ISOLATION AND PROPAGATION OF LACTIC ACID BACTERIA FROM FERMENTED MAIZE FLOUR FOR THE OPTIMIZATION OF THE FERMENTATION PROCESS

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(BSc. FOOD SCIENCE AND TECHNOLOGY)

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SAFETY AND QUALITY OF THE UNIVERSITY OF NAIROBI

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY

FACULTY OF AGRICULTURE

2019

DECLARATION

This dissertation is my original work and has not been presented for a degree award in any other university.

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To my Parents, for being my inspiration and motivation to always do my best in everything I do and to always soar higher, for their hard work just to be able to send me to school, for their patience, love and undying support, for always being there for me in times of laughter and when things were not so great, for their teachings and guidance which made me become as to what I am now.

ACKNOWLEDGEMENT

I take this wonderful opportunity to extend my appreciation to those who made it possible to complete this Masters Dissertation. First l would like to thank the Almighty God for giving me strength and ability to understand, learn and complete this Dissertation.

I would like to express my sincere gratitude to my Supervisors, Prof. S.K. Mbugua, Prof. Michael. W. Okoth and Dr. George .O. Abong' for providing their valuable guidance, comments and suggestion that produced this research work.

I greatly appreciate the Chemistry and Microbiology lab Technicians; Jared Omondi, Catherine Ngunju, Monica Njoroge and James Ouma for their assistance during analysis of the samples and their creditable inputs throughout the course of the project.

I also thank Duke Gekonge , Oliver Otieno and Joshua Ombaka for shairing their time and effort in giving me piece of advice and support during statistical data Analysis. They always helped me out whenever I needed assistance. I appreciate their ideas, help and good humour.

My heartfelt thanks to Joan Waluvengo. Her friendly nature and support has always made me feel at ease and kept me going.

A special mention of thanks to the retired Chief Technologist, Mr. Jeremiah M'thika. His untimely support and encouragement shall always be remembered.

I would like to recognize the important role of my beloved husband Cosmas Langat. This work would not have materialized without his financial support. His constant support will remain my motivation throughout my life.

A special declare goes to my Children Evans Kimetto, Emma Chepchumba, Cedric Earl and my Nephew Victor for their Patience, Love, Understanding and Moral support. Their inconvenient help shall always be remembered.

Last but not least, I express my profound gratitude to my Mother, Elizabeth for her spiritual support and encouragement. 'I salute you Mum'.

TO GOD BE THE GLORY!

ABBREVIATIONS AND ACRONYMNS

LAB	Lactic Acid Bacteria
MRS	Man, Rogosa, Sharpe
FAO	Food and Agriculture Organization
DRBC	*Dichloran Rose-Bengal Chloramphenicol Agar*
PCA	Plate Count Agar

OPERATIONALDEFINITIONS

Fermentation: A metabolic process by which the sugars in the food are converted into acids, gases or alcohol by microorganisms.

Fermentative bacteria: The bacteria that are involved in the fermentation process.

Isolates: Refers to bacterial strains that have been separated from a mixed population of living microbe in the food.

Starter culture: A microbiological culture that carries out fermentation in food.

Mkarango: A roasted fermented maize flour product that is a consumed especially in the Western Region of Kenya.

Inoculum: Microbial culture that is introduced into the food to facilitate the fermentation process.

PA: Phytic acid

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GENERAL ABSTRACT

Production of traditional fermented foods especially lactic acid fermented foods has long been practiced for ages in Africa with a variety of products being produced. Lactic fermentation improves the digestibility of proteins and starch, bioavailability of micronutrients, palatability of porridges as well as imparting antimicrobial properties in them. Spontaneous fermentation of the maize flour products involves mixed culture microorganisms, of which only a given microbial community exists under specific conditions. This has resulted in variation in product quality and low standards with no known shelf-life with varied sensory attributes. The right environmental conditions have not been created for these organisms to thrive and proliferate for the fermentation process. Therefore, this study isolated and characterized fermentative bacteria to optimize the fermentation process to improve the uniformity in quality. The study was therefore specifically carried out to formulate and evaluate use of starter cultures and at the same time characterize lactic acid bacteria from traditionally fermented thick porridge (*Mkarango*) responsible for its sensory attributes. To prepare *Mkarango*, the composite flours were mixed in various ratios and the mixture was kneaded into a consistent paste and allowed to ferment at the ambient temperatures (15-25^{0}C) for about 2 days, the mixture was decanted and then roasted for 30 minutes. The physico-chemical characteristics was determined following standard procedures by AOAC procedures while Microbial profiles in terms of LAB, *Enterobacteriaceae*, Yeast and molds were evaluated through a ten-fold serial dilutions of fermented cereal flour samples at each day of fermentation. The population of Lactic acid bacteria and yeast was enumerated on plate count agar and Oxytetracycline Glucose Yeast Extract Agar respectively. Cultures of lactic acid bacteria (*L. plantarum,* and *L. brevis* and yeast culture (*S. cerevisiae)* isolated earlier from fermented *Mkarango* were used to formulate starter cultures which were either tested alone or in a mixture for their ability to ferment maize flour in order to produce *Mkarango*. Microbial

quality and mineral contents of *Mkarango* was also determined following recommended standards

Results showed that pH, titratable acidity and tannin content of *mkarango* were significantly ($p \leq 0.05$) influenced by the period of fermentation, the type of flour and the interaction of the two. The phytic acid content was only influenced significantly ($p \leq 0.05$) by the period of fermentation and ranged from 8.5 to 10.1 for the initial period but significantly reduced after the first and the second day. Increasing the period of fermentation considerably increased the titratable acidity to 0.397% from 0.1% while reducing the pH averagely from 5.6 to 5.34.The LAB, yeast and mould and total viable counts significantly increased with increased period of fermentation whereas the population *Enterobacteriaceae* reduced. The use of starter culture bacteria in combination with yeasts decreased steeping time of the maize flour and produced acceptable pH results and increased the quantity of the titratable acidity. The 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 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 sensory attributes varied for the different products and the overall acceptability was highest 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 respectively.

The use of starter culture bacteria in combination with yeasts decreased fermentation time of the maize flour and produced acceptable pH and increased the quantity of the titratable acidity thus creating an environment not conducive for enteric bacterial growth thereby increasing the safety

and shelf life of the products. Based on the microbial activity exhibited by the isolates, the best starter organism for production of *Mkarango* consisted of mixed cultures of LAB and yeast. Quality of *mkarango* is influenced by more than one single culture. The effect of fermentative action on the nutritional and physical attributes of *mkarango* is dependent on the period of storage and the type of flour used.

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Fermentation has the origin of its word from Latin, *Fervere*, which was defined by Louis Pasteur as life with no air. It is defined as a metabolic process that derives energy from organic compounds without involving an exogenous oxidizing agent (Bourdichon *et al.*, 2012). Fermentation has been employed greatly in many parts of the world, Africa included, to produce both commercial and home-based products. Production of traditional fermented foods especially lactic acid fermented foods has long been practiced for ages in Africa with a variety of products being produced (Franz *et al.*, 2014). Some of the foods that undergo fermentation in Africa include; fermented porridges, beverages (alcoholic and non-alcoholic), fermented meat, cereals such as breads and pancakes, fermented fish, vegetables, tubers, legumes, fermented dairy products and condiments (Marshall and Mejia, 2012; Oyewole and Isah, 2012). Fermented porridge is one of the most common fermented cereals. In Kenya, fermented cereals especially from maize include fermented porridge, local brew (*busaa)* and grain fermentation to produce beer.

Fermentation can be employed to diversify the production of various products from maize. Most of the products of maize flour fermentation in Kenya undergo wild fermentation in a natural process (Assohoun *et al.*, 2013). One of these is locally roasted maize flour commonly known by its local name *mkarango*. This product is popular in the Western region of Kenya where it is used in different ways. *Mkarango* is mostly made through wild fermentation thereby has a varied quality. The process of which is known to pose health risks as it is unhygienic and time-consuming, with the resultant product quality being inconsistent (Abegaz, 2013; Kivanç and Funda, 2017).

This study sought to isolate and characterize the fermentative bacteria used in this process which will help optimize this process and improve the uniformity in quality. Wild or spontaneous fermentation is defined as a process of letting natural bacteria available in a product to initiate fermentation process (Chaves-Lopez *et al.*, 2014). Spontaneous fermentation of the maize flour products involve mixed culture microorganisms, of which only a given microbial community under specific conditions determine product quality (Okoronkwo, 2014). However, the right environmental condition should be created for these organisms to thrive and proliferate such that bacteria and yeast present in the product controls the fermentation process. Therefore, the results will depend on the species that dominate fermentation. Spontaneous fermentation can have excellent quality and superb taste. Nevertheless, it might fail at times, even in ideal conditions. This is because spontaneous fermentation by nature is irregular (Nwachukwu *et al.*, 2010) and the process also takes longer time.

1.2 Statement of the Problem

The production of fermented maize flour is widely practiced in Western region of Kenya where its diverse use include being consumed as a snack, locally known as *mkarango*, and as an ingredient in the fermentation of local alcoholic brew, *busaa.* Even though this product is popular among its consumers and is claimed to have a shelf-life of more than 3 months, its preparation is by spontaneous fermentation that is dependent on the natural inoculum from the environment. This has resulted in variation in product quality with no standard. The fermentation time currently vary from 4 to 6 days resulting into a range of quality in the product. The production of this product is yet to be standardized and its true shelf-life is unknown. The sensory attributes of the product are also varied as a result of the non-standardized preparation procedures. The industrial production and commercialization of this product as a functional food

and source of healthy probiotic bacteria has not been carried out due to limited knowledge in terms of its production. Lactic acid bacteria has been used for preservation of fermented and cooked maize flour products for a long time, and various of strains have been reported to be antagonistic to pathogens and spoilage organisms related with those products. In contrast, there has been no investigation on the use of pure strains of LAB starter culture to improve the quality of *Mkarango* (maize product).Spontaneous fermentation has varied quality attributes and is done by wide spectrum microorganisms yet only a few microorganisms determine the product quality (Abegaz, 2013). Therefore the current study seeks to evaluate and isolate specific microorganisms involved in its fermentation with aim of developing starter culture and establish the shelf life of fermented maize flour.

1.3 Justification of the Study

The product, *mkarango* is produced through spontaneous fermentation, which results in various quality attributes. Wide spectrum of microorganisms causes spontaneous fermentation yet only a few determine the product quality. Isolation, characterization and identification of these microorganisms sought to establish particular environmental conditions that specific microbial communities will determine product quality, thus making the production process more predictable. In order to develop bio-preservation strategies for *Mkarango*, suitable LAB species must be identified for use in the enhancement of safety and quality of Maize products. The antagonistic properties of LAB originating from the production of metabolites that are antimicrobial such as organic acids (lactic and acetic), hydrogen peroxide, and antimicrobial peptides (bacteriocins). The study sought to improve the quality of *mkarango* through the optimization of the fermentation process through use of a pure starter culture in order to ensure a uniform product in terms of its quality attributes. This can greatly contribute towards the

modernization of techniques of production of fermented maize flour based products. The study will also avail traditional fermented products to be used as nutrient sources when there is food insecurity and improve the quality of fermented *mkarango* in terms of nutrients content, thus provide nutritional benefits to its consumers. The pure cultures will also encourage the adoption of this process outside the Western region of Kenya thereby encouraging diversification of maize flour products.

1.4 Study Aim

The aim of this study was to contribute towards product diversification that enhances the national food security status.

1.5 Purpose

To develop a starter culture that is used to optimize fermentation process of maize flour product to produce a quality product.

1.6 Objectives

1.6.1 General objective

To develop a fermentative lactic acid bacteria starter culture for improvement of traditional dry cereal product production process.

1.6.2 Specific objectives

1. To characterize quality attributes of traditionally fermented *mkarango* in terms or pH, titratable acidity, sugar content, organic acid content and ethanol content.
2. To isolate and characterize LAB from traditionally fermented *mkarango* in terms of morphological and biochemical characteristics.
3. To formulate lactic acid bacteria starter culture for improvement of fermented roasted maize flour *(mkarango)* production process.

4. To evaluate microbial, nutritional and sensory quality of fermented dried roasted maize meal*(mkarango)*

1.7 Hypothesis

The overall hypothesis of the study is that the quality attributes of *mkarango* produced from optimized fermentation process is significantly different from that of *mkarango* produced from spontaneous fermentation specifically:

1. There is no difference in the quality attributes of the different *mkarango* samples.
2. The microbial isolates from the various samples are not different from each other.
3. The microbial isolates have no effect on the process parameters of the fermentation process.
4. The sensory acceptability and the shelf-stability of the fermented maize products produced through pure lab starter culture process has no difference from that of the traditional one.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fermentation

Fermentation overly involves a process by which energy-yielding microbial metabolism occurs whereby a carbohydrate is partially oxidized and organic carbohydrate acts as an electron acceptor (Adesulu and Awojobi, 2014). The scientific identification of microorganisms by Van Leeuwenhoek and Hooke in 1665 and the discoveries by Louis Pasteur that gave credibility to the fact that microorganisms were in existence has helped develop advanced techniques of fermentation (Bourdichon *et al.,* 2012). However, fermentation was long in practice before the development of pasteurization, a process invented by Louis Pasteur (Chaves-Lopez *et al.*, 2014). History shows that fermentation has been in used as a method of preservation for as early as 7000 years ago Fermentation is a relatively cost-effective and cheap process and is aimed at enhancing food safety and shelf-life. Currently fermentation is done at the household and at the industrial basis, large-scale application (Bourdichon *et al.*, 2012; Chaves-Lopez *et al.*, 2014). Most of the fermentation processes done in the small-scale or at household level are spontaneous. Both the industrial and household fermentation processes have been exploited to produce products which have varieties of taste, flavor, aroma and texture (Alexandraki *et al.*, 2014).

Fermentation has both food and non-food applications. Food that have been subjected to fermentation include dairy products and non-dairy products including cereals, soybeans, fruits and vegetables, fish, beverages and meat (Rhee, Lee and Lee, 2011; Adesulu and Awojobi, 2014). Fermentation has several advantages in food, for example, enrichment of the food in

terms development of flavors, aromas and textures in food, preservation of the food by increasing its acidity, addition of nutrients such as protein, essential amino acids, essential fatty acids and vitamins, detoxification of the food; and decrease in cooking times of food. Fermentation has been used both at the household level under traditional techniques and the large scale industrial processes. The large scale industrial process entails controlled processes of microbial action (Alexandraki *et al.*, 2014). Some of the microorganisms that have been used in the industrial fermentation in food include lactic acid bacteria in yoghurt and fermented cereals, *Acetobacter* and *Gluconobacter* bacteria in beverages, yeast in bread (Oyewole and Isah, 2012; Jayabalan *et al.*, 2014; Tamang *et al*., 2016). The industrial fermentation process has undergone technological advancements that single strain pure cultures have been used in fermentation processes such as *S. cerevisiae* (Chambers and Pretorius, 2010). The aim of fermentation in food processing is to convert bulky, perishable and frequently inedible raw materials into safer, more shelf-stable and more palatable foods or beverages (Alexandraki *et al.*, 2014). The indigenous fermentation techniques applied in the across the world have heavily relied on wild cultures from the environment from which bacteria such as lactic acid bacteria and yeast have been isolated (Maundu *et al.*, 2013; Chaves-Lopez *et al.*, 2014).

Apart from the lactic acid bacteria (Lactobacillus), some of the microorganisms which have been used in the fermentation of foods include; Alkali bacterium, *Lactococcus*, *Enterococcus*, *Tetragenococcus* ,*Carnobacterium*, *Streptococcus*, Oenococcus, *Weissella*, *Leuconostoc*, *Pediococcus*, and *Vagococcus* (Tamang *et al*., 2016). Some of the yeast and moulds such as Saccharomyces have been used in fermentation of foods too (Hatoum *et al*., 2012).

2.1.1 Application of fermentation in the production of fermented maize flour products

The fermentations process basically occurs spontaneously in traditional methods, either through back-slopping or through using starter cultures (Forschungsgemeinschaft,2010).Spontaneous fermentation involves colonization of the food through growth of an indigenous microbiota which at the initial stages is mainly lactic acid bacteria (LAB) but would later have a variety of species of yeasts and at times moulds though their growth in some foods is limited by the fact that they can only grow aerobically (Alexandraki *et al.*, 2014). LAB produces lactic acid, bacteriocins, hydrogen peroxide and carbon dioxide which have antimicrobial properties and thus restrains the growth of pathogenic microorganisms and also reduces the sugar content (Saranraj *et al*., 2013). The yeasts in the microbiota produces aroma components and alcohols while the in case moulds are also involved, they produce intra- and extracellular proteolytic and lipolytic enzymes and thereby both influence the flavor and texture of the product (Alexandraki *et al.*, 2014). Fermentation involves a metabolic process where sugars are converted into to acids, gases or alcohol. The process mainly occurs in anaerobic conditions while to some extent such as in *Saccharomyces cerevisiae* the process can happen in aerobic conditions (Hagman and Piškur, 2015).

Optimization of traditional fermentation processes in order to improve quality of the products is currently on the rollout to improve the quality of the products (Yahyaoui *et al.*, 2017). The traditional technologies as they are currently are inefficient and produces low product yields and variable product quality (Nduko et al., 2017). These technologies tend to also have long

fermentation time due to below optimum fermentation conditions for the microorganism thus a lower product quality.

Some of the fermented maize flour based products include *Uji* (porridge) and *Busaa* in Kenya, *kenkey*, *banku*, *ogi* and *koko* from Ghana and Nigeria (Chelule, Mokoena and Gqaleni, 2010; Yahyaoui *et al.*, 2017).*Ogi*which is a traditional fermented maize flour based product mainly in Nigeria among the Yoruba tribe (Egwim *et al.*, 2013). The traditional preparation of this product mainly employs spontaneous fermentation as shown in **Figure 2.1.** It starts with the soaking of maize grains in water for 1-3 days. The soaked maize grains are wet-milled and sieved to help remove the bran, hulls and germ (Achi and Ukwuru, 2015). The residue in the sieve is used as animal feed while the filtrate is subjected to fermentation for 2-3 days to yield *Ogi*.

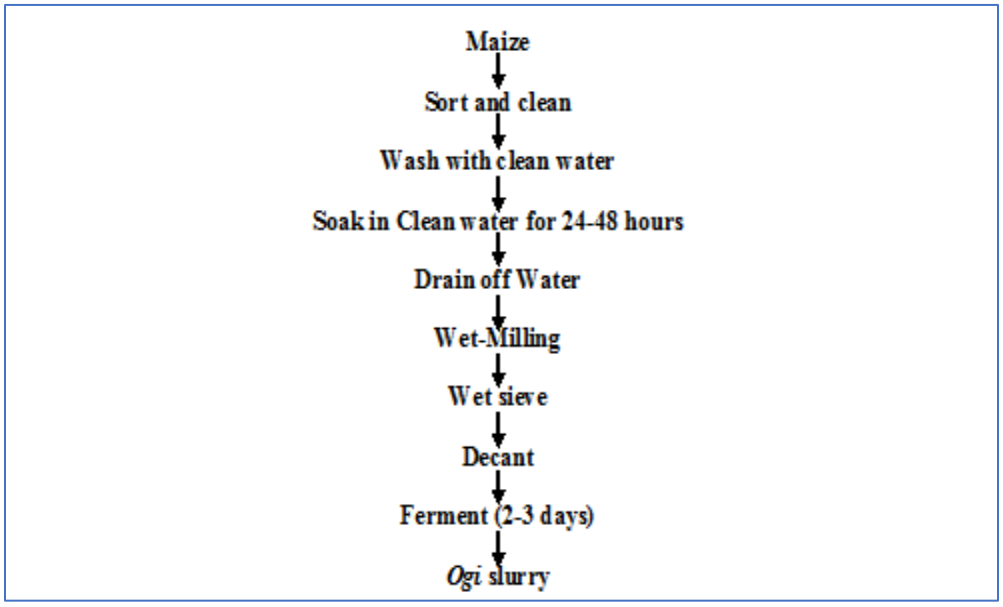

Figure 2.1: Production of Ogi

Kenya has indigenous technology for the production local brew, *busaa*, in the process where fermented roasted maize flour, *mkarango* or *tsimbale*, is used (Maundu *et al.*, 2013). These products are a consumed as traditional products in the Western region of Kenya. The *mkarango* is an intermediate product in the making of *busaa*. *Mkarango* is made by addition of water into

milled maize at the ratio of (1:2v/w) respectively. This mix is kneaded into a consistent paste and allowed to ferment for a period of 2-3 days at the ambient temperatures upon which it was decanted and fried for 30 minutes to make *mkarango* or *tsimbale* among the Isukha community (Maundu *et al.*, 2013). This mixture is mixed with yeast prepared from the finger millet at the ratio of 10:1 and then fermented further for two days. The product will be sieved and the filtrate is the *busaa* (Kirui *et al.*, 2014).

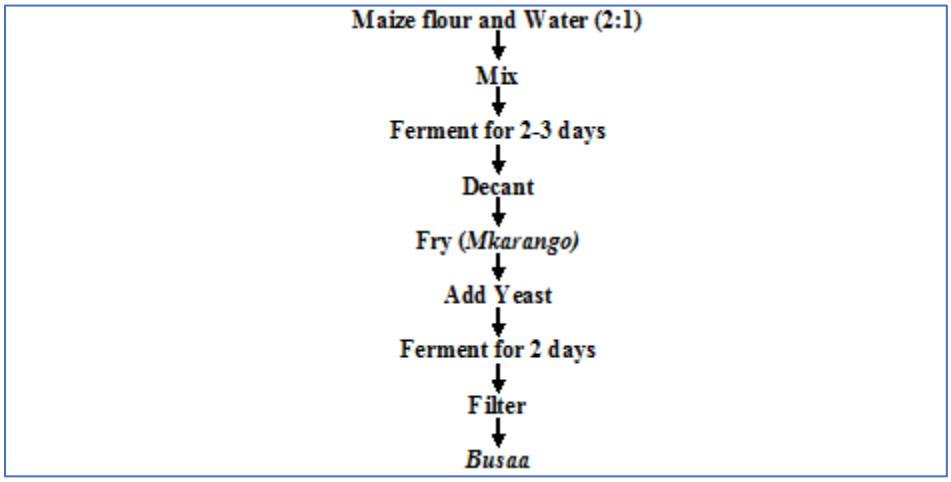

Figure 2.2: Traditional preparation of *busaa*

2.2 Isolation of Fermentative Microbes for Food Processing

The ancient technology of fermentation has undergone modification from spontaneous to controlled fermentation process targeting to achieve optimum product quality (Soro-Yao *et al.*, 2014). Microbial cultures which were originally acting as part of wild cultures in traditional spontaneous fermentation of food are isolated then identified for further use as starter cultures in optimized fermentation processes to produce a product with a more superior quality (Wakil et al., 2014). Many of these wild cultures have mixed microbial cultures in terms of strains and species with varied microbial activity, thus the fermentation parameters such as fermentation time are also varied (Kivanç and Funda, 2017). The microorganisms in spontaneous fermentation pose a

threat even in terms of shelf stability of the product as if the microorganisms are not properly managed they can result into food spoilage (Onyeze *et al.,* 2013).

Development of industrial starter cultures first begins with spontaneous fermentation of the food product (Bao *et al.,* 2011). The isolated microbes are usually subjected to morphological, biochemical and physiological characterization; upon which they are subjected to proliferation under suitable conditions to develop starter cultures (Fleck *et al.,* 2012). Further tests such as gene analysis using quantitative polymerase chain reaction (qPCR) to identify the sub-species and strain for these microorganisms (Osuret *et al.*, 2016).

In as much as cereal products are the most exploited in the fermentation process, the only commercially available starter cultures for industrial production of cereal fermented products is sourdoughs commercial cultures (Brandt, 2014). The developed starter cultures are usually stored frozen to avoid further growth that can result into spoilage; enhances stability of the starter culture (Akabanda *et al.,* 2014; Westerik *et al.,* 2016).

2.3 Lactic Acid Bacteria

Lactic Acid Bacteria are gram positive and anaerobic for they lack the cytochrome system (Kumar, 2011). The microorganisms are also non-sporeforming and catalase-negative *(Patil et al.,* 2010). The bacteria are greatly fastidious and strictly fermentative(Hayek and Ibrahim, 2013). The microorganisms are Generally Recognized as Safe (GRAS) in food (Hatoum, Labrie and Fliss, 2012). This group of microorganisms comprises both the rods and cocci bacteria: *Lactobacillus, Lactococcus* and *Streptococcus*. This group of microorganisms has greatly been exploited in the fermentative processes to produce fermented products both traditional and industrial (Soro-Yao *et al.*, 2014). Lactic Acid Bacteria was initially isolated from milk and was later isolated from other fermented products such as meat, beverages, vegetables and bakery

products (Beasley, 2004). LAB ferment the sugars in the product to produce acids thereby lower the pH of the food, prolong product stability and storability, improve product flavor through production of some aromatic compounds and improve the product texture (Fleck *et al.*, 2012).

The lactic acid bacteria have had a wide application in food fermentation (Das and Goyal, 2012). Traditional fermented foods have been used as sources of pure fermentative LAB to be used in optimized industrial fermentation processes. Fermented foods are perceived as pleasant tasting and with greater keeping quality due to the spoilage bacteria inhibitory action of LAB (Patil, Pal and Ramana, 2010). The production of high quality product, calls for isolation and purification of the predorminant LAB (Fleck *et al.*, 2012). The culture can be freeze-dried to enhance its storage. Some of the Lactic acid bacterial species that are common in fermented foods include *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Lactococcuslactis*, *Lactobacillus salivarius*, *Lactobacillus paraplantarum*, *Lactobacillus amylolyticus*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Leuconostoc mesenteroides*, *Weissella confuse* and *Streptococcus gallolyticus* (Quinto *et al.*, 2014; Soro-yao *et al.*, 2014; Sornplang and Piyadeatsoontorn, 2016). The over two hundred isolated and identified lactic acid bacteria can be classified into four genera: *Leuconostoc*, *Lactococcus*, *Enterococcus* and *Lactobacillus* (Wang *et al.*, 2016).

2.4 Benefits of Fermentation of Maize Flour

Fermentation of maize flour results into production of functional foods which have bioactive components that have beneficial health effects to the consumers (Achi and Ukwuru, 2015). By the fact that fermented maize flour is subjected to lactic acid bacteria (LAB), antimicrobial properties are induced into the maize flour (Ali, 2010). During fermentation, lactic acid, acetic

acid, ethanol and other antimicrobials are formed by the microbiota and these are responsible for inhibition of pathogenic microorganisms that can grow in food (Avit *et al.*, 2014; Ojokoh *et al.*, 2015). The LAB are probiotics and enhance the microflora in the gut and this is known to be beneficial to the human beings as microflora is important in the digestion process and provision of important nutrients such as the vitamin K (Ali, 2010). The fermentative action of lactic acid bacteria result into formation of bioactive substances in the fermented foods which prevent the initiation, promotion and development of allergies and diseases (Achi and Ukwuru, 2015).

Maize flour fermentation has enhanced dietary diversity among many communities; improved the shelf life, texture, taste and aroma of products; improved on the nutritional value and digestibility of the fermented product; and has lowered the content of antinutrients such as phytic acid in the maize flour products (Achi and Ukwuru, 2015). The fermentation of maize has also increases the total soluble solids and non-protein nitrogen and thus improves the nutritional value of the content (Egwim *et al.*, 2013).Fermentation in maize flour also decreases the antinutrients in the maize flour. The abundant antinutrients in maize flour is the phytic acid which reduces the bioavailability of many micronutrients (Assohoun *et al.*, 2013; Adesulu and Awojobi, 2014). The fermentative action by the bacteria reduces the phytic acid in the maize flour (Mahesh *et al.*, 2015).

CHAPTER THREE
PHYSICO-CHEMICAL CHARACTERISTICS OF TRADITIONALLY FERMENTED AND ROASTED MAIZE MEAL (*MKARANGO)*

3.1 Abstract

Mkarango is made from a diversity of cereal flours including maize, sorghum and millet which relies mainly on spontaneous fermentation that defines its quality. The current study sought to establish the chemical attributes of traditionally fermented *mkarango* that makes it a delicacy among its consumers. An experiment with 8 by 2 factorial arrangement was formulated where the effect of the treatments on Physico-chemical attributes of the product were investigated. Composite flours made from maize, millet and sorghum at different ratios of A-1:0:0, B-2:0:1, C-3:0:1, D-4:0:1, E-8:1:0, G-6:1:0, F-4:1:0, H-8:2:1, I-6:2:1 and J-4:2:1 were subjected to fermentation. The pH, titratable acidity, phytic acid and tannin contents were then determined according to AOAC standard procedures. Results showed that pH, titratable acidity and tannin content of *mkarango* were significantly ($p \leq 0.05$) influenced by the period of fermentation, the type of flour and the interaction of the two. The phytic acid content was only influenced significantly ($p \leq 0.05$) by the period of fermentation and ranged from 8.5 to 10.1g/kg for the initial period but significantly reduced after the first and the second day. Increasing the period of fermentation considerably increased the titratable acidity to 0.397from 0.1w/w while reducing the pH averagely from 5.6 to 5.34. Whole maize based *mkarango* had the highest decline in the tannin and phytic acid contents. The composite flours comprising of all the three cereals had the least pH values. The effect of fermentative action on the nutritional and physical attributes of *mkarango* is dependent on the period of storage and the type of flour used.

Keywords: anti-nutrients, *Mkarango*, pH, titratable acidity

3.2 Introduction

Mkarango is a traditional fermented roasted cereal flour, a well-known cereal product in the Western regions of Kenya (Mwizerwa *et al*., 2018). The product is an intermediate product in the preparation of the traditionally fermented alcoholic beverage known as *busaa* (Kirui, et al., 2014). Cereal-based beverages have been promoted as a non-dairy option for their probiotic rich property (Enujiugha and Badejo, 2017). The spontaneous fermentation of cereals has largely been attributed to the Lactic acid bacteria (LAB) (Nwachukwu, et al., 2010). The traditional fermentation of these cereal products involves a wide spectrum of these microorganisms, however, only a few of these microorganisms determine the quality of these products.

The consumption of cereals is high in Africa thus their integral role in efforts aimed at eradicating food and nutrition insecurity (Laurent-Babot and Guyot, 2017). The fermentation process of these cereals is aimed at ameliorating the quality of these products, including their organoleptic properties and acceptability. Traditionally fermented cereal products have been exploited as weaning foods across communities because of their health promoting properties (Soro-Yao, et al., 2014). Adavachi (2017) reported preference of *mkarango* as a weaning food among the children to the tune of 10% in the Western region of Kenya. The health promoting probiotics have been harnessed through consumption of these fermented cereals. However, it remains imperative that in order to increase consumption of these products, their production must be expanded and promoted.

Mkarango is made from a diversity of cereals including maize, sorghum and millet. The production of *mkarango* mainly relies on spontaneous fermentation that so highly defines its quality. The evaluation of the chemical attributes of this product is yet to be established in any documented literature. The study sought to establish the chemical attributes of traditionally

fermented *mkarango* to indicate the chemical profile of the product that so makes it highly likeable among its consumers.

3.3 Materials and Methods

3.3.1 Acquisition of materials

Maize, sorghum and millet were bought from local retail market, milled at the local Posho mill and taken to the pilot plant at the Department of Food Science, Nutrition and Technology (DFSNT) in the University of Nairobi for product formulation.

3.3.2 Preparation of traditionally fermented *mkarango*

The preparation of *mkarango* was done as shown in **Figure 3.1.**The composite flours were mixed as shown in **Table 3.1.** The milled composite flour and water were mixed at the ratio 2:1 respectively. The mixture was kneaded into a consistent paste and allowed to ferment at the ambient temperatures (15-25^{0}C) for about 2 days. The mixture was decanted and roasted for 30 minutes to make *Mkarango*. The ratios were informed by the antinutrient contents such as tannins that are in high concentrations and conspicuous color in sorghum and millet but not in maize.

Table 3.1: Composite flour used in preparation of *mkarango*

Sample treatments	**Ratio of cereal in composite flour**		
	Maize	**Millet**	**Sorghum**
Sample A	1	0	0
Sample B	2	0	1
Sample C	3	0	1
Sample D	4	0	1
Sample E	8	1	0
Sample F	6	1	0
Sample G	4	1	0
Sample H	8	2	1
Sample I	6	2	1
Sample J	4	2	1

3.3.3Analytical methods

3.3.3.1 Determination of tannin content of *mkarango*

The content of tannin in the samples was determined following the procedures by (AOAC, 1990) method 9.098 and the calculation was done following the formula and the Absorbance was read at 725nm.

$$Constant\ (14.5) \times\ Absorbance \times\ 100 \times \frac{50}{2} \times \frac{100}{0.5} = (\frac{7250mg \times Absorbance)\ \mu g}{100g}$$

3.3.3.2 Determination of phytic acid content

Phytic acid content was determined according to (AOAC, 2006) method 986.11 with slight modifications. A sample of 0.5g was weighed and petroleum ether used to defat the sample. The determination was done in duplicate and absorbance reading done at 500nm.

3.3.3.3 Determination of titratable acidity of *mkarango*

Titratable acidity was determined as per the AOAC (2005) method number 920.124.

3.3.3.4 Determination of pH of *mkarango*

The pH of the samples was determined according to (AOAC, 2005) method number 981.12. The pH meter was standardized using the 7.0 and 4.0 pH buffer standard solution. The pH value was read and recorded to the nearest tenth of a whole number.

3.3.4 Statistical analysis

The data was analyzed using Genstat version 15. Descriptive statistics such as mean and standard deviation were generated for the variables. Statistical test for significance was done using two-way ANOVA with the p-value$\leq$ 0.05. The Fischers' LSD was used to separate means that were statistically different at p-value $\leq$0.05.

3.4 Results and Discussion

Different composite cereal flour, the period of fermentation and the interaction of the two factors affected the pH, titratable acidity and tannin content of the resultant *mkarango* product whereas the phytic acid was only influenced by the period of fermentation at, $p \leq 0.001$ (**Table 3.2)**.

Table 3.2: Mean square values for ANOVA analysis of variation in chemical attributes

Source of variation	pH	Titratable acidity	PA (mg/100g)	Tannins (mg/100g)
Sample	0.428	0.053	0.128	5.439
Period of fermentation	0.989	0.890	43.275	7.098
Sample x Day	0.122	0.038	0.031	0.016
Error	0.003	0.000	0.001	0.000

The results indicate that increasing the length of the period of fermentation decreases the pH of the product **(Table 3.3)**. *Mkarango* made from *c*omposite flours of the three cereals, maize, millet and sorghum, had significantly ($p \leq 0.05$) lower pH values (4.65-4.99) compared to the maize product (5.98). Fermenting all the composite flour for additional two days rather than one day decreased the pH further. The findings agree with the results from similar studies by Alka*et al*. (2012)and Marko *et al*. (2014)where increasing the period of fermentation decreased the pH further. The pH readings of the cereals after a day of fermentation are supported by findings by Kingamkono *et al*. (1997) who found that after a day of fermentation of cereals, a pH ≥ 5.2 is achieved. The fermentation of cereals mainly involve lactic acid bacteria (Kohajdova and Karovicova, 2007; Uzochukwu, *et al.*, 2016). Lactic acid bacteria utilize fermentable sugars in the cereals to release lactic acid; that is responsible for the reduction of the pH of the cereals. The LAB have the ability to break down starch to fermentable reducing sugars thus the continued fermentation action beyond the first day (Marko *et al*., 2014).

Table 3.3: pH of traditionally fermented *mkarango*

Samples	Period of fermentation	
	Day 1	**Day 2**
A	5.97±0.01[a]	5.98±0.01[a]
B	5.80±0.01[a]	6.15±0.21[a]
C	5.82±0.01[a]	5.85±0.07[a]
D	5.70±0.02[b]	5.35±0.01[a]
E	5.61±0.03[b]	5.10±0.01[a]
F	5.62±0.03[b]	5.22±0.02[a]
G	5.59±0.04[b]	5.15±0.02[a]
H	5.64±0.04[b]	4.96±0.01[a]
I	5.45±0.07[b]	4.65±0.04[a]
J	5.35±0.06[b]	4.99±0.01[a]
Averages	5.65±0.18[b]	5.34±0.48[a]

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

Increasing the period of fermentation resulted into elevated levels of titratable acidity ($p \leq 0.05$) (**Table 3.4).**The product that was made from wholly maize flour had the highest titratable acidity values of 0.48±0.47% ($p \leq 0.05$). Fermentation of the whole maize flour for two days as compared to one day resulted into a higher titratable acidity values as compared to the composite flours. Maize flour based *mkarango* had the highest titratable acidity (0.481±0.473%). The titratable acidity increased with decreasing pH in all the cereal flours. The findings concur with those of Onuoha*et al*., (2017) who reported that spontaneous fermentation of pearl millet increased the titratable acidity. Wakil and Ajayi (2013) also reported that maize based *ogi* would have higher titratable acidity than the sorghum based *ogi* but similar to that of millet based ogi. Fermentation

in cereals generate organic acids which is reflected as increase in the titratable acidity (Ojokoh, 2009).The most abundant organic acid in the *mkarango* is the lactic acid which is generated from the LAB fermentative action on the cereals.

Table 3.4: **Titratable acidity of traditionally fermented *mkarango* (%)**

Samples	**Period of fermentation**	
	Day 1	**Day 2**
A	0.071±0.001[a]	0.890±0.014[a]
B	0.016±0.001[a]	0.190±0.014[b]
C	0.080±0.014[a]	0.185±0.007[b]
D	0.011±0.001[a]	0.314±0.001[b]
E	0.051±0.056[a]	0.335±0.004[b]
F	0.107±0.002[a]	0.314±0.001[b]
G	0.106±0.003[a]	0.341±0.001[b]
H	0.186±0.004[a]	0.403±0.004[b]
I	0.175±0.007[a]	0.497±0.003[b]
J	0.185±0.006[a]	0.502±0.003[b]
Averages	0.099±0.065[a]	0.397±0.198[b]

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

Fermentation of cereals resulted into a significant ($p \leq 0.05$) reduction in the anti-nutritional factors in the cereals **(Table 3.5 and 3.6)**. Increased period of fermentation for three days resulted in the decline of phytic acid from 89% to 84% and tannin content from 88% to 83.7%. The findings are supported by similar results as reported by Roger*et al*.(2015) and Ojha *et al*. (2018) whose study on the fermentation of corn and sorghum respectively; the results revealed that fermentation reduced the phytates and tannin contents. LAB cultures have phytase enzymes that is known to break down the phytic acid into inorganic phosphates (Coulibaly *et al*., 2011; Osman, 2011). Reduction of anti-nutritional factors has been promoted as a way of ameliorating nutritional quality of products known to be high in anti-nutritional factors. Cereals (Maize, Millet

and Sorghum) high in anti-nutritional factors are recommended that further food processing that would even entail fermentation to improve their nutritive value for possible use in weaning diet (Sherrif, 2017).

Table 3.5: Phytic acid content of traditionally fermented *mkarango*

Samples	Period of fermentation			% loss of tannins after two days of fermentation	Averages
	Day 0	Day 1	Day 2		
A	10.06±0.08^{a}	3.29±0.04^{b}	1.19±0.00^{a}	88.2	4.85±4.15^{a}
B	9.05±1.18^{b}	3.07±0.04^{c}	1.19±0.06^{a}	86.9	4.44±3.71^{a}
C	9.58±0.48^{ab}	3.18±0.04^{c}	1.23±0.00^{a}	87.2	4.66±3.91^{a}
D	9.90±0.03^{a}	3.49±0.02^{ab}	1.30±0.00^{a}	86.9	4.90±4.00^{a}
E	9.16±1.11^{b}	3.13±0.04^{c}	1.17±0.00^{a}	87.2	4.49±3.76^{a}
F	9.92±0.00^{a}	2.94±0.00^{c}	1.09±0.00^{a}	89.0	4.65±4.17^{a}
G	9.56±0.48^{ab}	3.33±0.06^{a}	1.25±0.00^{a}	86.9	4.71±3.87^{a}
H	9.55±0.46^{ab}	3.32±0.04^{a}	1.22±0.00^{a}	87.2	4.70±3.88^{a}
I	8.59±1.78^{b}	3.82±0.06^{a}	1.40±0.00^{a}	83.7	4.60±3.37^{a}
J	8.95±1.19^{a}	3.64±0.08^{a}	1.38±0.00^{a}	88.2	4.66±3.52^{a}
Mean	9.43	3.32	1.24	87.1	4.7
LSD	0.74	0.20	NS	NS	NS

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

Table 3.6: Tannin content of traditionally fermented *mkarango*

Samples	Period of fermentation			% loss of tannins after two days of fermentation	Averages
	Day 0	Day 1	Day 2		
A	3.77±0.77^{c}	1.45±0.00^{d}	0.65±0.01^{a}	82.76	1.95±1.49^{a}
B	3.61±0.42^{c}	4.20±0.00^{a}	3.40±0.00^{a}	5.82	3.74±0.42^{e}
C	3.59±0.56^{c}	2.39±0.00^{c}	1.59±0.00^{a}	55.71	2.52±0.93^{b}
D	4.07±0.00^{b}	2.39±0.00^{c}	1.63±0.06^{a}	59.95	2.70±1.12^{bc}
E	3.73±0.68^{c}	3.41±0.00^{b}	2.20±0.00^{a}	41.02	3.11±0.78^{d}
F	4.12±0.00^{b}	3.26±0.00^{c}	2.43±0.04^{a}	41.02	3.27±0.75^{d}
G	3.71±0.62^{c}	1.81±0.00^{d}	1.01±0.00^{a}	72.78	2.18±1.27^{ab}
H	3.65±0.57^{c}	2.39±0.00^{e}	1.59±0.00^{a}	56.44	2.54±0.96^{c}
I	5.62±0.52^{a}	5.08±0.00^{a}	4.26±0.01^{a}	24.2	4.32±0.69^{f}
J	3.57±0.44^{c}	1.59±0.00^{d}	0.79±0.01^{a}	77.87	1.98±1.30^{a}
Mean	3.94	2.79	1.95	**51.75**	**2.83**
LSD	0.44	0.84	NS	17.34	0.56

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

3.5 Conclusion

The fermentative action reduces the pH, phytic acid and tannin contents of the products while increasing the titratable acidity. This impact is largely dependent on the period of fermentation and the type of flour that is used as the ingredient. The use of maize produces largest decline in anti-nutritional factors and the highest titratable acidity values.

CHAPTER FOUR

MICROBIAL PROFILE AND GROWTH IN TRADITIONALLY FERMENTED *MKARANGO*

4.1 Abstract

Mkarango is a cereal product that is widely consumed in the Western region of Kenya. The production process is not standardized and there is variation in the product quality. The current study sought to evaluate the microbial profile of traditionally fermented *mkarango* and to isolate and characterize the LAB cultures that are involved in the fermentation process. Recipes of *mkarango* adopted from different region in the Western Kenya with the composites of maize: millet: sorghum ratios of A-1:0:0, B-2:0:1, C-3:0:1, D-4:0:1, E-8:1:0, F-6:1:0, G-4:1:0, H-8:2:1, I-6:2:1 and J-4:2:1 respectively and subjected to fermentation for different hours were developed. Microbial profiles in terms of LAB, *Enterobacteriaceae*, Yeast and moulds and total viable counts were evaluated. The LAB cultures were purified and evaluated for morphological and biochemical characteristics. The LAB, *Enterobacteriaceae*, yeast and moulds and total viable counts were significantly ($p \leq 0.05$) influenced by the period of fermentation and the type of composite flour used. The LAB, yeast and mould and total viable counts significantly ($p \leq 0.05$) increased with increasing period of fermentation whereas with increasing period of fermentation the *Enterobacteriaceae* significantly ($p \leq 0.05$) reduced. Composite flour *mkarango* dough had significantly ($p \leq 0.05$) higher LAB counts than the maize based *mkarango* dough. The LAB cultures that were identified in the *mkarango* were the heterofermentative group including *L. paramesenteroides, L. brevis, L. lactis, L. plantarum* and *L. fermentum*. The study concluded that the quality of *mkarango* is influenced by more than one single culture. It is recommended

that the relationship between the different cultures be established for future prospects of developing pure starter cultures.

4.2 Introduction

Lactic acid bacteria (LAB) are the abundant and primary agents of fermentation of most cereal products such as maize, sorghum and millet in Africa (Soro-yao, et al., 2014). LAB cultures have been used in indigenous technologies across African countries to produce various cereal products of varied importance to the cultural settings of these communities. The nutritional and other quality benefits such as extended shelf-life have been induced in most cereal products through fermentation (Okoronkwo, 2014). Food fermentation is an inexpensive food preservation technique and is thus widely practiced across Africa (Mokoena et al., 2016). LAB induced fermentation of cereals in Africa is majorly spontaneous as most communities rely on inoculation by LAB cultures from either environment or an already fermented product. The products in this case are known to have a mixed quality, which is way off the optimum quality (Rhee et al., 2011). Thereby, commercialization of these products has been low.

The LAB isolated from various traditionally fermented cereal products have been proven to be responsible for the quality and palatability of these products through their fermentative activity that produces lactic acid and other organic acids and some bacterial inhibitors (Mokoena et al., 2016). Studies have documented the use of such starter cultures to improve sensory properties and reduce the use of food additives in fermented beverages (Peyer et al., 2016). The benefits of LAB isolates are not restricted to product quality alone, they also tend to have a transferrable probiotic effect on the consumers. A study by Angmo et al. (2016)found that LAB isolates from fermented cereal based beverages had an *in vitro* probiotic potential to varying degrees.

The isolation and commercialization of starter cultures has been employed for possible commercialization of such products (Nwachukwu et al., 2010). LAB cultures that induce fermentation in cereals are isolated based on their performance and quality of product under optimal conditions. A study by Simsek et. al. (2017) on a traditional fermented cereal product in Anatolia known as Tarhana, recommended the use of *L. alimentarius, L. plantarum*, and *L. brevis* as starter cultures from a very broad spectrum of microorganisms for possible commercialization of the product. This has the important effect of improving the product quality. Another study by Mwizerwa et al. (2018) on maize based *mkarango* reported a rich LAB profile of up to 5.97 log CFU/ml. However, the study fell short of comparing the microbial profile in the maize based *mkarango* from that made of other cereals such as millet, sorghum and other composite flours of maize that have been identified as ingredients too in the production of this product. For standardization of food production procedure, it is important to establish any possible similarities and differences in the fermentation of different cereals for this product. The objective of the present study was to characterize LAB cultures from different cereals in terms of morphological and biochemical characteristics, thereby establishing similarities and differences in the fermentative action of these cereals.

4.3 Materials and Methods

4.3.1 Acquisition of materials

Maize, sorghum and millet were bought from local retail market, milled at the local posho mill and taken to the microbiology Laboratory at the Department of Food Science, Nutrition and Technology in the University of Nairobi for product formulation.

4.3.2 Preparation of traditionally fermented *mkarango*

The cereal flours were mixed as shown in **Table 3.1.** The milled composite flour and water were mixed at the ratio 2:1 respectively. The mixture was kneaded into a consistent paste and allowed to ferment at the ambient temperatures (15^0C-25^0C) for a period of 2-3 days. The mixture was decanted and roasted for 30 minutes to make *mkarango*.

4.3.3 Isolation and characterization of LAB from fermented *mkarango*

4.3.3.1 Microbiological analysis

During spontaneous fermentation of selected cereals, samples were collected for analysis at an interval of 0, 12 and 24h until the end of fermentation. The appropriate ten-fold serial dilutions of fermented cereal flour samples (25 ml) at each day of fermentation period were prepared in 0.85% sodium chloride for the enumeration of microbial population (Thongruck., et al. 2017). For total bacteria count, Plate Count Agar media (HiMedia Laboratories, India) was used and incubated at 37°C for 24-48 hrs ,for the LAB count MRS agar (RCI Labscan Limited, Thailand) containing 0.01% (w/v) cycloheximide (RCI Labscan Limited, Thailand), 0.01% (w/v) sodium azide (Thermo Fisher Scientific Inc., Australia) and 0.01% (w/v) bromocresol purple incubated in anaerobic jars at 37°C for 24-48 hrs under anaerobic condition was used; for *Enterobacteriaceae* count VRBD Agar was used and for yeast count Potato Dextrose Agar(HiMedia Laboratories, India) pH 3.5% incubated at 37°C for 24-48 hrs was used. To determine the LAB distribution in fermented cereal flour, bacterial colonies that characteristically showed yellow zone on the plate were selected at random from each plate with 30-300 colonies and streaked on MRS agar. The procedure was repeated in order to purify the

isolates. The isolation procedure was done for three microorganisms that have been previously reported as dominating natural fermentation process of cereal flour.

4.3.3.2 Identification of lactic acid bacteria

For identification of Lactic acid bacteria, isolates from the plates having discrete colonies were taken and sub-cultured severally on MRS agar until pure colonies were obtained. They were then subjected to the following tests.

4.3.3.3 Morphological characteristics

LAB strains were examined microscopically for cellular morphology and then subjected to Gram stain phenotype. Strains were observed visually by different sizes and color appearance of their colonies (Menconi *et al*., 2014)

4.3.3.4 Catalase test

Catalase test was done according to the method described by Mwizerwa *et al*. (2018). Using a sterile inoculating wooden stick, a colony of isolated presumptive lactobacillus was aseptically picked from different plates and placed on a sterile slide after which a drop of 3% hydrogen peroxide solution was added on the cells. Formation of bubbles immediately indicated the presence of catalase in the cells.

4.3.3.5 Gram staining test

Isolates which were catalase-negative were Gram stained and observed under light microscopic with a magnification of 100x.

4.3.3.6 Motility test

The method by Mwizerwa et al. (2018) was used. Sulfide indole motility (SIM) medium was used for testing for motility. The overnight culture isolates were aseptically picked using a sterile wire needle inoculated by stabbing the centre of a tube containing sterile SIM medium. After 24 h, the motility of the bacteria was checked by observing the spreading growth in the incubated SIM medium tubes.

4.3.3.7 Sugar fermentation test

The sugar fermentation test was done according to method used by Oyedeji et al.(2013). Microbial cultures that were gram positive, catalase negative and non-motile rods were subjected to sugar fermentation. The carbohydrate fermentation test was done on the following sugars: Galactose, Sucrose, Melibiose, D-Glucose, Lactose, Galactose, Raffinose, Mannose, L-arabinose, D-xylose, Rhamnose, Cellobiose and Fructose. An overnight culture of Lactobacilli isolate grown on Tryptone Soy Agar (TSA) was used to test for the fermentation of the above different sugars. Nutrient broth was supplemented by each sugar (1%). As per the method by Berłowska et al., (2016) 26 sterile tubes of the solution were inoculated with loopful of isolated Lactobacilli and incubated at 37^0C for 24 hours. Then 2 drops of Phenol red were put on each tube. Tubes which changed to yellow indicated acid production hence positive for sugar fermentation and tubes which maintained the red color were negative for sugar fermentation. A non-inoculated medium was used as control.

4.4 Results and Discussion

4.4.1 Microbial profile of *mkarango* dough

The microbial profile of the *mkarango* was significantly ($p \leq 0.05$)influenced by the period of fermentation, the type of flour and the interaction between the type of flour and the period of fermentation as shown in **Table 4.2**.

Table 4.1: ANOVA analysis of microbial counts of *mkarango*

Source of variation	Lactic acid bacteria counts	Total viable count	Yeast and moulds counts	Enterobacteriaceae counts
Sample	0.2710	0.93	0.28	0.40
Hours	138.500	64.68	45.83	6.09
Sample x Hours	0.2050	2.01	0.47	0.87
Residual	0.0001	0.02	0.00	0.00

The lactic acid bacteria counts significantly ($p \leq 0.05$) increased with increasing period of fermentation as shown in **Table 4.3**. The wholly maize flour based *mkarango* dough had the least average LAB counts ($5.12 \pm 2.35 \log cfug^{-1}$). Fermentation of *mkarango* dough increased the microbial counts from 3.48-4.45 $\log cfug^{-1}$ to 8.13-9.32 $\log cfug^{-1}$.In their study, Mwizerwa et al. (2018) reported that the optimal increase in microbial counts in maize based *mkarango* dough is achieved in the third day of its fermentation. Muyanja et al.(2004) in their study of *bushera*, sorghum based fermented product, also reported a progressive increase in LAB counts during the 96 hours fermentation period. A study that focused on the fermentation of pearl millet based dough found that the LAB counts were a bit lower than those found in the present study (Onuoha, Orukotan, & Ameh, 2017). The difference in the microbial counts in the fermentation of these cereals is majorly due to the type of cereal used and the fermentative conditions that were observed. Generally in the cereal fermentation, it has been noted that the process mainly

entails the action of LAB culture (Gabaza et al. 2018; Muyanja et al. (2004).Adavachi (2017) reported the consumption of *mkarango* to be up to 14% of the households in some communities in Kenya. Promotion and expansion of the consumption of this product would enable the consumers to harness the health-promoting probiotic-rich property in them. The product can be promoted as a functional food and promoted in strategies aimed at promoting food security

Table 4.2: Lactic acid bacteria counts of traditionally fermented *mkarango* dough(cfug-^{1})

Sample	**Period of fermentation (hours)**			**Average**
	0	**24**	**48**	
A	3.48±0.00^{a}	3.73±0.01^{b}	8.16±0.01^{c}	5.12±2.35^{a}
B	3.58±0.01^{a}	3.86±0.01^{b}	8.74±0.02^{c}	5.39±2.60^{c}
C	3.71±0.00^{a}	4.66±0.00^{b}	8.23±0.04^{c}	5.53±2.13^{f}
D	4.13±0.01^{a}	4.59±0.01^{b}	8.41±0.04^{c}	5.71±2.10^{h}
E	3.67±0.01^{a}	4.38±0.01^{b}	8.58±0.01^{c}	5.54±2.38^{f}
F	3.86±0.00^{a}	4.05±0.00^{b}	9.32±0.01^{c}	5.74±2.77^{i}
G	3.73±0.00^{a}	3.92±0.00^{b}	8.76±0.00^{c}	5.47±2.55^{d}
H	3.65±0.00^{a}	3.72±0.01^{b}	8.13±0.01^{c}	5.16±2.30^{b}
I	3.74±0.00^{b}	4.42±0.00^{a}	8.81±0.00^{c}	5.51±2.24^{e}
J	4.45±0.00^{a}	4.05±0.01^{b}	8.49±0.01^{c}	5.68±2.19^{g}
Average	3.80±0.29^{a}	4.14±0.34^{b}	8.52±0.35^{c}	

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

The period of fermentation, the type of flour and the interaction of the two significantly ($p \leq 0.05$) Influenced the total viable counts as shown in **Table 4.4**. Increasing the fermentation period significantly ($p \leq 0.05$) increased the Total viable counts in the fermented *mkarango* dough. Mwizerwa et al. (2018) reported similar results as upon 5 days of fermentation, *mkarango* dough had a total viable count averaging 7.70 log CFU g^{-1}.Similar findings were reported by Henshaw et al. (2016) as aerobic counts had significant increase in the first 48 hours of fermentation.

Adebayo and Aderiye (2007) reported results for cereal dough that differed with the findings of the present study as a decline of 20.3-37.84% in the aerobic counts was noted after fermentation period of 96 hours. Microbial profile from traditionally fermented cereal products differ depending on the environmental factors and process parameters that are used. Traditionally, fermented cereal based dough have aerobic counts within the microbial profile(Izah, Kigigha, & Okowa, 2016), some of which possess fermentative action. However, total counts to the tune of 9.82 log CFU g^{-1} could present product safety challenges and thus would require proper heat treatment to ensure safety of the final product.

Table 4.3:**Total viable counts of traditionally fermented *mkarango* dough (logcfug^{-1})**

Sample	**Period of fermentation (hours)**			**Average**
	0	**24**	**48**	
A	5.47±0.71^{b}	3.57±0.00^{a}	8.56±0.03^{c}	5.87±2.28^{a}
B	6.05±0.00^{b}	5.38±0.01^{a}	7.74±0.02^{c}	6.39±1.09^{d}
C	6.32±0.01^{b}	5.81±0.01^{a}	8.38±0.03^{c}	6.83±1.22^{f}
D	3.60±0.01^{a}	5.71±0.00^{b}	8.61±0.01^{c}	5.97±2.25^{ab}
E	6.72±0.00^{b}	5.94±0.00^{a}	7.55±0.03^{c}	6.74±0.72^{ef}
F	6.37±0.01^{b}	5.92±0.01^{a}	8.40±0.00^{c}	6.89±1.19^{f}
G	6.64±0.01^{b}	5.34±0.01^{a}	8.66±0.01^{c}	6.88±1.50^{f}
H	4.65±0.43^{a}	4.97±0.01^{b}	8.79±0.00^{c}	6.13±2.07^{bc}
I	6.06±0.00^{b}	5.12±0.01^{a}	8.72±0.02^{c}	6.63±1.67^{e}
J	3.19±0.01^{a}	5.48±0.01^{b}	9.82±0.00^{c}	6.17±3.01^{c}
Mean	5.51±1.25^{b}	5.32±0.68^{a}	8.52±0.60^{c}	

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

The yeast and mould counts of all the composite and maize flour based *mkarango* significantly ($p \leq 0.05$) increased with increasing period of fermentation. This is because the media was amended by addition of tartaric acid to adjust the pH to 3.5%.In the fermentation of sorghum based cereal dough, Ali and Mustafa (2009) reported a similar trend in the yeast and mould counts as they increased with increasing period of fermentation. Teniola et al.(2005)also observed a similar trend of growth of yeast with increasing period of fermentation of ogi; however he observed that the moulds were rather inhibited by the fermentative action of LAB. Yeast are known to be tolerant to acidic pH generated through the process of fermentation and are at times responsible for distinctive sensory qualities of these fermented cereals. Some of the mould species that have also been isolated from the fermentation of cereal dough include *Aspergillus* and *Penicillium*, pointing towards the survival of these microbes in the fermented cereal dough (Osamwonyi & Wakil, 2012).

Table 4.4: Yeast and moulds counts of traditionally fermented *mkarango* (log CFU g^{-1})

Sample	Period of fermentation (hours)			Mean
	0	24	48	
A	2.68 ± 0.01^{a}	4.40 ± 0.00^{b}	5.50 ± 0.01^{c}	4.19 ± 1.27^{b}
B	2.34 ± 0.01^{a}	3.88 ± 0.01^{b}	6.13 ± 0.02^{c}	4.11 ± 1.71^{a}
C	2.55 ± 0.00^{a}	4.72 ± 0.01^{b}	5.38 ± 0.00^{c}	4.22 ± 1.32^{c}
D	3.20 ± 0.00^{a}	4.56 ± 0.00^{b}	5.49 ± 0.00^{c}	4.42 ± 1.03^{g}
E	3.28 ± 0.00^{a}	3.94 ± 0.01^{b}	5.53 ± 0.00^{c}	4.25 ± 1.04^{d}
F	2.91 ± 0.00^{a}	3.51 ± 0.01^{b}	6.46 ± 0.01^{c}	4.29 ± 1.70^{e}
G	2.98 ± 0.01^{a}	4.08 ± 0.01^{b}	6.64 ± 0.00^{c}	4.56 ± 1.68^{i}
H	3.47 ± 0.00^{a}	3.44 ± 0.01^{b}	6.09 ± 0.00^{c}	4.33 ± 1.36^{f}
I	3.24 ± 0.00^{a}	3.81 ± 0.00^{b}	6.43 ± 0.01^{c}	4.49 ± 1.52^{h}
J	3.68 ± 0.01^{a}	4.37 ± 0.01^{b}	6.49 ± 0.00^{c}	4.84 ± 1.31^{j}
Mean	3.03	4.0	6.01	

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

The *Enterobacteriaceae* counts in the *mkarango* dough significantly ($p \leq 0.001$) increased with increasing period of fermentation (Table 4.6).*Mkarango* dough made from whole maize flour had significantly ($p \leq 0.001$) higher *Enterobacteriaceae* counts. This is because whole maize are rich in starch which on fermentation they are converted into simple sugars that are readily available to be utilized by microorganisms as food, hence it's faster growth rate. At 48 hrs, the number of *Enterobacteriaceae* decreases due to an inhibitory effect of the fermentation process through lactic acid production by lactic acid bacteria. Muyanja *et al*. (2003) reported similar results in the study of sorghum based beverages for in as much as an increase from 5.9 to 7.8 log cfu g^{-1} was realized in the coliform count after 24 hours of fermentation, a sharp decline to 4.0 log cfu g^{-1} occurred with extended fermentation for 48 hours. Another study by Abegaz (2014) relates that *Enterobacteriaceae* may initiate the fermentation process of beverages but continued fermentation process would have the LAB counts increase greatly while the *Enterobacteriaceae* are inhibited. *Enterobacteriaceae* and other coliforms can compromise the safety of products thus the inhibition of this class of microbes enhances product safety.

Table 4.5: ***Enterobacteriaceae* counts in traditionally fermented *mkarango* (logcfug^{-1})**

Sample	**Period of fermentation (hours)**			**Mean**
	0	**24**	**48**	
A	5.42±0.00^{c}	3.51±0.01^{a}	3.95±0.00^{b}	4.29±0.89^{g}
B	4.38±0.01^{c}	3.98±0.00^{b}	3.51±0.00^{a}	3.96±0.39^{de}
C	3.86±0.01^{b}	4.41±0.01^{c}	3.62±0.00^{a}	3.96±0.36^{de}
D	3.53±0.23^{a}	3.68±0.00^{b}	3.47±0.01^{a}	3.56±0.14^{b}
E	5.10±0.01^{c}	3.76±0.01^{b}	1.67±0.01^{a}	3.51±1.55^{a}
F	5.19±0.01^{c}	3.79±0.01^{b}	3.05±0.00^{a}	4.01±0.97^{e}
G	3.32±0.03^{a}	3.63±0.01^{b}	3.68±0.01^{b}	3.54±0.17^{ab}
H	5.25±0.01^{c}	3.27±0.01^{a}	3.78±0.01^{b}	4.10±0.92^{f}
I	4.35±0.04^{c}	3.87±0.03^{b}	3.56±0.01^{a}	3.93±0.36^{d}
J	4.28±0.02^{c}	3.74±0.01^{b}	3.52±0.01^{a}	3.84±0.35^{c}
Mean	4.47±0.73^{c}	3.76±0.29^{b}	3.38±0.63^{a}	

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

4.4.3.8 Morphological and biochemical characteristics of LAB from *mkarango* dough

As per the procedure used by Mwizerwa *et al*. (2018), the LAB cultures in the maize based *mkarango* dough was identified as *L. acidophilus.* From the other composite cereal flour based *mkarango* dough; *L. paramesenteroides, L. brevis, L. lactis, L. plantarum* and *L. fermentum* were identified (table 4.7). The LAB cultures were from the heterofermentative group and their fermentative action would generate a mixture of alcohol, lactic acid and carbon dioxide gas (Keles& Demirci, 2011). Studies by Olonisakin et al. (2017) and Onwuakor et al. (2014) concluded that the fermentation of cereals such as sorghum, maize and millet involved the action of heterofermentative bacteria. The study points to a mixture of more than one culture as the determinant of product quality in *mkarango*. This was also established by Mwizerwa et al. (2018) who found a multiplicity of LAB cultures in isolates from *mkarango.*

Table 4.6: Morphological and biochemical characteristics of LAB cultures from *mkarango* dough

Parameter	**Sample (maize, millet and sorghum) composites**									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Grams stain	+	+	+	+	+	+	+	+	+	+
Motility test	-	-	-	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	+	-	+	+	+	+	-	+
Xylose	-	-	+	-	+	+	+	+	-	+
Raffinose	+	±	+	-	+	+	+	+	±	+
Cellobiose	-	-	+	-	+	+	+	+	-	+
Lactose	+	-	-	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	-	+	+	-	-	-	-	+	-
Maltose	+	+	+	+	+	+	+	+	+	+
Mannitol	+	±	+	+	+	+	+	+	+	+
Sucrose	-	-	+	+	+	+	+	+	+	+
	L. acido	*L. p* *L. brevis*	*L.lactis*	*L. p*	*L. ferm*	*L. ferm*	*L. ferm*	*L. ferm*	*L. plant* *L. acid*	*L. ferm*

4.5 Conclusion

Mkarango fermentation mainly involves LAB cultures. Growths of *Enterobacteriaceae*, yeast and moulds and aerobic bacteria also occur in *mkarango*. The high aerobic counts in *mkarango* compromise the safety of the product. The standardization of the product would call for proper thermal treatment to reduce the aerobic counts to acceptable levels.

CHAPTER FIVE

FORMULATION OF LACTIC ACID BACTERIA STARTER CULTURE FOR IMPROVEMENT OF FERMENTED ROASTED MAIZE FLOUR *(MKARANGO)* PRODUCTION PROCESS

5.1 Abstract

Mkarango is a thick roasted porridge produced from maize flour and consumed mainly in the Western regions of Kenya. This study was done in order to characterize and develop fermentative Lactic acid Bacteria (LAB) for *Mkarango* production process so as to improve the safety and quality of Starter cultures of lactic acid bacteria (*Lactobacillus brevis, Lactobacillus plantarum and Pediococcus pentoceus*) isolated from native Cereal flours. The population of Lactic acid bacteria and yeast was enumerated on plate count agar and oxytetracycline Glucose Yeast Extract Agar respectively. The microbes were tested alone or in a mixture for their ability to ferment maize flour in order to produce *Mkarango*. Baker's yeast and milk were also incorporated to simulate the normal environment used during traditional fermentation. Yeasts mixed with lactic acid bacteria (LAB) had a modest effect on the final acidity while all species of Lactic acid bacteria fermented maize flour slurry as judged by the lowering of pH from 6.04 to 4.14 – 4.62 and the increase of the titratable acidity from 0.14% to 0.46–0.63% (w/w, lactic acid) in 12 h. Lactic acid production significantly ($p \leq 0.05$) reduced at a faster rate in maize flour samples fermented by *L. plantarum* or *L. brevis* in co-culture with baker's yeast. Most volatile flavour compounds were produced by *L. pentoceus* and baker's yeast in co-culture with either *L. plantarum* or *L. brevis*. The use of starter culture bacteria in combination with yeasts decreases steeping time of the maize floor and produced acceptable pH results and increased the quantity of the titratable acidity. A mixed culture of LAB and yeast are considered the best starter organism for production of *Mkarango*

Keywords: Cereal fermentation, Lactic acid bacteria, *Mkarango*, Starter cultures, Yeasts

5.2 Introduction

Maize (*Zea mays*) constitute vital ingredient for most common dishes for majority of the Kenyan population. The common maize dishes include thick porridge (ugali), and porridge (De Groote *et al*., 2010). Maize provides caloric needs for majority of consumers in both urban and rural dwellers (Nyoro *et al*., 2007). Fermentation of cereals is one of the oldest traditional methods of food preparation and preservation (Katongole, 2008). In many African societies, fermented foods constitute huge part of the diet which consists of sour milk, cassava, fish and cereals. In many African societies, the traditionally fermented foods served functions in societal ceremonies such as marriage, funeral where they are served as drinks.

Fermentation is a chemical anaerobic process where carbohydrates are converted to carbon dioxide (CO_2) and alcohol. The conversion of carbohydrates into CO_2 and alcohol with the help of yeast is also called fermentation (Adavachi, 2014). Natural lactic fermentation improves the values of cereals (Kunyanga, 2006). Uji slurry from wet milled maize is nutritionally superior to the slurry made from finely sifted maize flour (Mbugua, 1987). Lactic fermentation improves the digestibility of proteins and starch, bioavailability of micronutrients, palatability of porridges as well as imparting antimicrobial properties in them. Steady metabolism involving LAB and yeasts is common in many foods, thus exploitation of substances such as starch that are otherwise non-fermentable and hence improving the microbial adaptability to complex food ecosystems (Gobbetti *et al*., 1994; Stolz *et al*., 1995; Gobbetti and Corsetti, 1997).

Lactic acid bacteria (LAB) and yeasts are the principal microorganisms in many African indigenous fermented foods (Kunene *et al*., 2000) but according to Sanni (1993), the use of starter cultures is an appropriate approach in controlling and optimization of fermentation

process therefore alleviating the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. There have been no LAB starter cultures for commercial use that are available for processing of traditional African foods (Holzapfel, 1997). The development of starter cultures is a prerequisite for establishment of small-scale industrial production of fermented foods in Africa (Sanni, 1993).LAB and yeasts are the major microorganisms common *mkarango* (Mugula *et al*., 2001). The use of isolated strains for cereal fermentation has been reported to minimize dry matter loss, enhance acid production thus reduction in pH levels, improve sensory quality and overall acceptability of different foods, and lastly enhance the nutritional quality of the product through the formation of preservative compounds or a reduction in mycotoxins, such as aflatoxins and fumonisins (Annan *et al*., 2003; Songré-Ouattara *et al*. 2010; Enwa *et al*. 2011; Ekwem 2014). In order to identify those that are function efficiently for the preparation of this product, their role needs to be investigated. Therefore, this study intended to develop a pure starter culture and clarify the role of selected species in their contribution to stability and safety of the product and to the formation of flavor compounds during fermentation in the preparation of *mkarango* within a short time of fermentation.

5.3 Materials and Methods

5.3.1 Sampling of maize floor

Maize grains were bought from the local retail markets then milled at the local posho mill and taken to the microbiology laboratory at the Department of Food Science, Nutrition and Technology at the University of Nairobi for product formulation and analysis.

5.3.2 Preparation of traditional fermented *Mkarango*

The milled maize flour and water were mixed in the ration 1:2 respectively. The mixture was kneaded into a consistent paste and allowed to ferment at the ambient temperature ((28-30^{o}C) for 2-3 days. The mixture was then decanted and roasted for 30 minutes to make *Mkarango.*

5.3.3 Starter cultures

Two cultures of lactic acid bacteria (*L. plantarum,* and *L. brevis* and yeast culture (*S. cerevisiae)* respectively isolated earlier from fermented *Mkarango* were used. The cultures were stored in 50% glycerol at –20^{o}C.

5.3.4 Preparation of inoculum

Stock cultures of *L. plantarum,* and *L. brevis* (lactic acid bacteria) and *S. cerevisiae* (yeasts) isolated earlier from fermented *Mkarango* were sub-cultured in MRS Broth, (MRS, Oxoid CM361) and Malt Extract Broth (Oxoid CM57) respectively. Serial dilutions were done by transferring 1 ml of the sub- cultures into 9 ml growth media which was then incubated for 24 h at 37^{o}C. The cells were then harvested by adding 900µl of distilled water to 100µl of culture to obtain 1:10 dilution ratio (Annan, 2013).The concentration of cells in each dilution was determined by counting under the microscope using the Thomas counting chamber and was determined by multiplying the number of cells by a factor of 10^4: (microscopic count x dilution factor x 10^4).Concentration of 10^7 cells/ml was obtained for LAB and 10^6 cells/ml were obtained for the yeast. For harvesting, the cultures for inoculation were then centrifuged at 5000 rpm for 15 minutes after which the supernatant was discarded and the cultures re-suspended in 4 ml sterile distilled water and centrifuged again. The supernatants were again discarded and the washed cultures reconstituted in sterile distilled water and used to inoculate the whole maize flour.

5.3.5 Fermentation with single starter cultures

For each of the fermentation trails 1.25kg of whole maize flour was soaked in 2.5litres water in 5L capacity plastic container. Either 10^7cfu/ml of lactic acid bacteria or 10^6cfu/ml of yeast was inoculated into the maize flour as single starter culture (*L. plantarum, L. brevis, S. cerevisiae*). The whole maize grains were left at ambient temperature (28-30^{o}C) for 12h and sampled at 0h, 8h, and 12h for determination of pH, titratable acidity and microbiological analysis. After steeping, the grains were recovered, milled and kneaded into dough. The dough was allowed to ferment for 12 hrs, this is because with pure starter culture bacteria acidification is always fast. Sampling was done at 0, 8 and 12h for determination of pH, titratable acidity and microbiological analysis.

5.3.6 Fermentation with combined starter culture

Maize flour of 2.5 kg batches were steeped in 5L water (1:2 w/v) in 10L capacity plastic container. Three separate batches were prepared by adding to the steep water and maize flour, cultures of *L. plantarum*, *L. brevis*, yeast in the following combinations *L. plantarum*+ whole maize flour, *L. brevis*+ maize flour + yeast and *L. pentoceous* + maize flour + milk. Yeasts cultures were inoculated to attain concentrations of 10^6cells/ml while LAB was inoculated to attain concentrations of 10^7 cells/ml. Samples of steeped water and fermenting maize flour were collected for analyses.

5.3.7 Chemical analyses

The titratable acidity was determined potentiometrically according to Nout *et al*. (1989) by titrating 10g maize flour slurry against 0.1 M Sodium hypochlorite using phenolphalein indicator. Acidity was calculated as percent (w/w) lactic acid equivalent. The pH was determined with a pH meter (PHM61, Radiometer, Copenhagen, Denmark) prepared with a glass electrode

(Orion 9102, Orion Research, Boston, MA, USA). The pH meter was calibrated against standard buffer solutions (Merck) at pH 4.0 and 7.0.

5.3.8 Statistical analyses

The collected data was subjected to analysis of variance (Genstat, 2015) and least square differences determined by Duncan's multiple range (LSD) test at 95%.

5.4 Results and Discussion

pH values ranged from 6.19 to 6.24 at the start of fermentation and reduced to between 4.4 and 5.7 after 12 hours of fermentation **(Table 5.1).** The most drastic drop in pH was achieved by the treatment combination of *L. brevis* + Maize Flour + Yeast after 12 hours of fermentation. The other treatment combination also reduced the pH levels but not to the desired levels. Results indicated a steady increase in titratable acidity with corresponding reduction in pH values during the fermentation process and this was more pronounced with treatment combination of *L. brevis* + Maize Flour + Yeast. The results concur with findings by Annan, (2013) and Wakil and Dobson, (2011) who reported reduced pH levels with increased amounts of titratable acid. This can be attributed to increase in growth of lactic acid bacteria and the likely utilization of free sugars by yeasts and lactic acid bacteria (Efiuvwevwere and Akona, 1995).The increase in acidity and resultant drop in pH during fermentation of cereals is as a result of production of lactic acid by fermentative organisms (Wakil and Dobson., 2011).Acidic environments favors proliferation of yeasts in foods and this environment is created by LAB while yeasts stimulates the increase in the number of bacteria, through provision of growth factors such as vitamins and soluble nitrogen compounds. The association of LAB and yeasts during fermentation may also contribute metabolites, which could impart taste and flavour to foods (Akinrele, 1970; Halm *et al*., 1993). The production of acids and other antimicrobial components in maize flour slurry during fermentation may promote or improve the microbiological safety and stability of the

products (Mensah *et al*., 1991).Lactic acid bacteria (LAB) and yeasts are the major microorganisms in the African fermented foods (Kunene *et al*., 2000). According to Sanni (1993) and Kimaryo *et al*. (2000), starter cultures are appropriate for the control and optimization of fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. When introducing starter cultures for fermentations factors such as improvement of processing conditions and product quality, desirable sensory attributes, safety and reduction of hygienic risks should be considered (Holzapfel, 1997). The knowledge gained with controlled starter culture is beneficial to those operating at a very small scale and practicing backslopping. *Mkarango* is fermented cereal flour thick slurry prepared either from maize, sorghum, millet or their combinations (Mugula *et al*., 2001).

The titratable acidity (TA) values at the initial stage (0 hours) ranged from 0.60 to 0.74 and after 12 hours of fermentation the levels ranged from 0.77 to 0.85 (**Table 5.1**). There were no significant differences between the treatments ($p \leq 0.05$). The TA averagely increased from 0.67% from the initial fermentation to 0.82% at the end of fermentation. Titratable acidity increased in all samples from all production runs with a corresponding decrease in pH. Titratable acidity was used to evaluate the rate of acidification during fermentation. The cause of the increase in acidity and consequent drop in pH during fermentation of cereals is as a result of production of lactic acid by fermentative microorganisms. Thus, lactic acid bacteria fermented foods contain lactic acid as preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity.

Table 5.1: Effect of three strains of LABs combined with maize flour on pH and titratable acidity of dried roasted thick porridge (*Mkarango*).

Treatments	0 Hours		12 hours	
	Ph	Titratable acid (%)	pH	Titratable acid (%)
L .plantarum + whole Maize flour	6.19[a]	0.60[a]	5.2[ab]	0.85[a]
L. brevis + Maize Flour + Yeast	6.21[a]	0.74[a]	4.4[b]	0.83[a]
L. pentoceus + Maize flour + Milk	6.24[a]	0.66[a]	5.7[a]	0.77[a]
Mean	6.2	0.67	5.1	0.82
LSD ($p \leq 0.05$)	0.13	0.12	0.8	0.11
CV (%)	1.1	9.0	8.0	6.6

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

A combination of flour and *L. p* and yeast resulted in reduced pH and produced high amounts of lactic acid **(Table 5.2).**A combination of *L. pentocious* and yeast demonstrated higher capacity to acidify maize flour and the fastest rate of acidification. The role of lactic acid bacteria in food processing is to preserve by converting sugars to organic acids thus a reduction in pH (Annan, 2013). Presence of yeast in the study had significant effect on fermentation. Halm *et al*., (1993) reported *Candida* and *Saccharomyces* species as the most frequent during maize fermentation. According to Obiri-Danso, (1994).*S. cerevisiae* is dominant during fermentation of maize dough. Yeasts are known to produce aromatic compounds that are likely to influence the sensory quality of fermented maize dough.

The main aim of choosing a starter culture is to produce food with consistent quality with a preserved sweet flavour and aroma (Glover et al., 2009). In the present study, there was decline in pH with corresponding increase in titratable acidity following treatment application of *L. brevis*. Fermentations with starter cultures of lactic acid bacteria are characterized by a drop in pH. Therefore a mixed culture inoculum of *L. brevis* and yeasts improved the conditions of the final products which resulted in increased rate of souring of the *Mkarango*. Overall,

Lactobacillus brevis demonstrated the fastest rate of acidification and this occurred during 0 h to 12 hours of fermentation.

Table 5.2: Effect of two strains of LABs combined with maize flour on pH and titratable acidity of dried roasted thick porridge (*Mkarango*)

Treatments	0 Hours		12 Hours	
	Ph	Titratable acid (%)	pH	Titratable acid (%)
Flour+ Milk+ L. b	6.08±0.03[a]	0.2±0.02[a]	4.17±0.08[b]	0.61±0.03[a]
Flour + yeast+ L. p	6.12±0.03[a]	0.2±0.02[a]	4.44±0.08[a]	0.51±0.03[b]
Mean	6.11	0.20	4.33	0.56
LSD ($P \leq 0.05$)	0.09	0.06	0.23	0.08
CV (%)	0.07	0.05	0.18	0.06

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

5.5 Conclusion

Increased population of lactic acid bacteria responsible for the fermentation resulted in more production of lactic acid and possible utilization of free sugars by yeast and lactic acid bacteria. Therefore lactic acid fermentation preserves food by converting sugars to organic acids thus causes a reduction in pH. The best starter culture responsible for fermentation was a combination of *L. brevis* and yeast which forms the desired pH value with increased volume of titratable acidity within the shortest time.

CHAPTER SIX

EVALUATION OF MICROBIAL AND NUTRITIONAL QUALITY OF FERMENTED DRIED ROASTED THICK PORRIDGE *(MKARANGO)*

6.1 Abstract

Fermented foods constitute diet in 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. The aim of this study was to evaluate the population of lactic acid bacteria, and sensory characteristics of dried roasted thick porridge (*mkarango*). 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. 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. Fermentation improves the nutritional qualities of food. 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.

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

6.2 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 (Belton and Taylor, 2004).Fermentation is an old form of food preservation in the world (Rhee et al., 2011). Maize are the major sources of carbohydrates in the Western part of the Country and also a source of vitamins, minerals like manganese, zinc, copper and magnesium and considerable iron but its bio- availability is low (Oyarekua, 2011). 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 (Dada and Muller 1970).

Foods can be fermented following different methods through alcoholic, lactic acid and alkali methods (Katongole, 2008). Alcoholic fermentation results in the production of ethanol and yeasts are the main organisms for beers as well as wine. However, lactic acid fermentation is mainly done by lactic acid bacteria and acetic acid producing bacteria. Fermentation is important as it 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 (Sindhu and Khertarpaul, 2001).

The process of fermentation through microorganisms is a complex process, this process is natural and involves a mixture of cultures of yeasts, bacteria and fungi (Katongole, 2008). Mostly used fermenting bacterial species include *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus* while the fungal genera include *Aspergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium* and *Trichothecium* while the most common fermenting yeast species is *Saccharomyces*, which is involved in alcoholic fermentation (Steinkraus, 1998; Nigatu, 1998; Lei, 2006).

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 (Perez-Chabel, *et al*., 2006). 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 (Badanirath, *et al*., 2009). The aim of this study was to study the growth of microorganisms in cereal based fermented products during fermentation period of 24 hours.

6.3 Materials and Methods

6.3.1 Preparation of samples

400 ml 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 aslurry prior to inoculation.

6.3.2 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. Inoculated samples were thoroughly mixed and incubated at 30^0C. At 0, 4, 8, 12, and 24 hours intervals of fermentation, the samples were withdrawn for microbial counts, pH and organic acids(Lactic acid) analysis. The experiments were replicated three times (Oguntoyinbo, 2008).

6.3.2.1Chemical analyses

The titratable acidity was determined potentiometrically according to Nout*et al*. (1989) 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 was determined with a pH meter (PHM61, Radiometer, Copenhagen, Denmark) equipped with a glass electrode (Orion

9102, Orion Research, Boston, MA, USA). The pH meter was calibrated against standard buffer solutions (Merck) at pH 4.0 and 7.0.

6.3.2.2Enumeration of LAB, *Enterobacteriaceae* and yeasts/molds

The pure bacterial cultures in flour pellets were inoculated into appropriate maize flour slurry. Duplicate samples of maize flour slurry (10 ml) were homogenized in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, 1000 ml distilled water, pH 7.0F0.2). The homogenate was decimal diluted and the relevant dilutions surface plated. For the enumeration of lactic acid bacteria (LAB)MRS agar (Merck) plates with 0.1% (w/v) natamycin were incubated anaerobically at 30^0C.*Enterobacteriaceae* was incubated on violet red bile glucose agar (VRBGA, Oxoid) at 37^0C and Yeasts were enumerated after incubation for 3–5 days at 25^0C on potato dextrose agar (PDA, Oxoid).

6.3.3 Sensory evaluation

A panel of 10 persons evaluated the sensory properties of the different fermented *Mkarango* samples. The panel consisted of trained staff and semi trained students from the Department of Food Science and Technology, University of Nairobi. Each parameter in sensorial analysis was evaluated using a 5 point hedonic scale. The panelists on sensory evaluation sheet did not have to mark on the basis of the intensity perceived 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.

6.3.4 Mineral content analysis

The analyses for essential minerals, zinc and iron were determined using AOAC 1997 method by Atomic Absorption Spectrophotometer (Perkin Elmer, model 402) method (Oladeji *et al*., 2018).

6.3.5 Statistical analyses

The data obtained were subjected to analysis of variance (Genstat, 2015) and mean differences determined by Duncan's multiple range or the least square difference (LSD) test ($P \leq 0.05$).

6.4 Results and Discussion

6.4.1 Chemical analysis

There was significant difference ($p \leq 0.05$) in the pH of cofermenting mixtures after 0, 4, 12 and 24 hours **(Table 6.1)** leading to acid production. Product D1, after 0, 4, 8 hours had the highest pH readings while treatment A1 had the least pH reading. However, the pH reading was from 5.9 to 5.6 after 8 hours. After 12 hours, product C1 recorded the highest pH reading while product F1 recorded 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 significantly ($p \leq 0.05$) in all the products 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). 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 (2008) 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. During fermentation, lactic acid bacteria produce lactic acid that causes reduction in pH due to increased colony forming units of lactic acid bacteria (Jay *et al*., 2005). Rapid decrease in pH is accompanied by intensive increase in lactic acid (Zdolec *et al*., 2008). The decrease in pH may be due to availability of nutrients in the products that may enhance the population of lactic acid bacteria and their subsequent production of lactic acid (Oyerakua, 2011).

Table 6.1: 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 ($P \leq 0.05$)	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 ($p \leq 0.05$).

6.4.2 Titratable acidity

There were significant differences ($p \leq 0.05$) for the products and fermentation time **(Table 6.2)**. 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). Similar results in maize steeping and dough fermentation by Kalui *et al*; 2009).Wakil and Daodu (2011).This observation could be due to the increased population of lactic acid bacteria responsible for fermentation which resulted in increased volume of lactic acid (Efiuvwevwere and Akona, 1995).

Table 6.2: 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 ($P \leq 0.05$)	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 column are not significantly different between the treatments using Fishers Protected LSD test ($p \leq 0.05$).

6.4.3 Microbial analysis

Table 6.3 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 ($p \leq 0.05$). 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 ($p \leq 0.05$) 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.* 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 increase with increase in fermentation time. The population of yeasts and mould were high and continued to increase 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 (Oyerakua, 2011). According to Hama, *et al. (*2009) lactic acid bacteria are stimulated by yeasts which act as a source of soluble nitrogen compounds and vitamin B.

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. There was a high population of lactic acid bacteria. 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 (Oyerakua, 2011) 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 available oxygen and produce carbon dioxide which is inhibitory to growth of probable microorganism involved in decay like *Enterobacteriaceae*

(Abegaz *et al*., 2002). The small population of *Enterobacteriaceae* might also be due to the fact their growth might have been inhibited by the presence of lactobacilli which produced lactic acid. (Omemu *et al*., 2007).

Table 6.3. 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 ($P \leq 0.05$)	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 ($P \leq 0.05$)	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 ($P \leq 0.05$)	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 using Fishers Protected LSD test ($p \leq 0.05$).

6.4.4 Sensory evaluation

The sensory analyses results of the products with different ingredients are presented in **Table 6.4**. Results of the final products showed that 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 combination of yeast, and *L. Plantarum+ L. brevis* was evaluated 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. The overall acceptability was highest for product prepared from Milk+ *L. Plantarum+ L. brevis* (2:1) which scored 4.7 on the 5-point hedonic scale. Sensorial evaluation of the final products showed that increased 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 arises from microbiological and physicochemical processes like enhanced acidification and proteolysis microorganisms added (Zdolec *et al*., 2008).

Table 6.4: 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.

6.4.5 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. 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. Significant difference was ($p \leq 0.05$) found in trace mineral contents in the sampled products. The results agree with those that were reported by Blair et al., (2009) 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 (2013), who reported lower iron value of 0.64 mg/100g and zinc value of 1.13 mg/100gfor 90% maize flour.

Table 6.5: 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 ($P \leq 0.05$)	0.2	2.2
CV (%)	0.2	19.7

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

6.4.6 Correlation analysis

According to correlation analysis, significant but negative relation was displayed between pH and titratable acid (-0.8463, $p \leq 0.05$) (Table 6). However, positive correlation was displayed between pH and *Enterobacteriaceae* (0.3977, $p \leq 0.05$). Isolation frequency of yeasts and molds negatively correlated with population of LABS and *Enterobacteriaceae* (-0.4062, -0.0639, $p \leq 0.05$). The observation of a negative correlation has been reported by Efiuvwevwere and Akona, (1995) and this was attributed to increased population of lactic acid bacteria that is responsible for fermentation of the *Mkarango* which had produced more lactic acid and likely utilization of free sugars by yeast and lactic acid bacteria

Table 6.5: 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	-

6.5 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.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General Discussion

Fermentation of maize flour results into production of functional foods which have bioactive components that have beneficial health effects to the consumers. During fermentation, lactic acid, acetic acid, ethanol and other antimicrobials are formed by the microbiota and these are responsible for inhibition of pathogenic microorganisms that can grow in food (Avit *et al.*, 2014; Ojokoh *et al.*, 2015).

Different composite cereal flours, the period of fermentation and the interaction of the two factors affected the pH, titratable acidity and tannin content. The results indicate that increasing the length of the period of fermentation decreases the pH of the product. The findings agree with the results from similar studies by Alka*et al*., (2012)and Marko *et al*., (2014) where increasing the period of fermentation decreased the pH further. Lactic acid bacteria utilize fermentable sugars in the cereals to release lactic acid that is responsible for the reduction of the pH of the cereals. Fermentation of cereals resulted in the reduction of anti-nutritional such as phytic and tannins. This agrees with the findings of Roger *et al*., (2015) and Ojha *et al*., (2018) who reported decreases volumes of tannins and phytic after fermentation of corn and sorghum respectively. LAB cultures have phytase enzymes that is known to break down the phytic acid into inorganic phosphates (Coulibaly *et al.*, 2011; Osman, 2011). Reduction of anti-nutritional factors has been promoted as a way of ameliorating nutritional quality of products known to be high in anti-nutritional factors.

The wholly maize flour based *Mkarango* had high levels of microbial counts. According to Mwizerwa et al. (2018) the optimal increase in microbial counts in maize based *mkarango*dough is realized in the third day of fermentation. Muyanja *et al*., (2004) reported a progressive increase in LAB counts during the 96 hours fermentation period. However, Onuoha, et al., 2017) found low LAB counts in fermented pearl millet based dough compared with our present study. The difference in the microbial counts in the fermentation of these cereals is mainly due to the type of cereal used and the fermentative conditions that were observed. Microbial profile from traditionally fermented cereal products differ depending on the environmental factors and process parameters that are used. Traditionally, fermented cereal based dough have aerobic counts within the microbial profile (Izah, Kigigha, & Okowa, 2016). The yeast and mould counts of all the composite and maize flour based *mkarango* increased with increasing period of fermentation. These results are comparable to those of Ali and Mustafa (2009). Yeasts are known to be tolerant to acidic pH generated through fermentation and are responsible for distinctive sensory qualities of these fermented cereals. Some of the mould species that have also been isolated from the fermentation of cereal dough include *Aspergillus* and *Penicillium*, pointing towards the survival of these microbes in the fermented cereal dough(Osamwonyi& Wakil, 2012). Starter cultures are appropriate for the control and optimization of fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. The knowledge gained with controlled starter culture is beneficial to those operating at a very small scale and practicing backslopping. A combination of *L. brevis* and yeast demonstrated higher capacity to acidify maize flour and the fastest rate of acidification. The role of lactic acid bacteria in food processing is to preserve by converting sugars to organic acids thus a reduction in pH (Annan, 2013). The main aim of

choosing a starter culture is to produce food with consistent quality with a preserved sweet flavour and aroma (Glover et al., 2009).

Sensorial evaluation of the final products showed that increased ratio of plantarum with corresponding reduction of brevis had positive impact on the sensory characteristics evaluated and on the overall quality of the product. Product samples were rich in trace minerals, iron and zinc contents. The results agree with those that were reported by Blair et al., (2009) who reported increased values for both zinc and iron. However, the results contradict the findings by Adeoti (2013) who reported lower iron value and zinc value in maize flour.

7.2 Conclusions

The fermentative action reduces the pH and phytic acid and tannin contents of the products while increasing the titratable acidity. However, this is largely dependent on the period of fermentation and the type of flour that has been used as the ingredient. Fermentation of cereals products involves the action of hetero fermentative bacteria and *Mkarango* fermentation mainly involves Lactic Acid Bacterial cultures. This study points to a mixture of more than one culture as the determinant of *Mkarango* product quality, since multiple LAB cultures were in isolates from *mkarango.* Increased population of lactic acid bacteria responsible for the fermentation resulted in more production of lactic acid and possible utilization of free sugars by yeast and lactic acid bacteria. Therefore, lactic acid fermentation in preserve food, by converting sugars to organic acids causes a reduction in pH. The lactic acid bacteria responsible for fermentation is *L. brevis* which forms the desired pH value with increased volume of titratable acidity. Growths of yeast and moulds and aerobic bacteria also occur in *mkarango.* 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

7.3 Recommendations

i. The best starter cultures for *mkarango* production consisted of *L. brevis* and yeast. Therefore, the LAB *L. brevis* is recommended for production and preservation of traditional foods

ii. Its recommended that high standardizations procedures be applied when preparing *mkarango* since there were high isolations of aerobic microbes which may interfere with safety of the product

iii. People should embrace *mkarango* prepared through controlled fermentation, since it is safe and nutritious for consumption.

iv. Additional research should be done on the potential effect of fermentation of cereal products on other elements and amount of vitamins

v. Additional research should also be done on the preservative activity of Lactic Acid Bacteria, yeasts and molds

vi. Molecular characterization of different lactic acid bacteria (LAB) should be done to confirm species of the dominant lactic acid bacteria and yeasts responsible for *Mkarango* fermentation.

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APPENDICES

Appendix 1.1: SENSORY EVALUATION QUESTIONNAIRE

Date.....

Gender.....

Respondent number.....

Kindly rate the sensory parameters 1 to 7

Sample / Parameters	Sample A	Sample B	Sample C	Sample D
Color				
Texture				
Aroma				
Taste				
Overall acceptability				

Score scale

1. Dislike very much
2. Dislike slightly
3. Dislike moderately
4. Neither like nor dislike
5. Like moderately
6. Like slightly
7. Like very much

Total score:

THANK YOU FOR PARTICIPATING