

# Evaluation of Microbial and Nutritional Quality of Fermented Dried Roasted Thick Porridge (*Mkarango*)

## ABSTRACT

Most of the products of maize flour fermentation in Kenya undergo wild fermentation in a natural process. One of these is locally roasted maize flour commonly known by its local name *mkarango* which is popular in the Western region of Kenya where it is used in different ways. *Mkarango* is mostly made through wild fermentation which is known to pose health risks as it is unhygienic and time-consuming, with the quality of the resultant product being inconsistent. The aim of this study was to evaluate the population of lactic acid bacteria, and sensory characteristics of dried roasted thick porridge (*mkarango*). Six different *mkarango* products made with addition of *Lactobacillus plantarum* and *Lactobacillus brevis* in different ratios were studied for microbial quality, mineral element content and sensory characteristics. Titratable acidity and pH properties of the products were also determined. These were done following recommended standards. After 24 hours of fermentation, products with Yeast+ *L. plantarum*+ *L. brevis* (1:2) and Milk+ *L. plantarum*+ *L. brevis* (1:2) had the highest pH values (5.12) while products with Milk+ *L. plantarum*+ *L. brevis* (2:1) had the lowest pH values (4.8). Yeast, molds, and Lactic Acid Bacteria had the highest in all the samples tested while *Enterobacteriaceae* the least. The overall acceptability of the product of the product prepared from Milk + *L. plantarum* + *L. brevis* (2:1) had the final score of 4.7 on the 5-point hedonic scale. The results of the trace elements zinc and iron ranged from 2.7mg/100g to 3.9mg/100g and 2.7mg/100g to 16.9mg/100g on the product respectively. Fermentation creates an environment that is conducive for the population of lactic acid bacteria. This results in increased volume of lactic acid that is responsible for improved nutritional quality of *Mkarango*.

*Key Words: Fermentation, Lactic acid Bacteria, mineral content, Mkarango, Sensory characteristics*

## 1. INTRODUCTION

Fermented foods constitute diets in many African communities, some of the most important ones in this group include 'gari' from cassava, 'ogi' and 'mahewu' from maize and 'kaffir' beer from sorghum. Fermentation is an important means of preserving and introducing variety into the diet, which often consists of staple foods such as milk, cassava, fish and cereals [1]. Fermentation is an old food preservation method that is used in the World [2]. Maize is a major source of carbohydrates, vitamins, manganese, zinc, copper, magnesium and iron which is available in low amounts [3]. Although cereals are deficient in essential amino acids and iron, fermentation of these cereals by lactic acid bacteria may improve the nutritional quality and sensory properties [4]. Foods can be fermented using different methods such as alcoholic, lactic acid and alkali methods [5]. Yeasts are the main organisms used in beer production as well as wine while alcoholic fermentation results in the production of ethanol. However, lactic acid fermentation is mainly

done by lactic acid bacteria and acetic acid producing bacteria. Fermentation reduces loss of raw materials, cooking time, improves protein quality carbohydrate digestibility and also enhances availability of micronutrients and eradication of toxic and ant-nutritional factors [6]

UNDER PEER REVIEW

The process of fermentation is a complex process involving cultures of yeasts, bacteria and fungi [5]. Mostly used fermenting bacterial species include *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Micrococcus* and *Bacillus*. Fungal genera include the following *Aspergillus*, *Paecilomyces*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichothecium* while the most common fermenting yeast species is *Saccharomyces*, which is involved in alcoholic fermentation [7-9]. Isolation and identification of specific microorganisms like lactic acid bacterial strains can be used for the improvement of nutritional and technological properties of various products [10]. Isolated strain of lactic acid bacteria have been reported to inhibit spoilage by other microorganisms, lengthen the shelf life of products and may therefore improve food safety. Lactic acid bacteria are food-grade microorganisms that are generally considered safe [11]. The study was carried out to study the growth of microorganisms in cereal based fermented products during fermentation period of 24 hours.

## **2. MATERIALS AND METHODS**

### **2.1 Preparation of samples**

Four (4) hundred grams of Maize flour were added into 1000 ml screw-capped bottle and 1000ml of distilled water were sterilized by autoclaving at 121<sup>0</sup>C for 15 minutes and cooled down to 30<sup>0</sup>C then mixed to make slurry prior to inoculation. The samples were coded A1 – Yeast+ *Lactobacillus plantarum*+ *Lactobacillus brevis*(1:1), B1- Milk *Lactobacillus plantarum*+ *Lactobacillus brevis*(2:1), C1- Yeast+ *Lactobacillus Plantarum*+ *Lactobacillus brevis* (1:2), D1- Yeast+ *Lactobacillus plantarum*+ *Lactobacillus brevis* (2:1), E1- Milk+ *Lactobacillus plantarum*+ *Lactobacillus brevis* (1:2), F1- Flour+ *Lactobacillus Plantarum*+ *Lactobacillus brevis* (1:1). The coded samples were separately fermented for 3-4 days after which they were roasted at 180<sup>0</sup>C until they were brown in colour.

### **2.2 Fermentation of maize flour slurry**

For controlled fermentation, maize flour slurry (1:2w/v) was inoculated with 3% of isolated Lactic Acid Bacteria inoculum in pellet form to initiate fermentation. After thoroughly mixing, the samples were incubated at 30<sup>0</sup>C and the microbial population were counted, pH and organic acids (Lactic acid) analysis was done after zero, four, eight, twelve, and twenty four hour intervals of fermentation. The experiments were replicated three times [12].

### **2.3 Chemical analyses**

The titratable acidity was determined potentiometrically according to Volmer *et al* [13] by titrating 10 g of maize flour slurry against 0.1 M NaOH using phenolphalein as an indicator. The acidity was calculated as percent (w/w) lactic acid equivalent. The pH meter (PHM61, Radiometer, Copenhagen, Denmark) equipped with a glass electrode (Orion 9102, Orion Research, Boston, MA, USA) was used to determine pH values. The pH meter was calibrated against standard buffer solutions (Merck) at pH 4.0 and 7.0.

## **2.4 Enumeration of LAB, *Enterobacteriaceae* and yeast/moulds**

Bacterial cultures in flour pellets were inoculated into suitable maize flour slurry. Duplicate samples of maize flour slurry (10 ml) were standardized in 90 ml sterile solution of peptone physiological saline (5g peptone, 8.5g NaCl, 1000ml distilled water, pH 7). The homogenate was serially diluted and the relevant dilutions,  $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  were used. For Lactic acid bacteria, 0.1ml of the relevant dilutions were surface plated on MRS agar (Merck) with 0.1% (w/v) natamycin previously prepared and left to solidify in a sterile condition. The plates were then incubated anaerobically at 37°C. For *Enterobacteriaceae*, 1ml of the homogenate of dilutions  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  were inoculated with violet red bile glucose agar medium (VRBGA, Oxoid) and incubated at 37°C. While for Yeast and moulds, 1ml of the homogenate from dilutions  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  were inoculated on potato dextrose agar (PDA, Oxoid) using pour plate technique and incubated at 25°C for 3–5 days. The plates with 30-300 cfu/ml were enumerated.

## **2.5 Mineral content analysis**

Mineral content such as zinc and iron were analyzed by Atomic Absorption Spectrophotometer (Perkin Elmer, model 402) method [14] the method of AOAC [15]

## **2.6 Sensory evaluation**

A panel consisting of 60 people was recruited to evaluate sensory properties of different fermented *Mkarango* samples. The panel comprised of trained staff (10) and semi trained students (50) from the Department of Food Science and Technology, University of Nairobi. The parameters were evaluated on 5 point hedonic scale. The panelists evaluated on the sheet by marking the intensity perceived where 5- Like very much, 4- Like a little, 3- like nor dislike, 2- Dislike a little, 1- Dislike very much. Prior to tasting, colour, appearance and smell were evaluated. The samples were marked with a code and the products were tasted and graded for colour, taste, flavor, mouth feel texture and overall flavor. Finally, the panelists graded the overall acceptability.

## **2.7 Statistical analyses**

The data of physiochemical properties and microbial analyses were analyzed using Genstat Version 15 and mean differences determined by Least square difference (LSD) test at 0.05 while Sensory data was coded and after the evaluation, mean values were calculated for each parameter and analysis done using SPSS version 20.

# **3. RESULTS**

## **3.1 Chemical analysis**

Significant differences ( $p \leq 0.05$ ) in the pH of cofermenting mixtures were observed after 0, 4, 12 and 24 hours (Table 1) leading to acid production. D1 (Yeast+ *L. plantarum*+ *L. brevis* (2:1), after 0, 4, 8 hours had the highest pH values 5.9, 5.8, 5.7 while A1 (Yeast+ *L. plantarum*+ *L. brevis* (1:1) had the lowest pH values 5.6, 5.4, and 5.2. However, the pH values ranged from 5.9 to

5.6 after 8 hours. After 12 hours, C1 (Yeast+ *L. plantarum*+ *L. brevis* (1:2) was found to have the highest pH value (5.29) while F1 (Flour+ *p L. plantarum*+ *L. brevis* (1:1) had the lowest pH value of 3.9. However, after 24 hours products E1 had the highest pH values (3.75) while F1 had the lowest pH value of 3.2. The pH values in all the products were significantly lower as the fermentation time continued to increase such that after 24 hours, average pH for all the products was 3.5. In general, after 24 hours of fermentation, products B1 and F1 were the best since they had the lowest pH readings (4.85 and 4.84 respectively) while C1 had the highest pH readings (5.14).

**Table 1:** pH values during different hours of controlled fermentation of different roasted thick porridge products (*mkarango*)

Treatments	Fermentation Period (hours)				
	Zero time	4	8	12	24
A1.Yeast+ <i>L.plantarum</i> + <i>L.brevis</i> (1:1)	5.65 <sup>d</sup>	5.41 <sup>e</sup>	5.26 <sup>e</sup>	4.75 <sup>b</sup>	3.69 <sup>a</sup>
B1.Milk <i>L. plantarum</i> + <i>L. brevis</i> (2:1)	5.73 <sup>cd</sup>	5.62 <sup>d</sup>	5.22 <sup>e</sup>	4.21 <sup>c</sup>	3.47 <sup>b</sup>
C1.Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	5.78 <sup>bc</sup>	5.69 <sup>c</sup>	5.56 <sup>b</sup>	5.29 <sup>a</sup>	3.40 <sup>c</sup>
D1.Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	5.94 <sup>a</sup>	5.85 <sup>a</sup>	5.67 <sup>a</sup>	4.29 <sup>c</sup>	3.50 <sup>b</sup>
E1.Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	5.85 <sup>ab</sup>	5.79 <sup>b</sup>	5.37 <sup>d</sup>	4.80 <sup>b</sup>	3.75 <sup>a</sup>
F1.Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	5.78 <sup>bc</sup>	5.74 <sup>bc</sup>	5.48 <sup>c</sup>	3.92 <sup>e</sup>	3.26 <sup>d</sup>
Mean	5.79	5.68	5.42	4.54	3.51
LSD (P = 0.05)	0.06	0.04	0.05	0.038	0.036
CV (%)	0.50	0.4	0.5	0.5	0.6

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ( $p = 0.05$ ).

### 3.2 Titratable acidity

There were significant ( $p \leq 0.05$ ) differences between the products and fermentation time (**Table 2**). After 4 hours of fermentation, product A1 had the highest amount (0.26) of TA acid while E1 had the lowest amount (0.15). However, at 8 hours product B1 had the highest amount (0.43) of TA while E1 had the lowest amount (0.26). Product F1 had the highest amount (0.50 and 0.55) of TA after 12 and 24 hours respectively. The amount of TA increased as the fermentation time was increasing. When the fermentation reached 24 hours the TA increased in all the products.

**Table 2:** Titratable acidity values of different roasted thick porridge at different intervals

Treatments	Fermentation Period (Hours)				
	Zero time	4	8	12	24
A1.Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	0.19 <sup>a</sup>	0.24 <sup>a</sup>	0.31 <sup>c</sup>	0.34 <sup>d</sup>	0.39 <sup>c</sup>
B1.Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	0.16 <sup>a</sup>	0.19 <sup>c</sup>	0.43 <sup>a</sup>	0.46 <sup>b</sup>	0.48 <sup>b</sup>
C1.Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	0.18 <sup>a</sup>	0.20 <sup>abc</sup>	0.25 <sup>d</sup>	0.28 <sup>e</sup>	0.34 <sup>c</sup>
D1.Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	0.16 <sup>a</sup>	0.19 <sup>bc</sup>	0.35 <sup>bc</sup>	0.41 <sup>c</sup>	0.49 <sup>b</sup>
E1.Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	0.11 <sup>b</sup>	0.15 <sup>d</sup>	0.26 <sup>d</sup>	0.32 <sup>d</sup>	0.51 <sup>ab</sup>
F1.Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	0.18 <sup>a</sup>	0.23 <sup>ab</sup>	0.38 <sup>b</sup>	0.50 <sup>a</sup>	0.55 <sup>a</sup>
Mean	0.17	0.2	0.33	0.38	0.46
LSD (P ≤ 0.05)	0.03	0.02	0.031	0.021	0.033
CV (%)	8.90	6.6	5.2	3.0	4.0

Values followed by the same letter within the same row are not significantly different between the treatments using Fishers Protected LSD test (p = 0.05).

### 3.3 Microbial Analysis

**Table: 3** shows the microbiological accounts of different roasted porridge products at different fermentation intervals/ There were significant differences in population of microbes in the products and fermentation time (p≤0.05). After zero hours of fermentation, the population of yeasts and molds were highest in product D1 (6.7) but least in C1 ( $5.6 \times 10^5$ ) while CFUs of LABs were highest in product E1 ( $6.9 \times 10^7$ ) and lowest in D1 ( $5.4 \times 10^7$ ). The *Enterobacteriaceae* was not detected in product E1 but highly contaminated product A1 ( $4.7 \times 10^5$ ). After eight hours of fermentation, the populations of different microbes significantly (p≤ 0.05) increased. The product D1 had the highest population of yeast and molds ( $7.9 \times 10^5$ ) but product F1 had the lowest ( $7.0 \times 10^5$ ) population. Product B1 had the highest population for both LABs ( $8.5 \times 10^7$ ) and *Enterobacteriaceae* ( $5.3 \times 10^5$ ) while products F1 had the least CFUs for LABs ( $6.5 \times 10^7$ ) while C1 had the least population for *Enterobacteriaceae* ( $2.6 \times 10^5$ )

Sixteen hours later, the CFUs of Yeasts and molds were highest in the product A1 ( $9.8 \times 10^5$ ) and lowest in B1 ( $9.3 \times 10^5$ ), those of LABs were highest in B1 and lowest in E1 ( $6.5 \times 10^7$ ) while those of *Enterobacteriaceae* were highest in product C1 ( $4.4 \times 10^5$ ) and lowest in B1 ( $2.6 \times 10^5$ ).

**Table 3.** Microbial counts of different roasted thick porridge at different fermentation intervals

Treatments/ Fermentation time	Microbes ( $10^5$ )		
<b>0 hours</b>	Yeasts/ molds	LABs	<i>Enterobacteriaceae</i>
A1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	6.39 <sup>d</sup>	5.89 <sup>d</sup>	4.73 <sup>a</sup>
B1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	6.00 <sup>c</sup>	6.00 <sup>c</sup>	4.10 <sup>ab</sup>
C1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	5.62 <sup>c</sup>	6.11 <sup>b</sup>	3.20 <sup>cd</sup>
D1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	6.71 <sup>a</sup>	5.42 <sup>f</sup>	0.00 <sup>e</sup>
E1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	6.40 <sup>b</sup>	6.89 <sup>a</sup>	2.50 <sup>d</sup>
F1:Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	6.03 <sup>c</sup>	5.54 <sup>e</sup>	3.51 <sup>bc</sup>
Mean	6.19	5.97	3.01
LSD (P ≤ 0.05)	0.031	0.045	0.49
CV (%)	0.3	0.4	9.0
<b>8 hours</b>			
A1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	7.59 <sup>b</sup>	7.28 <sup>c</sup>	3.66 <sup>b</sup>
B1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	7.03 <sup>d</sup>	8.46 <sup>a</sup>	5.25 <sup>a</sup>
C1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	7.39 <sup>c</sup>	6.83 <sup>d</sup>	3.35 <sup>c</sup>
D1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	7.96 <sup>a</sup>	7.63 <sup>b</sup>	2.63 <sup>d</sup>
E1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	7.12 <sup>d</sup>	6.98 <sup>d</sup>	2.83 <sup>d</sup>
F1:Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	7.04 <sup>d</sup>	6.47 <sup>e</sup>	5.10 <sup>a</sup>
Mean	7.35	7.27	3.8
LSD (P ≤ 0.05)	0.11	0.11	0.102
CV (%)	0.8	0.8	1.5
<b>16 hours</b>			
A1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	9.85 <sup>a</sup>	7.27 <sup>d</sup>	2.83 <sup>e</sup>
B1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	9.25 <sup>e</sup>	8.45 <sup>a</sup>	2.69 <sup>f</sup>
C1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	9.54 <sup>b</sup>	7.82 <sup>b</sup>	4.39 <sup>a</sup>
D1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	9.44 <sup>c</sup>	7.04 <sup>e</sup>	3.29 <sup>c</sup>
E1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	9.41 <sup>d</sup>	6.53 <sup>f</sup>	3.02 <sup>d</sup>
F1:Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	9.41 <sup>d</sup>	7.56 <sup>c</sup>	3.96 <sup>b</sup>
Mean	9.48	7.45	3.36
LSD (P ≤ 0.05)	0.011	0.034	0.021
CV (%)	0.1	0.2	0.3

Values followed by the same letter within the same column are not significantly different between the treatments.

### 3.4 Mineral elements composition of the flour samples

The quantity of mineral elements varied from 2.7mg/100g to 3.9mg/100g and 2.7mg/100g to 16.9mg/100g for zinc and iron respectively (Table 4). Different products had different contents of zinc and iron, product F1 (maize flour + *L. plantarum*+ *L. brevis*(1:1) had the highest quantity of zinc while E1 (Milk+*L. plantarum*+ *L. brevis*(1:2) had the least amount. However, for iron C1 (yeast+*L. plantarum*+ *L. brevis*(1:2) had the largest amount of iron while A1 (Yeast+ *L. plantarum*+ *L. brevis*(1:1) had the least amount of iron.

**Table 4:** Mineral elements composition in various products treated with various isolates in different ratios.

Sample products	Zinc (mg/100g)	Iron (mg/100g)
A1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	3.2±0.03 <sup>b</sup>	2.7±0.03 <sup>d</sup>
B1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	3.2±0.05 <sup>b</sup>	7.5±0.08 <sup>c</sup>
C1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	3.4±0.05 <sup>b</sup>	16.9±0.81 <sup>a</sup>
D1:Yeast+ <i>L.Plantarum</i> + <i>L.brevis</i> (2:1)	3.2±0.005 <sup>b</sup>	7.6±0.17 <sup>c</sup>
E1:Milk+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:2)	2.7±0.06 <sup>c</sup>	10.6±0.2 <sup>b</sup>
F1:Flour+ <i>L.Plantarum</i> + <i>L.brevis</i> (1:1)	3.9±0.06 <sup>a</sup>	8.9±0.44 <sup>b</sup>
Mean	3.3	9.0
LSD (P≤ 0.05)	0.2	2.2

Values followed by the same letter within the same row are not significantly different between the treatments using Fishers Protected LSD test ( $p \leq 0.05$ ).

### 3.5 Sensory evaluation

The sensory analyses results of the products with different ingredients are presented in **Table 5**. According to the panelists, the product B1 had better taste, colour, flavour, mouth feel and texture compared to other products. The addition of Milk+ *L. Plantarum*+ *L. brevis* in the ratio 2:1 impacted positively on sensory properties of the *Mkarango* and improved the overall acceptability of *Mkarango*. Furthermore, *Mkarango* product produced by recipe of yeast, and *L. Plantarum*+ *L. brevis* was recorded as inferior product. Overall, *Mkarango* product produced with Milk+ *L. Plantarum*+ *L. brevis* in the ratio 2:1 was accepted (4.7) by the majority of the panelist.



**Table 5:** Sensory evaluation of fermented roasted maize flour (*Mkarango*) food produced after 24hrs of fermentation.

Sample Products	N	Taste	Colour	Flavor	Mouth feel	Texture	Overall Acceptability
A1:Yeast+Plantarum+Brevis(1:1)	10	4.3±0.82	3.7±0.95	3.6±1.07	3.1±1.37	3.4±1.35	3.6±0.84
B1:Milk+Plantarum+Brevis(2:1)	10	4.7±0.48	4.5±0.53	4.4±0.51	4.4±0.69	4.2±0.42	4.7±0.48
C1:Yeast+Plantarum+Brevis(1:2)	10	4.1±0.87	3.0±1.15	3.0±1.24	3.4±1.51	3.0±1.41	3.1±0.87
D1:Yeast+Plantarum+Brevis(2:1)	10	3.7±0.48	3.7±1.33	3.7±1.33	3.3±1.05	3.3±0.82	3.4±0.96
E1:Milk+Plantarum+Brevis(1:2)	10	4.2±0.63	3.3±1.33	3.5±1.43	3.2±1.22	3.6±1.17	3.6±1.35
F1:M.Flour+Plantarum+Brevis(1:1)	10	4.5±0.53	4.4±0.52	4.3±0.67	4.2±0.63	4.2±0.63	4.3±0.48
Total	60	4.3±0.70	3.7±1.12	3.75±1.15	3.6±1.19	3.6±1.09	3.7±1.01
F Value		2.758	3.31	2.267	2.41	2.246	4.566
Sig		0.027	0.011	0.061	0.048	0.063	0.002

Grade scale 1–5.

#### 4. Discussion

After 24 hours of fermentation, product with Yeast+ *L. plantarum*+ *L. brevis* (1:2) and Milk+ *L. plantarum*+ *L. brevis*(1:2) had the highest pH readings (5.12) while product with Milk+ *L. plantarum*+ *L. brevis*(2:1) had the least pH readings (4.8). The pH significantly dropped in all the products as the fermentation time continued to increase hence inhibited bacterial growth. These results agree with the findings by Katongole [5] who reported decrease in pH level with increased fermentation time such that after 48 hours the products had lowest pH levels of about 3.5. Lactic acid bacteria produce lactic acid as a byproduct that causes reduction in pH hence favoring growth and multiplication of lactic acid bacteria during fermentation [15]. Rapid decrease in pH is accompanied by intensive increase in lactic acid [16]. These may be as a result of the availability of nutrients in the products that enhances the population of lactic acid bacteria and therefore results in increase in production of lactic acid [3]

The population of yeast/molds, and LABs were the highest in all the samples while *Enterobacteriaceae* was the least. The initial counts of the microbes were least but continued to rise over time with prolonged fermentation time. The population of yeasts and mold were high and continued to proliferate with increase in fermentation time. However, yeast and molds are known not to play considerable role in fermentation and therefore may be considered as contaminants. However, microbial combinations between the lactic acid bacteria and yeasts may play significant role in the nutritional content and sensory characteristics of the end product

[3].According to Hama, *et al* [17]. Lactic acid bacteria are stimulated by yeasts which act as a source of soluble nitrogen compounds and vitamin B.

The population of lactic acid bacteria was high in the dough *Mkarango*. The predominance of these acid producing bacteria may be due to secretion of lactic acid which creates an environment that is not conducive for the growth of other bacteria [3] and yeast. However, in the present study, the population of yeast was not affected by the acid producing bacteria. Lactic acid bacteria is the main microorganism involved in fermentation. The population of yeasts and molds continued to increase with increase in fermentation time while those of *Enterobacteriaceae* remained very low in all the fermentation periods. The increase in population of yeasts and molds may be due to the fact that these microorganisms utilize oxygen present and in return produce carbon dioxide which inhibits the growth of microorganisms such as *Enterobacteriaceae* involved in decay like [12] and again the low population could be due to the presence of lactobacilli which produced lactic acid [18]

The results showed that the product samples were rich in trace minerals, iron and zinc contents which were high ranging from 2.7mg/100g to 3.9mg/100g and 2.7mg/100g to 16.9mg/100g for zinc and iron respectively and the different products had different contents of zinc and iron. There was significant difference in trace mineral contents in the sampled products. The results agree with those that were reported by Blair *et al.*, [19] who reported values between 40.0 and 84.6 mg/kg for iron and 17.7 and 42.4 mg/kg for zinc. The results of the present study contradict findings by Adeoti [20] who reported lower iron value of 0.64 mg/100g and zinc value of 1.13 mg/100g for 90% maize flour.

The overall acceptability was highest for product prepared by combining *Mkarango* and Milk+ *L. plantarum*+ *L. brevis* in the ration 2:1 which scored 4.7 on the 5-point hedonic scale. Increase in the ratio of plantarum with corresponding reduction of brevis had positive impact on the sensory characteristics evaluated and on the overall quality of the product, Improvement of sensory parameters is due to enhanced acidification and proteolysis that arises from microbiological and physicochemical processes [21]

## **5. CONCLUSION**

It was confirmed that increase in lactic acid bacteria results in increased production of lactic acid thus creates an environment not conducive for the growth of enteric bacteria thereby increasing the safety and shelf life of the products. Results in this study also showed that, fermentation results in improvement of a product with increased amount of trace elements, and both bacteria produced significantly improve nutritional quality of maize flour product.

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