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Microbiological dynamics involved in cereal-porridge production using maize and sorghum

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Abstract

Cereal-porridge('ogi') was produced by spontaneous fermentation using maize and sorghum substrates. The microbiological dynamics involved were monitored over a period of 48h fermentation. Bacteria, yeasts and moulds were isolated. Based on the morphological, cultural and biochemical test results, the aerobic bacterial isolates were identified as *Proteus vulgaris, Proteus mirabilis, Klebsiella* sp*, Staphylococcus aureus, Lactobacillus* sp*, Pseudomonas* sp*, Citrobacter* sp*, Bacillus* sp*, Proteus* sp*, Shigella* sp*, and Escherichia coli*. The Lactic acid bacteria were *Lactococcus* sp*, Enterococcus* sp*, Lactobacillus fermentum*, *Lactobacillus* sp. The yeast isolates were 2 strains of *Saccharomyces cerevisiae*, one other *Saccharomyces* sp and a *Candida* sp. The moulds were *Aspergillus niger, Aspergillus flavus, Rhizopus* sp and *Penicillium* sp. The lactic acid bacteria (LAB) isolated were 2 strains of Lactococcus *lactis*, 2 *Enterobacter* spp, 5 strains of *Lactobacillus fermentum* and 1 other *Lactobacillus* sp. The initial total viable aerobic bacterial count at 0h in maize, sorghum and maize-sorghum blend were 4.6×10^4, 7.3×10^4 and 2.4×10^5cfu/ml respectively. The growths rose to peaks of 6.5×10^7 and 3.9×10^7cfu/ml at 24h in maize and maize-sorghum blend, respectively. A Peak of 4.7×10^7cfu/ml was attained at 36h in sorghum. Coliform bacteria and moulds growths in the three samples attained peaks of growth at 12h and reduced till there was no growth by 48h. Lactic acid bacteria and yeasts increased in numbers till the end of fermentation. The initial pH value at 0h was lowest in maize-sorghum blend sample (5.43) and highest in maize (5.75). Final values at 48h were 3.76, 3.78 and 3.75 in maize, sorghum and maize-sorghum blend samples respectively. There were no significant differences between the microbial growth patterns, changes in pH, total titratable acidity (TTA) and amylase enzymatic activities in maize, sorghum and maize-sorghum blend samples during fermentation.

Keywords: Maize; Sorghum; Fermentation; Lactic acid bacteria, Yeast

1. Introduction

Microorganisms play dual roles (beneficial and harmful) in food products, especially during storage and processing. In the fermentation industry, the food products produced is as a result of the type, age and the composition of the microorganisms employed. However, microbial populations and diversity play a role in the products of fermentation.

'Ogi' is a traditional fermented cereal-porridge consumed on a large scale among the low- income group in Nigeria, especially the nursing mothers, convalescent and weaning infants. It is usually produced by soaking of maize, sorghum or millet, followed by blending, sieving and the fermentation of the starchy cake. The traditional fermentation process occurs as a result of microbial inoculation from the environment [1, 2].

Microorganisms found in food products occur through several means namely; exposure, handling, and use of contaminated materials for preparation. The following genera of microorganisms are known to participate in the fermentation of steeped maize for '*ogi*' production: *Staphylococcus*, *Escherichia, Pseudomonas, Enterococcus, Klebsiella,*

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Bacillus, Lactobacillus, Leuconostoc, Clostridium, Corynebacterium, Streptococcus, Micrococcus and *Citrobacter*(bacteria), *Aspergillus, Saccharomyces, Penicillium, Candida, Rhizopus, Fusarium, Mucor, Geotrichum* and *Rhodotorula*(fungi). *Saccharomyces cerevisiae* and several species of the genus *Lactobacillus* are found in the fermentation of maize for '*ogi*' production and to a lesser extent, the species of the genera, *Enterococcus, Klebsiella, Micrococcus, Clostridium* and *Citrobacter*[3,4].

The presence of contaminants(unspecified microorganisms) complicate the control of the fermentation process [5, 6]. Moreover, it is important to monitor the presence of pathogenic bacteria during 'Ogi' production by fermentation in order to prevent problems associated with them. Pathogenic bacteria such as *Escherichia coli*, *Salmonella spp* and *Bacillus cereus* have often been associated with fermentation processes in foods [7]

This study aimed to ascertain the microbial diversity associated with maize and sorghum fermentation for the production of 'ogi 'and monitor changes in the microbial populations.

2. Materials and methods

2.1. Processing of Samples

Maize and sorghum grains were separately winnowed, sorted out from debris, stones and chaff and then dried in the sun for three days before storage at room temperature in plastic containers. A modified wet-milling laboratory method developed and reported by Ojokoh [8] was adopted for the processing of the grains into paste. Three different types of samples were prepared as follows:

- Maize only, labeled as MZS
- Sorghum only, labeled as SWM
- Maize and sorghum blend (1:1, $^{w}/_{w}$) labeled as MES.

2.2. Fermentations of Samples

After wet-milling and sieving, the different samples were labeled and allowed to sediment and ferment in tightly-covered containers, for 48h. The analyses were carried out at 12h intervals, starting at 0h[9]

2.3. Microbiological Analysis

2.3.1. Determination of Microbial Counts in the Samples

Ten-fold serial dilutions of the sedimented "ogi" paste were made using 0.1% (w/v) peptone water in test tubes and 0.1ml of appropriate dilutions were inoculated in duplicates by spread plate technique on various culture media as follows:

- Plate count agar (PCA: OXOID, England) for growth of total aerobic viable bacteria. The plates were incubated at 37°C for 24 h.
- MacConkey agar (MAC: OXOID, England) for growth of coliforms. The plates were incubated at 37°C for 24 h.
- Potato Dextrose agar (PDA: OXOID, England) for growth of fungi (yeast and moulds). The medium was acidified by the addition of lactic acid (0.1%, v/v). The plates were incubated at 30˚C for 72 h.
- de Man, Rogosa and sharpe's agar (MRS: OXOID, England), for the growth of lactic acid bacteria. The plates were incubated anaerobically in Gallenkamp anaerobic jars for 48h at 30°C.

Enumeration was done at the end of incubation. The total viable microbial numbers per millilitre of sample(cfu/ml) were determined by multiplying the mean values of the duplicate counts by the dilution factor.

2.3.2. Isolation and Characterization of Isolates

Colonies of different cultural characteristics from the culture plates after plate counts were picked and sub-cultured twice on appropriate fresh sterile media for purification and isolation by streaking to obtain pure cultures. The characterization of bacteria was carried out using standard microbiological procedures of Cheesbrough [10] and Erkmen [11]. Cultural macroscopic observations were made. These included colony size, shape, consistency, colour and type of mycelia (for moulds).

The criteria for characterization and identification included the cultural characteristics, Gram reaction, lactophenol cotton-blue stain(for moulds), ethanol tolerance test (for yeasts) spore-staining, motility and biochemical tests (coagulase, oxidase, catalase, citrate-utilization, triple sugar, iron, fermentation of carbohydrates, indole, methyl red, Voges-proskauer, urease and nitrate reduction) for bacteria. The lactic acid bacteria (LAB) were further characterized based on their growth at different levels of sodium chloride (NaCl) concentration, pH and temperature.

2.4. Chemical Analyses

2.4.1. Determination of Lactic Acid (%)

The titration method of Aneja [12] was adopted. The lactic acid bacteria were first sub-cultured on MRS Agar and incubated at 37°C under anaerobic conditions for 48h, before the titration.

2.4.2. Determination of pH

Each sample was homogenized by stirring with a sterile glass rod and the pH determined directly using a pH meter (WEALTEC, USA).

2.4.3. Determination of Temperature

Each sample was stirred to homogenize and the temperature measured using a thermometer (MERCK, Germany).

2.4.4. Total Titratable acidity (TTA)

Changes in percent TTA during fermentation were determined as described by AOAC [13].

2.5. Amylase Assay

The method of Junge *et al.* [14] was adopted for the assay.

2.6. Data Analysis

The data obtained from the study were subjected to statistical analyses. The SPSS 23 package was used to separate mean values and Analysis of Variance (ANOVA) to determine the differences in the changes in microbial populations.

3. Results

3.1. Microbial Flora of the cereal-porridge('Ogi') Paste Samples

The different types of microorganisms that were isolated from the paste samples are shown in Tables 1-4. Based on the morphological, cultural and biochemical test results, the samples contained bacteria, yeasts and moulds. The aerobic bacterial isolates included *Proteus vulgaris, Proteus mirabilis, Klebsiella* sp, *Staphylococcus aureus, Lactobacillus* sp, *Pseudomonas* sp, *Citrobacter* sp, *Bacillus* sp, *Proteus* sp, *Shigella* sp, and 4 strains of *Escherichia coli*. Lactic acid bacteria were 2 strains of Lactococcus *lactis,* 2 strains of *Enterococcus sp, 5 Lactobacillus fermentum strains* and 1 other *Lactobacillus sp*. The yeast isolates were 2 strains of *Saccharomyces cerevisiae* and one other *Saccharomyces sp* and a *Candida sp*. The moulds included 2 strains of *Aspergillus niger, Aspergillus flavus, Rhizopus* sp and *Penicillium* sp.

3.2. Changes in Viable Aerobic Bacterial Counts during Fermentation

The results showed that the freshly prepared paste samples contained a high bioload. The total viable aerobic bacterial counts in the maize, sorghum and maize-sorghum blend paste samples at 0h were $4.6x10^4$, $7.3x10^4$, $2.4x10^5$ cfu/ml respectively. The population increased to a peak at $6.5x10^7$cfu/ml (maize), $2.6x10^7$ (sorghum) and $3.9x10^7$ (maize-sorghum) at 12h. Thereafter, a decline of growth occurred in all the samples till the end of fermentation at 48h, however, the decline was slower in the sorghum sample. Generally, the growths in maize-sorghum blend were higher than in other samples (Figure 1).

Table 1 Aerobic bacterial isolates

Isolate Code	Cultural Characteristics	Gram reaction	Cell Morphology	Citrate	TSIA				Lactose	Sucrose	glucose	Mannitol	Indole	MR	VP	Motility	Oxidase	Catalase	Urease	Coagulase	Probable organism
					Butt	Slant	H_2S	Gas													
PCA 1	2mm, milky, circular, spreading, weeping, circular, mucoid	-ve	Rods	-	A	A	+	+	-	A	A	-	+	+	-	+	-	+	+	-	*Proteus vulgaris*
2	5mm, weeping, circular mucoid raised milky,	- ve	Rods	+	A	A	-	+	-	-	A	+	-	-	+	-	-	+	+	-	*Klebsiella* sp
3	2mm, circular, milky, mucoid	-ve	Rods	-	A	A	-	+	A/G	-	A/G	+	+	+	-	+	-	+	-	-	*Escherichia coli*
4	1mm, circular, milky – golden	+ve	cocci	+	B	A	-	-	-	A	A	-	-	-	+	-	-	+	-	+	*Staphylococcus aureus*
5	0.5mm (minute) circular, golden	+ve	cocci	+	A	A	-	-	A	A	A	-	-	-	+	-	-	+	-	-	*Lactobacillus* sp
6	3mm, raisedcentre Milky-yellow, rough edges	-ve	Rods	-	B	A	+	+	A	-	-	-	+	-	+	-	-	-	+	-	*Pseudomonas* sp
7	3mm, yellow, circular, raised	-ve	Rods	+	A	A	+	+	A	A	A	-	-	-	-	+	-	-	+	-	*Proteus mirabilis*
8	5mm, white, mucoid, circular	-ve	Rods	-	B	-	+	+	A/G	A/G	A/G	+	-	+	-	+	-	+	-	-	*Citrobacter* sp
9	2mm, dull peach, surface wrinkle, spreading	+ve	Large rods	-	-	-	-	-								+		+			*Bacillus* sp
MAC 1	1mm, deep pink, circular, raised	-ve	Rods	+	B	A	-	+	A/G	A	A/G	+	+	+	-	+	-	+	-	-	*Escherichia coli*
2	1mm, light pink, circular, raised	-ve	Rods	+	A	A	-	-	A/G	A	A/G	+	+	+	-	+	-	+	-	-	*Escherichia coli*
3	1mm, Milky centre, pink edged, mucoid	-ve	Rods	+	A	A	-	-	A	A	A	+	+	+	-	+	-	+	-	-	*Escherichia coli*

4	3mm, light pink, circular, mucoid	-ve	Rods	+	A	A	-	+	A	A	+	+	+	-	+	-	+	+	-	-	*Escherichia coli*
5	1mm, transparent, milky-pink	-ve	Rods	-	A	A	+	+	A	A	A/G	-	+	-	-	+	-	+	+	-	*Proteus* sp
6	2mm, circular, raised, milky peach	-ve	Rods	+	B	A	-	-	A	A	A/G	-	-	-	-	-	-	-	-	-	*Shigella* sp

LEGEND: PCA: Isolates from plate count agar; MAC: Isolates from MacConkey agar

Table 2 Lactic acid bacterial isolates

Isolate Code	**Gram**	**Cell Morphology**	**oxidase**	**catalsase**	**Motility**	**D-Glucose**	**Ribose**	**Sucrose**	**Lactose**	**Maltose**	**Fructose**	**Raffinose**	**Arabinose**	**Rhaminose**	**Xylose**	**Mannitol**	**Galactose**	**Salicin**	**Sorbitol**	**Mannose**	**Starch**	**Trehalose**	**Indole**	**Nitrate red.**	**Nacl (%)**					**pH**				**Temp.**			
																									2	**3**	**4**	**6.5**	**10**	**4.4**	**7.2**	**.9.3**	**9.6**	**30°C**	**40°C**	**45°C**	**Probable organism**
LAB 1	+ve	Cocci	-	-	-	A/G	A/G	A/G	A/G	A	A	A/G	A	A	-	A	A/G	A/G	A	A	-	A/G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
LAB 2	+ve	Cocci	-	-	-	A	A	A	A	A	A	-	-	A	A	-	+	-	-	A	+	+	-	-	+	+	+	+	-	+	+	-	-	+	-	-	*Lactobacillusfermentum*
LAB 3	+ve	Cocci	-	-	-	A	A	A	-	A	A	-	A	-	-	-	A	A	-	A	-	A	-	+	+	+	+	+	-	+	+	+	+	+	+	+	Lactobacillus*lactis*
LAB 4	+ ve	Cocci	+	+	+	A	A	A	A	A	A	A	A	-	A	-	A	A	-	-	-	A	-	-	+	+	+	+	+	-	+	+	+	+	+	-	*Lactobacillusfermentum*
LAB 5	+ ve	Cocci	-	-	-	A	A	A	-	A	A	-	A	-	-	-	A	A	-	A	-	A	-	+	+	+	+	+	-	+	+	+	+	+	+	+	*Lactobacillus*fermentum
LAB 6	+ ve	Cocci	-	-	-	A	A	A	A	A	A	A	A	A	-	A	-	-	A	A	A	A	-	-	+	+	+	+	-	+	+	-	-	+	+	+	*Enterobacter*sp
LAB 7	+ ve	Cocci	-	+	-	A	A	A	A	A	A	-	-	-	-	A	A	A	A	A	-	A	-	-	+	+	+	+	-	+	+	-	-	+	+	-	*Lactobacillus fermentum*
LAB 8	+ ve	Cocci	-	-	+	A	A	A	A	A	A	A	A	A	A	A	A	A	-	-	A	-	A	A	+	+	+	+	+	+	+	+	+	+	+	+	*Enterococcus* sp
LAB 9	+ve	Cocci	-	-	+	A	A	A	-	A	A	A	A	-	A	A	A	A	A	A	+	A	-	-	+	+	+	+	-	+	+	+	-	+	+	-	*Lactobacillus fermentum*
LAB10	+ ve	Cocci	-	+	-	A	A	A	A	A	A	-	-	-	-	A	A	A	A	A	-	A	-	-	+	+	+	+	-	+	+	-	-	+	+	-	*Lactobacillus* sp

NB: Isolates LAB1-LAB5, were isolated from maize sample, while isolates LAB5-LAB10 were isolated from sorghum sample

Table 3 Yeast isolates

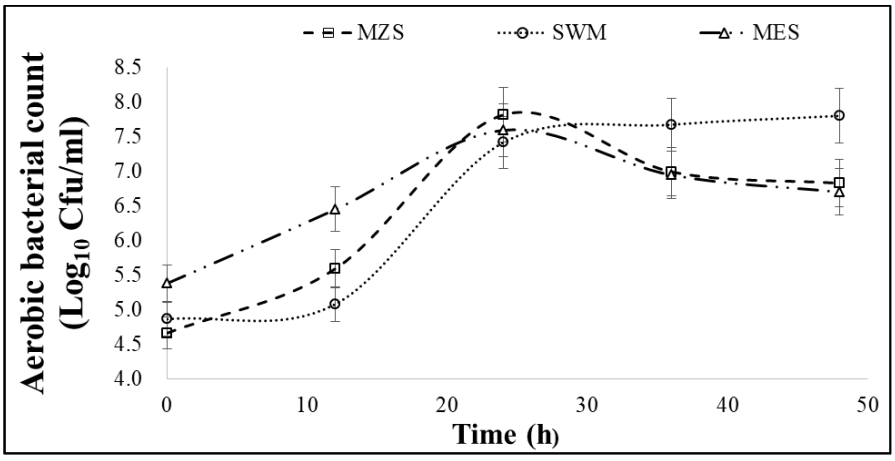
Isolate Code	Cultural Characteristics	Gram Stain	Shape	Sugar Fermentation						Ethanol tolerance	Probable organism
				Glu.	Malt.	Galact.	Sucr.	Lact.	Fruct.		
Y1	2mm, mucoid, milky, rough edges	+	Oval	+	+	-	+	-	+	+	*Saccharomyces Cerevisiae*
Y2	1mm, yellow round edge	+	Oval	+	+	-	-	-	+	+	*Saccharomyces* sp
Y3	3mm, dry surface, pointed centre, smooth edges	+	Oval	+	+	-	+	-	-	-	*Candida* sp
Y4	1mm pale milky round, smooth edges	+	Oval	+	+	-	+	-	+	+	*Saccharomyces cerevisiae*

ND: Each yeast isolate was isolated from both maize and sorghum samples

Table 4 Mould isolates

Isolate Code	Cultural Characteristics	Microscopy	Probable Organism
M1	5mm, whitish, green centre	Bi-seriated vesicle with smooth conidia. Non septate conidiophores arising from thick hyphae cells	*Aspergillus flavus*
M2	5mm, dark brown powdery surface, tan on the reverse	Spherical vesicle mop-like head of conidia	*Aspergillus niger*
M3	5mm, black powdery surface, cracked on the reverse	Spherical vesicles, mop-like head of conidia	*Aspergillus niger*
M4	3mm Yellowish, cotton wool-like colony with white reverse	Dark round sporangium containing oval brown spores, with long, unbranched sporangiophores, terminating in a collumella	*Rhizopus* sp
M5	1cm, white velvet-like colony with raised centre, white reverse	Branched conidiophores with brush-like conidia head	*Penicillium* sp

Note: Each type of mould was isolated from the three samples

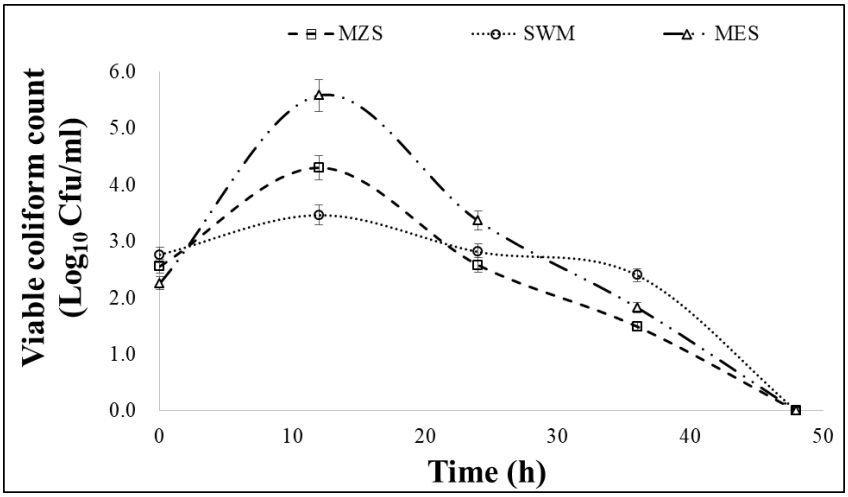


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 1 Changes in viable aerobic bacterial counts during fermentation

3.3. Changes in Viable Coliform Counts during Fermentation

The coliform counts were 3.4 x 10^2 in maize, 5.8 x 10^2 in sorghum and 1.8 x 10^3 in maize- sorghum blend respectively at 0h. These increased and had peaks of growth at 12h, with the highest peak in the maize- sorghum blend(MES). Afterwards there were rapid declines in population till the end at 48h. There were no growths of coliforms at 24h in any of the samples (Figure 2).

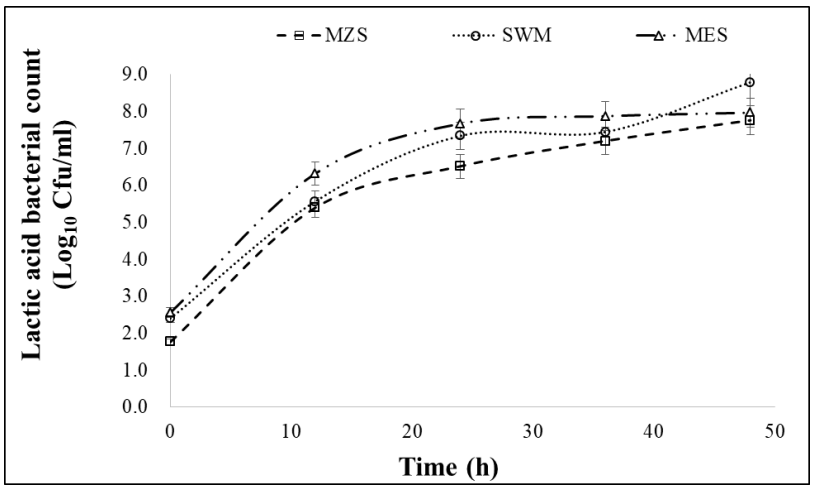


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 2 Changes in viable coliform counts during fermentation

3.4. Changes in Lactic Acid Bacterial (LAB) Counts during Fermentation

The LAB growth ranged from 5.8 x 10^1cfu/ml (maize), 2.5 x 10^2cfu/ml (sorghum) and 3.6 x 10^2cfu/ml(maize-sorghum blend) at 0h to 5.8 x 10^7cfu/ml (maize), 6.2 x 10^8cfu/ml (sorghum) and 9.3 x 10^7cfu/ml (maize-sorghum blend) at 48h. Increase in growth occurred throughout the period of fermentation without any decline. However, growths were slow after 24h. Growths in maize-sorghum blend (MES) were generally better than in maize and sorghum samples (Figure 3).

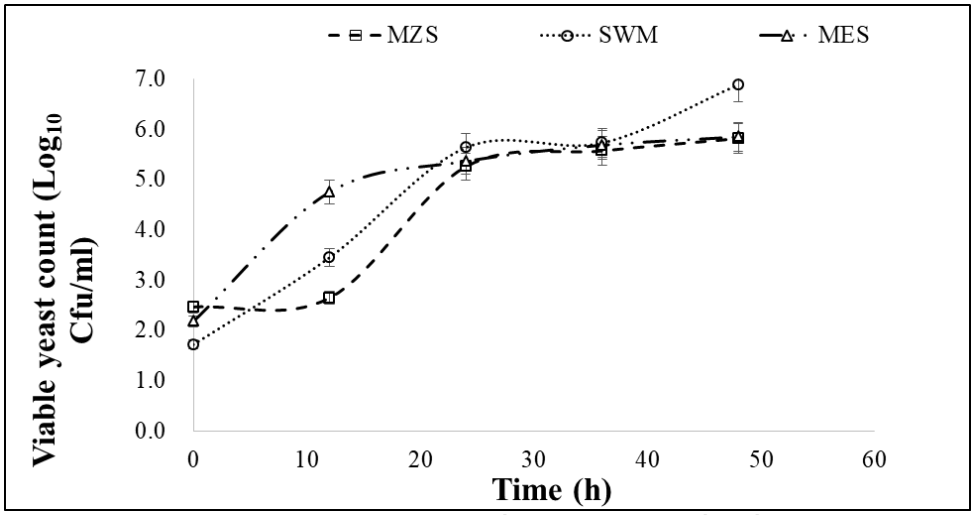


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 3 Changes in Lactic acid bacterial counts during fermentation

3.5. Changes in Viable Yeasts Counts during Fermentation

The growths of yeast in the three samples continued throughout the period fermentation. At 0h, there were 2.9 x 10^2cfu/ml, 5.2 x 10^1cfu/ml and 1.5 x 10^2cfu/ml in maize, sorghum and maize-sorghum samples respectively. By 48h, the numbers had increased up to 6.5 x 10^5cfu/ml, 7.6 x 10^6cfu/ml 7.0 x 10^5cfu/ml in maize, sorghum and maize-sorghum blend respectively (Figure 4). The growths in the maize-sorghum blend sample (MES) were generally better.

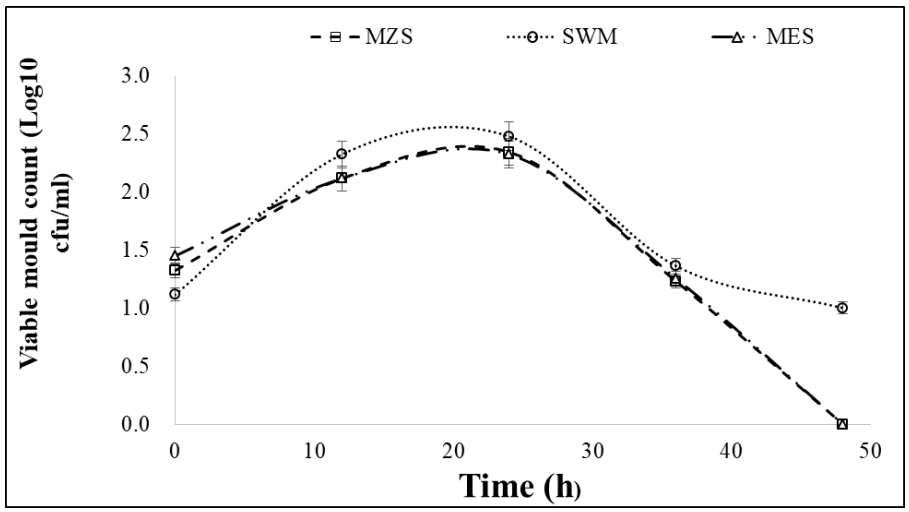


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 4 Changes in viable yeast counts during fermentation

3.6. Changes in Viable Moulds Counts during Fermentation

Mould populations in the three samples were low from 0h till the end of the fermentation, but were generally higher in the sorghum sample (SWM). At 0h, the counts were 2.1 x 10^1 in maize, 1.3 x 10^1 in sorghum and 2.8 x 10^1cells/ml in maize-sorghum growth. The peak of growths was at 24h, when they increased to 2.2 x 10^2, 3.0 x 10^2 and 2.1 x 10^2 cells/ml in maize, sorghum and maize-sorghum blend respectively. However, by 48h, there were no more growths in maize and maize-sorghum blend samples, while there were only a few cells(10cells/ml) in the sorghum samples (Figure 5).



LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 5 Changes in viable mould counts during fermentation

3.7. Percentage Lactic Acid Production by Lactic Acid Bacteria

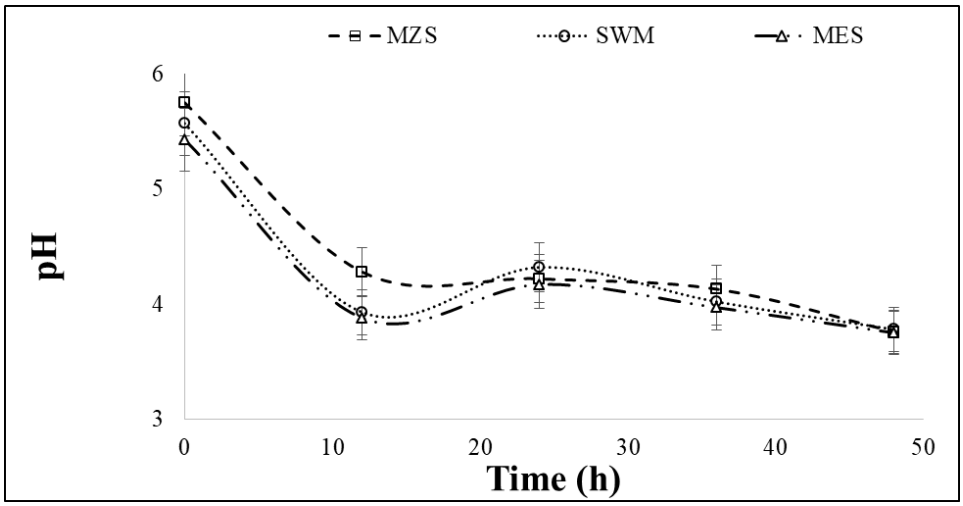
The percentage production of lactic acid by the different lactic acid bacterial isolates is as shown in Table 5. Two strains of *Lactobacillus fermentum* produced the highest amounts (1.54% and 1.24%). The least amount was produced by one of the strains of *Lactococcus* sp. Other percentage amounts in descending order were 1.22, 0.95, 0.60, 0.45, 0.28 and 0.27, produced by Lactococcus *lactis*, *Enterobacter* sp, *Lactobacillus fermentum*, an *Enterobacter* sp, *Lactobacillus fermentum* and a *Lactococcus lactis* respectively.

Table 5 Percentage lactic acid production by lactic acid bacteria isolates

Isolate code	Name of Lactic acid bacterium	Lactic acid produced (%)
B1	*Lactococcus lactis*	1.22 (± 0.00)
B2	*Lactobacillus fermentum*	0.58 (± 0.212)
B3	*Lactococcus lactis*	0.27 (± 0.141)
B4	*Lactobacillus fermentum*	0.60 (± 0.282)
B5	*Lactobacillus fermentum*	1.54 (± 0.141)
B6	*Enterobacter* sp.	0.95 (± 0.707)
B7	*Lactobacillus fermentum*	1.24 (± 0.354)
B8	*Enterobacter* sp.	0.45 (± 0.424)
B9	*Lactobacillus fermentum*	0.28 (± 0.141)

3.8. Changes in pH during Fermentation

The changes in pH in the three samples during the fermentation are shown in Figure 6. The initial pH values ranged from 5.43 in maize-sorghum blend to 5.75 in maize at 0h. The pH values in all the three samples decreased with time of fermentation till the least were obtained at 48h. However, after a decrease at 12h to pH 4.28, 3.93, 3.88 in maize, sorghum and maize-sorghum blend respectively, there was a slight increase at 24h. The final values at 48h were 3.76 in maize, 3.78 in sorghum and 3.75 in maize-sorghum blend.

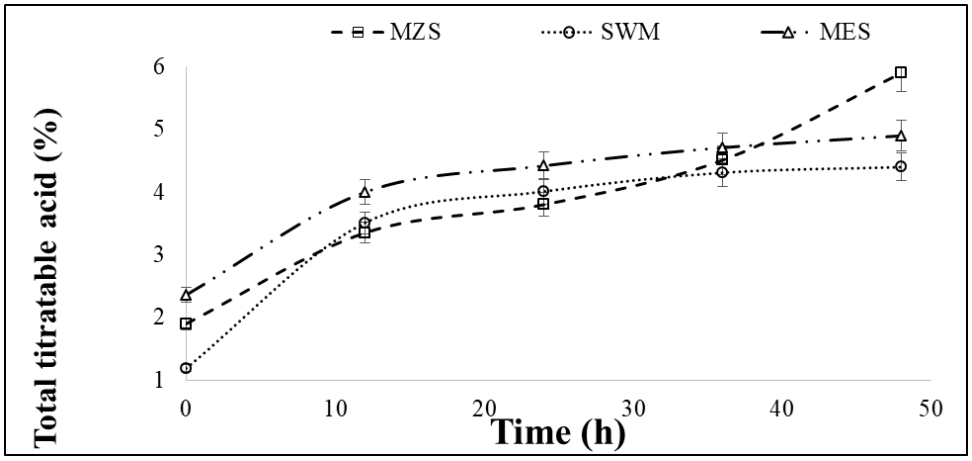


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 6 Changes in pH during fermentation

3.9. Changes in Total Titratable Acidity (TTA) during Fermentation

The changes in TTA during the fermentation are shown in Figure 7. The values increased with time in all the three samples till the end of fermentation. Increases were more in the maize sample than in the sorghum and maize-sorghum blend samples. The values at 0h in the three samples were 1.90%, 1.19% and 2.36% in maize, sorghum and maize-sorghum samples respectively. The highest value of 5.90% was obtained in the maize sample at 48h.

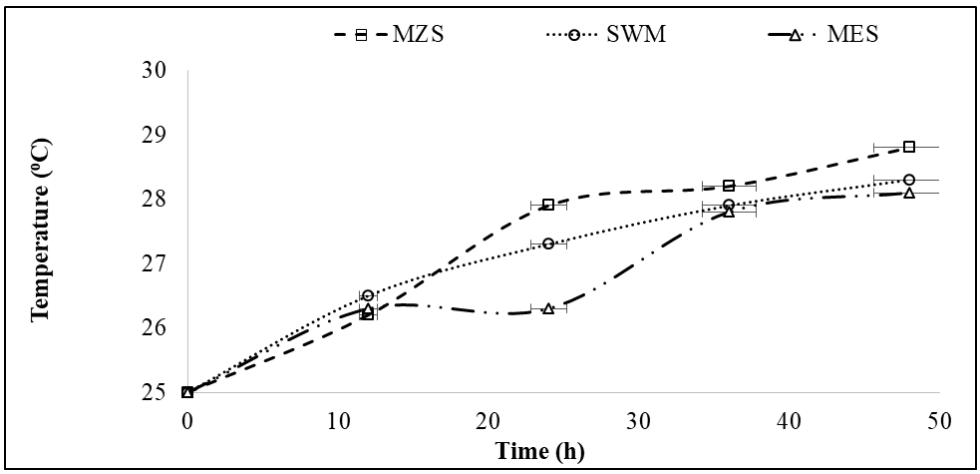


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 7 Changes in total titratable acidity during fermentation

3.10. Changes in Temperature during Fermentation

Temperature changes during fermentation are shown in Figure 8. The temperatures for the three samples were the same, 25°C at 0h. The temperature increased slightly throughout the fermentation period, to close values of 28.8°C, 28.3°C and 28.1°C in maize, sorghum and maize-sorghum blends respectively.

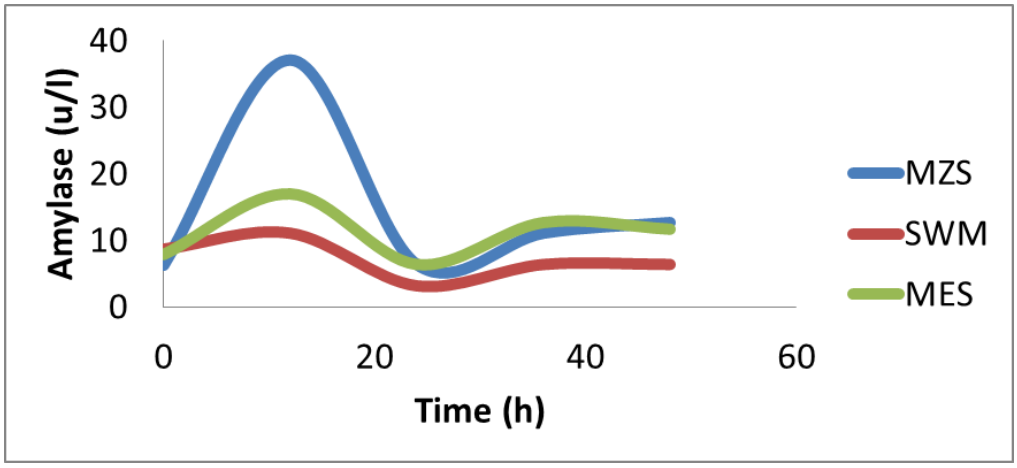


LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 8 Changes in temperature during fermentation

3.11. Amylase Activity Changes

The activity of enzyme, amylase during fermentation is shown in Figure 9. The enzyme activity peaked at 12h in all the samples, with the highest activity occurring in maize (37.1u/l). At 0h, the values were 6.3, 8.7, and 7.9u/l in maize, sorghum, and maize-sorghum samples respectively. At 24h, there were reductions in amylase activity in all the samples after which slight increases occurred till the end of fermentation when the activity was 7.1 in maize, 4.9 in sorghum and 7.0u/l in maize-sorghum sample.



LEGEND: MZS = Maize; SWM = Sorghum; MES = Maize and Sorghum

Figure 9 Changes in amylase activity during fermentation

4. Discussion

Maize and Sorghum are cereals which are rich in fermented carbohydrates that could support a wide range of microorganisms such as bacteria, yeasts and moulds. The activities of some of these microorganisms are exploited during the fermentation of these cereals to produce "Ogi", a semi- solid breakfast or weaning ceral-porridge meal in Nigeria [15, 16]. The microbiological dynamics involving microbial succession, microbial flora, lactic acid production by the LAB and changes in some fermentation conditions, such as temperature, pH, TTA, enzyme activity were studied. The chemical and sensory qualities of the product usually depend on the type of the microorganism, the available nutrients and fermentation factors [17].

The cereals (maize and sorghum) had a high microbial load, made up of bacteria, yeast and moulds. During the fermentation, it was possible to obtain a total bacterial load as high as 6.5 x 10^7cfu/ml in maize, 2.6 x 10^7cfu/ml in sorghum and 3.9 x 10^7 in maize –sorghum blend samples at the peak of growth at 12h. This is an indication that the cereals are good substrates for microbial fermentation. The result is similar to that obtained by Ojokoh [8] andOmemu [15]. The high biaload is not unexpected in view of methods of harvesting and processing of the grains and possible contaminations of the grains during storage. The growth of microorganism occurring in the paste may be due to the wide range of ecological conditions provided by the wet paste. The conditions include water activity, pH, oxygen and chemical components [16[.

Among the fungi, the yeasts were more in number and increased in population throughout the period of fermentation. This finding is similar to that obtained by Ojokoh [8]. It is believed that there is a kind of symbiotic association existing between the yeasts and lactic acid bacteria (LAB). The yeasts are believed to provide growth factors for the bacteria, while the lactic acid bacteria provide the yeasts with a favorable acidic environment [18]. The lactic acid bacteria isolated from the samples were mainly *Lactobacillus* spp and *Lactococcus* spp. In previous reports *Lactobacillus fermentum* was also commonly isolated[18].

The pH changes of the various pastes are comparable to those obtained by Agu and Aluyah [19] in which pH 5.1 and 4.2 were recorded at 0h and 12h respectively at room temperature. The microorganisms metabolized the nutrients in the paste, particularly the carbohydrates and produced acids which resulted in the reduction of pH. This was easily observed by the increasing values of total titratable acidity(TTA) with time. As the acidity increased, coliform bacteria and moulds reduced in number and eventually died by 48h. On the other hand, the lactic acid bacteria, which produced the acids, and the acid-tolerant yeasts continued to increase in number till the end of fermentation. These changes also brought about the souring of the paste, which usually gives the characteristic sour taste to' Ogi' [20].

As the microorganisms multiplied, the temperature of the paste became slightly raised due to heat production as a result of metabolic activities [21]. However, the temperature difference between 0h and 48 was not more than 3^0C and this is similar to the rise in temperature of the fermenting bean mass during tempeh production from soy bean. The extent of the rise in temperature is believed to be related to the number of microorganism in the substrate and quantity of the substrate [22].

The peak of amaylase enzymatic activity coincided with the peak of growth of the total aerobic bacteria which had a peak at 12h. The increase in enzyme levels and activities may therefore be due to increased metabolism of the microorganisms [21]. Then as some of the organisms die off, the enzymatic levels also reduced. The changes in pH, TTA and the enzymatic activity of the paste were related to the pattern of the growth of the microorganism. However, comparison of these factors and the growth patterns showed that there were no significant differences between their changes with time of fermentation in the various pastes of maize, sorghum or maize –Sorghum blend samples.

5. Conclusion

The microbial community dynamics show that Lactic acid bacteria and yeasts predominate during the fermentation period. The lactic acid bacteria reduced the pH of the medium sufficiently to mitigate the proliferation of the contaminating bacteria and fungi.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists.

References

[1] Odunfa SA. African fermented foods. In: Microbiology of Fermented Foods (Wood, B.J. (Ed.)). Elsevier Applied Science Publishers, New York. 1985; 25 – 42.

[2] Steinkraus KH. Indigenous fermented foods involving an acid fermentation. Handbook of Indigenous Fermented Foods, 2nd edn. New York: Marcel Dekker, Inc. 1996; 111 – 347.

[3] Izah SC, Kigigha IT, Okowa IP. Microbial quality of fermented maize Ogi (a cereal product) and options for overcoming constraints in production. Biotechnology Research. 2016; 2: 81 – 93.

[4] Ijabadeniyi AO. Microorganisms associated with Ogi traditionally produced from three varieties of maize. Research Journal Microbiology. 2007; 2: 247 – 253.

[5] Teniola OD, Odunfa SA. The effects of processing methods on the levels of lysine, methionine and the general acceptability of ogi processed using starter cultures. International Journal of Food Microbiology. 2001; 63: 1 – 9.

[6] Olukoya DK, Ebigwei SI, Olasupo NA, Ogunjimi AA. Production of Dogik: an improved ogi (Nrian fermented weaning food). With potentials for use in diarrhoea control. Journal of Tropical Pediatrics. 1994; 40: 108 – 113.

[7] Lawal AK, Oyedoyin OB, Olatuniji OO. Fate of pathogenic bacteria during fermentation of cereal porridge (Ogi) – A weaning food formula. Nigerian Food Journal. 2009; 27: 19 – 26.

[8] Ojokoh AO. A comparative study on the effect of traditional and improved methods of fermentation in the production of Ogi food. Oriental Journal of chemistry. 2009; 25(3): 471 – 476.

[9] Ali AA, Mustafa MM. Use of Starter Cultures of Lactic Acid Bacteria and Yeasts in the Preparation of Kisra, a Sudanese Fermented Food.ParkistanJournal of Nutrition. 2009; 8: 1349-1353.

[10] Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Ed. Cambridge University Press, UK. 2006.

[11] Erkmen O. Basic methods for the Microbiological Analysis of food. Nobel Publishing, Gaziantep University, Turkey. 2015.

[12] Aneja KR. Experiments in microbiology, plant pathology and Biotechnology, 4th Ed. New Age International (P) Limited Publisher. New Delhi, India. 2003; 410-411.

[13] AOAC. Official Methods of Analysis (18th Edition). Association of Official Analytical Chemists, Washington D.C. 2005.

[14] Junge W, Troge B, Klein G, Popper W, Gerber M. Evaluation of a new assay for pancreatic amylase: Performance characteristics and estimation of reference intervals. Clinical Biochemistry. 1989; 22(2):110-114.

[15] Omemu AM. Fermentation dynamics during production of ogi, a Nigerian fermented cereal porridge. Report and Opinion. 2011; 3: 8 – 17.

[16] Onwuakor CE, Nwaugo VO, Nnaji CJ, Emetole JM. Effect of varied culture conditions on Bacteriocin Production of four Lactobacillus species isolated from locally fermented maize (ogi). Global Journal of Medical Research, Microbiology and Pathology. 2014; 14: 1 – 7.

[17] Modu SH, Laminu H, Nkama I. Production, Chemical and Sensory Properties of ogi from different Pearl Millet Varieties. Journal of Biological Sciences. 2005; 5(2): 103 – 106.

[18] Bintsis T. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiology. 2018; 4(4): 665-684.

[19] Agu H, Aluyah E. Production and chemical analysis of weaning food from maize, dsorghum, soybean and fluted pumpkin seed flour. Nigerian food journal. 2005; 22:1.

[20] Sheriff MK, Butt MS, Sheriff HR, Nasir M. Sensory evaluation and consumer Acceptability; in book: Handbook of Food Science and Technology. Research Gate. 2017; 362 -386.

[21] Okafor N, Okeke B. Modern Industrial Microbiology and Biotechnology, 2nd Ed. CRC Press, USA. 2018.

[22] Adegbehingbe KT. Microbiological and nutrient studies of fermented cooked Lima bean seeds. Global Journal Biology, Agriculture and. Health Sciences. 2014; 2: 94 – 101.