Original Research Article

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

ABSTRACT

Aims: The aim of this study was to evaluate the population of lactic acid bacteria, and sensory characteristics of dried roasted thick porridge (*mkarango*).
Methodology: Five products with addition of *L.plantarum* and *L. brevis* in different ratios were studied for microbial quality, mineral element content and sensory characteristics. Some physicochemical properties of the products were also determined. The microbial counts and mineral contents determinations were done following recommended standards.
Results: 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 readings (5.12) while products with Milk+ *L. plantarum*+ *L. brevis* (2:1) had the least pH readings (4.8). The population of yeast / molds, and LABs were the highest in all the samples while *Enterobacteriaceae* was the least. The overall acceptability was maximum for product prepared from Milk + *L. plantarum* + *L. brevis* (2:1) which scored 4.7 on the 5-point hedonic scale. The results show that the product samples were rich in trace minerals, zinc and iron contents which ranged from 2.7mg/100g to 3.9mg/100g and 2.7mg/100g to 16.9mg/100g.
Conclusion: Fermentation improves the nutritional qualities of food by increasing the population of lactic acid bacteria thus increase in volume of lactic acid. This creates an environment that is not conducive for enteric bacterial growth thereby increasing the safety and shelf life of the products.

Key Words: Fermentation, Lactic acid Bacteria, Mkarango, Sensory

1. INTRODUCTION

Fermented foods constitute diets in many African communities and are 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 form of food preservation in the world [2]. Maize is a major source of carbohydrates, vitamins, manganese, zinc, copper, magnesium and considerable iron but its bio-availability is low [3]. Although cereals are deficient in essential amino acids and iron, fermentation of these cereals by lactic acid bacteria may improve the nutritional level and sensory properties [4]. Foods can be fermented following different methods through 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, and cooking time, improves protein quality and carbohydrate digestibility and also enhances availability of micronutrients and eradication of toxic and ant-nutritional factors [6]

The process of fermentation done by microorganisms 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*, the fungal genera are *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 selection 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 hundred grams quantities of Maize flour in 1000 ml screw-capped bottles and 1000ml of distilled water were sterilized separately by autoclaving at 121^0C for 15 minutes and cooled down to 30^0C then mixed to make slurry prior to inoculation. The samples were coded A1 –Yeast + *L. plantarum* +*L. brevis* (1:1), B1- Milk *L. plantarum* + *L. brevis* (2:1), C1- Yeast+ *L. Plantarum* + *L. brevis* (1:2), D1- Yeast + *L. plantarum* + *L. brevis* (2:1), E1- Milk + *L. plantarum* + *L. brevis* (1:2), F1- Flour + *L. Plantarum* + *L. brevis* (1:1)

2.2 Fermentation of maize flour slurry

For spontaneous fermentation, maize flour slurry (1:2w/v) was inoculated differently with 3% of LAB inoculum in pellet form to initiate fermentation. After thoroughly mixing the samples they were inoculated at 30^0C and after zero, four, eight, 12, and 24 hour intervals of fermentation, the microbial counts within the samples were counted, pH and organic acids (Lactic acid) analysis was done. 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 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 yeasts/molds

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 7.0F0.2). The homogenate was decimal diluted and the relevant dilutions surface plated. Lactic acid bacteria were enumerated in plates with (LAB)MRS agar (Merck) and 0.1% (w/v) natamycin and were incubated anaerobically at 30^0C.*Enterobacteriaceae* was incubated at 37^oC on violet red bile glucose agar (VRBGA, Oxoid) while Yeast was incubated for 3–5 days at 25^0C on potato dextrose agar (PDA, Oxoid) then enumerated.

2.5 Sensory evaluation

A panel consisting of 10 people evaluated the sensory properties of different fermented *Mkarango* samples. The panel comprised of trained staff and semi trained students 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.

2.6 Mineral content analysis

The analyses for essential minerals, zinc and iron were determined using AOAC [14] method by Atomic Absorption Spectrophotometer (Perkin Elmer, model 402) method [15]

2.7 Statistical analyses

The data of chemical analysis and microbial tests were analyzed with Genstat Version 15 and mean differences determined by Duncan's multiple range or the least square difference (LSD) test (P=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. Interpretation of the data was made by inspection of the scores

3. RESULTS

3.1 Chemical analysis

Significant differences (p = 0.05) in the pH of cofermenting mixtures were observed after 0, 4, 12 and 24 hours (Table 1) leading to acid production. Product D1 (Yeast+ *L. plantarum* + *L. brevis* (2:1), after 0, 4, 8 hours had the highest pH readings while treatment A1 (Yeast+ *L. plantarum* + *L. brevis* (1:1) had the least pH reading. However, the pH reading was from 5.9 to 5.6 after 8 hours. After 12 hours, product C1 (Yeast+ *L. plantarum 0*+ *L. brevis* (1:2) recorded the highest pH reading while product F1 (Flour+ p *L. plantarum* + *L. brevis* (1:1) had the least pH readings. However, after 24 hours products E1 and A1 had the highest pH readings while C1 recorded the least pH readings. The pH levels in all the products significantly (p = 0.05) dropped 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+*L. plantarum*+*L.brevis*(1:1)	5.65^{d}	5.41^{e}	5.26^{e}	4.75^{b}	3.69^{a}
B1.Milk *L. plantarum*+*L.brevis* (2:1)	5.73^{cd}	5.62^{d}	5.22^{e}	4.21^{c}	3.47^{b}
C1.Yeast+*L.Plantarum*+*L.brevis* (1:2)	5.78^{bc}	5.69^{c}	5.56^{b}	5.29^{a}	3.40^{c}
D1.Yeast+*L.Plantarum*+*L.brevis* (2:1)	5.94^{a}	5.85^{a}	5.67^{a}	4.29^{c}	3.50^{b}
E1.Milk+*L.Plantarum*+*L.brevis* (1:2)	5.85^{ab}	5.79^{b}	5.37^{d}	4.80^{b}	3.75^{a}
F1.Flour+*L.Plantarum*+*L.brevis* (1:1)	5.78^{bc}	5.74^{bc}	5.48^{c}	3.92^{e}	3.26^{d}
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

Significant (p = 0.05) differences were observed for the products and fermentation time (Table 2). After four hours of fermentation, product A1 had the highest amount of titratable acid while E1 had the least amount. However, eight hours later product B1 had the highest amount of titratable acidity while E1 had the least amount. Product F1 had the highest amount of titratable acid after 12 and 24 hours. The amount of titratable acid continued to increase with increased fermentation time such that after 24 hours the products had the highest amount of titratable acid (0.46). Averagely, product F1 had the highest amount of titratable acid (0.37) while C1 had the least amount.

Table 2: Titratable acidity values during different hours of controlled fermentation of different roasted thick porridge products

Treatments	Fermentation Period (Hours)				
	Zero time	4	8	12	24
A1.Yeast+*L.Plantarum*+*L.brevis*(1:1)	0.19^{a}	0.24^{a}	0.31^{c}	0.34^{d}	0.39^{c}
B1.Milk+*L.Plantarum*+*L.brevis* (2:1)	0.16^{a}	0.19^{c}	0.43^{a}	0.46^{b}	0.48^{b}
C1.Yeast+*L.Plantarum*+*L.brevis* (1:2)	0.18^{a}	0.20^{abc}	0.25^{d}	0.28^{e}	0.34^{c}
D1.Yeast+*L.Plantarum*+*L.brevis* (2:1)	0.16^{a}	0.19^{bc}	0.35^{bc}	0.41^{c}	0.49^{b}
E1.Milk+*L.Plantarum*+*L.brevis* (1:2)	0.11^{b}	0.15^{d}	0.26^{d}	0.32^{d}	0.51^{ab}
F1.Flour+*L.Plantarum*+*L.brevis* (1:1)	0.18^{a}	0.23^{ab}	0.38^{b}	0.50^{a}	0.55^{a}
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 changes in population of yeasts/molds, LABs and *Enterobacteriaceae* during the fermentation period. 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 but least in C1 while CFUs of LABs were highest in product E1. The CFUs of *Enterobacteriaceae* in product E1 was not detected compared with other product samples. 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 but product F1 had the least population while product B1 had the highest population for both LABs and *Enterobacteriaceae* while products A1 was the least for LABs and D1 and C1 had the least population for *Enterobacteriaceae.*

Sixteen hours later, the population of Yeasts and molds were highest in the product A1, those of LABs were highest in B1 while those of *Enterobacteriaceae* were highest in product C1. The reason for the high counts of *Enterobacteriaceae* even at the lower pH and high titratable acidity could be the post contamination both from the environment, personnel and the equipment used for collecting the sample for analysis. It is important to note that the population of *Enterobacteriaceae* as the least in all the products while yeasts and molds were the highest.

Table 3. Viable cell counts during controlled fermentation of different roasted thick porridge maize products after 0, 8 and 16 hours of fermentation

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

The sensory analyses results of the products with different ingredients are presented in Table 4. The addition of Milk+ *L. Plantarum*+ *L. brevis* in the ratio 2:1 impacted positively on sensory properties of the *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 4: 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.

3.5 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 5). Different products had different contents of zinc and iron, product maize flour + *L. plantarum* + *L. brevis* (1:1) had the highest quantity of zinc while Milk + *L. plantarum* + *L. brevis* (1:2) had the least amount. However, for iron, yeast + *L. plantarum*+ *L. brevis* (1:2) had the largest amount of iron while Yeast + *L. plantarum* + *L. brevis* (1:1) had the least.

Table 5: Mineral elements composition in various products treated with various isolates in different ratios.

Sample products	Zinc (mg/100g)	Iron (mg/100g)
A1:Yeast+*L.Plantarum*+*L.brevis*(1:1)	3.2±0.03^{b}	2.7±0.03^{d}
B1:Milk+*L.Plantarum*+*L.brevis*(2:1)	3.2±0.05^{b}	7.5±0.08^{c}
C1:Yeast+*L.Plantarum*+*L.brevis*(1:2)	3.4±0.05^{b}	16.9±0.81^{a}
D1:Yeast+*L.Plantarum*+*L.brevis*(2:1)	3.2±0.005^{b}	7.6±0.17^{c}
E1:Milk+*L.Plantarum*+*L.brevis* (1:2)	2.7±0.06^{c}	10.6±0.2^{b}
F1:Flour+*L.Plantarum*+*L.brevis*(1:1)	3.9±0.06^{a}	8.9±0.44^{b}
Mean	3.3	9.0
LSD (P≤ 0.05)	0.2	2.2
CV (%)	0.2	19.7

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).

3.5 Relationship between pH, titratable acid and microorganisms isolated from *Mkarango*

There was significant but negative relationship between pH and titratable acid (-0.8463, p = 0.05) (Table 6). However, positive correlation was displayed between pH and *Enterobacteriaceae* (0.3977, p = 0.05). Isolation frequency of yeasts and molds negatively correlated with population of LABS and *Enterobacteriaceae* (-0.4062, -0.0639, p≤ 0.05).

Table 6: Correlation analysis between pH, titratable acid and microorganisms isolated from *Mkarango*

	pH	Titratable acid	Yeast/ molds	LABS	*Enterobacteriaceae*
pH	-				
Titratable acid	-0.8463	-			
Yeast/molds	0.0607	-0.3198	-		
LABS	-0.4993	0.3413	-0.4062	-	
Enterobacteriaceae	0.3977	-0.4067	-0.0639	-0.1035	-

4. Discussion

After 24 hours of fermentation, products Yeast+ *L. plantarum* + *L. brevis* (1:2) and Milk+ *L. plantarum*+ *L. brevis*(1:2) had the highest pH readings (5.12) while 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 which is inhibitory to 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 that causes reduction in pH due to increased colony forming units of lactic acid bacteria during fermentation [15].Rapid decrease in pH is accompanied by intensive increase in lactic acid [16].The decrease in pH may be as a result of nutrients availability 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 overtime 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 a.l*[17]. *L*actic acid bacteria are stimulated by yeasts which act as a source of soluble nitrogen compounds and vitamin B.

There 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 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]again the low population could be due to the presence of lactobacilli which produced lactic acid [18]

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 [19]

The results show that the product samples were rich in trace minerals, iron and zinc contents 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., [20]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 [21] who reported lower iron value of 0.64 mg/100g and zinc value of 1.13 mg/100gfor 90% maize flour.

5. CONCLUSION

It was also confirmed that increase in lactic acid bacteria results in increase of lactic acid thus creates an environment not conducive for enteric bacterial growth thereby increasing the safety and shelf life of the products. Results in this study show that fermentation produced a better product, and both bacteria produced significant improvement in nutritional and sensory quality of maize product.

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