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Microorganisms Associated with Ogi Traditionally Produced from Three Varieties of Maize

A.O. Ijabadeniyi
Department of Microbiology, Federal University of Technology,
P.M.B 704, Akure, Nigeria

Abstract: Aim of this study is to determine the microbial load, isolate and identify microorganisms associated with indiginous fermentation of the different maize varieties. Traditional method of preparation was used to produce ogi using three maize varieties; white local (WM), yellow (YM) and quality protein maize (QPM) varieties. The maize grains were steeped for 72 h, wet milled, sieved and fermented for 48 h separately at 30±2°C by the maize natural microflora. The number and type of microorganisms per mL of the fermenting substrates were estimated daily for the period of the fermentation. The isolated microorganisms were characterized using conventional methods. Molds isolated from the fermenting maize varieties were *Aspergillus niger*, *Penicillium* sp., *Mucor mucedo*, *Rhizopus stolonifer* and a yeast, *Saccharomyces cerevisiae*. The bacteria that were isolated were *Corynebacterium* sp., *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Clostridium bifermentans* and *Staphylococcus aureus*. During the secondary fermentation, the microorganisms had been reduced to *Lactobacillus plantarum*, *Lactobacillus fermentum* and yeast, *Saccharomyces cerevisiae*. Total viable bacteria counts during the fermentation of QPM ranged from 2.0×10^5 to 3.8×10^6 cfu mL^{-1}. For YM it ranged from 4.0×10^5 to 4.0×10^6 cfu mL^{-1} and WM; 5.0×10^5 to 4.0×10^6 cfu mL^{-1}. Fungal counts during the fermentation of QPM ranged from 1.0×10^3 to 3.6×10^4 cfu mL^{-1}. For YM it ranged from 1.0×10^3 to 3.6×10^4 cfu mL^{-1} and WM; 2.0×10^3 to 4.0×10^4 cfu mL^{-1}. *Lactobacillus* sp. and *Saccharomyces cerevisiae* were responsible for the quality characteristics of ogi. QPM had a reduced number and type of microorganisms compared to other two varieties.

Key words: Ogi, traditional fermentation, maize, microorganisms, bacteria, moulds

INTRODUCTION

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, pickled vegetables, sausages, cheeses, yogurts, vegetable protein amino acid/peptide sauces and pastes with meat like flavours, leavened and sour-dough breads (Steinkraus,1997).

Nigeria is endowed with a wide range 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 produce (Latunde-Dada, 2000). One common example of indigenous fermented foods in Nigeria is ogi.

Ogi porridge has a smooth texture similar to a hot blancmange and a sour taste reminiscent of yoghurt. Its colour depends on the colour of the cereal used; cream or milk white for maize, reddish brown for sorghum and dirty grey for millet (Moss *et al*., 1984; Onyekwere *et al*., 1989). Ogi is used

as a generic name, but in most states of Nigeria it refers to maize ogi. Sorghum ogi and millet ogi are known as ogi baba and ogi gero respectively. However, in some parts of the northern states of Nigeria, ogi which is referred to as furah is either made from sorghum or millet. In western and eastern parts of Nigeria however ogi is majorly prepared from maize (Onyekwere *et al*., 1989).

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).

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 foods (Onyekwere *et al*., 1989).

The objective of this study is to determine the microbial load, isolate and identify microorganisms associated with indigenous fermentation of the different maize varieties; white local farmer s variety, yellow farmer's variety and an improved variety, quality protein maize.

MATERIALS AND METHODS

This study was carried out at the Department of Microbiology, Federal University of Technology from March 2003 to September 2003. White local and yellow farmers varieties of maize grains were purchased at Oba market, Akure, Nigeria while Quality Protein Maize (QPM, EV. 8363- SRBC3) grains were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Maize grains were steeped for 72 h, wet milled and sieved and fermented for 48 h separately at 30±2°C by the maize natural microflora. The number and type of microorganisms (bacteria and fungi) per mL of the fermenting substrate was estimated daily for 72 h by pour plate method using the serial dilution technique. Nutrient agar and Potato dextrose agra were used for the isolation of bacteria and moulds, 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.

RESULTS AND DISCUSSION

Table 1 shows the biochemical characteristics of bacteria isolated from the fermenting substrates. Six major types of bacteria were identified during the primary fermentation (steeping). They were *Clostridium bifermentans*, *Corynebacterium* sp., *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Leuconostoc mesenteriodes* and *Staphylococcus aureus*. *Lactobacillus plantarum* and *Lactobacillus fermentum* were the only bacteria identified during the secondary fermentation.

Five different fungal species were isolated during primary fermentation (steeping). The fungi were *Penicillium* sp., *Mucor mucedo*, *Rhizopus stolonifer*, *Aspergillus niger* and *Saccharomyces cerevisiae*. *Saccharomyces* cerevisiae was the only fungus isolated during the secondary fermentation.

Table 2, 3 and 4 show the frequency of occurrence of microbial isolates during the primary fermentation (steeping) of the maize varieties. At zero hour, *Corynebacterium* sp., *Lactobacillus plantarum*, *Aspergillus niger* and *Saccharomyces cerevisiae* were isolated in all the three varieties. *Clostridium bifermentans*, *Staphylococcus aureus*, *Mucor mucedo*, *Penecillium* sp. and *Rhizopus stolonifer* were isolated from YM and WM. *Leuconostoc mesenteroides* was isolated from QPM and YM. After 24 h of steeping or primary fermentation, *Corynebacterium* sp., *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Saccharomyces cerevisiae* were isolated from the three maize varieties.

Table 1: Morphological and biochemical characteristics of bacteria isolated from fermenting substrates

Probablesolaited identity	Cit	Ur	Ni red	Ind	Xyl	Sor	Fru	Arab	Lac	Mal	Su	Man	Glu	O/F test	G (a)	GIA	M	Ca	Spores	Acid fast	Shape of cells	GS	IS
Clostridium bifermentans	ND	-	+	+	-	+	+	+	-	+	-	-	+	NT	+ AG	-	+	+	+ cen.oval	-	Rod	+	1
Corynebacterium sp.	ND	-	+	-	-	+	-	+	+	+	+	+	-	NT	-	+	-	+	-	-	Rod	+	2
Lactobacillus fermentum	ND	ND	-	-	-	-	-	+	+	+	+	-	+	F	+ AG	+	-	-	-	-	Rod	+	3
Lactobacillus plantarum	ND	+	+	-	-	+	+	+	+	+	+	+	+	F	+ AG	+	-	-	-	-	Long rod	+	4
Leuconostoc mesenteroides	ND	-	-	-	+	+	+	+	+	+	+	+	+	F	+ AG	+	-	-	-	-	Rod	+	5
Staphylococcus aureus	-	+	+	-	-	+	+	+	+	+	+	+	+	F	+ A	+	-	+	-	-	cocci	+	6

Cit = Citrate; Ur = Urease; Ni. Red = Nitrate Reduction; Ind = Indole; Xyl = Xylose; Sor = Sorbito; Fru = Fructose; Arab = Arabinose; Lac = Lactose; Mal = Maltose; Su = Sucrose; Man = Mannitol; Glu = Glucose; G (a) = Glucose (Acis); GIA = Growth in air; Ca = Catalase; G.S = Gram staining; AG = Acid and gas production; F = Fermentation; ND = Not Determined; NT = Not Testable; - = Negative; + = Positive; IS = Isolate Number

Table 2: Occurrence of microorganisms during steeping of QPM

	Time (h)			
Isolates	0	24	48	72
Clostridium bifermentans	-	-	-	-
Corynebacterium sp.	+	+	-	-
Lactobacillus fermentum	+	+	+	+
Lactobacillus plantarum	+	+	+	+
Leuconostoc mesenteroides	+	+	-	-
Staphylococcus aureus	-	-	-	-
Aspergillus niger	+	-	-	-
Mucor mucedo	-	-	-	-
Penecillium sp.	-	-	-	-
Rhizopus stolonifer	-	-	-	-
Saccharomyces cerevisiae	+	+	+	+

+: Positive; -: Negative

Table 3: Occurrence of microorganisms during steeping of YM

	Time (h)			
Isolates	0	24	48	72
Clostridium bifermentans	+	-	-	-
Corynebacterium sp.	+	+	-	-
Lactobacillus fermentum	+	+	+	+
Lactobacillus plantarum	+	+	+	+
Leuconostoc mesenteroides	+	+	-	-
Staphylococcus aureus	+	-	-	-
Aspergillus niger	+	+	-	-
Mucor mucedo	+	+	-	-
Penicillium sp.	+	-	-	-
Rhizopus stolonifer	+	-	-	-
Saccharomyces cerevisiae	+	+	+	+

+: Positive; -: Negative

Table 4: Occurrence of microorganisms during steeping of WM

	Time (h)			
Isolates	0	24	48	72
Clostridium bifermentans	+	+	-	-
Corynebacterium sp.	+	+	-	-
Lactobacillus fermentum	+	+	+	+
Lactobacillus plantarum	+	+	+	+
Leuconostoc mesenteroides	-	-	-	-
Staphylococcus aureus	+	-	-	-
Aspergillus niger	+	+	-	-
Mucor mucedo	+	+	-	-
Penecillium sp.	+	-	-	-
Rhizopus stolonifer	+	-	-	-
Saccharomyces cerevisiae	+	+	+	+

+: Positive; -: Negative

Clostridium bifermentans, *Aspergillus niger* and *Mucor mucedo* were isolated from the three varieties. *Clostridium bifermentans*, *Aspergillus niger* and *Mucor mucedo* were isolated from YM and WM. *Leuconostoc mesenteroides* was isolated from QPM and YM. The same results were after 48 and 72 h of primary fermentation or steeping in all the three maize varieties. *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Saccharomyces cerevisiae* were the only microorganisms isolated from QPM, YM and WM.

Table 5 shows the frequency of occurrence of microbial isolates during the secondary fermentation of the three maize varieties. During the zero, 24 and 48 h of fermentation, only *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Saccharomyces cerevisiae* were isolated.

Table 5: Occurrence of microorganisms during the secondary fermentation of QPM, YM and WM

	Time (h)		
Isolates	0	24	48
Clostridium bifermentans	-	-	-
Corynebacterium sp.	-	-	-
Lactobacillus fermentum	+	+	+
Lactobacillus plantarum	+	+	+
Leuconostoc mesenteroides	-	-	-
Staphylococcus aureus	-	-	-
Aspergillus niger	-	-	-
Mucor mucedo	-	-	-
Penecillium sp.	-	-	-
Rhizopus stolonifer	-	-	-
Saccharomyces cerevisiae	+	+	+

+: Positive; -: Negative

Table 6: Total bacteria counts during the steeping of maize varieties (10^5cfu mL^{-1})

	Maize varieties		
Time (h)	White maize	Yellow maize	Quality protien maize
0	5×10^5	4×10^5	2×10^5
24	9.0×10^5	5×10^5	3×10^5
48	16×10^5	16×10^5	12×10^5
72	18×10^5	17×10^5	16×10^5

The initial count of bacteria (immediately after steeping) was highest in white maize (WM) (Table 6), with 5×10^5 cfu mL^{-1} while it was 4×10^5 and 2×10^5 cfu mL^{-1} for yellow maize (YM) and quality protein maize (QPM), respectively. After 24 h of steeping or primary fermentation, WM still had the highest bacterial load of 9.0×10^5 cfu mL^{-1}. The bacterial load after 48 h in both YM and WM was 16×10^5 cfu mL^{-1}. Generally, a sharp increase in bacterial load was obtained after 24 h of steeping, this slowed down after 48 h and a slight difference in bacteria load was observed after 72 h of steeping or primary fermentation.

At zero hour (beginning of secondary fermentation) WM had the highest bacterial count of 1.8×10^6 cfu mL^{-1} while QPM and YM were 1.6×10^6 and 1.7×10^6 cfu mL^{-1}, respectively (Table 7). There was really no big difference between microbial load of the three maize varieties at the beginning of secondary fermentation. After 24 h of secondary fermentation, WM still had the highest bacterial load of 3.6×10^6 cfu mL^{-1}. The bacterial load after 48 h of secondary fermentation in both YM and WM was 4.0×10^6 cfu mL^{-1} while that of QPM was 3.8×10^6 cfu mL^{-1}.

Table 8 shows changes in fungal counts during the 72 h of steeping or primary fermentation of the three maize varieties. For QPM it was, 1.0×10^3, 2.0×10^3, 3.0×10^3 and 3.0×10^3 cfu mL^{-1}, for YM, it was 1.0×10^3, 3.0×10^3, 3.5×10^3 and 4.0×10^3 cfu mL^{-1} and while for WM, it was 2.0×10^3, 3.0×10^3, 3.5×10^3 and 4.0×10^3 cfu mL^{-1} at zero hour and after 24, 48 and 72 h, respectively.

The fungal count at zero hour in both YM and WM was 4.0×10^3 cfu mL^{-1} (Table 9) while QPM had 3.0×10^3 cfu mL^{-1}. The fungal count after 48 h in both QPM and YM was 3.6×10^4 cfu mL^{-1} while WM had 4×10^4 cfu mL^{-1}.

Microorganisms responsible for the fermentation of the maize varieties have been mentioned. None of the molds was isolated after 24 h of primary fermentation or steeping possibly because of the change in the pH of the fermenting substrates. *Staphylococcus aureus* was isolated from YM and WM at zero hour of steeping or primary fermentation. This could be as a result of the contamination from handling by the market women. *Staphylococcus aureus* was not isolated from QPM. After 24 h and beyond of steeping or primary fermentation, *Staphylococcus aureus* was no longer found in YM and WM possibly because of the change in the pH of the fermenting substrates.

Table 7: Total bacteria counts during the secondary fermentation of maize varieties (10^6cfu mL^{-1})

	Maize varieties		
Time (h)	White maize	Yellow maize	Quality protien maize
0	1.8×10^6	1.7×10^6	1.6×10^6
24	3.6×10^6	2.8×10^6	2.2×10^6
48	4.0×0^6	4.0×10^6	3.8×10^6

Table 8: Total fungal counts during the steeping of maize varieties (10^3cfu mL^{-1})

	Maize varieties		
Time (h)	White maize	Yellow maize	Quality protien maize
0	2.0×10^3	1.0×10^3	1.0×10^3
24	3.0×10^3	3.0×10^3	2.0×10^3
48	3.5×10^3	3.5×10^3	3.0×10^3
72	$4.0\text{x }10^3$	4.0×10^3	3.0×10^3

Table 9: Total fungal counts during the secondary fermentation of maize varieties (10^4cfu mL^{-1})

	Maize varieties		
Time (h)	White maize	Yellow maize	Quality protien maize
0	0.4×10^4	0.4×10^4	0.3×10^4
24	2.8×10^4	2.5×10^4	2.0×10^4
48	4.0×10^4	3.6×10^4	3.6×10^4

Lactobacillus sp. and *Saccharomyces cerevisiae* predominated among the microorganisms after 48 h of steeping or primary fermentation and during the secondary fermentation. This connotes that *Lactobacillus* sp. and *Saccharomyces cerevisiae* might be responsible majorly for the sour taste reminiscent of yoghurt.

The presence of *Lactobacillus* sp. in ogi according to several workers makes ogi a good source of bacteriocins and they have also identified *Lactobacillus* isolates as an important microflora of African fermented foods (Odunfa *et al*., 1996; Olasupo *et al*., 1995; Olasupo *et al*., 1997). Also Olukoya *et al*. (1994) have produced a special ogi that were able to stop the growth diarrhea causing bacteria.

It should be noted that some microorganisms that have been implicated in previous studies were not isolated. According to Onyekwere *et al*. (1989) not all microorganisms of ogi fermentation are always found in all fermentations.

QPM had the least bacterial count. The proper treatment it was subjected to at IITA, Ibadan, Nigeria could have been responsible for this observation. The bacterial counts in all the samples increased with the period of steeping or primary fermentation and secondary fermentation for all the three varieties; although WM had the highest count throughout the period of traditional fermentation or processing of ogi. This may be because the bacterial count in WM was highest on the first day of steeping or primary fermentation.

In conclusion, the microorganisms associated with the traditional fermentation of maize ogi have been observed from this work although some of them may be contaminants, *Lactobacillus* sp. and *Saccharomyces cerevisiae* played a major role right from the beginning of steeping to the end of fermentation.

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