



Profiling of Microbial Content and Growth in Fermented Maize Based Products from Western Kenya

HERVE MWIZERWA^{1,5*}, GEORGE OOKO ABONG¹, SAMUEL KURIA MBUGUA¹,
MICHAEL WANDAYI OKOTH¹, PATRICK GACHERU¹, MAINA MUIRU²,
BRENDA OBURA³ and BENNIE VILJOEN⁴

¹Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya.

²Department of Plant Science and Crop Protection, University of Nairobi, Nairobi, Kenya.

³Ministry of Public Health and Sanitation, P. O. Box 30016-00100, Nairobi, Kenya

⁴Department of Microbial, Biochemical and Food Biotechnology, Bloemfontein, University of Free State, South Africa.

⁵National Agricultural Export Development Board, Kigali, Rwanda.

Abstract

In most parts of Africa, the process of fermentation is not controlled and does not adhere to good manufacturing practices, therefore spoilage and pathogenic microorganisms can alter the quality of the end product and may cause foodborne illness. Traditional fermented products are mostly processed in an environment which creates a selection of microorganisms that produce the desired end product. In an attempt to find *Lactobacilli* which have probiotic properties and can be used in the development of starter culture for controlled fermentation of cereal products, the microbial populations of maize flour, overnight soaked dough, fermented cooked porridge, *Mkarango* and *Busaa* were enumerated and the inherent lactobacilli isolated. The microbial and biochemical profiles of the 6 days spontaneous *Mkarango* fermentation process were determined. The total viable count was 6.93 log cfu/g for fermented cooked porridge, 7.70 log cfu/g in *Mkarango* and 8.58 log cfu/g for *Busaa*. *Lactobacilli* counts were higher in maize flour with 7.43 log cfu/g while *Enterobacteriaceae* were lower in *Mkarango*. The highest moulds and yeasts counts were observed for *Busaa*, 7.25 log cfu/g. The *lactobacilli* isolates from fermented maize based products from western Kenya were predominantly *Lactobacillus fermentum* and *Lactobacillus Plantarum*. During fermentation time, *Lactobacilli* increased from 6.62 to 12.46 log cfu/g after 3 days of fermentation. From day 4, an increase in moulds and yeast count was observed, varying from 8.42 to 10.53 log cfu/g. *Enterobacteriaceae* count decreased from 5.99 log cfu/g on day 1 to less than 1 log cfu/g on day 6. Titratable acidity increased from 0.32% to 0.73% on day 5. Inversely, the pH of *Mkarango* decreased sharply from 6.64 to 3.64 on day 5 and slightly increased on the last day of fermentation. The microbial status of finished fermented maize based products is predominated by *Lactobacilli* and their isolates are predominantly *Lactobacilli* especially *Lactobacillus fermentum* and *Lactobacillus Plantarum* though further molecular tests are needed to confirm the species.





Article History

Received: 24 January 2018

Accepted: 24 August 2018

Keywords

Fermentation,
Lactobacilli,
Maize based products,
Microbial profile.

CONTACT Herve Mwizerwa  mwizerwaherve@gmail.com  Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya.



© 2018 The Author(s). Published by Enviro Research Publishers.

This is an  Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).

Doi: <http://dx.doi.org/10.12944/CRNFSJ.6.2.25>

Introduction

Fermentation is among the oldest methods of food preservation¹. Traditional fermented foods have their functionalities enhanced through development of flavours, aromas and textures, and are preserved through acidic, alcoholic or alkaline fermentations and enriched with protein and many nutrients^{2,3}. African fermented maize based products include Uji (porridge) and *Busaa* in Kenya, kenkey, banku, ogi, and koko from Ghana and Nigeria^{4,5}. Microorganisms are found in fermented foods as a result of indigenous microbiota of substrates, utensils and containers or added as starter culture⁶. The major microorganisms implicated in fermentation of maize products include lactic acid bacteria (LAB) such as *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus alimentarius*, *Lactococcus lactis*, *Enterococcus faecium*, *Leuconostoc mesenteroides* and *Pediococcus spp* as well as yeasts⁷⁻⁹. Traditional fermentation depends on inoculation of earlier fermented product; however, commercial starter cultures are currently available to ensure constancy and reliability of processes and products¹⁰. Traditional fermented products are mostly processed in a non-sterile environment which creates a selection of microorganisms that produce the desired end product however there is an increased risk of spoilage and unsafe products^{11,12} as a result of uncontrolled fermentation. The present study contributes towards determining the microbial status of fermented maize products from Western Kenya and isolating inherent *lactobacilli* which can be used in the development of starter culture for a controlled fermentation of *Mkarango* and other cereal products. This may result in enhanced fermentation process for maize products leading to optimization of their desired qualities as well as identifying the *lactobacilli* with probiotic properties. In Western part of Kenya, maize is a staple food of great socio-economic importance and a source of income and employment for millions of farming families in the region^{13,14}. In the present study, the microbial populations of maize flour, overnight soaked dough, fermented cooked porridge, *Mkarango* and *Busaa* were enumerated and the naturally present *lactobacilli* isolated. The microbial and biochemical profiles of the 6 days

spontaneous *Mkarango* fermentation process were also determined.

Materials and Methods

Sampling of Fermented Maize Based Products

Fermented maize based products were sampled from Kakamega and Homa Bay Counties in western Kenya where the population predominantly consumes maize moreover, maize is a staple food of great socio-economic importance and a source of income and employment^{13,14}. Five fermented maize based products: flour, overnight soaked dough, spontaneously fermented cooked porridge, *Mkarango* (roasted fermented maize flour) and *Busaa* (anaerobically fermented alcoholic beverage from maize) were sampled from five randomly selected producers, each providing all the five products. A total of 25 samples (15 from Kakamega and 10 from Homa Bay) were analysed. Samples were aseptically packed and taken to University of Nairobi for Laboratory analysis.

Enumeration of Total Viable Count of Fermented Maize Based Products

Total viable count (TVC) was determined on Plate count agar (PCA, HMEDIA M091-500G, India). The plates were incubated at 37°C for 48 hours as per AOAC Method 966.2315. The plates with 30-300 colonies were considered.

Enumeration of *Lactobacillus*

The *Lactobacillus* were enumerated on the deMan, Rogosa and Sharpe agar (MRS, 1.10661.0500, Merck KGaA, Germany) which was incubated anaerobically in gas pack jar at 30°C for 72 hours as per the method by De Man *et al.*,¹⁶. The colonies with different shape, size and morphology were sub-cultured on MRS until pure clear colonies observed. Identification was done by gram staining and biochemical tests.

Enumeration of Yeast and Moulds of Fermented Maize Based Products

Yeast and moulds were enumerated on Potato Dextrose Agar (PDA, HMEDIA M096-500G, India) as per FDA¹⁷. The medium was acidified with 10% tartaric acid to pH 3.5 and incubated at 30°C for 5 days. The petri dishes containing less than 150 colonies were counted. For identification of yeast

and moulds, areas of fungal growth were selected and observed under a microscope.

Enumeration of *Enterobacteriaceae* of Fermented Maize Based Products

By the use of pour plate technique, Violet red bile agar (VRBA, HMEDIA M049-500G, India) on which glucose (10%) was added was used to enumerate *Enterobacteriaceae*. as per method by ISO 21528-2¹⁸. Petri dishes were incubated at or 37°C for 24 hours. The plates containing less than 150 typical colonies were counted using a colony counter. Pink to red colonies were isolated and used for biochemical confirmation, oxidase and glucose fermentation tests as per ISO 21528-2¹⁸. For oxidase test, isolated colonies were streaked on filter paper containing oxidase reagent. A positive detection was observed by colour change within 10 seconds. For glucose fermentation test, using an inoculation needle, selected colonies were stabbed into tubes of glucose agar and incubated at 37°C for 24 hours. The positive reaction was indicated by yellow colour development.

Characterization of *Lactobacilli* in Fermented Maize Based Products

In order to characterize *Lactobacilli*, the confirmation and biochemical tests were conducted as described in the literature¹⁹⁻²¹. Colonies of *Lactobacilli* were examined for cell morphologies, cell arrangement, Gram staining, spore formation and catalase test. Rod shaped, Gram positive, catalase negative and non- spore forming isolates were purified and characterised by biochemical tests (sugar fermentation, growth in 6.5% Sodium chloride and motility test) and the isolates were identified in reference to Bergey's Manual of Determinative of Bacteriology as per Holt *et al.*,²².

Gram Staining Test and Cell Morphology

Colonies of *lactobacilli* formed on MRS were examined for Gram staining using Gram staining kit (SKU-Pack Size, SIGMA ALDRICH, USA) as described by Pyar and Peh²³. The colour, morphology and arrangement were observed under light microscopy (LABOMED, USA) using oil immersion objective.

Spore Formation Test

The method by Goyal *et al.*,²⁴ was used for endospore formation test. A smear of *lactobacilli*

isolate was aseptically made on a slide and heat fixed. The slides were placed on a boiling water bath and a primary stain (malachite green) was applied and for 5 minutes then rinsed with water until clear water is observed. The counter stain (safranin) was applied on slide for 20 s and rinsed with water again and the slides were blot dried then observed under the light microscope (LABOMED, USA). Red coloured (non-spore forming) isolates were kept and subjected to further tests.

Catalase Test

Catalase test was performed as per the method described by Kale²⁵. An isolated presumptive *lactobacillus* colony was streaked on a slide and a drop of 3% hydrogen peroxide was added. The absence of oxygen effervescence indicated the negative response.

Purification of *Lactobacilli* Isolates from Fermented Products from Western Kenya

Colonies of *lactobacilli* with different features were streaked on MRS (1.10661.0500, Merck KGaA, Germany) and incubated at 30°C. The pure cultures were then streaked on Tryptone Soy Agar (TSA) from which they were used for other tests. The isolates were stored in brain heart infusion (BHI) supplemented with yeast extract to avoid their damage at lower temperature.

Sugar Fermentation Test for *Lactobacilli*

An overnight culture of each isolate on Tryptone Soy Agar (TSA) was used to test the fermentation of different sugars. The carbohydrate fermentation test was done on the following sugars: D-Glucose, Lactose, Galactose, Mannitol, Mannose, L-arabinose, D-xylose, Cellobiose, Rhamnose and Fructose. Nutrient broth was supplemented by each sugar (1%). As per the method by Reiner²⁶ sterile tubes of the solution were inoculated with loopful of isolated *Lactobacilli* and incubated at 37°C for 24 hours after which each tube was inoculated with 2 drops of Phenol red and incubated for 24 hours. Tubes in which red colour changed to yellow indicated fermentation of sugars and acid production. An uninoculated medium was used as control.

Growth of *Lactobacilli* in 6.5% Sodium Chloride

As per Bhardwaj *et al.*,²⁷ the isolates were inoculated in MRS broth having NaCl concentration of 6.5%.

The tubes were observed for the presence or absence of growth.

Motility Test for *Lactobacilli*

The method by Ramírez-chavarín *et al.*,²⁸ was used. Sulfide indole motility (SIM) was used as motility medium. The isolates were inoculated into the centre of a tube containing SIM medium by stabbing method. The motility of the bacteria was checked by observing the spreading growth in the incubated tubes.

Microbial Changes During Fermentation of Maize Dough used to Make *Mkarango*

Mkarango is a roasted fermented maize flour from western Kenya. It can be served as such or used further for *Busaa* making. *Mkarango* was produced as per Aka *et al.*,⁴. The white maize flour was mixed with water (45%) to form the stiff dough. The mixture was well covered and left at ambient temperature to ferment for six days

The total viable count, *Lactobacilli*, *Enterobacteriaceae*, moulds and yeasts counts were monitored daily as described above.

Determination of pH and Titratable Acidity During Maize Dough Fermentation

The pH of fermenting dough was measured using a pH meter (pH 315i, WTW82362 Weilheim, Germany). The pH meter was calibrated using buffers of pH 4 and 7. Titratable acidity of fermenting dough was determined as per method by Kunyanga *et al.*,¹⁶. A sample of 10 ml was titrated with 0.1 N NaOH using

phenolphthalein as an indicator. The titratable acidity was expressed as percent lactic acid.

Statistical Analysis

Samples collected from Western Kenya producers were analysed in a completely randomized block design where a producer was considered as a block in order to analyse samples which have received the same treatment. Five producers (5 blocks) provided five products making 25 total number of sample. All samples were tested in duplicate and the mean values were recorded.

The Analysis of Variance (ANOVA) was performed and the level of significance was evaluated at $p \leq 0.005$; separation of means was done using Duncan test. In order to determine the microbial and biochemical changes during fermentation, three replications of fermentation were made and the mean values with standard deviations were reported.

Results

Microbial Composition of Maize based Fermented Products from Western Kenya

Microbial content of maize based products analysed is shown in Table 1. Total plate counts significantly ($p \leq 0.05$) varied among the samples. The highest values were observed in overnight soaked dough (9.47 log cfu/g) and the lowest were found in fermented cooked porridge (6.93 log cfu/g). On the other hand, *Lactobacilli* count differed significantly ($p \leq 0.05$) among the products ranging from 5.12 log cfu/g in fermented cooked porridge to 7.51 log cfu/g in roasted maize flour (*Mkarango*).

Table 1: Microbial content (log cfu/g) of selected maize based fermented products from Western Kenya

Parameters	Flour	Overnight Soaked dough	Fermented Cooked porridge	Roasted (<i>Mkarango</i>)	<i>Busaa</i>
TVC	8.82 ±0.77 ^a	9.47 ±0.16 ^a	6.93 ±1.76 ^b	7.70 ±1.54 ^b	8.58±0.36 ^a
<i>Lactobacilli</i>	7.43 ±0.76 ^a	7.51 ±0.38 ^a	5.14±2.32 ^b	5.97±2.21 ^d	7.38 ±0.59 ^a
Mould and Yeast	5.16±0.37 ^b	7.08±0.82 ^a	4.64±1.41 ^c	5.94±1.36 ^b	7.25±0.16 ^a
<i>Enterobacteriaceae</i>	4.87±0.12 ^b	6.16 ±0.64 ^a	4.48±1.31 ^c	4.84±2.21 ^b	4.99±0.94 ^b

TVC: Total Viable Count, Values are mean ± standard deviation, Values with different superscript in the same row are significantly different ($p \leq 0.05$).

Mould and yeast counts significantly ($p \leq 0.05$) varied within the products, with the highest counts (7.25 log cfu/g) being observed in *Busaa* and lowest counts (4.64 log cfu/g) in fermented cooked porridge. *Enterobacteriaceae* counts were not significantly different ($p > 0.05$) in flour, porridge, *Mkarango* and *Busaa*.

Characterization of *Lactobacilli* from Fermented Maize Based Products

Table 2 shows the presumptive *lactobacilli* isolates from maize based products from Western Kenya and their response to biochemical tests. Presumptively, fifty four (54) isolates of *Lactobacilli* were obtained from 25 samples collected from Western Kenya as

well as from controlled laboratory fermentation. After morphological and biochemical test, 16 isolates were identified to be probably *Lactobacillus fermentum*, 10 isolates to be *Lactobacillus Plantarum*, 6 isolates to be *Lactobacillus cellibiosis*, 5 isolates to be *Lactobacillus heliviticus* and 4 isolates to be *Lactobacillus casei*. Other isolates were presumptively *Lactobacillus lactis*, *Lactobacillus mesontoroides* and *Lactobacillus acidophilus* with 3 isolates each. *Lactobacillus fermentum* were the most predominant in all products with 29.62% of the isolates followed by *Lactobacillus plantarum* (18.51%). *Lactobacillus copophilus* and *Lactobacillus brevis* were fewest (3.70%). Further molecular tests are needed to confirm these isolates.

Table 2: Biochemical test results of *Lactobacillus* isolates

Isolate Code	Number	XY	GL	LC	FR	GAL	RH	CL	MN	MA	AR	6.5% S	MOT	GR	CAT	Probable Species
A3, A6, A7, A8, A9, B1, B2, B4, B6, B9, C1, C4, C5, D1, D5, E1,	16	+	+	+	+	+	-	+	+	+	+	+	-	+	-	<i>L. fermentum</i>
A2, A4, A5, C2, C8, C9, C10, D2, E4, E9	10	-	+	+	+	-	-	+	+	+	+	-	-	+	-	<i>L. plantarum</i>
B8, B10, D9, E6, E10,	6	+	+	+	+	-	-	+	-	+	-	+	-	+	-	<i>L. cellibiosis</i>
B3, B5, B11, C6, D11	5	-	+	+	+	-	-	+	-	-	-	-	-	+	-	<i>L. heliviticus</i>
A1, A11, C3, D3	4	+	+	+	-	+	+	+	-	-	+	+	-	+	-	<i>L. casei</i>
B7, E5, E7	3	+	+	-	+	+	+	+	+	+	+	-	-	+	-	<i>L. lactis</i>
D10, E3, E8	3	+	+	+	+	-	-	+	-	-	+	-	-	+	-	<i>L. mesontoroides</i>
C7, D4, D6	3	-	+	+	+	+	+	-	+	+	-	+	-	+	-	<i>L. acidophilus</i>
D7, D8	2	+	+	+	+	+	-	-	-	-	+	+	-	+	-	<i>L. copophilus</i>
A10, E2	2	+	+	+	+	+	-	-	-	-	+	+	-	+	-	<i>L. brevis</i>

+= Positive results, -= Negative Results, L= *Lactobacillus*, XY= D-xylose, GL=D-Glucose, LC= Lactose, FR=Fructose, GAL=Galactose, RH= Rhamnose, CL= Cellobiose, MN= Mannitol, MA= Mannose, AR= L-arabinose. S= Sodium Chloride, MOT= Motility, GR= Gram staining, CAT= Catalase, L.= *Lactobacillus*, A=Isolates from *Busaa*, B= Isolates from overnight soaked, C=Isolates from roasted dough (*Mkarango*), D= Isolates from fermented cooked porridge and E= isolates from maize flour.

Microbial Changes During Fermentation Process of *Mkarango* Dough

Figure 1 illustrate the change in microbial content of fermenting *Mkarango* dough. *Lactobacilli* were

observed to significantly ($p \leq 0.05$) increase from the beginning (6.62 log cfu/g) up to the third day of fermentation (12.46 log cfu/g) followed by a slow decrease from fourth to the sixth day.

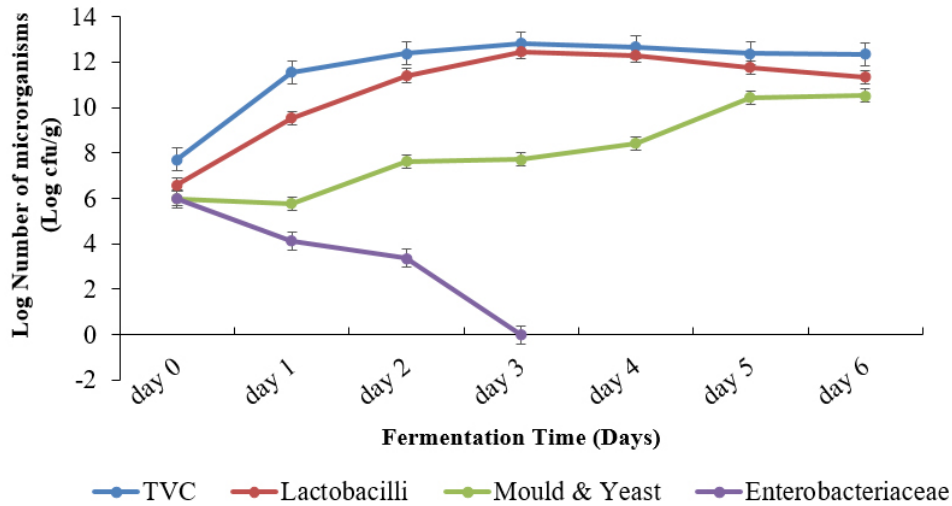


Fig. 1: TVC, Lactobacilli, Mould and Yeast and Enterobacteriaceae counts during fermentation
TVC: Total Viable Count, The bars indicate standard error of means

A significant ($p \leq 0.05$) decrease in *Lactobacilli* was observed on the sixth day (11.32 log cfu/g). Moulds and yeasts significantly ($p \leq 0.05$) increased from the fourth up to sixth day of fermentation rising from 8.42 log cfu/g to 10.53 log cfu/g. The *Enterobacteriaceae* were found to gradually decrease significantly ($p \leq 0.05$) during the fermentation period dropping from 5.99 log cfu/g to 1.0 log cfu/g.

Titrateable Acidity and pH of Fermenting Mkarango Dough

Titrateable acidity and pH change during fermentation of maize dough is shown in Figure 2. Titrateable acidity and pH were monitored during fermentation cycle of *Mkarango* dough at the interval of 24 hours (1 day) for 6 days.

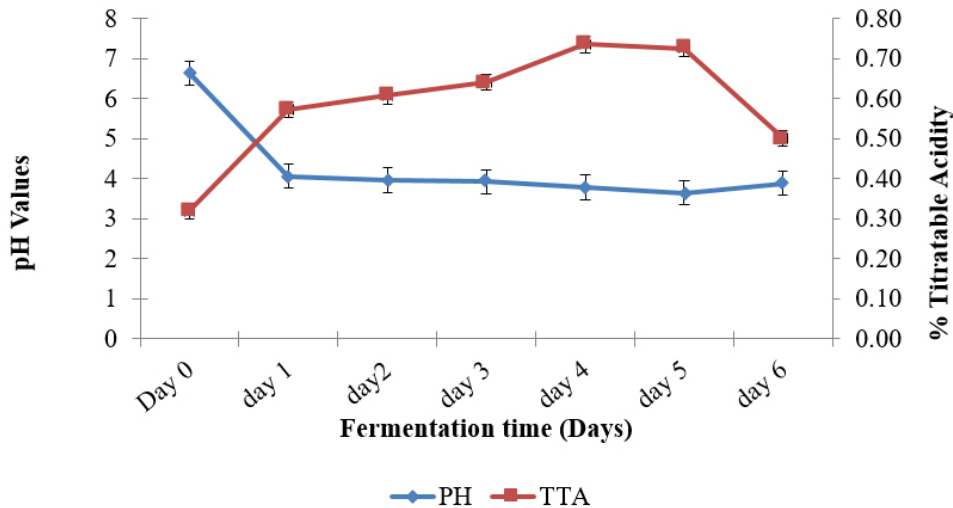


Fig. 2: Titrateable acidity and pH changes during fermentation of maize dough
TTA: Total Titrateable Acidity, The bars indicate standard error of means

The pH significantly ($p \leq 0.05$) decreased right from day 1 to day 5. It decreased from pH 6.64 on day 1 and steadily decreased throughout the fermentation process to 3.64 on day 5. The pH of the dough slightly increased on the sixth day. Titratable acidity significantly ($p \leq 0.05$) increased from 0.32% up to 0.73% on the fifth day and it significantly ($p \leq 0.05$) decreased on the sixth day.

Discussion

Microbial Composition of Fermented Products from Western Kenya

The highest total viable count was observed in the overnight soaked dough and lowest count in fermented cooked porridge. This reduction of microbial load may be attributed to the effect of heat treatment during cooking which is known to have a lethal effect on microorganisms where many non-sporulating bacteria are inactivated at temperatures above 55°C²⁹. The traditional fermentation in the village has little or no control of the microbial growth; this explains the elevated number of microorganisms that was found in all the products. The low pH of the fermented products and the heat treatment by roasting and/or cooking would make these products safe for consumption. However, poor hygienic conditions in which they are processed and handled may lead to the introduction of other microorganisms including pathogenic ones. The levels of *Enterobacteriaceae* which are hygiene indicators were high in all analysed products. Health Protection Agency³⁰ set 10⁴ cfu/g (4.0 log cfu/g) as the upper limit for *Enterobacteriaceae* content in all ready-to-eat foods placed on the market. The presence of *Enterobacteriaceae* shows that a failure occurred during processing and their absence indicates that proper hygienic conditions were maintained during the food manufacturing process³¹. *Enterobacteriaceae* include many pathogens especially *Salmonellae*, *Shigella dysenteriae*, *Yersinia enterocolitica* and *Escherichia coli* that may cause foodborne illness in people who regularly consume these foods³¹.

Yeast and mould count was most numerous in *Busaa*. It has been reported by Kirui *et al.*,³² that *Busaa* production involves conditions (ingredients, moisture and ambient temperature) which favour the growth of yeast and mould. Other products analysed

contained different counts of yeast and moulds. Most moulds and yeasts have low resistance to heat and do not survive thermal processes in low-acid foods and when found in processed foods, it is an indicator of poor processing or contamination³³. Moulds and yeasts prevalence in fermented *Mkarango* (Roasted) from Western Kenya could be attributed to the fact that during and after roasting, the processors continuously touch the dough with their hands in order to make uniform size roasted product. This may introduce other microorganisms including moulds and yeasts. It could also be attributed to poor storage conditions after production.

Lactobacilli count was high in all analysed fermented products and the highest values were observed in overnight soaked dough. This may be attributed to their natural presence in maize flour and on the availability of all growth requirements including moisture and sugars. The values in the present study are similar to those of Ogonnaya and Chidinma³⁴ in their studies on Akamu, a traditional fermented maize food (7.50 log cfu/g after 24 hours of fermentation) and Omemu3 (7.91 log cfu/g) in Ogi. It has been reported that ingestion of live cells of some strains of lactobacilli in suitable amounts confer a number of positive physiological effects on the host including maintenance of a healthy and equilibrated intestinal flora and enhanced resistance to intestinal infections³⁵.

Characterization of *Lactobacilli*

Predominance of *Lactobacillus fermentum* and *Lactobacillus plantarum* isolates was also observed by Ijabadeniyi³⁶, Adegbehingbe³⁷ and Atter *et al.*,³⁸ who reported *Lactobacillus plantarum* and *Lactobacillus fermentum* to be the most abundant microorganisms in ogi (fermented gruel from maize), Masa (fermented food product produced from maize), mawe (fermented maize meal from Benin) and burukutu (traditional beer in Ghana). *Lactobacilli fermentum* is considered as an essential microorganism for maize fermentation and avails more starch for other organisms³⁹. None of the isolate was motile. This is an indicator that the isolates were *lactobacilli* since lack of motility is a characteristic of lactic acid bacteria especially the *Lactobacilli acidophilus*^{23,25}. However, it has been reported that certain species of *Lactobacilli* spp like *Lactobacilli*

curvatus NRIC 0822 are motile and it was discovered that they contain smaller flagellum with peritrichous organisations⁴⁰.

There was different behaviour toward growth of lactobacilli in 6.5% salt concentration. Most of the isolates were able to grow in such high concentration. Cai *et al.*,⁴¹ reported that lactic acid bacteria grow better in environment of less than 5% Sodium chloride however, some species such as *Lactobacillus casei* and *Lactobacillus fermentum* have shown the ability to grow in a medium of 7% Sodium chloride. Salt is an important additive that finds application in food processing and preservation⁴⁰, therefore the ability to growth in saline medium provide lactobacilli a wide range of application especially in fermented food. The present findings are also in accordance with the report that many species of lactobacilli have the ability to withstand high salt concentrations and this makes them useful in food processing compared to other species⁴². All isolates were able to ferment at least five different sugars. The breakdown of sugars into organic acid is important in preservation of fermented food and an indicator that they can feature into starter culture strain formulation⁴³. This is in agreement with the findings of Mithun *et al.*,¹⁹, Akinleye *et al.*,²¹ and Bhardwaj *et al.*,²⁷ who reported the fermentation of simple sugars into acid by lactic acid bacteria.

Microbial and Acidity Changes During Fermentation of Mkarango

The dough fermentation process was predominated by *Lactobacilli*. This is in line with other studies done on similar fermented products which reported that *Lactobacillus* bacteria were the predominant microorganisms involved in the fermentation of products like Ogi^{20,21}, Masa³⁷ and Gari⁴⁴.

Lactic acid bacteria grow in many foods and quickly decrease the pH to 3.5 or less and competing microorganisms can no longer grow¹⁶. Moreover, *Lactobacilli* are able to produce hydrogen peroxide and antibiotics that have a significant effect on other organisms that would, otherwise, cause food spoilage⁴⁵. This may be related to the observed gradual decrease of the *Enterobacteriaceae* during fermentation dropping from 5.99 log cfu/g to less than 1 log cfu/g at the third day of fermentation.

Yeasts and moulds have also been observed to grow during fermentation of maize flour. Yeasts are widely spread in the environment and are mostly found in liquid foods that have sugars and they survive in a wide range of pH, pH 2 to above pH 9¹⁷. The starch content of maize flour may have served as a substrate and the acidity produced by breakdown of sugars by *Lactobacilli* might have restricted their growth since they survive in medium pH values less than 3.38, which was the lowest observed during fermentation.

The pH of the fermented dough decreased and the titratable acidity increased correspondingly. This acidic condition of the product could be due to the lactic acid produced by *Lactobacilli* which were found predominant during maize dough fermentation¹⁶. On the other hand, the observed increase in pH and a decrease in titratable acidity towards the end could be explained by the facts that moulds, which were shown to be growing rapidly towards the end of fermentation (6th day), have the ability to consume acids, which increase the pH and lower the titratable acidity of food products⁴⁶.

Conclusion

The microbial status of finished fermented maize based products is predominated by *Lactobacilli* and a significant number of *Enterobacteriaceae* whose growth is restricted by acidic environment. The presumptive isolates from fermented maize products from western Kenya are predominantly *Lactobacilli* especially *Lactobacillus fermentum* and *Lactobacillus plantarum*. However, further molecular tests are recommended to confirm these isolates so that they can be used in selection of starter culture for cereal products.

Acknowledgments

We acknowledge the contribution of the National Commission for Science Innovation and Technology (NACOSTI) of Kenya and the Department of Food Science, Nutrition and Technology at the University of Nairobi for facilitating the current research.

Conflict of Interest

The author declares no conflict of interest.

References

1. FAO. Traditional Fermented Food and Beverages for Improved Livelihoods. (Marshall E, Meija D, eds.). Rome, Italy: *Food and Agriculture Organization*;2012.
2. Ogbonnaya N, Bernice Chidinma C. Studies on Akamu, a traditional fermented maize food. *Rev Chil Nutr.*2012;**39**:180-184.
3. Omemu AM. Fermentation dynamics during production of ogi, a Nigerian fermented cereal porridge. *Rep Opin.*2011;**3**(4):8-17.
4. Aka S, Konan G, Fokou G, Dje Koffi M, Bonfoh B. Review on African traditional cereal beverages. *Am J Res Commun.*2014;**2**(5):103-153.
5. Gwartz JA, Garcia-Casal MN. Processing maize flour and corn meal food products. *Ann N Y Acad Sci.*2014;**1312**(1):66-75.
6. Tamang JP, Watanabe K, Holzapfel WH. Review: Diversity of microorganisms in global fermented foods and beverages. *Front Microbiol.*2016;**7**(377):1-28.
7. Muyanja CMBK, Narvhus JA, Treimo J, Langsrud T. Isolation, characterisation and identification of lactic acid bacteria from bushera: A Ugandan traditional fermented beverage. *Int J Food Microbiol.*2003;**80**(3):201-210.
8. Mohania D, Nagpal R, Kumar M, *et al.*, Molecular approaches for identification and characterization of lactic acid bacteria. *J Dig Dis.*2008;**9**(4):190-198.
9. FAO/WHO. Taxonomy and Physiology of Lactic Acid Bacteria, Effects and Function on Nutrition. Report of a Joint FAO/WHO Expert Consultation on Evaluation on Health and Nutritional. Piacenza, Italy;2001.
10. Wang X, Ren H, Wang W, *et al.*, Effects of Inoculation of Commercial Starter Cultures on the Quality and Histamine Accumulation in Fermented Sausages. *J Food Sci.*2015;**80**(2):M377-M384.
11. Galati A, Oguntoyinbo FA, Moschetti G, Crescimanno M, Settanni L. The Cereal Market and the Role of Fermentation in Cereal-Based Food Production in Africa. *Food Rev Int.*2014;**30**(4):317-337.
12. Wood BJB. Microbiology of Fermented Foods. 2nd ed. Glasgow, UK: Blackie Academic & Professional;1998.
13. Mignouna DB, Mutabazi K, Senkondo EM, Manyong VM. Adoption of a New Maize and Production Efficiency in Western Kenya. In: Joint 3rd African Association of Agricultural Economists (AAAE) and 48th Agricultural Economists Association of South Africa (AEASA) Conference. Cape town, South Africa;2010.
14. Ndwiga J, Pittchar J, Musyoka P, *et al.* Constraints and Opportunities of Maize Production in Western Kenya: A Baseline Assessment of Striga Extent, Severity, and Control Technologies. Ibadan, Nigeria; 2013.
15. Official Methods of Analysis of AOAC International. Method 996.23;2000.
16. Kunyanga CN, Mbugua S, Kangethe E, Imungi J. Microbiological and Acidity Changes During the traditional production of kirario: an indigenous kenyan fermented porridge produced from green maize and millet. *African J Food Agric Nutr Dev.*2009;**9**(6): 1419-1435.
17. FDA. Yeasts, Molds and Mycotoxins. In: Bacteriological Analytical Manual. Center for Food Safety and Applied Nutrition;2001.
18. ISO 21528-2. Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count method. In: Microbiology of Food and Animal Feeding Stuffs. Geneva, Switzerland: International Organization for Standardization; 2004.
19. Mithun S, Dipak V, Sheela S. Isolation and Identification of lactobacilli from raw milk samples obtained from Aarey Milk Colony. *Int J Sci Res Publ.*2015;**5**(1):1-5.
20. Oyedeji O, Ogunbanwo ST, Onilude AA. Predominant Lactic Acid Bacteria Involved in the Traditional Fermentation of Fufu and Ogi, Two Nigerian Fermented Food Products. *Food Nutr Sci.*2013;**4**(11):40-46.
21. Akinleye OM, Fajolu IO, Fasare AK,

- Osanyinpeju OS, Aboderin AO, Salami OO. Evaluation of Microorganisms at Different Stages of Production of Ogi in Alimosho Community, Area Southwest, Lagos, Nigeria. *Am J Res Commun.*2014;**2**(10):215-230.
22. Holt JH, Krieg NR, Sneath PH a., Staley JT, Williams ST. Bergey's manual of determinative bacteriology ninth edition. *Eur J Paediatr Neurol.*1994;**13**(6):560.
23. Pyar H, Peh KK. Characterization And Identification of *Lactobacillus Acidophilus* Using Biolog Rapid Identification System. *Int J Pharm Pharm Sci.*2013;**6**(1):189-193.
24. Goyal R, Dhingra H, Bajpai P, Joshi N. Characterization of the *Lactobacillus* isolated from different curd samples. *African J Biotechnol.*2012;**11**(79):14448-14452.
25. Kale PS. Isolation and identification of bacteria from curd and its application in probiotic chocolate. *Eur J Exp Biol.*2014;**4**(6):95-97.
26. Reiner K. Carbohydrate Fermentation Protocol. Sterling, USA: John Wiley & Sons, Ltd; 2012.
27. Bhardwaj A, Monica P, Sangu KPS, Kumar S, Dhewa T. Isolation and Biochemical Characterization of *Lactobacillus* species Isolated from Dahi. *Res Rev A J Dairy Sci Technol.*2012;**1**(2):1-14.
28. Ramírez-chavarín norma I., Wachter-rodarte carmen, Pérez-chabela m. L. Characterization and Identification of Thermotolerant Lactic Acid Bacteria Isolated From Cooked Sausages As Bioprotective Cultures. *J Muscle Foods.*2010;**21**(3):585-596.
29. Paéz R, Lavari L, Vinderola G, *et al.*, Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion. *Food Res Int.*2012;**48**(2):748-754.
30. Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. London, UK; 2009.
31. Baylis C, Uyttendaele M, Joosten H, Davies A, Heinz HJ. The *Enterobacteriaceae* and Their Significance to the Food Industry. Brussels, Belgium: International Life Sciences Institute; 2011.
32. Kirui MC, Alakonya AE, Talam KK, Tohru G, Bii CC. Total aflatoxin, fumonisin and deoxynivalenol contamination of busaa in Bomet county, Kenya. *African J Biotechnol.*2014;**13**(26):2675-2678.
33. Garnier L, Valence F, Mounier J. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. *Microorganisms.*2017;**5**(3):42.
34. Ogonnaya N, Chidinma CB. Studies on Akamu, a traditional fermented maize food. *Rev Chil Nutr.*2012;**39**(2):180-184.
35. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD. Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. *World J Dairy Food Sci.*2010;**5**(1):39-46.
36. Ijabadeniyi AO. Microorganisms Associated with Ogi Traditionally Produced from Three Varieties of Maize. *Res J Microbiol.*2007;**2**(3):247-253.
37. Adegbehingbe KT. Production of Masa Using Maize-Sorghum Blends. *Int J Sci Res.*2014;**3**(8):484-489.
38. Atter A, Obiri-Danso K, Amoah-Awua WK. Microbiological and chemical processes associated with the production of burukutu a traditional beer in Ghana. *Int Food Res J.*2014;**21**(5):1769-1776.
39. Chaves-López C, Serio A, Grande-Tovar CD, Cuervo-Mulet R, Delgado-Ospina J, Paparella A. Traditional Fermented Foods and Beverages from a Microbiological and Nutritional Perspective: The Colombian Heritage. *Compr Rev Food Sci Food Saf.*2014;**13**(5):1031-1048.
40. Cousin FJ, Lynch SM, Harris HMB, *et al.*, Detection and genomic characterization of motility in *Lactobacillus curvatus*: confirmation of motility in a species outside the *Lactobacillus salivarius* clade. *Appl Environ Microbiol.*2015;**81**(4):1297-1308.
41. Cai Y, Ohmomo S, Ogawa M, Kumai S. Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage. *J Appl Microbiol.*1997;**83**:307-313.
42. Abhijit Chowdhury, Nur Hossain, Nure Jannatul Mostazir, Fakruddin, Morsaline Billah, Monzur Morshed Ahmed. Screening of *Lactobacillus* spp. from Buffalo Yoghurt for

- Probiotic and Antibacterial Activity. *J Bacteriol Parasitol*.2012;**3**(8):1-5.
43. Halima Z-K, Nour-Eddine K. Statistical optimization of lactic acid production by *Lactococcus lactis* strain, using the central composite experimental design. *African J Biotechnol*.2014;**13**(45):4259-4267.
44. Kostinek M, Specht I, Edward VA, *et al.*, Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. *Syst Appl Microbiol*.2005;**28**(6):527-540.
45. Hertzberger R, Arents J, Dekker HL, *et al.*, H₂O₂ production in species of the *Lactobacillus acidophilus* group: a central role for a novel NADH-dependent flavin reductase. *Appl Environ Microbiol*.2014;**80**(7):2229-2239.
46. Hassan R, El-Kadi S, Sand M. Effect of Some Organic Acids on Some Fungal Growth and Their Toxins Production. *Int J Adv Biol*.2015;**2**(1):1-11.