH of the fermented products would make them safe for consumption. Appropriate safety measures and good manufacturing practices will ensure good quality of product.

Key words: Ogi, fermentation, moulds, maize, Lactic acid bacteria and yeasts.

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INTRODUCTION

The traditional preparation of maize Ogi involves soaking of maize in water for 1 to 3 days followed by wet milling and sieving to remove bran, hulls and germs (Akinrele et al; 1970, Akingbala et al., 1981; Odunfa, 1985). The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented (for 2-3 days) to yield Ogi, which is sour, white starchy sediment (Odunfa, 1985).

Maize products are the cheapest and readily available fermented foods for infants and young adults in most tropical countries (Torre et al, 1991). They are important energy food rich in carbohydrates and with traces of vitamins,

proteins and minerals (Achternberg et al., 1994; FAO, 2009) and are natural antioxidants (Eaton and Nelson, 1991). The importance of vitamins as antioxidants was aptly discussed by Singh and Sachan (2011).

Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet. Fermented foods have been with us since humans arrived on earth. They will be with us far into the future as they are the source of alcoholic foods/beverages, vinegar, picked vegetables, sausages, cheeses, yogurts, vegetable protein, amino acid/peptide sausages, cheese, yogurts, vegetables protein amino acid/peptide sauces and pastes with meat like flavours, leavened and sour-dough breads (Steinkraus, 1997).

Nigeria is endowed with a wide rage of fermentable indigenous staple foods that serve as raw materials for agro allied-cottage industries. These industries utilize small-scale equipment and provide alternative equipment for rural communities while adding value to such local porridge (Latunde -Dada, 2000). One common example of indigenous fermented foods in Nigeria is Ogi.

The wet fermented porridge is prepared and consumed as Ogi; Akamu and Akassan among the Yorubas, Ibos and Hauses in the west, east and northern Nigeria, respectively (Praveen and Hafiz, 2003). These maize products are common in seemingly poor and impoverished communities across the developing countries (Inyang and Idoko, 2006). These fermented products are largely from zea mays, Oryza Sativa, Sorghum Valgare and Tritiecum aestivum. Their production is often by small-scale enterprise undertaken by unskilled female attendants (Aminigo and Akungbale, 2004).

The inclusion of Fumonisin and at aflatoxins in maize (Jesperson et al., 1994) and other cereal products (Fandohan et al; 2005, Shepherd et al; 2002) has been linked to certain species of fungi including Aspergillus, Penicillium, Rhizopus and Fusarium by Omemu et al; (2005).

The cinogenic effects of this contamination were extensively discussed by Shepherd et al., (2002). Few of these toxins including FBI are associated with

high degree of cancer in rats and humans. Hendricks (1999) and Barug et al. (2004) warned of the health implication of consuming mycotoxins contaminated maize products. Fandohan et al. (2005) Warned of the danger of using the supernant of Ogi as solvent to extract active ingredients from traditional herbal plants because of probable high level of Fumonis. A positive correlation between the level of aflaxoxins and the incidences and severity of kwashiorkor in infants Adhikari et al., 1994 has been established. They also discovered a significantly low hemoglobin level, longer oedema and increased infection rate in children that were positive for aflatoxins.

Ogi is either consumed as porridge (pap) or as a gel-like product (Agidi) by a very large number of Nigerians. Pap however is the most important traditional food for weaning infants and the major breakfast cereal for adults especially the low income earners that cannot afford imported baby food (Onyekwere et al., 1989).

The objective of this study is to determine the critical points of contamination in the preparation of Ogi, isolate and characterized microorganism associated with indigenous fermentation of the different maize varieties.

MATERIALS AND METHODS

Collection of samples: This study was carried out in Alimosho Local Government in Lagos State between March, 2013 to June, 2014. There varieties of maize were purchased at the two difference local government markets, they were brown local, white local and yellow farmers.

For this study, three difference water reservoirs were used plastic tanks, drums and metal tanks maize grains, steep water from mashed grains, paste, filtrate from paste and fermented slurry (wet pap).

Swaps from fungi nails, clothes and cooking utensils were collected using most sterile swab stick.

The maize grains were sieved to remove pebbles and dirts and subsequently soaked in water for 3 days. The soft grains were mashed, milled and sieved using Muslin cloth. The solid paste was diluted with water and left to ferment for 48-72 hrs. The surface water was decanted and the sediment (wet sturry) was collected in a bowl and allowed to stand for 10-12hrs to sufficiently solidified.

The solidified slurry was portioned into small units or cubes and wrapped in leaves or polythene bags ready for sale.

Media preparation: The number and type of microorganisms (bacteria, yeast and fungi) per ml, of the fermenting substrate was estimated daily for 72hrs by pour plate method using the serial dilution technique. The techniques described by Arora and Arora (2008) and Willey et al., (2008) were employed in preparing the Potato Dextrose Agar (PDA) and Mueller Hitton Agar to obtain pure cultures of the fungal and bacterial isolates, respectively.

The isolated organisms were characterized by using conventional methods (Cowan and Steel, 1985; Barnett and Hunter, 1972). The pH of the fermenting substrates was measured daily on a Jenway pH meter standardized with the appropriate buffers.

The pH of the Ogi sample were determined manually, using JENWAY 3016 digital pH meter; Standardization (calibration) of the pH meter was done using buffer solution of pH 7 and 4. The samples were determined at 72hrs intervals.

The titratable acid (TA) of Ogi samples were analyzed at the same time interval by titrating 0.lm NoaH solution and methyl red as an end point indicator. The titre value of each homogenate was determined as percentage lactic acid consuming definite volume of 0.ml NaoH.

Biochemical tests analysis: The techniques described by Cheesbrough (2000) were employed for the Gram staining while the citrate utilizing, hydrogen sulphide production test and sugar fermentation and oxidase tests were as, respectively described by Mac Williams (2009) and Cowan and Steel (2002). The

isolates were later identified using Bergey's manual described by Brenner et al; (2005). Confirmation of coliforms was as described by Willey et al; (2008), using selective and differential media such as Mac Conkey and Mannitol salt agar to analyses for E. coli and Klebsiella spp and Staphylococcus aureus, respectively. The presumptive test in broth was streaked out on Mueller Hilton agar and incubated at 37^0c for 48hrs, for morphologically characterize the isolates.

RESULTS

Seven bacterial and eight fungi species were identified in this study:

In case of bacterial isolate, Lactobacillus plantarum has the highest value (10.31%) while staphylococcus aureus and lactobacillus fermentatum were (9.28%) each respectively. Aspergillus niger has the highest occurrence in fungi isolated (13.4%) followed by Aspergillus flavus (11.4%) while Fusarium oxysporium and Pencillium spp were (10.3%) and (5.2%) respectively (table 1).

The bacteria isolated were Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella species and Corynebacterium species. Lactobacillus plantarum and lactobacillus fementum were the only bacteria identified during secondary fermentation.

Eight different fungal species were isolated during primary fermentation (steeping). The fungi were penicillim spp., Rhisopus stolonifer, Mucor mucedo, Aspergillus niger, Candida albicans, Aspergillus flavus, Saccharomyces cervisiae and Fusarium oxysporium.

Table 2 shows the biochemical characteristics of bacteria isolated from the fermenting substrates.

Table 3 shows the duration of storage, pH and titratable acid of fermented different Ogi. As the titratble acid increase the pH value decreases. Table 4 and 5, show the total bacteria and fungi count during the steeping of maize varieties.

Table 1: Percentage of microorganism isolated from the three varieties of maize

Group	Microorganism	Number	%
Bacteria	Lactobacillus fermentum	9	9.28
	Lactobacillus plantarum	10	10.31
	Pseudomonas aeruginosa	4	4.12
	Staphylococcus aureus	9	9.28
	Escherichia coli	6	6.19
	Klebssiella spp Kl.ebs	5	5.15
	Corynebacterium spp	2	2.06
Fungi	Aspergillus flavus	11	11.34
	Aspergillus niger	13	13.40
	penicillum spp	5	5.15
	Fursarium oxysporium	10	10.31
	Rhizopus stolonifer	3	3.09
	Mucor mucedo	3	3.09
Yeasts	Saccharomyces cervisiae	3	3.09
	Candida albicans	4	4.12
Total		97	100%

Table 2: Morphological and biochemical characteristics of bacteria from fermenting substances

Bacteria isolated	Cit	Ur	NiRed	Ind	Xul	Sor	Fru	Arab
Corynebacterium spp	ND	*	+	*	*	+	+	+
Lactobacillus fermentatum	ND	ND	*	*	*	*	*	+
Lactobacillus plantarum	ND	+	+	*	*	+	+	+
Staphaureus aureus	*	+	+	*	*	+	+	
Kleb spp								
Esherichia coli								
Pseudmonas aeruginosa								

Bacteria isolated	Lac	Mal	Su	Man	Glu	G/a	G/A	Ca
Corynebacterium spp	*	+	*	*	+	TAG	*	+
Lactobacillus fermentatum	+	+	+	*	+	TAG	+	*
Lactobacillus plantarum	+	+	+	+	+	TAG	+	*
Staphaureus aureus								
Kleb spp								
Esherichia coli								
Pseudmonas aeruginosa								

Corynebacterium Rod – Shape

Lactobacillus fermentatum Rod – Shape

Lactobacillus plantarum Rod – Shape

Staphylococcus aureus Cocci Shape

Cit = Citrate; Ur = Urease; NiRed = Nitrate Reduction; Ind = Indole; Xyl = Xylose;

Sor = Sorbitaol, Fru = Fructose; Arab = Arabinose, Lac = Lactose; Mal = maltose;

Su = Sucrose; Man = Mannitol; Glu = Glucose; G(a) = Glucose (Acid); G/A= Growth in air; Ca = Catalase.

Table 3: Duration of storage, pH and titratable acid of fermented Ogi

Duration of storage for days	0	3	6	9	12	15
pH	6.4	6.1	6.0	5.1	5.2	5.0
Titratable	0.13	0.14	0.17	0.24	0.33	0.39

Mean pH value = 5.0 ±1

Mean titratable acid value = 0.01 ±1

Table 4: Total bacteria counts during the steeping of maize varieties (10^5 CFC – ML^{-1})

Time (h)	White maize	Maize varieties yellow maize	Brown maize
0	$6.0x10^5$	$4x10^5$	$2x10^5$
24	$8.0x10^5$	$6x10^5$	$4x10^5$
48	$15.0x10^5$	$14x10^5$	$11x10^5$
72	$17.0x10^5$	$16x10^5$	$15x10^5$

Table 5: Total Fungai counts during the steeping of maize varieties (10^3 CFC Ml^{-1})

Time (h)		**Maize varieties**	
	White maize	yellow maize	Brown maize
0	$2.0x10^3$	$1.0x10^3$	$1.0x10^3$
24	$3.5x10^3$	$2.5x10^3$	$2.0x10^3$
48	$4.0x10^3$	$3.5x10^3$	$3.0x10^3$
72	$4.5x10^3$	$4.0x10^3$	$3.0x10^3$

DISCUSSION

The outbreak of infections and communicable diseases in tropical parts of the world in primarily as a result of food poisoning due to microbial contamination (Jay, 2005). They are often responsible for acute gastroenteritis, abdominal discomfort, pain and diarrhea in infants and young adults (WHO; 2010, Kimmonon et al; 1999). A total of 15 micro-organisms were obtained from fermenting maize steep for Ogi production, seven bacterial and eight fungi, Lactobacillus plantarum 10 (10.31%), Lactobacillus fermentum 9 (9.28%), Staphylococcus aureus 9 (9.28%),. Aspergillus niger and Aspergillus flavus were 13 (13.40%) and 11 (11.34%) respectively. The isolates were subjected to various morphological, physiological and biochemical characterization. The characterization exhibited by the isolates were compared with those of standard strains for their identification (Sneath et al; 1986; Sharpe; 1997). The presence of moulds at the initial stage of fermentation of maize for Ogi production and the subsequent elimination had been reported previously (Jespersen et al., 1994; Omemu et al; 2007a). mucoracea fungi have roles in initial phase of fermentation mostly in sacharification of the substrates (Thapa and Tamang, 2004). The researchers also confirmed the major involvement of Lactobacillus bacteria in Ogi fermentation. They were obligatory or facultative heterofermentative rods and cocci which were present throughout the process. From the beginning to the end of the fermentation, their number increased 2-4 log units. Their growth was followed by the acidification of the product.

An interesting finding in this study is the fact that, in this work the L. plantarum and Aspergillus niger had the highest percentage of 10.31% and 13.40% respectively, which is in line with other studies on other types of fermented products reported that lactobacillus bacteria were the predominant micro-organisms involve in the fermentation of products like Kenkey, Gari, Agbelima, with L. plantarum being identified most often (Amoa-Awua et al;1996; Kostinek et al; 2005).

The pH of fementing maize grains dropped from 6.4 to 5.0 by the end of the 15 days. Correspondingly, the titratable acidity increased from 0.13± 0.01 to 0.39± 0.01 after 15 days. The cause of the increase in acidity and consequent drop in pH during fermentation of cereal was likely due to utilization of free sugars by yeast and lactobacillus bacteria (Efiuvwevwere and Akona, 1995; Zvauya et al; 1997). The total sugar contents decrease significantly ($P< 0.05$) throughout the fermentation. This due to a maximum break down of starch substrates to reducing sugars by amylolytic ezymes produced by some of the fermenting organisms (Nout and Aidoo, 2002).

The initial count of bacteria (immediately after stepping) was highest in white maize (WM) (Table 4), with $6x10^5$ CFU ml^{-1} while it was $4x10^5$ and $2x10^5$ CFU Ml^{-1} for yellow maize (YM) and Brown maize (BM), respectively. After 24^{th} of steeping or primary fermentation, WM still had the highest bacterial load of $8.0x10^5$ CFU ml^{-1}. The bacterial load after 48h in both YM and WM was $14x10^5$ and $15x10^5$ CFU ML^{-1} respectively. Generally, a sharp increase in bacterial load was obtained after 24h of steeping, this slowed down after 48hr in both YM and WM was $14x10^5$ and $15x10^5$ CFU Ml^{-1} respectively. Generally, a sharp increase in bacterial load was obtained after 24h of steeping, this slowed down after 48hr and a sight difference in bacteria load was observed after 72hr of steeping or primary fermentation.

Table 5 shows changes in fungal counts during the 72h of steeping or primary fermentation of the three maize varieties. For BM was, $1.0x10^3$, $2.0x10^3$, $3.0x10^3$ and $3.0x10^3$ CFU ML^{-1}, for YM, it was $1.0x10^3$, $2.5x10^3$, $3.5x10^3$ and $4.5x10^3$ CFU Ml^{-1} at zero hour and after 24, 48, 72h respectively.

The yeast count also increased during the fermentation of maize for Ogi-production. The coexistence and symbiotic association between Lactic acid bacteria and yeast in Africa traditional fermentated products have been reported by several authors (Jespersen et al., 1994; Omemu et al., 2007a). Besides their role in the build up of typical flavour of fermented product. Some yeast has been reported to show enymatic ability may contribute to

breaking down maize starch and also allow better access to nutritionally essential minerals (Amoa-Awua et al., 1996; Omemu et al; 2007b).

CONCLUSION

Water samples were major sources of microbial contamination of products at all the stages of production. Despite the unhygienic wet-milling and wet-sieving processes involved in the traditional preparation of Ogi, the low pH of the fermented products would make them safe for consumption. Appropriate safety measures and good manufacturing practices will ensure good quality of product.

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Alimosho is the largest Local Government Area located in the Ikeja Division of Lagos State in Nigeria. It has about 1,288,714 inhabitants residing in its environs according to a 2006 census. This however was disputed by the Lagos State Government who claimed that the population figure of the largest local government area is more than 2 million. Due to Alimosho LGA enormous size as seen in the Lagos state map, it has now been subdivided into Local Community Development Areas (LCDA) after the administration of Bola Ilori (last local government chairman of the old Alimosho Local Government). The Alimosho is a Local Government Area in the Ikeja Division, Lagos State, Nigeria. Alimosho is regarded as the largest Local Government Area in Lagos state. It has now been subdivided between several Local Community Development Areas (LCDA). The six sub-divisions created out of the old Alimosho are Agbado/Oke-odo LCDA,Ayobo/Ipaja LCDA, Alimosho LG, Egbe/Idimu LCDA, Ikotun/Igando LCDA and Mosan Okunola LCDA. The LGA contains the urban area of Akowonjo. Alimosho was established in 1945 and it was under the then western region. Majority who dominate Alimosho were the Egbados. The area is very rich