EFFECT OF KENYAN FERMENTED MILK ON *ESCHERICHIA COLI*

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ABSTRACT

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Different kinds of fermented milks have been used as probiotics in many societies for many centuries. To investigate the effect of Kenyan fermented milk on *Escherichia coli*, a starter culture obtained from one family of the Gusii community of southwestern Kenya, was used to make traditionally fermented milk using 2 % pasteurized milk. Approximately equal populations of *E. coli* were introduced into equal replicate volumes of Kenyan fermented milk and commercial yogurt samples. The inoculated samples were incubated at 37 °C and populations of *E. coli* were determined after every two hours for a period of eight hours. Results indicated significant decline ($p \leq 0.01$) in *E. coli* population within the first two hours and the bacteria was not detectible in both Kenyan fermented milk and yogurt after four hours. The inhibitory effect of the Kenyan fermented milk was significantly ($p \leq 0.01$) higher than that of commercial yogurt and it had no detectible *E. coli* after the first two hours of incubation.

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CHAPTER 1. INTRODUCTION

Introduction

This research paper is studying on one aspect of African fermented milk for use as a probiotic. Traditionally fermented milk, using a starter culture obtained from one family of the Gusii community of the southwestern part of Kenya, was produced and its effects on the growth of *E. coli* were determined. The fermentation process has been used in preservation of milk in many African communities for many years. Milk fermentation technique has been passed on from generation to generation without significant changes. However, methods of fermentation vary from community to community resulting in fermented milks that differ in terms of flavor and texture.

FAO (1990) estimated that the annual average milk consumption in African pastoralist communities to be about 19-30 Kilograms per capita, which is way below the recommended 62.5 Kilograms per capita. Low levels of consumption could be due poor milk production by milk producing animals, which constitute the African zebu cattle and goats. African weather and tropical diseases also influence productivity of the animals. On the other hand, many African people do not appreciate fresh pasteurized milk. This could be due to the fact that about 80% of African people are lactose intolerant. A condition characterized by inability to digest lactose as result of deficiency of lactase enzyme (Lore, 2003). According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, 2003), people with lactose intolerance experience cramps, bloating, gas and diarrhea soon after consuming foods containing lactose.

The fermentation process, depending on the type of microorganisms and temperature conditions used, results in different kinds of fermented milk products. In rural Kenya, pasteurized milk is allowed to ferment spontaneously in sanitized calabashes or gourds. The process of calabash/gourd sanitization varies from one community to another. Some smoke the inside of the gourd to kill pathogenic and spoilage microorganisms. In other cases the sanitized gourd is rinsed with ashes of certain woods like *Acacia busia* and *Olea africana*, whereas other communities simply sanitize the gourd with hot water before introducing the pasteurized milk and letting it ferment. These different ways of gourd sanitization combined with different environmental conditions result in fermented milks with different attributes in terms of texture, acidity, flavor and color. For these reasons there are almost as many different fermented milks as there are different communities/tribes in Kenya. Examples of these are *Mursik* from the Kalenjin and *amabere amaruranu* from the Gusii tribes respectively. Some tribes in Africa do not pasteurize milk before letting it ferment resulting in a wider variation in quality of fermented milk products. In addition to the processes, fermented milk products in Africa differ in characteristics from one region to another depending on indigenous microflora in the environment (Savadogo et al, 2004; Adebesin et al, 2001).

Lactic acid bacteria and other microorganisms in the milk convert lactose into lactic acid during fermentation, which in turn reduces the pH below the isoelectric point of most of milk proteins. This causes formation of a coagulum/curd of gel-like consistency. Apart from the conversion of lactose into lactic acid with subsequent change in texture, the resulting sour flavor makes fermented milk a desirable product to consume.

Not many experiments have been done to isolate and identify the kinds of microorganisms involved in spontaneously fermented milk in Africa, (Savadogo et al, 2004). Families within different communities of Africa have maintained same starter culture for generations by using previously fermented milk to initiate fermentation of new milk. In Kenya rural families at the farms maintain this practice. Some dairy processing plants in Kenya using specific lactic acid bacteria produce similar fermented milk products namely: *Maziwa lala (Mala^{TM})* and yogurt, which are very popular to urban residents (Lore, 2003).

According to Branca & Rossi (2002), fermented milks are not nutritionally any different from unfermented milk. A number of health benefits have been attributed to consumption of fermented milks, which include: enhanced bioavailability of iron and calcium as well as being an excellent source of protein and phosphorous, control of undesirable pathogens in the gastrointestinal tract, improved immune response, enhanced lactose digestion, detoxification action, control of serum cholesterol and lowering blood pressure in hypertensive individuals (Nakazawa and Hosono, 1992; Branca and Rossi, 2002; Lore, 2003; Chuayana Jr. et al, 2003 & Seppo et al, 2003). Due to the fact that there are numerous types of fermented milks, more studies need be conducted to provide more information about these enormous health benefits.

Fermented milks are part of that class of foods that contain live microorganisms referred to as probiotics which when consumed provide beneficial health effects to the body (Tamime, 2002). It has been postulated that probiotic microorganisms provide protection against gastrointestinal pathogens and toxins. Their mode of action include: adhering to intestinal lining and inhibiting pathogen growth, production of exopolysaccharides which protect intestinal lining, production of antimicrobial chemicals known as bacteriocins and reduction of intestinal pH by lactic acid production (Tamime, 2002). Studies have been conducted to demonstrate antimicrobial effects of some commercially fermented milk products against a number of food pathogens such as *Salmonella* spp, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Enterobacter aerogenes* (Eguchi et al, 1998; Asahara et al, 2001; Savadogo et al, 2003; Chuayana Jr., 2003).

Further research needs to be done on these antimicrobial effects considering the fact that there are many types of fermented milk products with varied strains of microorganisms involved in fermentation. Information obtained from such studies will contribute to development of strategies for combating foodborne diseases. According to Jones and Gerber (2001) about 76 million foodborne infections, with the illnesses costing about 23 billion dollars annually, occur in the United States alone.

Statement of the study

While growing up in the Gusii community in Kenya, this author observed that young children whose diet included a daily intake of fermented milk seldom suffered diarrheal infections. Many diarrheal diseases in children and adults result from foodborne infections and intoxications. The purpose of this study was to determine the antimicrobial effect of one kind of Kenyan fermented milk on *E. coli.* Some strains of *E. coli* cause food borne infections and intoxications in humans. The study was conducted by producing traditionally fermented Kenyan milk (amabere amaruranu) using a starter culture that was obtained from one family of the Gusii tribe of Kenya. In this study, antimicrobial effects of Kenyan Fermented milk were compared to those of commercial plain yogurt, which is consumed in the western world.

Objectives of the study

The objectives of this study were to:

1. produce Kenyan fermented milk using the starter culture from the Gusii community of Kenya.
2. determine the general microbial quality of Kenyan Fermented milk
3. determine and compare the effect of Kenyan Fermented Milk to that of Commercial Plain Yogurt on the growth of *Escherichia coli.*

Significance of the study

It is anticipated that this study will contribute to the body of information about traditionally African fermented milk varieties to help consumers and researchers gain deeper understanding and use the information when making specific decisions about such products. In Africa most of the fermented milk products rely on spontaneous fermentation techniques and for this reason the microbial composition of starter cultures of these products are relatively unknown. African fermented milk products might possess significant health benefits as probiotic food to humans but they are difficult to commercialize. Therefore attributes of traditionally African fermented milks need more exploration.

Limitations of the study

This study was conducted as an academic project to fulfill the requirements for the author's Masters Degree and the experiments that were conducted were based on materials available in the Microbiology Laboratory at the Biology Department of the University of Wisconsin-Stout. The author's financial constraints were also a significant limiting factor. Therefore the design of the experiments that were conducted was exploratory, time-limited for a two-credit project.

Definition of terms

1. Amabere amaruranu: Fermented milk in Gusii tribal language.

2. Antimicrobial: Any substance that destroys or suppresses proliferation of microorganisms.

3. Bacteriocins: Proteins produced by certain strains of bacteria, for destroying closely related bacteria (Mitsuoka, 1978 pp 105).

4. Exopolysaccharides: Complex sugars secreted by microorganisms to cover their cell walls when surrounding conditions are harsh.

5. Homofermentation: A fermentation process in which only one type of compound is produced as the end product.

6. Heterofermentation: A fermentation process in which a mixture of two or more different compounds are produced as end products.

7. Maziwa lala (Mala^{TM}): Fermented milk in Kiswahili language.

8. Mursik: Fermented milk in Kalenjin tribal language.

9. Spontaneous fermentation: A process in which a food material is allowed to ferment using naturally inherent fermentative microorganisms.

CHAPTER 2. LITERATURE REVIEW

Introduction

Use of milk from animals for direct consumption and making different kinds of dairy products, has been demonstrated in different societies as part of their cultural heritage for many years (Nakazawa & Hosono, 1992). As the physiological effects of fermented milk are getting clearer as a result of information obtained from research, the value of such products is much more likely to be appreciated by consumers. This chapter will review various publications on different aspects of fermented milks including milk fermentation technology and the importance of fermented milks to humans.

Milk

Sources of milk

The main sources of milk are domesticated ruminant animals such as cows, goats, camels and sheep. Worldwide population of domestic ruminants and corresponding milk production is shown in Table 1. According to Marth and Steele (1998), there are about 3 billion domesticated ruminants in the world, of which dairy cattle are the major producers of milk for human consumption.

Table 1: Worldwide population of domesticated ruminants and milk production
(Source: Food and Agricultural Organization (1993) as quoted by Marth & Steel, 1998. p. 2).

Species	Population (10^6 head)	Milk production (10^6 metric tons)
Cattle	1,284	
Dairy cattle	225	455.0
Sheep	1,138	7.8
Goats	574	9.6
Buffalo	147	0.5

Composition of milk

Major constituents of milk are water, fat, protein, carbohydrate (in the form of lactose) and mineral matter or ash. Milk composition varies depending on the type of feeds given to the animals, type of animal and breed (Marth & Steel, 1998). Average composition of milk from different domesticated animals is shown in Table 2.

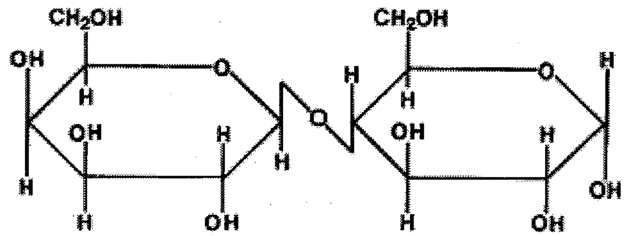
Table 2: Mean composition of milk from domestic ruminants (Source: Bondi (1983) as quoted by Marth & Steel, 1998. p. 8)

	Percent by weight in milk of		
Component	Cow	Goat	Sheep
Fat	3.5	4.5	7.4
Protein	2.9	2.9	5.5
Lactose	4.9	4.1	4.8
Ca	0.12	0.13	0.20
P	0.10	0.11	0.16

Water constitutes 80-87 % of milk. Milk fat is present in milk in the form of small globules dispersed in milk plasma. The fat molecules comprise triglycerides consisting of glycerol esterified with fatty acid chains with 4-20 carbon atoms. Milk protein comprises casein, which constitutes about 82%–86% of total milk protein and whey proteins or globulins. The casein complex is dispersed in milk in the form of colloidal suspensions or micelles, while whey proteins are present as a solution (Marth & Steel, 1998; O'Connor & Tripathi, 1995). Lactose is the major carbohydrate in milk that is made up of two monosaccharides: D-glucose and D-galactose linked by an α-1,4 glycosidic bond as illustrated in the chemical equation and structural formula shown in Figure 1.

$$\underset{\text{D-glucose}}{C_6H_{12}O_6} + \underset{\text{D-galactose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + \underset{\text{Water}}{H_2O}$$

Figure 1: Structural formula of lactose



Nutritional value of milk

Milk is an excellent source of major nutrients essential for human development including: proteins, carbohydrate, fat, minerals and vitamins especially A, D, E and K. Milk protein is rich in essential amino acids making milk and milk products very important constituents of the human diet. Carbohydrate in milk is in the form of lactose, which is difficult to digest in lactose intolerant individuals. Lactose intolerant individuals, especially young children cannot enjoy the nutritional benefits of consuming milk. Clinical signs of lactose intolerance include bloating and gas production as a result of lactose breakdown of undigested lactose by bacteria in the lower portion of the gastrointestinal (GI) tract. Consumption of fermented milk has been shown to suppress clinical signs of lactose intolerance with enhanced nutritional and health benefits (Branca & Rossi, 2002).

Milk Fermentation

Definition

Fermentation refers to enzymatically controlled anaerobic breakdown of a carbohydrate to an organic acid or to carbon dioxide and alcohol (O'Connor & Tripathi, 1995). Fermentation should be distinguished from putrefaction, which refers to decomposition of food constituents manifested by unpleasant changes in flavor as a result of uncontrolled growth of microorganisms (Nakazawa & Hosono, 1992).

Why there is variety in fermented milks

There are hundreds of fermented milk preparations which differ in terms of microorganisms and methods used in their preparation (Nakazawa & Hosono, 1992; O'Connor & Tripathi, 1995). Attempts have been made to classify fermented milks and not one of them has been adopted to be the standard method for classification. In some studies, classification has been based on the type of fermentation: those using only lactic fermentation such as yogurt, sour cream and buttermilk on one hand, and those that use alcoholic fermentation involving use of yeasts, as well as lactic acid bacteria as in kefir and koumiss milks on the other (Nakazawa & Hosono, 1992). Other parameters that can be used to classify fermented milks include: texture, gas production during fermentation and the types of bacteria used in the starter culture, according to Kurmann (1984); Kosikowski (1977); Gordin (1980); and Tamime & Robinson (1978) as quoted by Nakazawa & Hosono (1992). Worldwide examples of fermented milk preparations and regions of origin are shown in Table 3. Fermentation of milk results in new products with variety in flavor, pleasant taste and more importantly, it helps to preserve the milk by suppressing spoilage and pathogenic microorganisms.

Table 3: Some fermented milk preparations and their region of origin
(Source: O'Connor & Tripathi, 1995)

Fermented milk product	Region of origin
Yogurt	Turkey
Laban	Middle East
Skyr	Iceland
Kefir	Balkans
Koumiss	Mongolia
Irgo, Ititu	Ethiopia
Mala	Kenya
Mursik	Kenya
Acidophilus milk	Europe

Microorganisms used in milk fermentation

The system of fermentation in milk is a complex one. It is based on enzymatic breakdown of lactose to produce lactic acid with subsequent lowering of milk pH. Preservation of dairy products by lactic fermentation is one of the oldest methods. Fermented milk is characterized by sour flavor and coagulation of milk protein. Microorganisms used in the preparation of fermented milks include lactic acid bacteria (LAB) species of genera streptococci, lactobacilli, enterococci, pediococci, bifidobacteria, yeasts and molds (See Table 4). Yeasts are responsible for the production of alcohol in kefir and koumiss milk products (O'Connor & Tripathi, 1995). Other compounds resulting from milk fermentation include citrates, acetates, short chain fatty acids (SCFAs), peptides, exopolysaccharides and bacteriocins. Bifidobacteria share some common characteristics with LAB, but they are phylogenetically unrelated. Bifidobacteria have a unique system of sugar fermentation.

Table 4: Microorganisms used in fermented milks (Source: Nakazawa & Hosono, 1992. p 7)

Genus	*Habit*	*Fermentation*	*Main species*
Streptococcus	Coccal chains	Homo	*S. cremoris, lactis, thermophilus*
Leuconostoc	Coccal pairs	Hetero	*L. citrovorum, mesenteroides*
Lactobacillus	Rods	Homo	*L. acidophilus, bulgaricus, casei, jugurti, lactis*
Bifidobacterium	Rods	Hetero	*B. bifidum, breve, longum*
Others			
Yeasts (*Torulopsis, holmii; Saccharomyces fragilis, cerevisiae, lactis; Candida pseudotropicalis*, etc.)			
Molds (*Geotrichum candidum*)			
Acetic acid bacteria (*Acetobacter acetii, rasens*)			

Types of lactic acid bacteria used in milk fermentation

Lactic acid bacteria involved in lactose fermentation are classified into two main groups as homofermentative and heterofermentative based on the pathway they use in sugar metabolism (See Table 4). The homofermen-tative types produce lactic acid from lactose. *Lactobacillus bulgaricus* and *Lactococcus lactis* are examples of homofermentative bacteria. The heterofermentative LAB metabolize glucose to produce carbon dioxide, alcohol, as well as acetic acid in addition to producing lactic acid. Common hetero-fermentative bacteria include strains of *Lactobacillus* and *Leuconostoc* species (O'Connor & Tripathi, 1995). The two metabolic pathways of LAB are illustrated in Figure 2.

Lactose Fermentation

The lactose depends on its mode of transportation into the cell. In some microorganisms lactose may enter the cells as free disaccharide molecules whereas in others it enters as sugar phosphates. That is, lactose is transported by a phosphoenolpyruvate (PEP) depending on the presence of the enzyme lactose permease or phosphotransferase system (PTS). In those bacteria where lactose enters the cytoplasm as free sugar, the enzyme β–galactosidase hydrolyses the sugar into glucose and galactose. Glucose and galactose are then metabolized in different pathways to yield lactates and other compounds (Marth &Steel, 1998; Salminen & von Wright, 1998). In the case where lactose enters the cell as a sugar phosphate, the lactose phosphate is hydrolyzed by the enzyme phospho-β-galactosidase to produce galactose-6-phosphate and glucose, which are then metabolized through tagatose-6-phosphate and Embden-Meyerhof pathways to yield lactates (Marth &Steel, 1998; Salminen & von Wright, 1998).

Starter cultures

Definition

A starter culture is any active microbial preparation that is intentionally added during product manufacture to initiate desirable changes (Marth &Steel, 1998).

Purpose of starter cultures

Starter cultures have a number of roles depending on the type of dairy product being manufactured. In fermented milk, it is their ability to produce lactic acid and low molecular weight compounds such as diacetyl contribute to flavor and aroma that is desirable. Their ability to rapidly produce acid not only helps in preservation of fermented milks, but also contributes to modification of product texture through formation of curd (Marth &Steel, 1998).

Spontaneous fermentation takes advantage of the actions of different strains of lactic acid bacteria naturally present in milk. Spontaneous fermentation is difficult to control. In some traditional African communities, some small amount of good quality spontaneously fermented product is set aside and later on added to pasteurized milk to initiate fermentation in order to maintain same quality (Lore, 2004).

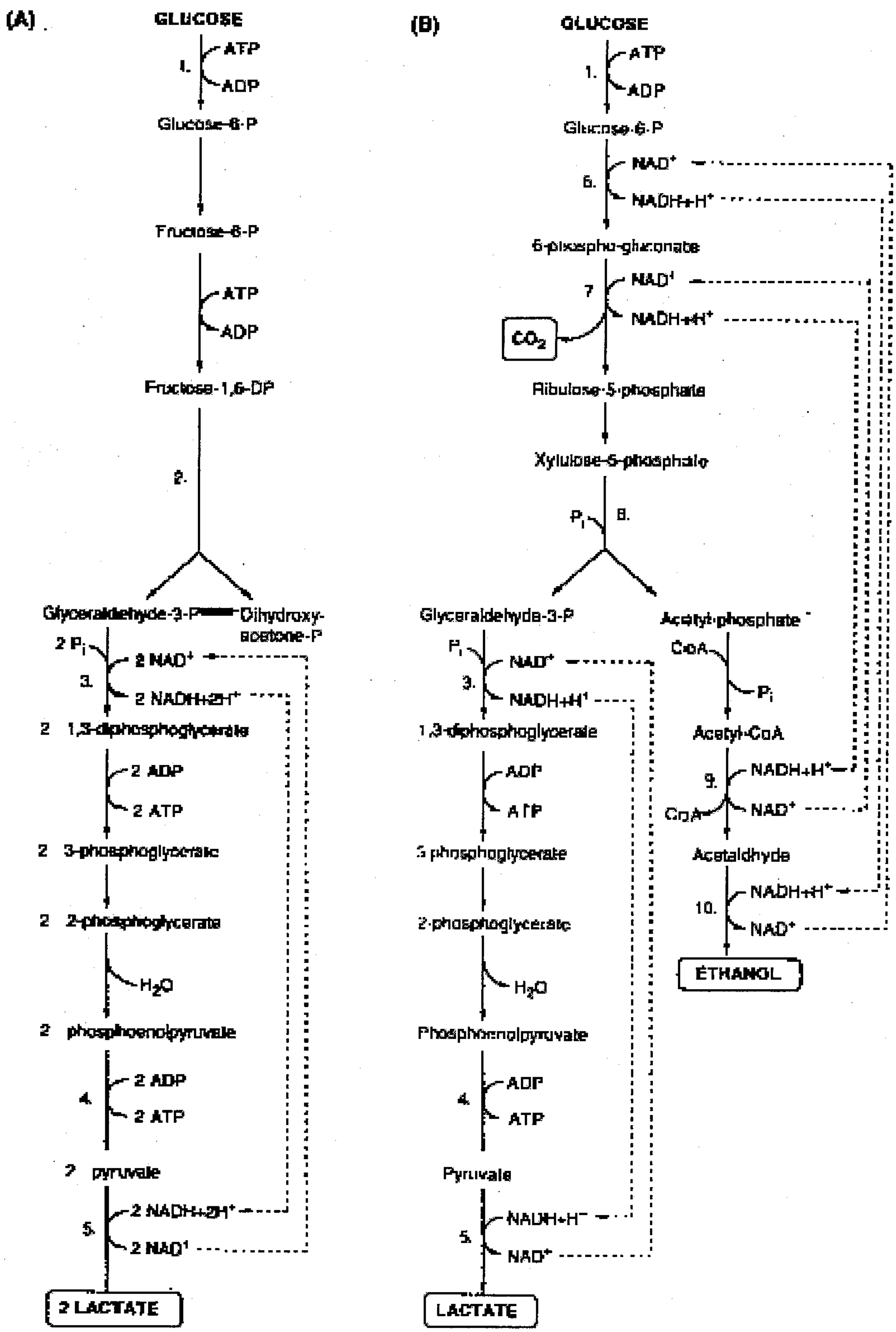


Figure 2: Fermentation pathways of glucose. (A) Homolactic fermentation (glycolysis, Embden-Meyerhof pathway); (B) heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway). (Source: Salminen & von Wright, 1998)

Types of starter cultures

The most common starter cultures used in production of fermented milks comprise different strains of LAB. The starter culture may consist of one strain of microorganism, referred to as single-strain culture; or a number of strains and/or species, referred to as multi-strain or mixed-strain culture (Marth &Steel, 1998; Robinson, 1981). Starter cultures are classified into two main categories as: mesophilic (optimal growth temp. 20-30° C) and thermophilic (optimal growth temp. 40-45° C), according to their optimal growth temperatures. Examples of mesophilic LAB include: some species of *Leuconostoc* and *Lactococcus*. Commonly used thermophilic LAB are: *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* sub species *bulgaricus* and *lactis*.

Manufacture of fermented milks

There are various methods used in the manufacture of fermented milk products depending on the type of product but they have many common features (Tamime, 2002; O'Connor & Tripathi, 1995). Figure 3 and Table 5 illustrate major steps and requirements for controlled preparation of some popular fermented milks.

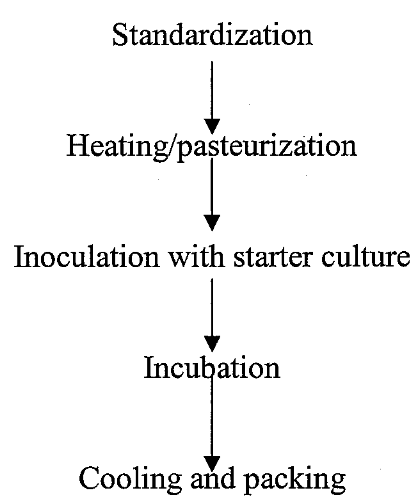


Figure 3: Fermented milk preparation flow sheet (Source: O'Connor & Tripathi, 1995)

Standardization step is done to adjust milk fat to a desired level as well as fortify milk with milk solids-non-fat to produce a product with better consistency. The next step is heating/pasteurization to kill pathogens and spoilage organisms, destroy bacteria that may compete with starter bacteria and give the final product desired consistency. The heating temperatures vary within ranges 65° C for 30 minutes and 72° C for 15 seconds or heating at near boiling temperatures at 85° C for 30 minutes and 95° C for 5 minutes for pasteurization. After heating, the milk is cooled to the required fermentation temperature (26° C for mesophilic starters and 43° C for thermophilic starters) before it is inoculated with starter. After inoculating the milk with desired starter culture, the milk is incubated at the desired temperature for a specified period or until it develops desired qualities. After incubation and packaging, cooling to refrigeration temperatures is done in order to suppress further fermentation while the product is in the market.

Table 5: Different fermented milks and fermentation features (Source: O'Connor &Tripathi, 1995).

Product	Milk type	Heating	Starter	Incubation
Yogurt	Cow	85° C (30 min.)	*S. thermophilus* *L. bulgaricus*	42° C (4-6 hrs)
Acidophilus	Cow	110° C (20 min.)	*L. acidophilus*	38° C (18-24 hrs)
Kefir	Cow Ewe Goat Horse	85° C (30 min.)	Saccharomyces and Torula yeast	22° C (12 hrs)
Koumiss	Horse Cow	70° C (30 min.)	*L. bulgaricus* and Torula yeast	27° C (18-24 hrs)

Yogurt

Nakazawa and Hosono (1992) defines yogurt as a coagulum obtained by lactic acid fermentation of milk or milk products by the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The proportion of the above microorganisms is usually at the ratio of 1:1 for rods to cocci, and addition of flavors and other additives is optional. According to the National Agricultural Statistics Service (2004), yogurt production in the United States increased by 238 % from 570 million pounds in 1980 to 2.39 billion pounds in 2003. This tremendous increase in production is probably due to sustained increase in information about functional effects of yogurt as a probiotic food product.

Manufacturing process

The basic steps of manufacture are illustrated in Figure 3 and Table 5 above. Two main types of yogurt, based on the system of manufacture, are set and stirred types (Robinson, 1981). The set type is made by carrying out the fermentation/incubation in retail containers, which is characterized by having continuous custard like semi-solid gel/coagulum. For the stirred type fermentation is carried out in bulk containers where the coagulum is broken by stirring before cooling and final packaging. Flavors may be added to the product to increase variety in yogurt types, and these include sugars and fruit flavorings. Post incubation processing also adds into more variety in yogurt products namely: liquid, solid/semi-solid, solid or frozen and dried yogurts (Robinson, 1981).

Health benefits of yogurt

Yogurt is one of many varieties of fermented milks. Some hypothesized potential health benefits of fermented milks are discussed later in this chapter. For yogurt starter culture bacteria *St. thermophilus* and *Lb. bulgaricus* do not survive through the stomach due to presence of gastric juices. Hence they do not possess probiotic properties unless other probiotic bacteria like *Bifidobacteria* or *Lb. acidophilus* are included in the yogurt starter culture. The most important physiological benefit of yogurt consumption is the improvement of lactose utilization in lactose intolerant individuals.

Quality control of fermented milks

Like in other food products, good manufacturing practices (GMPs) must be applied in order to produce good quality fermented milk products. This is done by ensuring that good quality starter culture, milk and other ingredients are used. The manufacturing environment should also comply to the set standards of hygiene. During the manufacturing process of fermented milk, various conditions involving specific temperatures of operation at different stages should be adhered to so as to maintain prescribed physical, chemical and sensory qualities of the product. Establishing a HACCP (hazard analysis critical control point) system in the manufacturing plant will also help ensure that all aspects of quality control at every stage of production are monitored and standardized.

Routine quality tests for fermented milks

Some of the routine quality control tests for fermented milks will include: pH and acidity tests, microscopic examination for starter cultures using gram's staining technique, coliform and fecal coliform tests (*E. coli* test), general viable counts, and yeasts and molds counts to determine gross contamination of ingredients and products (Robinson, 1981). Some studies have shown that that coliforms have the capability of surviving through the fermentation process (Savadogo et al, 2004). Therefore, coliform tests are important for fermented milks produced from un-pasteurized raw milk.

Coliform tests

According to the Standardard Methods for examination of Dairy Products (Marshall, 1992) as quoted by Marth &Steel (1998), coliforms are a group of bacteria that include all aerobic and facultatively anaerobic, gram negative, non-spore-forming rods capable of fermenting lactose and producing gas at 32° C or 35° C within 48 hours. These microorganisms are used as a measure of sanitary processing and handling conditions of raw milk and other milk products. Their presence serves as an indication of fecal contamination due poor sanitary conditions and unhygienic handling of products during processing; implying a high likelihood of the presence of enteric pathogens such as *E. coli* and Salmonella (Marth &Steel, 1998). Many strains of coliforms are usually present in the natural environment and their presence in food may not necessarily be an indication of fecal contamination. For this reason fecal coliform tests with respect to *E. coli* are regarded as a better indication of recent fecal contamination (Robinson, 1981).

Escherichia coli

In the Shorter Bergey's Manual of Determinative Bacteriology (Holt, 1977), *E. coli* bacteria are defined as gram-negative, straight rods that appear singly or in pairs, motile by peritrichous flagella or are non motile. *E. coli* is a member of the family Enterobacteriaceae whose natural habitat is the gastro intestinal tract of animals. Being the dominant species in human feces, *E. coli* are used as indicator bacteria for indirect evidence of recent fecal contamination (Labbe & Garcia, 2001). They are aerobic, facultatively anaerobic, non-sporeforming rods capable of fermenting lactose to produce acid and gas. As a genus, *Escherichia* is classified based on IMViC tests (indole production, methyl red, Voges-Proskauer and Citrate utilization). Other biochemical reactions and serological reactions are used to distinguish between different strains of the *E. coli* (Labbe & Garcia, 2001).

Whereas, *E. coli* is generally regarded as harmless, some strains have been found to be virulent to individuals with compromised immunity, especially young children and the elderly. There are six major groups of pathogenic *E. coli,* namely: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregatiive (EAEC), enterohemorrhagic (EHEC) and diffusely adherent (DAEC). In these groups, serotype O157: H7 is the most widely implicated in worldwide gastrointestinal illnesses (Labbe & Garcia, 2001). Depending on the strain, symptoms of disease resulting from pathogenic *E. coli* infections include: mild diarrhea, fever, vomiting and abdominal cramps (Labbe & Garcia, 2001).

Probiotics

Definition

Probiotic microorganisms are live microbial food supplements that are beneficial to health (Salminen & von Wright, 1998). They include LAB mainly from the *Lactobacillus* group such as *Lb. acidophilus, Lb. casei*, *Lb. reuteri*; yeasts and molds (Tamime, 2002).

In Table 6 are some examples of probiotic microorganisms used in making fermented milks. One very important characteristic requirement that distinguish probiotic microorganisms in the usual starter cultures is that they should have the abilty to withstand gastric acid and bile salts; and multiply the large intestine in order to produce their probiotic effects in humans (Tamime, 2002).

Table 6: Some probiotic microorganisms used during production of fermented milks (Source: Tamime, 2002)

Genera	Microbial species
Lactobacilus	*Lb. acidophilus* *Lb. casei* *Lb. rhamnosus* *Lb. johnsonii* *Lb. helveticus* *Lb. delbrueckii* subsp. *bulgaricus* *Lb. gasseri* *Lb. plantarum* *Lb. paracasei* subsp. *Paracasei* and subsp. *tolerans* *Lb. reuteri*
Pediococcus	*P. acidilactici*
Bifidobacterium	*B. bifidum, breve, longum, adolescentis, infantis, lactis, animalis*
Lactococcus	*Lc. Lactis* subsp. *lactis*
Enterococcus	*E. faecium, faecalis*
Saccharomyces	*S. sabolardii*

Impact of Probiotics and fermented milks to human health

Potential nutritional and physiological benefits of fermented milks and probiotics have been widely studied. One of these benefits is the improvement of lactose utilization in individuals with lactose intolerance (Lore, 2004; Marth & Steele, 1998). Other benefits which are initiated by activities of probiotic micro-organisms in the gastrointestinal tract include: control of intestinal infections, control of cholesterol, improvement of immune system and anticarcinogenic activity (Marth & Steele, 1998). Potential beneficial effects would be achieved by consumption of a variety of fermented dairy products since there are different types of starter cultures used in different products. Table 7 illustrates a summary of some of the benefits of probiotic microorganisms.

Table 7: Some of the health promoting activities of probiotics/fermented milks in humans (Source: Tamime, 2002)

Action/Effect	Alleged health benefit
In digestive tract	Active against *Helicobacter pyroli* Enhanced lactose digestion Stimulation of the intestinal immunity Stabilization of Crohn's disease Stimulation of intestinal peristalisis
On intestinal microflora	Improves balance between microbial populations (e.g. increase in fecal bifidobacteria) Decrease in fecal enzyme activity Colonization of the intestinal tract Reduced carrier time for *Salmonella spp.*
On diarrhea	Prevention/treatment of acute and of Rotavirus diarrheas Prevention of antibiotic induced diarrhea Treatment of relapsing of *Clostridium difficille* diarrhea
Other effects	Improved immunity to disease Suppression of some cancers Reduction in serum cholesterol Reduction in hypertension

Antimicrobial effects of fermented milks

Mitsuoka (1978), reports that administration of antibiotics results in disturbance of normal balance of intestinal flora, in that bacteria with normal dormant pathogenicity such as streptococci, *Staphylococcus aureus*, *Proteus*, yeasts and molds become virulent. Some studies have shown that consumption of fermented milks helps restore balance in the intestinal micro flora to prevent such a negative impact of antibiotics (Asahara et al, 2001). A number of studies have shown that some fermented milks/fermented milk starter cultures possess antimicrobial effects against a number of pathogenic microorganisms (Asahara et al, 2001; Chauyana et al, 2003, Savadogo et al, 2004; Eguchi et al, 1998).

Studies on antimicrobial effects of fermented milks

In a study conducted by Asahara et al (2001), to determine the effect of oral administration of fermented milk on mice that had been injected with a lethal dose of 5-fluorouracil (400mg/kg); a dose of 5-fluorouracil induced an abnormal increase of *E. coli* in the GI tract of the mice. Results indicated that oral administration of fermented milk had protective effect in mice against *E. coli* proliferation. Another study by Chuayana et al (2003) to determine antimicrobial effects of probiotics isolated from different milk products indicated that the isolates varied in their antimicrobial activity against *S. aureus*, *E. coli, P. aeruginosa*, *S. typhi*, *Serratia marcescens* and *Candida albicans*. Eguchi et al (1998) conducted a study to determine bactericidal effect of liquefied yogurt product against *Salmonella enteritidis, Salmonella typhi* and *Vibrio parahemolyticus*. Results indicated that the yogurt had a considerable bactericidal effect against the pathogens within the first two hours. Bactericidal effects were fastest on VP compared to SE and ST. Further tests indicated that bactericidal effects were due to the production of lactic acid and not due to reduction in medium pH alone. In their study with Burkina Faso fermented milk, Savadogo et al (2004) isolated and identified eight strains of bacteriocin-producing lactic acid bacteria. The bacteriocins obtained from the bacteria were found to exhibit antibacterial activity against *Enterococcus fecalis, Bacillus cereus, Staph aureus* and *E.coli*. Inhibitory effects were greatest on gram-positive indicator bacteria.

CHAPTER 3. METHODOLOGY

Materials and Equipment

Plain Old Home™ yogurt made from pasteurized grade-A non-fat milk containing live active cultures of Acidophilus and Bifidus, and 2% Kemps™ pasteurized milk were purchased from a grocery store in Menomonie Wisconsin. The 2% Kemps™ pasteurized milk was used in the production of Kenyan fermented (KF) milk. The starter culture for making the fermented milk was obtained from one family of the Gusii community of the southwestern region of Kenya where this author was born. The starter culture was kept in frozen storage at temperatures of -23° C to -25° C prior to its use.

Potassium dihydrogen phosphate and 1N NaOH used for the preparation of the phosphate dilution water were obtained from University of Wisconsin-Stout Microbiology Laboratory chemical storage section. 3M™ Petrifilm™ EC, Petrifilm™ PC and Petrifilm™ Y/M plates for enumeration of *E. coli*, total bacterial counts and yeasts and molds respectively, and plastic sample spreading devices as well as interpretation guides for the Petrifilm™ plating method were obtained from University of Wisconsin-Stout Microbiology Laboratory media storage section having been donated by 3M Inc., St. Paul MN.

The HI 98127™ pH meter from Hanna Instruments Inc. Woonsocket RI, was used for pH determinations. Other equipment included: the AP–402 electronic weighing balance from Denver Instruments, the 818 Low Temperature Illuminated Incubator from Precision Scientific Inc., SI–16 Scientific Isothermal Sterilizer from Steris Inc., Mentor OH, a refrigerator and a standard colony counter. Pipettors and sterile disposable pipet tips were used for all transfers of bacteria, media and samples.

Methods

Production of Kenyan Fermented Milk

The Kenyan fermented milk frozen starter culture that had been resuscitated by thawing at a temperature of 21-25° C, was used to inoculate pasteurized milk. The specific microbial constitution of the Kenyan fermented milk starter culture was not determined. The milk was originally produced by spontaneous fermentation, and the experimenter found that it was a mixed starter culture comprising different kinds of lactic acid bacteria and yeasts.

The 2% pasteurized milk was heated to about 100° C and cooled to 40° C to 45° C in a sanitized plastic jerry can. The starter culture was added to the milk at the rate of 1mL starter culture to 10 mL milk. The milk was allowed to ferment at a temperature of 23-25° C for 48 hours or longer until it attained a pH less than 4.0 before being cooled to refrigeration temperatures of 0-8° C to stop the fermentation.

Preparation and inoculation of samples with E. coli

A known strain/species of *E. coli* from a frozen agar slant stock culture was inoculated in a test tube of sterile tryptic soy broth (TSB) and incubated at 37° C for 24 hours. Serial dilutions were made to reduce estimated number of cells to about 10^4 per milliliter as illustrated in the procedure below:

1. Using a sterile loop *E. coli* in a stock culture was aseptically transferred from slant into a tube of sterile TSB. The cap was replaced and the tube was vigorously shaken to distribute bacteria in the medium. After inoculation the broths were incubated for 24 hours at 37° C.
2. Serial dilutions of 24 hr old *E. coli* culture broths were done using sterile phosphate buffer as follows:

Transfer 1mL EC culture into 99 mL sterile buffer (10^{-2} dilution)

↓

1 mL of 10^{-2} dilution into 99 mL sterile buffer (10^{-4} dilution)

↓

2.5 mL 1 mL of 10^{-4} dilution into 250 mL fermented milk/yogurt/TSB

Figure 4: Serial dilution preparation and medium inoculation flow sheet

Two and half milliliters of the diluted culture were used to inoculate 250 mL triplicate samples of Kenyan fermented milk, plain yogurt and 2 % pasteurized milk (as illustrated in step 2 above), to introduce approximately equal numbers of cells into each aliquot sample at the beginning of the experiment. The same amount was added to 250 mL sterile TSB as a positive control. Uninoculated 250 mL sterile TSB was set up for use as a negative control. The pH of the samples and media were determined at the beginning and at the end of the experiment using the HI 98127™ pH.

Plating and Incubation

To determine the general microbial quality of the 2% pasteurized milk, Kenyan fermented milk and plain yogurt samples, 1mL appropriate decimal dilutions selected to yield plates with 25-250 colonies per plate were transferred to 3M™ Petrifilm™ EC, Petrifilm™ PC and Petrifilm™ Y/M plates for the enumeration of *E. coli*, total bacterial counts and yeasts and molds respectively. The PC & EC plates were incubated at 37° C for 24 hours, while Y/M plates were incubated at 21° C for 5 days. Also inoculated (TSB medium with *E.coli*) and uninoculated (TSB medium without *E.coli*) sterile medium were plated in the same way to make the positive and negative controls as well as helping assess the quality of the media.

Appropriate dilutions of inoculated samples and controls were made using buffered sterile blanks and plated on 3M™ Petrifilm™ EC plates. The plates were incubated at 37° C to determine the initial number of *E. coli* population in the samples and the controls at 0 hour. The inoculated samples and controls were then placed in the incubator at 37° C and dilutions were plated on 3M™ Petrifilm™ EC plates after every two hours of incubation to determine change in population in the samples and the controls with time.

Reading Results

Reading of results was done as directed by Petrifilm™ manufacturer's interpretation guide. Using a standard colony counter, blue colonies with gas around them in the 20 cm^2 growth area were counted as presumptive *E. coli* colonies. For those plates containing more than 150 colonies, colonies in a few representative small squares were counted and determined the average number of colonies per square. The average count was multiplied by 20 to determine the count in the growth area. The average number of colonies in consecutive dilutions was calculated before determining the average bacterial count per mL of sample. The number of colony forming units (cfu) per mL of sample was calculated by multiplying the total count in the growth area by the dilution factor.

Statistical analysis

Data were analyzed using SPSS™ statistical program version 11.0 (SPSS Inc. Chicago, Illinois). Two-way analysis of variance (ANOVA) was done on replicate data to determine the effect of growth medium (Kenyan fermented milk and plain yogurt) and interactions between medium and time. Numeric data were converted to Log_{10} before being analyzed.

CHAPTER 4. RESULTS AND DISCUSSION

Introduction

The aim of this study were: to produce Kenyan fermented milk using the starter culture from the Gusii community of Kenya, determine the general microbial quality of Kenyan fermented milk and determine and compare the effect of Kenyan Fermented Milk to that of Commercial Plain Yogurt on the growth of *Escherichia coli.*

Kenyan fermented milk with an average pH of 3.6 was produced. It took 60 hours for the pH of Kenyan fermented milk to drop down from 6.7 to 3.6. The rate of fermentation was dependent on amount of starter culture per unit amount milk and incubation temperature used. Initial pH of pasteurized 2% milk; plain yogurt and tryptic soy broth were 6.7, 4.1 and 7.2 respectively (Table 10).

Microbiological quality analysis

Results of microbiological analysis of fermented milk, yogurt, 2% pasteurized milk and tryptic soy broth (TSB) based on aerobic plate counts, *E. coli* counts and Y/M counts are summarized in Table 8. Results indicate that *E. coli* was not detectable in the sterile broth, milk (both fermented and unfermented) and yogurt. Absence of E. coli in the different growth media was a very important aspect of this study as it influenced subsequent experiments.

Results revealed that total bacterial counts in Kenyan fermented milk were about 3.3 x 10^2 cfu/mL based on two replicate samples. Yeasts and molds counts on Kenyan fermented milk were 9.3 x 10^2 cfu/mL all of which were yeasts from visual characteristics. General bacteria were not detectable in yogurt and sterile TSB but 2 % pasteurized milk had 5.5 x 10^1 based on two replicate dilutions. Yeast and molds were not detectable in 2 % pasteurized milk, yogurt and sterile TSB.

Kenyan fermented milk had higher counts for aerobic bacteria and Y/M as anticipated. This was probably because the starter culture used had been made through spontaneous fermentation indicating that many varieties of lactic acid bacteria as well as lactic acid tolerant bacteria and yeasts were present. In contrast with the Fulani traditionally fermented milk from Burkina Faso described by Savadogo et al (2004), Kenyan fermented milk had relatively very low counts of aerobic bacteria and yeasts/molds. This is because the Fulani do not pasteurize their milk before fermentation. Pasteurization/heat treatment before addition of starter culture helped reduce the general microbial load of milk as well as destroying pathogenic and spoilage microorganisms.

Table 8: Aerobic plate, E. coli and yeasts & molds counts in different dilutions of tryptic soy broth negative control (uninoculated TSB), Kenyan fermented milk, plain yogurt, 2% pasteurized milk and tryptic soy broth positive control (TSB inoculated with *E. coli*)

Medium		Aerobic plate count (APC)				EC count	Y/M count			
		10^{0}	10^{-1}	10^{-2}	Average	10^{0}	10^{-0}	10^{-1}	10^{-2}	Average
TSB negative control	1	<1			<1	<1	<1	<10		<1
	2	<1					<1	<10		
	3	<1					<1	<10		
KF-Milk	1	TNTC	--		#3.3×10^{2}	<1	TNTC	TNTC	18	9.3×10^{2}
	2		25				TNTC	TNTC	3	
	3			40			TNTC	TNTC	7	
Plain-Yogurt	1	<1			<1	<1	<1	<10		<1
	2	<1					<1	<10		
	3	<1					<1	<10		
2% Pasteurized Milk	1	25	5		#5.5×10^{1}	<1	<1	<10		<1
	2	24	1				<1	<10		
	3	<1	<10				<1	<10		
TSB positive control	1	TNTC			*5.6×10^{2}	<1	<1	<10		<1
	2	--	560				<1	<10		
	3	--	TNTC				<1	<10		

Average of duplicate dilution counts
* Average of single sample count
TNTC = Too numerous to count

Determination of effect Kenyan fermented milk on E. coli

The growth pattern of *E. coli* in Kenyan fermented milk and other media is illustrated in Table 9 and figure 5 below. The results indicate that it took an average of two hours for both Kenyan fermented milk and plain yogurt to inhibit the growth of *E. coli*. *E. coli* population in 2 % pasteurized milk and TSB, which served as controls increased logarithmically throughout the eight hours of observation. The suppression of E.coli growth by both Kenyan fermented milk and plain yogurt is in line with studies conducted by Savadogo et al (2004), Chuayana et al (2003) and Eguchi et al (1998) which determined that fermented milk had antimicrobial effects on different foodborne pathogens. In fact, the study by Eguchi et al (1998) determined that fermented milk inhibited pathogenic bacteria within the first two hours and they were not detected at all after four hours. *E. coli* was non detectible in both Kenyan fermented and plain yogurt after four hours.

Table 9: Growth pattern of *E. coli* in Kenyan fermented milk, plain yogurt, pasteurized milk and tryptic soy broth

	Colony forming units counted after time (in hours)				
Medium	0	2	4	6	8
Kenyan fermented milk	950	110	<100	<100	<100
	1990	575	<100	<100	<100
	2175	175	<100	<100	<100
Plain yogurt	1710	1220	550	<100	<100
	3765	1360	<100	<100	<100
	4125	880	<100	<100	<100
Pasteurized 2% milk	6625	6450	297000	5120000	$<10^7$
	4915	5390	140500	64000000	217000000
	6120	7950	179000	TNTC	326000000
TSB positive control	4220	25100	380000	TNTC	TNTC
	7040	8700	215000	21000000	720000000
	5000	6630	325000	18400000	1100000000

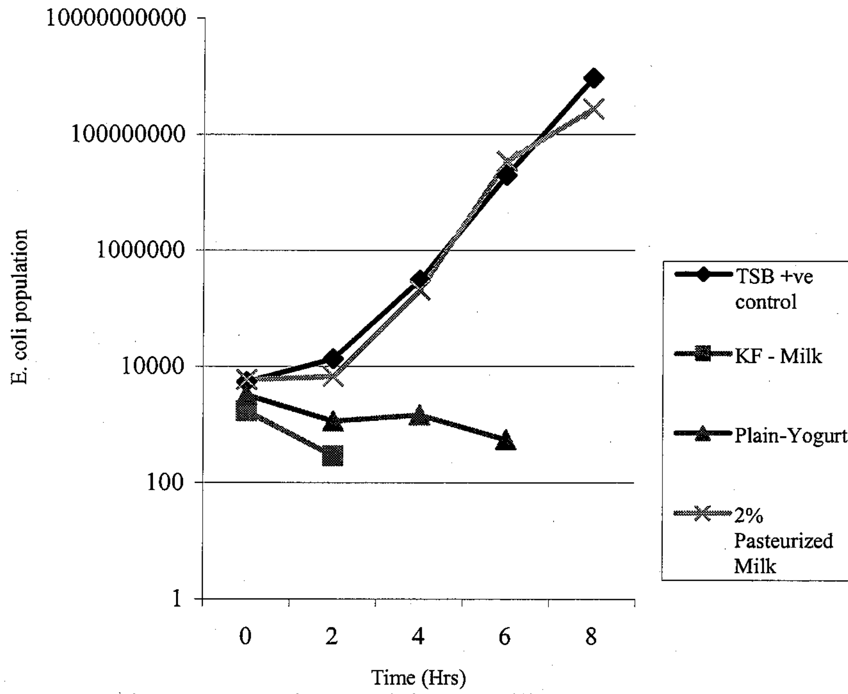


Figure 5: *E. coli* growth in KF-milk, plain yogurt, 2% pasteurized milk and trypticase soy broth (TSB)

Results indicated that initial pH of the growth medium affected the initial bacterial cell population introduced into the medium (see Table 10). The findings of Eguchi et al (1998), indicated that a sufficient reduction in pH of a growth medium inhibited microbial activity. Their findings suggested that the observed microbial inhibition was specifically due to presence of lactic acid in the medium. This is confirmed by the growth pattern of E. coli in different media shown in Figure 5. There was exponential growth of E. coli in 2 % pasteurized milk and tryptic soy broth even when medium pH was decreasing with time (Table 10). On the other hand, E. coli growth in Kenyan fermented milk and yogurt, both of which contained lactic acid, was suppressed within the first two hours of incubation. Unbuffered 3M™ Petrifilm™ plates were used in this experiment, which might have also influenced the initial microbial population on the inoculated plates. The data collected during the experiment is shown in Appendix, Tables A, B, C, D and E.

Table 10: Effect of initial growth medium pH on *E. coli* growth

Medium	Average initial pH	Average pH after 8 hours	Average initial EC population (CFU/mL)	Average time for EC inhibition (Hrs)
KF-Milk	3.6	3.4	1705	2
Plain-Yogurt	4.1	3.9	3200	2.5
2% Pasteurized milik	6.7	6.3	5887	No inhibition
TSB positive control	7.2	5.6	5420	No inhibition

Statistical analyses were performed using $\log_{10}$ the number of CFU counted for the average replicate counts from Kenyan fermented milk and yogurt media during the first two hours of the growth experiment (Table 11). Results indicated significant differences ($p \leq 0.01$) in the rate of *E. coli* inhibition between the two products (see Table 12). The inhibitory rates by the two products were significantly different ($p \leq 0.01$) as time increased.

The Kenyan fermented milk had a higher inhibitory effect on *E. coli* than yogurt probably because it had a lower pH due to the presence of more lactic acid. There could have been other bacterial inhibitory components in the Kenyan fermented milk that were not present in yogurt. This was probably due to the presence of more varied microorganisms in Kenyan fermented milk starter culture.

Table 11: Mean count and standard deviation of log10 of *E. coli* counts when separated medium (Kenyan fermented milk/plain yogurt) and time (0 hours /2 hours)

Medium of growth	Time	N	Mean	Std. Dev.
KF-milk	0 hours/initial	3	3.2047	.19749
	2 hours	3	2.3480	.37047
	Total	6	2.7764	.53912
P-yogurt	0 hours/initial	3	3.4747	.21.28
	2 hours	3	3.0548	.09840
	Total	6	3.2648	.27288
Total	0 hours/initial	6	3.3397	.23488
	2 hours	6	2.7014	.45676
	Total	12	3.0206	.48064

Table 12: Two-way ANOVA on $\log_{10}$ *E. coli* counts using medium (Kenyan fermented milk/plain yogurt) and time (0 hours /2 hours)

			Sum of squares	df	Mean square	F	Sig.
LG_{10}-EC	Main effects	(Combined)	1.938	2	0.969	16.840	0.001
		Medium	0.716	1	0.716	12.437	0.008*
		Time	1.222	1	1.222	21.242	0.002*
	2-way interactions	Medium*Time	0.143	1	0.143	2.486	0.154
	Model		2.081	3	0.694	12.055	0.002
	Residual		0.460	8	0.058		
	Total		2.541	11	0.231		

Note: * indicates significance at 0.01 level.

CHAPTER 5. SUMMARY AND CONCLUSION

Summary and Conclusion

This was an exploratory study on traditionally fermented milk using the starter culture obtained from the Gusii community of Kenya. The study attempted to determine the general microbial quality of the traditionally fermented milk as well its effect on *Escherichia coli*. The results were compared to similar experimental tests on commercial plain Yogurt. The tests indicated that both products had pHs of 3.6 and 4.1 for Kenyan fermented milk and plain yogurt respectively, which were sufficiently low enough to suppress most pathogenic microorganisms. No coliforms or fecal coliforms were detected in either product. The Kenyan fermented milk had initial higher general bacterial and Y/M counts than yogurt.

Experimental results indicated that Kenyan fermented milk had a higher inhibitory effect on *E. coli* compared to commercial plain yogurt. This inhibitory effect was significant in the first two hours and by the fourth hour no *E. coli* was detectible in both Kenyan fermented milk and yogurt (see Appendix Table C).

Some errors were detected in the course of conducting this study and these resulted in some instances where there were no counts or too numerous to count (TNTC) results in some plates. The differences in the initial sample counts resulted from using Petrifilm™ which were unbuffered. In order to get accurate results sterile buffered diluent was used to make dilutions, and it was not possible to get a zero count.

This study recommends that further tests need be conducted on the Kenyan fermented milk starter culture to determine the type and characteristics of microflora present. Further studies on the Kenyan fermented milk starter culture are recommended to determine specific antimicrobial components produced and their effects on various foodborne pathogens.

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APPENDIX

Tables with raw data collected during experiments

Table A: Initial *E. coli* count before incubation (0 Hour EC count)

Medium		EC count at dilution and average count per mL			
		10^{-1}	10^{-2}	Average count/mL	Average count/mL
TSB –ve control	1	<10		<10	$<10^1$
	2	<10		<10	
	3	<10		<10	
KF-Milk	1	70	12	950	1.7×10^3
	2	78	32	1990	
	3	125	31	2175	
Plain-Yogurt	1	142	20	1710	3.2×10^3
	2	163	59	3765	
	3	285	54	4125	
2% Pasteurized Milk	1	505	82	6625	5.9×10^3
	2	473	51	4915	
	3	524	70	6120	
TSB +ve control	1	344	50	4220	5.42×10^3
	2	608	80	7040	
	3	400	60	5000	

Table B: *E. coli* count after two hours incubation at 37 °C (2 Hour EC count)

Medium		EC count at dilution and average count per mL			
		10^{-1}	10^{-2}	Average count/mL	Average count/mL
TSB -ve control	1	<10		<10	$<10^1$
	2	<10		<10	
	3	<10		<10	
KF-Milk	1	1	1	110	2.9×10^2
	2	25	9	575	
	3	15	2	175	
Plain-Yogurt	1	74	17	1220	1.15×10^3
	2	112	16	1360	
	3	116	6	880	
2% Pasteurized Milk	1	360	93	6450	6.6×10^3
	2	318	76	5390	
	3	540	105	7950	
TSB +ve control	1	TNTC	251	25100	1.35×10^4
	2	660	108	8700	
	3	536	79	6630	

Table C: *E. coli* count after four hours incubation at 37 °C (4 Hour EC count)

Medium		EC count at dilution and average count per mL					
		10^{-0}	10^{-2}	10^{-3}	10^{-4}	Average count/mL	Average count/mL
TSB –ve control	1	<1				$<10^0$	$<10^0$
	2	<1				$<10^0$	
	3	<1				$<10^0$	
KF-Milk	1		<100	<1000		$<10^2$	$<10^2$
	2		<100	<1000		$<10^2$	
	3		<100	<1000		$<10^2$	
Plain-Yogurt	1		9	2		1450	*1.45×10^3
	2		<100	<1000		$<10^2$	
	3		<100	<1000		$<10^2$	
2% Pasteurized Milk	1			74	52	297000	2.06×10^5
	2			81	20	140500	
	3			138	22	179000	
TSB +ve control	1		TNTC	380		380000	3.07×10^5
	2		TNTC	215		215000	
	3		TNTC	325		325000	

* Average of single sample count
TNTC = Too numerous to count

Table D: *E. coli* count after six hours incubation at 37 °C (6 Hour EC count)

		EC count at dilution and average count per mL						
Medium		10^{-0}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Average count/mL	Average count/mL
	1	<1					$<10^0$	
TSB –ve control	2	<1					$<10^0$	$<10^0$
	3	<1					$<10^0$	
	1		<100	<1000			$<10^2$	
KF-Milk	2		<100	<1000			$<10^2$	$<10^2$
	3		<100	<1000			$<10^2$	
	1		1	1			550	
Plain-Yogurt	2		<100	<1000			$<10^2$	5.50×10^2
	3		<100	<1000			$<10^2$	
2% Pasteurized	1			TNTC	504	52	5120000	
Milk	2			TNTC	TNTC	640	64000000	*3.46×10^7
	3			TNTC	TNTC	TNTC	$> 10^8$	
	1			TNTC				
TSB +ve control	2				TNTC	210	21000000	1.97×10^7
	3				TNTC	184	18400000	

* Average of duplicate dilution counts
TNTC = Too numerous to count

Table E: *E. coli* count after eight hours incubation at 37 °C (8 Hour EC count)

Medium		EC count at dilution and average count per mL								
		10^{0}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Average count/mL	Average count/mL
TSB –ve control	1	<1							$<10^{0}$	
	2	<1							$<10^{0}$	$<10^{0}$
	3	<1							$<10^{0}$	
KF-Milk	1		<100	<1000					$<10^{2}$	
	2		<100	<1000					$<10^{2}$	$<10^{2}$
	3		<100	<1000					$<10^{2}$	
Plain-Yogurt	1		<100	<1000					$<10^{2}$	
	2		<100	<1000					$<10^{2}$	$<10^{2}$
	3		<100	<1000					$<10^{2}$	
2%-Pasteurized Milk	1				TNTC	TNTC	$<10^{6}$	$<10^{7}$	$<10^{7}$	
	2				TNTC	TNTC	194	24	2.17×10^{8}	*2.72×10^{8}
	3				TNTC	TNTC	392	26	3.26×10^{8}	
TSB +ve control	1		TNTC	TNTC					$>10^{3}$	
	2						TNTC	72	7.2×10^{8}	*9.25×10^{8}
	3						TNTC	113	1.1×10^{9}	

* Average of duplicate dilution counts
TNTC = Too numerous to count