MICROBIAL SAFETY OF TRADITIONAL CAMEL MILK PRODUCTS FROM NORTH EASTERN KENYA AND FUNCTIONAL CHARACTERIZATION OF SELECTED LACTIC ACID BACTERIA

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This thesis is my original work and has never been submitted for an award of a degree in any other university

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DEDICATION

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ACRONYMS AND ABBREVIATIONS

AFDPs	African fermented dairy products
ASALs	Arid and Semi-Arid Lands
CFU	Colony Forming Units
DGGE	Denaturing Gradient Gel Electrophoresis
EFSA	European Food Safety Authority
FDA	U.S. Food and Drug Administration
GRAS	Generally Recognized as Safe
LAB	Lactic Acid Bacteria
mL	Milliliters
NaCl	Sodium chloride
PCR	Polymerase Chain Reaction
QPS	Qualified Presumption of Safety
ST	*Streptococcus thermophillus*
uL	Micro liters

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GENERAL ABSTRACT

African fermented dairy products serve as a major food and the main source of nutrition in many African rural communities. These products are mostly made through an artisanal process of spontaneously fermenting raw milk especially amongst the pastoral communities and play a vital role in their diet. The spontaneous fermentation process results in products having inconsistent quality and risk due to the unpredictable microbial inhabitants which are hazards to the consumer. African *Streptococci* strains which have been found to be predominant in most African fermented dairy products can be utilized in milk fermentation to help maintain the health benefits, cultural background, enhance productivity for communities, and general acceptance of the products. Camel milk and milk products are part of the principal food sources for communities in North Eastern Kenya who use traditional raw camel milk fermentation as a means of milk preservation and value addition.

The study was carried out to assess the microbial quality and safety of fermented camel milk product (*Suusac*) from North Eastern Kenya with reference to selected pathogens namely *E. coli, S. aureus, Shigella,* and *Klebsiella spp,* using standard analytical methods. Twenty-eight samples (n=28) of *Suusac* from different areas of the region sold in informal markets at Eastleigh in Nairobi were aseptically collected at the sales points. *E. coli, S. aureus, Shigella spp,* and *Klebsiella spp* were detected in 100 %, 63.09 %, 88.1 %, and 77.4 %, of the samples respectively. The mean $\log_{10}$ counts for *E. coli, S. aureus, Shigella spp,* and *Klebsiella spp* were 3.135, 2.576, 2.784 and 3.138, $CFUmL^{-1}$, respectively. There is a potential public health concern posed by *Suusac* which is sold for direct consumption due to the presence of the life-threatening bacterial pathogens. Training on food hygiene, improving production technology, and implementing the food legislations along the value chain can minimize the risk.

The practical functionality of selected African *Streptococci* strains; African type *Streptococcus thermophillus, Streptococcus infantarius* subspecies *infantarius* variety *CJ18* and *Streptococcus infantarius* subspecies *infantarius* variety *CCUG* in dairy fermentation was done. Each of the three strains, a combination of *S. thermophillus* and *CJ18* were inoculated in pasteurized camel and cow milk samples at a rate of 3 % v/v. The samples were then incubated at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C, and 45 ^{o}C for 9 hours with the analysis done after every 3 hours for pH and titratable acidity. Viscosity was done after incubating for 9 hours and then cooling product for 10 hours. Sensory analysis for mouth feel, sourness and general acceptability was done by 12 panelists on the potential cultures which showed a competitive advantage.

The results varied depending on the type of milk, sample strain, incubation time and temperature. Titratable acidity of camel milk was from 0.42±0.03 to 0.83±0.03 while that of cow milk ranged from 0.89±0.010 to 0.97±0.010. The average pH of camel milk ranged from 5.880±0.020 to 4.150±0.010 while that of cow milk ranged from 5.20±0.02 to 4.12±0.02. The viscosity of camel milk ranged from 18.6±0.755 to 29.44±0.906 while that of cow milk ranged from 19.77±0.37 to 59.64±0.49. From the pH, acidity and viscosity profiles, the combined strain of *S.thermophillus* and *CJ18* at 45 ^{o}C gave the best results for a potential starter culture. Sensory analysis results showed that the combined strain gave a better product in cow milk than camel milk and this could have been as a result of the difference in milk composition.

From the practical technological properties, the mixed strains of African type *Streptococcus thermophillus* and *Sii CJ18* where found to be suitable for starter culture development in camel and cow milk fermentations. Their functional properties can be used to innovate and develop new products, enhance their safety and nutritional benefits while retaining the traditional qualities.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Livestock production for milk and meat has been increasing from year to year with a large population being kept by pastoralists in arid and semi-arid lands (ASALs) of Kenya. Animals kept include cattle, goats, sheep, and camels. Camels form an integral part of the herd for supporting the livelihood in pastoral communities. East Africa harbors an estimated population of 11 million camels kept in ASALs. This population yields over two-thirds of the world's annual camel milk volume of 1.3 million tonnes. Production of camel milk in Kenya was estimated to be over 340 million liters in 2007 (Musinga *et al*., 2008). Only 12 % of this total is sold in rural and urban markets, with the bulk of raw milk (10 %) being sold to the rural markets. The remaining portion (88 %) does not reach the market of which 38 % is used by pastoralists as their food and 50 % is usually wasted due to spoilage (Akweya *et al*., 2012).

Camel milk has three times more vitamin C and ten times iron content than cow's milk with substantial amounts of vitamins B (Arrowal *et al*., 2005). High vitamin C content is attributed to higher synthetic activity in the mammary tissues during early phases of lactation that declines with the advance in the lactation period (Mal and Pathak, 2010). Camel milk is, therefore, the best source of vitamin C for the population in ASALs of Kenya. The overall dietary quality of camel milk is contributed by the high amount of unsaturated fatty acids (Konuspayeva *et al*., 2009). African fermented foods (AFD) serve as the main source of nutrition in many African rural communities with lactic acid bacteria as an integral component of these foods (Anukam and Reid, 2009). Spontaneously fermented milk products such as *Suusac* (fermented camel milk) in Kenya and Somalia or *Gariss* in Sudan are made through back-slopping by continuous use of fermentation vessels and play a key role in the pastoralists diet (Farah *et al*., 2007).

Suusac is prepared by spontaneous fermentation of raw milk at ambient temperatures (26-29 ^{o}C) for 24-48 hours in smoked guards (Lore *et al*., 2005). Fermentation is known to improve nutritional quality, digestibility and shelf-life stability of foods (Motarjemi, 2002). Most pastoral communities in Kenya consume traditionally fermented milk as their main food supply (Mathara *et al*., 2004). "*Kule Naoto''*, a traditionally fermented milk product by the Maasai community, possesses therapeutic properties for the prevention of diarrhea and constipation in nomadic communities (Mathara *et al*., 2004).

Lactic acid bacteria (LAB) are generally regarded as safe due to their long history of safe use and are Gram-positive bacteria, which ferment milk sugars with lactic acid as the major metabolic end product, hence play a major role in fermented dairy products production (Airidengcaicike *et al*., 2010; Liu *et al*., 2004). LAB play an important role in food fermentations which include; improving shelf life and beneficial influence on the sensory and nutritional properties of the food (Gawad *et al*., 2010). Consumers are in demand of high quality, safe products and therefore the need to explore new microbial strains with novel properties. This study aimed at assessing the prevalence of various pathogens with reference to *E. coli, S. aureus, Shigella spp,* and *Klebsiella spp*. in fermented camel milk from North Eastern Kenya. The functionality of a potential starter culture from African *Streptococci* to process fermented camel and cow milk product was investigated as a way of value addition to camel milk while reducing losses, foodborne health cases and improving the quality and safety.

1.2 Statement of the problem

Most fermented dairy products in Kenya are as a result of spontaneous fermentation, which is a process that may lead to lack of consistency in the quality of the products and also contribute to

the proliferation of unsafe microorganisms in milk posing a health hazard to consumers. The spontaneous fermentation process of dairy products is slow and the desired acidity of the fermented product may not be achieved. Small-scale milk vendors and farmers require standard starter culture that will not only shorten the duration of dairy products development but will also be beneficial to their health.

There is limited microbiological data on Africa fermented dairy products which require further research before arriving at a conclusion on the standard processes and safety of fermented dairy products (FDPs). *Streptococcus thermophillus (ST)* is the only species approved for use in dairy fermentations by European Food Safety Authority (EFSA) Panel on Biological Hazards, 2016 but in Africa, traditional dairy fermentations are predominated by other strains like African dairy *Streptococcus infantarius* sub *spp. infantarius* (*Sii*). Consumers in ASALs of Kenya require consistent high quality and safe FDPs as a way of value addition and reduction of losses due to spoilage. This study aimed at solving the problems by developing and testing the functionality of selected pure strains of African *Streptococcus* which are indigenous LAB from fermented camel milk to make an acceptable quality fermented product using a standard manufacturing process.

1.3 Justification

There has been an increasing concern about food safety and food security especially in developing countries like Kenya. Fermented dairy products are a major contributor to food security in the northern parts of Kenya. The fermentation processes for these products are in many cases spontaneous and they depend on microbes from the environment to carry out the fermentation process (Nduko *et al*., 2017). The fermentation process is slow and therefore the acid production is slow resulting to the survival of initial undesirable microorganisms. The milk

is also often of low quality due to unhygienic handling resulting in spoilage microorganisms which are health hazards (Jans and Njage, 2009). There is a need to develop pure starter cultures for Africa fermented dairy products which are friendly to the environment and will not give a different taste from the conventional FDP while ensuring safety. *Sii* has adaptation to dairy fermentations similar to that of *S. thermophillus*, it's predominant and has high levels of distribution in Africa FDPs, yet not classified by the Qualified presumption of safety (QPS) of the EFSA nor have the status of GRAS (U.S. Food and Drug Administration FDA, 2017; EFSA Panel on Biological Hazards, 2016). Therefore, their role in milk fermentations requires more investigations in order to develop a starter culture with well-designed technological application.

Consumers in ASALs of Kenya require consistent high quality and safe FDPs as a way of value addition and reduction of losses due to spoilage. This can be achieved through developing and testing the functionality of pure strains from the indigenous LAB to make an acceptable quality fermented product using a standard manufacturing process hence the reason for this study.

1.4 Objectives

1.4.1 Main objective

To determine the microbial safety of fermented camel milk in the final markets and evaluate the technological characteristics of African *Streptococci* strains as starter cultures in controlled fermentation of camel milk to improve food security.

1.4.2 Specific objectives

1. To determine the quality and safety of the fermented camel milk products (*Suusac*) in North Eastern Kenya with reference to *E. coli, S. aureus, Shigella spp,* and *Klebsiella spp.*

2. To evaluate the technological functionality of African dairy *Streptococcus* as starter culture in camel and cow milk fermentation.

1.5 Research questions

1. What are the technological properties of different combinations of African *Streptococci* starter culture strains in dairy products development?

1.6 Hypothesis

1. There is technical application potential of African type *Streptococci* found in fermented milk products from Northern Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Traditional Fermented milk products in Kenya

Fermentation is one of the oldest traditional methods of food processing and preservation which has evolved as a way of imparting flavors and aroma to foods such as milk, cereals and tubers (Chelule *et al*., 2010). Fermented foods are deemed to have improved texture, flavour, prolonged shelf-life, the bioavailability of nutrients and reduced or absence of anti-nutrients and toxic substances (Motarjemi, 2002). According to Nduko *et al.,* (2017), most of the food consumed by man is fermented food and contributes to between 20-40 % of the global food supply.

In Africa, several fermented food products have been stated, which include milk and milk products among others (Nduko *et al*., 2017). In Kenya, traditional fermentation is used at the household level. The raw materials used include milk, bananas, cereals, honey, and sugarcane among others (Mathara *et al*., 2004; Nyambane *et al*., 2014). The most common is fermentation of milk which is believed to have the ability to promote good health. There are many different ways of fermenting milk adopted by different communities that result in differences in flavor, texture and the composition of microorganisms (Nduko *et al*., 2017). The most common Kenyan traditional fermented milk products are discussed below.

2.1.1 *Mursik*

Mursik is a product of spontaneous fermentation of cow's milk which is common to the Kalenjin community. The milk is prepared and left in gourds to ferment for a period of three to five days or sometimes more depending on the desired sensory preferences (Nduko *et al*., 2017).Upon fermentation ,the gourd is shaken to ensure the product has a smooth uniform consistency. It is

consumed by breast feeding mothers and initiates, as it is believed to boost immune system (Muigei *et al*., 2013; Mathara, 1999)

2.1.2 *Kule naoto*

Kule naoto is a fermented milk product made by the Maasai community in Kenya. It is prepared from unpasteurized cow's milk using spontaneous fermentation that lasts for about five days (Mathara *et al*., 2004). A gourd is made from a dried fruit of a plant known as *Lagenaria siceraria*. A burning stick of *Olea africana* tree is used to rub the calabash leading to break up of charcoal inside the calabash and mixed with fresh cow's blood (Mathara, 1999). The stick is rubbed on the calabash continuously three times and the gourd closed with a cap (Mathara *et al*., 2004).After fermentation the product is shaken to improve texture before consumption. The Maasai's believe that it has therapeutic value for prevention or treatment of diarrhea and constipation (Mathara, 1999).This product is not consistent in quality and safety is not assured (Mathara *et al*., 2004).

2.1.3 *Suusac*

Suusac is fermented camel milk that is consumed by the communities that have inhabited the arid and semi-arid areas of Kenya, for instance, the Somali. Preparation of *Suusac* is through spontaneous fermentation of camel milk which is carried out in gourds treated with smoke (Lore *et al*., 2005).The camels are milked directly into a gourd that has been cleaned, smoothed and treated with smoke. It was found out that the smoke improved colour, taste and improves the shelf-life by up to 20 days. The milk used is usually raw without any kind of heating. *Suusac* fermentation is carried out for a period of one to two days and this takes place at room temperature of between 26-29 ^{o}C (Lore *et al*., 2005).After fermentation, the top fatty layer is removed and the product is ready for consumption having a shelf life of one week at room

temperature. *Suusac* is usually characterized by astringent taste as well as a smoky flavor and it's often unhygienic. The lactic acid of *Suusac* range between 0.52-0.71 % and pH is between 3.6-4.4 (Njage and Wangoh, 2008).*Suusac* varies in quality due to the uncontrolled nature of spontaneous fermentation and also there is a knowledge gap of the nature and the interactions of microorganisms contributing to the fermentation (FAO, 1990).

2.1.4 *Amabere amaruranu*

Amabere amaruranu is a fermented dairy product that is consumed among the members of the Kisii community in Kenya. In this, milk from a cow is heated to boiling and then held at that temperature for about 10 minutes followed by cooling for 10 to 20 minutes. The milk is then allowed to ferment at room temperature between 10-32 ^{o}C, by adding a small portion of previously prepared *Amabere amaruranu* (Nyambane *et al*., 2014).The milk provides nutrients to children and the elderly. The study by Nyambane, Thari, Wangoh and Njage (2014), identified most of the microorganisms in *Amabere amaruranu*, particularly the bacteria which could have probiotic potential.

2.2 Importance of fermented milk and milk products in Kenya

Foodborne diseases are one of the major public health concerns in both developing and developed countries although the problem is more rampant in developing countries. The number of diarrhea cases among children in developing countries shows the magnitude of the food safety problem (Motarjemi, 2002). The agents causing food-borne diseases range from viruses, parasites to bacteria like *Staphylococcus aureaus*. Food contamination is caused by unhygienic handling of food by handlers, flies, and dust among others. Food may also be responsible for harboring chemical hazards like heavy metals. Anti-nutritional factors like phytates and tannins are present in food and they may interfere with the absorption of other nutrients or digestion

(Motarjemi, 2002). Due to these, fermentation becomes an important process for ensuring food safety and mostly in developing countries.

Fermentation is a food processing technology that applies many processes in food, in order to render it safe for human consumption. Some of the benefits of fermentation include inhibition of microbial growth and toxin production by bacteria in food (Adams and Nicolaides, 1997). It is also a method of preserving food especially in households in which refrigeration is not affordable (Motarjemi, 2002). Fermentation improves digestibility of food, taste and flavor. Fermentation is also known to inhibit the growth and replication of pathogenic microorganisms in food thus ensuring food safety, especially in developing countries since it is an affordable way of ensuring food safety and thus available to the whole population (Motarjemi, 2002).

2.3 The microbiology of traditional African milk products

Traditional milk products in Kenya are fermented spontaneously in gourds. On the other hand, modern techniques of milk fermentation involve the use of starter cultures to produce consistent and safe products with increased shelf-life as opposed to those spontaneously fermented (Nduko *et al*., 2017). In some communities, fermentation is carried out by the use of raw milk and this may lead to safety concerns while in other products like *Mursik*, the milk is boiled before fermentation (Anukam and Reid, 2009). Lactic acid bacteria (LAB) are the main microorganisms involved in the fermentation of various products. *Mursik*, *Kule naoto,* and *Amabere amaruranu* involve *Lactobacilli* as the predominant microorganism during fermentation while in *Suusac*, *Leuconostoc* is the predominant microorganism, although *Lactobacillus* is also present (Nduko *et al*., 2017). Most studies involving the microbiology of milk have shown the predominance of the LAB.

Many species of LAB are present in milk and these include; *Lactococcus lactis*, *Lactobacillus*, *Streptococcus bovis*/ *Streptococcus equinus* complex (SBSEC), *Enterococcus* and yeast (Jans *et al*., 2017). *Lactococcus lactis*, a Mesophillic LAB has been isolated in FDP in Kenya and other African countries. It is involved in the fermentation of lactose leading to the formation of diacetyl which is important for food preservation and flavor development (Teuber, 2009).

Lactobacillus species have been isolated in FDPs. The most identified subspecies are *Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus delbrueckii* (Jans *et al*., 2017). *Enterococcus* species represent milk contaminants. Most of the species of *Enterococci* represent part of the intestinal microbiota of birds and mammals and are also found in water and plants (Ludwig *et al*., 2009). *Enterococci faecalis* and *Enterococci faecium* are the most identified subspecies in milk products and their presence in milk is an indicator of fecal contamination from either animal or human sources (Quigley *et al*., 2013). *Enterococcus* has been associated with human infections and this renders it unsafe for the production of safe products although some strains have been incorporated in the production of cheese since they contribute to the development of aroma and flavor (Ogier and Serror, 2008).

In both raw and FDPs, yeast is underestimated. Various studies involving the presence of microorganisms in African fermented dairy products have reported the presence of yeast (Sserunjogi, 1999; Beukes *et al*., 2001). Yeast plays significant roles in product development during fermentation of dairy products mainly through metabolism of lactose, lipolysis, enzyme degradation and proteolysis which together leads to flavor development (Quigley *et al*., 2013). The species of yeast mostly identified in raw milk and are important in dairy product

development include *Saccharomyces cerevisiae*, *Debaryomyces*, *Issatchenkia*, *Geotrichum*, *Kluyveromyces* and *Candida* (Quigley *et al*., 2013).

Members of the *Streptococcus spp* are always identified in milk and milk products across the world. Those that are commonly identified in Africa include; *Streptococcus thermophillus*, *Streptococcus salivarius*, *Streptococcus infantarius* subspecies *infantarius* (*Sii*), *Streptococcus gallolyticus*, and *Streptococcus agalactiae* (Jans *et al*., 2017). Of these, only *Streptococcus thermophillus* is approved for its use in dairy processes. However, AFDPs are dominated by *Sii* rather than *Streptococcus thermophillus.* There is, therefore, a need for an investigation on the taxonomic and phylogenic characteristics of *Streptococcus infantarius* subspecies *infantarius* and its function in the fermentation of dairy products (Jans *et al*., 2017). *Sii* is a member of the SBSEC complex which is mainly associated with pathogenic microorganisms and the predominant LAB in FDPs. The role of *Sii* in milk fermentation was not well known until it was isolated as the predominant LAB in cow and camel fermented milk in Kenya, Somalia and Cote d'Ivoire (Jans *et al*., 2012; Jans *et al*., 2013; Wullschleger *et al*., 2013). The first discovery of *Sii* in FDPs was in Sudan, in the year 2008 (Abdelgadir *et al*., 2008).

Genomic analyses on *Sii* isolates have revealed an adaptation to lactose metabolism that is parallel to that of S. *thermophillus*. The common ancestor of *S. thermophillus* strains is believed to have lived between 3,000 - 30,000 years ago based on genome decay and this is approximately when human dairy activity started (Bolotin *et al.*, 2004; Fox, 1993). In East Africa Camels were introduced around 2,500 years ago (Epstein, 1971; Mikesell, 1955) and the less genome decay in *CJ18* may be attributed to the start of fermentation of camel milk which came later (Jans *et al*., 2013). The analysis of the African strain of *Sii* that is *CJ18* has also revealed more dairy adaptations and events involving close function that is still parallel to the adaptation of *S.*

thermophillus to the dairy niche. *Sii* has therefore been found to carry a partial additional gal-lac operon consisting of genes lac*S* and lac*Z* and exhibiting phenotypic lactose/galactose exchange as *S. thermophilus* (Jans *et al*., 2013). *Sii* has not been classified as GRAS (generally recognized as safe). Its occurrence in intestinal tracts of humans and animals, together with its presence in FDPs requires research to identify its phylogeny and host associations and the ability to move in different ecological niches and hosts (Jans *et al*., 2015). Further research on the functional analysis of *Sii* is required to ensure that innovations like the developing of starter cultures with optimization of the manufacturing processes are implemented based on facts from research.

2.4 Starter cultures

Starter cultures are preparations containing high numbers of live microbial strains inoculated in food either as single, multiple or mixed species to overcome and dominate the existing flora with the aim of contributing to flavor, texture, taste, enhanced preservation, digestibility, nutrition and detoxification of the final product (Robinson, 2005). Starter cultures help improve and accelerate predictable fermentation processes, which leads to improved product safety and reduced hygienic risks (Kimaryo *et al*., 2000).

In developed countries, the technology on starter culture design has been used to produce products like enzymes and microbial cultures which in most cases are imported to developing countries including Kenya (Marshall and Mejia, 2012). Even the starter cultures that are used in the development of products like yogurt in Kenya are usually imported from developed countries. There is a growing need to incorporate Probiotic bacteria in the fermentation of dairy products. The traditionally fermented dairy products are a source of these microorganisms but the opportunities are yet to be exploited (Reid *et al*., 2014; Franz *et al*., 2014).

In dairy fermentations, the starter cultures used belong to Lactic Acid Bacteria (LAB) Family, a collection of functionally related organisms which are all fermentative, gram-positive, nonmotile bacteria and generally have no functional catalase. LABs convert lactose and other sugars in milk to lactic acid (Rhee *et al.*, 2011)

The LAB contains a lot of probiotics like *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus* which are GRAS (Massod *et al*., 2011). Therefore, products like *Mursik*, *Kule naoto* and the other traditionally fermented dairy products contains Probiotic strains of bacteria and efforts towards characterizing, purifying and incorporation of these bacteria into fermented food products would be a way of improving the wellbeing of Kenyans especially those that are vulnerable (Nduko *et al*., 2017). High throughput technologies involving the use of starter cultures can lead to products with improved flavor and aroma. According to Valyasevi and Rolle (2002), Thailand has used these technologies in the production of fermented soy sauce. The process involved in the production of the soy sauce takes a shorter time and there is control of parameters like humidity and temperature in the Fermenter (Valyasevi and Rolle, 2002). These processes have led to enhanced safety and consistency of the soy sauce.

2.5 Traditional fermentation technologies in Kenya

In Kenya, production of traditional fermented foods is carried out through spontaneous fermentation. Some of the fermented food products include; milk, honey, vegetables, cereals, and fruits among others (Nduko *et al*., 2017). This technology has shown vast growth in the food industry because of low cost in energy, infrastructure and the wide acceptance of traditionally fermented food products in Kenya (Tamang and Samuel, 2010). Demand for fermented food products is rising due to the health benefits associated with these products, migration, and

urbanization of the people (Franz *et al*., 2014). The increased demand by consumers calls for the improvement in the safety and quality of fermented food products.

Due to the rising demand in fermented food products by consumers, there is a need for the improvement of the technologies involved in fermentation, in order to ensure the production of consistent safe, quality products that are widely accepted by the community (Nduko *et al*., 2017). Traditional spontaneous fermentation of food products has many limitations which include; low yields, inefficiency, and production of products with varying quality standards (Chilton *et al*., 2015). In addition, there are concerns regarding the safety of products of spontaneous fermentation due to the presence of pathogenic bacteria and chemical toxins produced by the bacteria. These concerns have been demonstrated by reports on the possible causes of cancer due to *Mursik* consumption in Kenya (Patel *et al*., 2013; Nieminen *et al*., 2013). Back-slopping involving the use of samples from the previously prepared batch have been used in the production of products like *Amabere amaruranu*. Although this process produces products that are inconsistent in quality and their safety not assured (Nduko *et al*., 2017).

Basing our arguments on fermentation technologies used in developed countries, a need to develop and describe bacteria and their characteristics during their interaction in the ecosystems is important in order to be able to develop starter cultures for production under controlled conditions and be able to exploit the Probiotic potential (Franz *et al*., 2014). Traditional fermented milk products in Kenya have been associated with various microorganisms (Nyambane *et al*., 2014; Digo, 2015; Nieminen *et al*., 2013). There is a growing interest in research on potential starter microorganisms from various milk and milk products (Ouadghiri *et al*., 2009; Wouters *et al*., 2002)

Most of the studies have used culture-dependent techniques in the identification of microorganisms. However, the use of synthetic media to cultivate microorganisms in the laboratory mostly turns out to be inaccurate and can finally lead to misleading conclusions (Hijum *et al*., 2013). According to Reid *et al.,* (2014) and Nduko, (2017), none of the studies have put together the techniques which are culture-dependent with the current high throughput techniques which are independent, in the microbial study for starter culture development and none of the products have incorporated a defined culture.

2.6 High-Throughput Screening Assays

Micro-organisms characterization using throughput technologies began with the use of Polymerase Chain Reaction (PCR) in profiling microorganisms in traditionally fermented food products using the 16S rRNA genes and Denaturing Gradient Gel Electrophoresis (DGGE) sequencing (Tamang *et al*., 2016). This sequencing has shown to be useful in differentiating different genera or species in fermentation. Amplification of 16S ribosomal RNA genes or gene fragments, which are conversed and universally found in every bacterial genome, is an example of a high throughput screening assay. Comparative genomics is used to identify marker sequences specific for a group of bacterial pathogens or species or serotypes. PCR primer pairs designed to specifically bind shared marker regions are used for large-scale screening assays set up for detection, amplification, and characterization of these genotypes from various samples. The screening assays provide information about the absence or presence of a genetic feature within a sample of unknown microbial composition. If the amplified PCR product is sequenced, the information can be used for phylogenetic analysis, that is, different isolated PCR products can be aligned to generate taxonomic trees that predict the evolutionary relationships between the different isolates (Hugenholtz *et al*., 1998).

2.7 Camel keeping in North Eastern Kenya

Kenya has only one-humped (dromedary) camels, which is an important component of the livestock sector in the arid and semi-arid lands (ASALs) in the northern part of Kenya where 66% of the population live below the poverty line (ADF, 2003). In the past, because of lack of regular census, Kenya's camel population was estimated at below the one million mark. However, according to the results of 2009 livestock census, the national camel population is estimated at 2.97 million (KNBS, 2010).

The dromedary breed of camel is multipurpose, as it is kept for transportation, meat and milk production. It is also a financial asset and security for pastoralists. It plays a key role in wealth and social status for pastoralists (Guliye *et al*., 2007). For example, customarily, camels are the most important indicator of wealth and a determinant of status within the Somali society (Mahmoud, 2010). In Kenya, camels are important livelihood assets for food security and wealth creation in the ASALs. Camels provide income to the household through the sale of milk, meat, hides, transport services, riding and tourism which is essential to pastoral subsistence economy (Glücks, 2007; Njanja, 2007).

Milk from camel is highly ranked as an important utilization at the household level because it is a source of food and income (Njanja, 2007; Guliye *et al*., 2007). To Somali pastoralists, camels act as security against drought and diseases (Farah *et al*., 2004). Pastoralists often sell camels when they have emergencies or urgent need for cash and not when prices are optimal, as they satisfy their status roles and other needs.

Isiolo County is the central point connecting other areas of North Eastern Kenya and it covers an area of 25,700 km^2 with 143,294 People, according to 2009 Population Census of which 73,694

are males and 69,600 females. It borders the following counties; to the north, it borders Marsabit, Wajir to the north-east, Kitui and Tana River to the south, Laikipia and Samburu to the west, Garissa to the south-east, Tharaka Nithi and Meru to the south-west. It is located between longitude 36° 50' and 39° 50' east of the Greenwich Meridian; 0° 05' south and 2° latitude north of Equator with Isiolo town as the county headquarter (ASDSP, 2014). The county has three sub-counties; Garbatulla, Merti and Isiolo, and further sub-divided into administrative units of 11 divisions, 22 locations, and 44 sub-locations.

Livestock production is the major economic activity of the county's economy with over 80 % of the population relying on livestock farming for their livelihood. Camels, goat, and cows are the main livestock kept. Isiolo County has approximately about 40,300 camels, mostly owned by Borana and Somali communities and produces about 50,000L of milk daily. It is estimated that 87.5 % of the produced camel milk is for home consumption or sold to locals in nearby trading centers while 12.5 % is supplied to Eastleigh, the main market in Nairobi (Musinga *et al*., 2008).

Camel milk production supports a significant proportion of the population in rural and urban areas of Isiolo County and should be at the top of the county's agenda (Yazan and Wasonga, 2015). Due to diminishing grazing land, water resources and the recurrent drought, rearing camels can serve as an alternative to livestock or crops to provide a pathway to more resilient livelihoods. The county has a great opportunity to enhance milk production, value addition, and marketing. Therefore the policymakers should consider allocating more resources to the camel milk subsector (Yazan and Wasonga, 2015).

CHAPTER THREE: MICROBIAL QUALITY AND SAFETY OF TRADITIONAL FERMENTED CAMEL MILK PRODUCT *SUUSAC* SAMPLED FROM DIFFERENT REGIONS IN NORTH EASTERN, KENYA

Abstract

The study was carried out to assess the microbial quality and safety of fermented camel milk product (*Suusac*) from North Eastern Kenya.

Twenty-eight samples (n=28) of *Suusac* from different areas of the region sold in informal markets at Eastleigh in Nairobi were aseptically collected at the sales points. The quality and safety of the *Suusac* with reference to selected pathogens namely *E. coli, S. aureus, Shigella spp,* and *Klebsiella spp* was evaluated using the standard analytical methods.

Escherichia coli were detected in all the samples while *Staphylococcus aureus* was detected in 63.09 % of the samples analyzed. *Shigella spp* was detected in 88.1 % of the samples analyzed and *Klebsiella spp* was detected in 77.4 % of the samples. The mean $\log_{10}$ counts for *E. coli, S. aureus, Shigella,* and *Klebsiella spp* were 3.135, 2.576, 2.784 and 3.138, $CFUmL^{-1}$, respectively. There is a potential public health concern posed by *Suusac* which is sold for direct consumption due to the presence of the life-threatening bacterial pathogens.

The *Suusac* being sold at Eastleigh market in Nairobi from North Eastern Kenya may be responsible for transmission of these pathogens to the consumers. Training on food hygiene, improving production technology, hygienic conditions and implementing the food legislations along the value chain can minimize the risk.

3.1 Introduction

There is rising public health concern associated with microbial food safety with reports implicating unpasteurized and raw camel milk products as major contributing factors to illnesses caused by foodborne pathogens (De Buyser *et al.*, 2001; Odeyemi, 2016).Traditional milk products in Kenya are fermented spontaneously in gourds while modern techniques of milk fermentation involve the use of starter cultures to produce consistent and safe products with improved shelf-life (Nduko *et al*., 2017). In some communities, fermentation is carried out by the use of raw milk and this may lead to quality and safety problems (Anukam and Reid, 2009). Concerns regarding the safety of products of spontaneous fermentation due to the presence of pathogenic bacteria and chemical toxins produced by the bacteria have been raised. These concerns have been demonstrated by reports on the possible causes of diseases due to *mursik* consumption in Kenya (Patel *et al*., 2013; Nieminen *et al*., 2013).

Suusac is fermented camel milk that is consumed by the communities that have inhabited the arid and semi-arid areas of Kenya. Preparation of *Suusac* is through spontaneous fermentation of camel milk which is carried out in gourds treated with smoke (De Buyser *et al.*, 2001). The camels are milked directly into a gourd that has been cleaned, smoothed and treated with smoke. It was found out that the smoke improved color, taste and improves the shelf-life by up to 20 days. The milk used is usually raw without any kind of heating. *Suusac* fermentation is carried out for a period of one to two days and this takes place at room temperature of between 26 and 29 ^{o}C.

A study carried out by Kaindi *et al*., in 2011 showed that, 25 % of the milk at Isiolo market and 75 % of the milk at the final market in Nairobi, was not acceptable. *Suusac* production is also

associated with unkonwn factors such as poor udder health, milking personnel practices like tying the quarters to prevent suckling by the calf, dusty milking environment, and lack of water,which may act as points of contamination. Various microorganisms have been reported in traditional fermented milk products in Kenya (Nyambane *et al*., 2014; Digo, 2015; Musinga *et al*., 2008) and at the same time there exists no information on the microbial quality and safety of *suusac*.

Therefore, the objective of this study was to determine the quality and safety of traditionally fermented camel milk product (*Suusac*) from North Eastern Kenya.

3.2 Materials and Methods

3.2.1 Study Site

The study was carried out in various regions in North Eastern part of the country which is camel milk producing zone. The area has an average temperature ranging between 12 and 28 ^{o}C and receives low rainfall ranging between 300 and 500 mm per year. However, milk samples from those areas were collected in Eastleigh, Nairobi County which is a major urban consumption center for camel milk. North Eastern Kenya has approximately about 40,300 camels, mostly owned by Borana and Somali communities and produces about 50,000L of milk daily. It is estimated that 87.5 % of the produced camel milk is for home consumption or sold to locals in nearby trading centers while 12.5 % is supplied to Eastleigh, the main market in Nairobi (Musinga *et al*., 2008).

3.2.2 Milk Sampling

A total of 28 milk samples were exhaustively collected from selected places in the seven Counties of North Eastern, Kenya; Isiolo (14), Tana River (5), Marsabit (1), Namanga (3),

Garissa (3), Moyale (1), Mandera (1) were collected for this study. Approximately 50mls of each sample was obtained from bulking containers in selected trader shops in Eastleigh. At the shops, 28 traders were selected. Each sample was coded numerically to show the County from where it was sourced.

3.2.3 Determination of pH of milk samples

pH determination was done using an electronic digital pH meter (Orion Research Inc., Cambridge, MA, USA) which was calibrated using a Buffer solution of pH 4 and 7 following the ISO 26323:2009(en) method. Samples of the camel milk were taken and analyzed for pH. Readings were taken after immersing the pH meter electrodes into the samples and steady values displayed.

3.2.4 Microbial analysis

3.2.4.1 *Staphylococcus aureus*

The ISO 6888-1:1999 method was used for enumeration of *Staphylococcus aureus*.

25 mL of milk sample were aseptically measured into a sterile jar and 225 mL of buffered peptone water added, and then mixed in the stomacher for 30 seconds. The homogenate was shaken and then 1.0 mL pipetted into a tube containing 9 mL of buffered peptone water to make 10^{-1}dilution. This was mixed carefully by aspirating 10 times with a pipette. Using the same pipette 1.0 mL was in turn transferred into another dilution tube containing 9 mL of buffered peptone water to make the 10^{-2} dilution. This was repeated up to 10^{-7}dilution.

0.1 mL of homogenate samples were pipetted onto the surface of previously dried Baird-Parker agar plates and spread with a sterile bent glass rod in triplicate. The plates were then incubated at 37 ^{o}C for 24 hours. The enumeration was done using colony counter for colony forming units

and expressed per mL of the sample ($CFUmL^{-1}$). The colonies were identified based on colour which was black and shiny, with narrow white margins, surrounded by clear zones extending into the opaque medium. The colonies were confirmed by conducting catalase, lipase test and glucose fermentation.

3.2.4.2 *Escherichia coli*

ISO 16649-2:2001 method was used for enumeration of *Escherichia coli.*

25 mL of milk sample were aseptically measured into a sterile jar and 225 mL of buffered peptone water added, and then mixed in the stomacher for 30 seconds. The homogenate was shaken and then 1.0 mL pipetted into a tube containing 9 mL of buffered peptone water to make 10^{-1}dilution. This was mixed carefully by aspirating 10 times with a pipette. Using the same pipette 1.0 mL was in turn transferred into another dilution tube containing 9 mL of buffered peptone water to make the 10^{-2} dilution. This was repeated up to 10^{-7}dilution.

0.1 mL of homogenate samples were pipetted on the surface of dried Hi-Crome agar plates in triplicate and spread with a sterile bent glass rod. The plates were incubated at 30 ^{o}C for 4 hours and then at 44 ^{o}C for 18 hours. Enumeration was then done using colony counter for colony forming units on colonies which had bluish-green coloration and expressed per mL of the sample ($CFUmL^{-1}$). The colonies were confirmed by conducting IMViC test (Indole test, Methyl Red test, Voges Proskauer test).

3.2.4.3 *Shigella spp*

The ISO 21567:2004 method was used for enumeration of *Shigella spp.*

25 mL of milk sample were aseptically measured into a sterile jar and 225 mL of buffered peptone water added, and then mixed in the stomacher for 30 seconds. The homogenate was

shaken and then 1.0 mL pipetted into a tube containing 9 mL of buffered peptone water to make 10^{-1}dilution. This was mixed carefully by aspirating 10 times with a pipette. Using the same pipette 1.0 mL was in turn transferred into another dilution tube containing 9 mL of buffered peptone water to make the 10^{-2} dilution. This was repeated up to 10^{-7}dilution.

0.1 mL of homogenate samples were pipetted on the surface of dried plates of XLD agar in triplicates and spread with a sterile bent glass rod. The plates were incubated at 37 ^{o}C for 24 hours. Enumeration was then done using colony counter for colony forming units and expressed per mL of the sample ($CFUmL^{-1}$). Counting was done on presumptive Shigella colonies which appeared uniformly red. The colonies were confirmed by conducting oxidase, urea agar test and IMViC test.

3.2.4.4 *Klebsiella spp*

The ISO 21528-2:2004 method was used for enumeration of *Klebsiella spp*.

25 mL of milk sample were aseptically measured into a sterile jar and 225 mL of buffered peptone water added, and then mixed in the stomacher for 30 seconds. The homogenate was shaken and then 1.0 mL pipetted into a tube containing 9 mL of buffered peptone water to make 10^{-1}dilution. This was mixed carefully by aspirating 10 times with a pipette. Using the same pipette 1.0 mL was in turn transferred into another dilution tube containing 9 mL of buffered peptone water to make the 10^{-2} dilution. This was repeated up to 10^{-7}dilution.

0.1 mL of homogenate samples homogenate samples were pipetted on the surface of dried plates of XLD agar in triplicates and spread with a sterile bent glass rod. The plates were incubated at 37 ^{o}C for 24 hours. Enumeration was then done using colony counter for colony forming units and expressed per mL of the sample ($CFUmL^{-1}$). IMViC test was done for confirmation.

3.2.5 Statistical analysis

Data obtained from the analysis was subjected to analysis of variance (ANOVA) using Genstat software 15th Edition.

3.3 Results

3.3.1 pH and Microbial Counts Isolated from the Milk Samples

The pH and presence of selected microbial pathogens found in *Suusac* samples (n=28) from different areas of North Eastern region of Kenya were summarized in Table 1. The pH values for the samples ranged from 4.17 to 4.95. Samples coded 8 from Tana River, 10 from Mandera and 15 from Isiolo had the highest pH values averaging 4.93 while sample coded number 6 from Tana River, 24 and 27 from Isiolo had the lowest pH values. However, in terms of sites, all samples from Mandera had the highest pH values while samples from Tana River had the least pH values.

Sample coded number 25 from Tana River had the lowest *E. coli* population count of 2.39 $\log_{10}$ $CFUmL^{-1}$, and the highest was sample number 27 from Isiolo which had a count of 3.41 $\log_{10}$ $CFUmL^{-1}$. *Klebsiella spp.* was not detected in five samples coded 9,10,13,21 and 23 from different parts of North Eastern Kenya but was present in the other samples. The highest count of 4.24 $\log_{10}$ $CFUmL^{-1}$, was in sample coded 20 from Isiolo. The highest number of *Shigella spp* was in sample number 27 from Tana River, which had an average count of 3.28 $\log_{10}$ $CFUmL^{-1}$, and was absent in five samples number 8,13,14, 23 and 25 from different areas of North Eastern Kenya. The highest number of *Staphylococcus aureus* was in sample number 27, and had a count of 2.86 $\log_{10}$ $CFUmL^{-1}$ and was absent in samples 3,5,6,8,11,15,18,19,21 and 25.

Table 3. 1: **Bacterial contamination in log_{10} $CFUmL^{-1}$ and pH of *Suusac* samples from North Eastern, Kenya**

Samples (n = 28)	Region	pH	Contamination Levels			
			E. coli	*Klebsiella spp*	*S. aureus*	*Shigella spp*
1	Isiolo	4.45	3.32±0.07^{ab}	3.42±0.09^{bc}	2.6±0.3^{abc}	3.02±0.11^{e}
2	Isiolo	4.44	3.39±0.08^{ab}	3.25±0.04^{bc}	2.1±0.17^{d}	2.92±0.21^{cde}
3	Namanga	4.58	3.53±0.07^{a}	3.16±0.06^{c}	ND	2.54±0.28^{abcd}
4	Garissa	4.48	3.13±0.15^{cd}	2.67±0.62^{d}	2.56±0.24^{abcd}	2.83±0.16^{abcd}
5	Namanga	4.60	3.26±0.09^{bc}	2.61±0.32^{d}	ND	2.5±0.35^{abcd}
6	Tana River	4.17	3.17±0.11^{bc}	3.13±0.12^{c}	ND	2.63±0.31^{abcd}
7	Tana River	4.24	3.33±0.07^{abc}	2.00±0.00^{e}	2.26±0.24^{c}	2.66±0.22^{abc}
8	Tana River	4.94	2.64±0.30^{fg}	2.39±0.35^{d}	ND	ND
9	Isiolo	4.56	2.75±0.18^{ef}	ND	2.62±0.33^{abc}	2.48±0.44^{abcd}
10	Mandera	4.95	3.07±0.10^{d}	ND	2.79±0.28^{abc}	2.46±0.15^{abcd}
11	Moyale	4.53	3.22±0.08^{bc}	2.46±0.15^{d}	ND	2.39±0.36^{a}
12	Garissa	4.56	3.31±0.06^{abc}	3.15±0.18^{c}	2.59±0.11^{abc}	2.92±0.21^{cde}
13	Tana River	4.42	2.77±0.07^{ef}	ND	2.67±0.19^{abc}	ND
14	Isiolo	4.38	3.39±0.07^{abc}	3.31±0.08^{bc}	2.56±0.24^{abcd}	ND
15	Isiolo	4.9	3.26±0.09^{bc}	3.57±0.06^{b}	ND	3.01±0.2
16	Isiolo	4.37	3.32±0.14^{abc}	3.38±0.11^{bc}	2.82±0.19^{ab}	2.48±0.44^{abcd}
17	Namanga	4.42	3.23±0.05^{b}	3.07±0.07^{c}	2.67±0.19^{abc}	2.54±0.47^{abcd}
18	Isiolo	4.75	2.39±0.36^{g}	3.06±0.26^{c}	ND	2.82±0.2^{abcd}
19	Isiolo	4.45	3.14±0.09^{cd}	3.06±0.14^{c}	ND	2.85±0.22^{bcde}
20	Isiolo	4.4	3.30±0.08^{abc}	4.24±0.06^{a}	2.64±0.3^{abc}	2.99±0.11
21	Marsabit	4.48	2.82±0.19^{e}	ND	ND	2.59±0.11^{abcd}
22	Isiolo	4.43	3.34±0.10^{abc}	3.26±0.15^{bc}	2.39±0.36^{bcd}	2.94±0.31^{de}
23	Garissa	4.41	2.93±0.20^{de}	ND	2.15±0.21^{d}	ND
24	Isiolo	4.21	3.26±0.09^{bc}	3.45±0.09^{bc}	2.48±0.44^{abc}	3.04±0.24^{e}
25	Tana River	4.43	2.39±0.36^{g}	2±0.00	ND	ND
26	Isiolo	4.32	3.37±0.11^{abc}	3.34±0.05^{bc}	2.63±.31^{abc}	3.23±0.07^{e}
27	Isiolo	4.28	3.41±0.10^{ab}	3.35±0.07^{bc}	2.86±0.17^{ab}	3.28±0.06^{e}
28	Isiolo	4.29	3.30±0.07^{abc}	3.24±0.09^{bc}	2.82±0.2^{ab}	3.17±0.12^{e}
LSD (P≤0.05)		4.48	0.25	0.36	0.43	0.44

*Value along a column whose superscripts are different letters are significantly different at P<0.05.

*Each value is mean ± standard deviation for triplicate experiments.

***ND**=*Not detected*

3.3.2 Summary of selected pathogens isolated from *Suusac* samples from North Eastern, Kenya

Summary of each pathogen from the samples is shown in Table 2**.** *Klebsiella spp* in the samples collected had a mean value of 3.138 $\log_{10}$ CFUmL^{-1}, with a maximum of 4.303 $\log_{10}$ CFUmL^{-1}, and a standard deviation of ±0.4738. Klebsiella was not detected in 22.6 % of the samples. *Escherichia coli* in the samples collected had a mean value of 3.135 $\log_{10}$ CFUmL^{-1}, with a maximum of 3.591 $\log_{10}$ CFUmL^{-1}and a standard deviation of ±0.325. *E. coli* was detected in all the samples analyzed. *Shigella spp* in the samples collected had a mean value of 2.784 $\log_{10}$ CFUmL^{-1}, with a maximum of 3.322 $\log_{10}$ CFUmL^{-1}, and a standard deviation of ±0.350. It was not detected in 11.9 % of the samples analyzed. *Staphylococcus aureus* in the samples collected had a mean value of 2.576 $\log_{10}$ CFUmL^{-1}, with a maximum of 3.041 $\log_{10}$ CFUmL^{-1}, and a standard deviation of ±0.300. It was not detected in 36.91 % of the samples analyzed (Fig. 1).

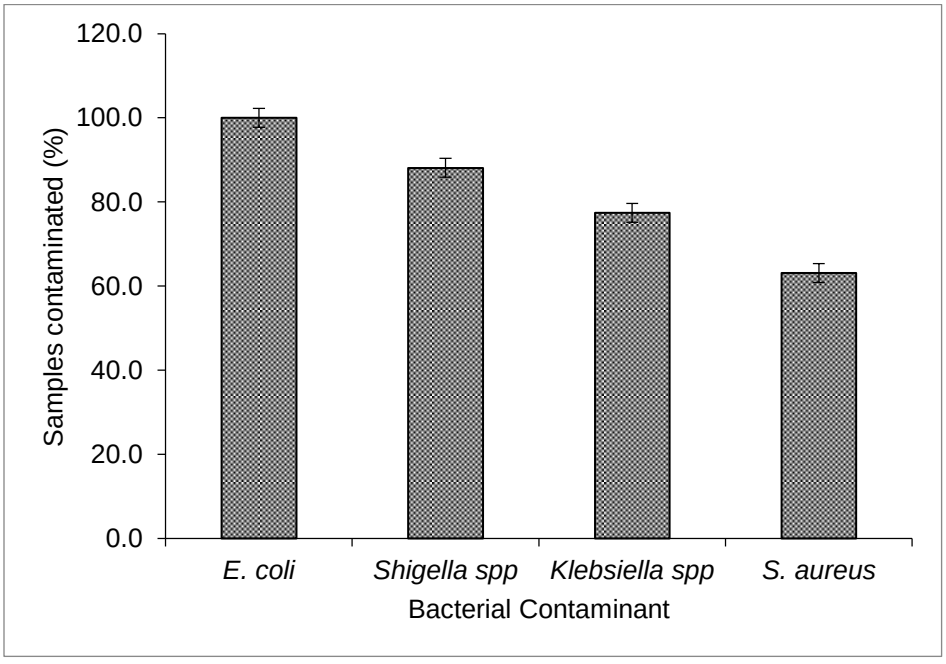

Figure 3. 1. Percentage of traditional fermented camel milk product samples from North Eastern, Kenya contaminated by various bacterial pathogens

Table 3. 2: Microbial analysis summary of selected pathogens from traditional fermented Camel milk product samples from North Eastern, Kenya

Spp	N	Mean	Standard Deviation	Minimum	Maximum
Klebsiella spp	65	3.138	0.4738	2.000	4.303
E. coli	84	3.135	0.325	2.000	3.591
Shigella spp	70	2.784	0.350	2.000	3.322
S. aureus	53	2.576	0.3000	2.000	3.041

* Data are mean values of triplicate samples.

* Units of bacterial counts are $\log_{10}$ $CFUmL^{-1}$

3.4 Discussion

The pH values for the samples ranged from 4.17 to 4.95. Samples number 8 from Tana River, 10 from Mandera and 15 from Isiolo had the highest pH values averaging 4.93 while sample number 6 from Tana River, 24 and 27 from Isiolo had the lowest pH values. The acidic nature of the milk samples could be due to production of lactic acid by microorganisms. The microbiological hazards present in traditionally fermented camel milk *(Suusac)* in the Kenyan main market was assessed by enumerating bacterial pathogens. The results show high contamination of *Suusac* with *E. coli, S. aureus, Shigella spp*, and *Klebsiella spp* which the study aimed at evaluating.

Being that *Suusac* is traditionally made from raw camel milk and is consumed directly without undergoing any processing, contamination may be common. The *Suusac* could have been contaminated along the market value chain. At the deliveries which also act as sales points, it is an open air market which is dusty, and has high human traffic. It is also dusty with open drainage lines and has flies. Hygienic condition of the food handlers is poor and they use opaque plastic

containers which are not easy to clean. There is no proper implementation of the public health rules and regulations. All these factors and poor hygienic practices can contribute to contamination of the *Suusac.*

Camel milk is believed to have therapeutic ability against many bacterial spp. due to the lytic action of lactoferrin and lysozyme present (Al-Majal *et al*., 2011; Rasheed 2017; Wernery *et al*., 2003), but it is still a significant source of human infections (El-Ziney *et al*., 2007, Matofari *et al*., 2007, Vanegas *et al*., 2009). The results indicated high level of microbial hazards in the products. *Klebsiella* spp was detected in 77.4% of the samples analyzed with an average of 3.138 $\log_{10}$ $CFUmL^{-1}$. The occurrence of this pathogen may be as a result of infection of the udder, poor hygiene of the handlers, cleaning, and disinfection of the S*uusac* containers (Bonfoh *et al*., 2006) .The pathogen is associated with pneumonia, intraabdominal infections, urinary tract and bloodstream infections to humans and animals (Peterson, 2006).

Escherichia coli was detected in many of the samples but according to KEBS standards (KS 941:2018), *Escherichia coli* should be totally absent in fermented milk. Various studies have shown *E. coli* O157:H7 is resistant to acid (Zhao *et al*., 1995), and it can survive for long periods of time in fermented milk products (Tsegaye and Ashenafi 2005 ; Bachrouri *et al.,* 2006, Şimşek *et al.*, 2006).There was a 36% prevalence of Shiga toxin-producing Escherichia coli (STEC) in camel milk with half of the isolates being from *Suusac* (Njage *et al*., 2010). In Zimbabwe, 100% of all naturally fermented milk had *E. coli* (Odeyemi,2016).The results indicate that *Suusac* could be an important medium for the transmission of pathogens to humans for instance strains of *E. coli* like O555, O111, O127 that cause infantile diarrhea, while others like 06:H16, O5:H11, and O25:H42 that produce potent enterotoxins capable of producing acute diarrhea.

The population of *Shigella spp* averaged 2.784 $\log_{10}$ $CFUmL^{-1}$ and was detected in 88.1% of the samples analyzed. When ingested, *Shigella spp* grows in the intestine, then lyses and release endotoxins causing an infection called shigellosis. *Shigella spp* existed in raw camel milk samples but not detected in any tested samples of fermented camel milk in Iran (Gran *et al*., 2003).Contamination of raw milk is usually from external sources (Yam *et al*., 2014).Therefore, the results from this study clearly indicate there was contamination of the *Suusac* during production, storage or at the sales point.

Staphylococcus aureus in the samples collected had a mean value of 2.576 $\log_{10}$ $CFUmL^{-1}$ and was detected in 63.1% of the samples analyzed. This showed the samples were highly contaminated with *S. aureus* which is an enterotoxins producer that causes gastroenteritis after consumption of contaminated food (Hennekinne *et al.,* 2012).This results concurs with those reported in Morocco where *S. aureus* was present in 30% of the samples with an average count of 2.32$CFUmL^{-1}$ (Loir *et al.*, 2003). Similarly, work done on *Roub*, a Sudanese traditionally fermented dairy product found out that *S. aureus* was present in 60% of the samples analyzed with a bacterial count of 6.18$CFUmL^{-1}$ (Ismaili *et al*., 2016). Another study found out that this microbe is the most commonly isolated from udder infections in camels and causes diseases to both humans and animals (Abdalla and Hussain,2010).Nosocomial and community-acquired staphylococcal infections are the most common cases reported in humans (Younan and Abdurahman, 2004) . Coagulase positive and negative *Staphylococci* are pathogens which cause mastitis in animals (Uemura *et al.,* 2004; Krishnamoorthy *et al.,* 2016).

Many factors along the informal *Suusac* production and market chain contribute to its quality and safety. The slow fermentation process of *Suusac* by traditional methods of production which is usually as a result of weak starter culture leads to contamination with pathogenic and toxigenic

bacteria, molds, and other unwanted changes in the milk (Chelule *et al.*, 2010). These pathogens have been found to grow faster than lactic acid bacteria (FAO, 1999). The environment is a contributing factor to cross-contamination of *Suusac*. Milking area is usually open and dusty hence possibility of contaminating the milk and milk containers with microorganisms from the soil, milking personnel or camel coat during milking (Abera *et al.*, 2016; Lore *et al*., 2005). The study was done in the months of July and August when it was extremely dry and dusty hence high levels of contamination from the dust.

Suusac is traditionally prepared from unpasteurized milk (Gran *et al.*, 2002). Traditional preparation methods can mitigate foodborne diseases. *Suusac*, flow through a long informal value chain in order to meet the increased demand from the urban areas, resulting in increased risk. There is increased handling of the product and also the informal end markets are in poor hygienic conditions. These informal markets are highly preferred by the poor and middle-class people because they are cheap, have trusted vendors who can give credit facilities but there is a high risk of product contamination from the dirty open drainages and dusty surroundings. There is no strict implementation of the food safety legislation hence this is a public health concern. Good quality water and proper sanitation are important if milk contamination is to be avoided (Lore *et al*., 2006). Containers should be cleaned with clean potable water to avoid contamination (Farah Z.,2004) but another study found out that water in the ASALs of Kenya is highly contaminated and scarce, thus difficult to improve the hygiene standards at the milking level (Knight-Jones *et al*., 2016).This could have been a contributing factor to the microbial contamination.

In this study, it was also found that plastic containers of five, ten and twenty liters which are opaque with narrow openings are used for handling, storage, and transportation of *Suusac*.

Therefore, this creates a problem in cleaning (Amenu *et al*., 2016; Bonfoh *et al*., 2006; Ahmed *et al*., 2010; Younan and Abdurahman, 2004) and therefore a contributing factor to the pathogenic contamination. Lactating camels with mastitis also contribute to foodborne pathogens and therefore, they can also be linked as a source of the pathogens (Oliver *et al*., 2005; Tesfaye *et al*., 2011;Kaindi *et al*., 2011). The poor microbial quality of *Suusac* was contributed by the many interactive factors discussed above. Therefore, production of *Suusac* with unpasteurized milk, with the poor hygienic conditions along the value chain as it is currently, poses potential public health risk as was reported in other studies (Karenzi *et al*.,2013; Farah *et al*., 2007).

3.5 Conclusion

The study concludes that the microbiological quality determined for *Suusac* was poor and of public health concern due to the presence of pathogens in the samples which according to KEBS standards (KS 941:2018) should be absent. This may be due to production processes, handling practices, storage vessels, selling method, and or sales environment.

The presence of life-threatening pathogens is a potential health risk which makes the product unfit for consumption. The *Suusac* being sold at Eastleigh market in Nairobi may be responsible for transmission of the pathogens to the consumers; therefore, there is a need for effective control.

3.6 Recommendations

Stringent measures along the fermented camel milk products value chain should be observed to safeguard the health of consumers and strengthen *Suusac* business by the informal producers and

vendors**.** Further study to determine the toxins produced by the *S. aureus* and *E. coli* present in the *Suusac* should be carried out.

CHAPTER FOUR: THE TECHNOLOGICAL FUNCTIONALITY OF AFRICAN *STREPTOCOCCI* STRAINS IN MILK FERMENTATION

Abstract

Streptococcus infantarius is predominant in most African fermented dairy products. However, little information is given on their technological properties. This study was carried out to determine the technological functionality of selected African *Streptococci* strains in dairy fermented products. Each of the strain, and a selected combination was inoculated in pasteurized camel and cow milk samples at a rate of 3 % v/v and then incubated at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C, and 45 ^{o}C for 9 hours. Analysis was done after every 3 hours for pH and titratable acidity while viscosity was done after incubation and cooling of the product. Sensory analysis for mouthfeel, sourness and general acceptability was done by 12 panelists. Titratable acidity for fermented camel milk ranged from 0.42±0.03 to 0.83±0.03 while that of cow milk ranged from 0.89±0.010 to 0.97±0.010 after incubation. The pH of camel milk ranged from 5.880±0.020 to 4.150±0.010 while that of cow milk ranged from 5.20±0.02 to 4.12±0.02. The cow milk had the highest viscosity level compared to camel milk. The levels of Titratable acidity and viscocity depended on the strain and incubation temperature. The African *Streptococci* strain isolated from fermented camel milk had good technological properties that are useful as starter culture for development of fermented milk products.

4.1 Introduction

In Kenya, production of traditional fermented dairy products like *Suusac* and *Mursik* is carried out through spontaneous fermentation. This technology has shown vast growth in the food industry because of low cost in energy, infrastructure, and the wide acceptance of traditionally fermented food products in Kenya (Tamang and Samuel, 2010). Demand for fermented food products is rising due to the health benefits associated with these products, migration, and urbanization of the people (Franz *et al*., 2014). This calls for the improvement in the safety and quality of the fermented food products. There is a need for the improvement of the technologies involved in fermentation in order to ensure the production of safe products which are consistent in quality and that are widely accepted by the community (Nduko *et al*., 2017). Several limitations have accrued in spontaneous fermentation of food products which include; low yields, inefficiency, and production of products with varying quality standards (Chilton *et al*., 2015).

Traditional milk products in Kenya are fermented spontaneously in gourds while modern techniques of milk fermentation involve the use of starter cultures to produce consistent and safe products with increased shelf-life as opposed to those spontaneously fermented (Nduko *et al*., 2017). In some communities, fermentation is carried out by the use of raw milk and this may lead to safety concerns while in other products like *Mursik*, the milk is boiled before fermentation (Anukam and Reid, 2009). Basing argument on fermentation technologies used in developed countries, a need to develop and describe bacteria and their characteristics during their interaction in the ecosystems is important in order to be able to develop starter cultures for production under controlled conditions and be able to exploit the Probiotic potential (Franz *et al*., 2014). There is a growing interest in research on potential starter microorganisms from various

milk and milk products (Ouadghiri *et al*., 2009; Wouters *et al*., 2002)**.** Lactic acid bacteria (LAB) are the main microorganisms involved in the fermentation of various products (Nduko *et al*., 2017).

Many species such as *Lactococcus lactis, Lactobacillus spp, Streptococcus bovis/ Streptococcus equinus* complex (SBSEC), *Enterococcus spp* and yeast are present in milk (Jans *et al*., 2017). Members of the *Streptococcus species* are always identified in milk and milk products across the world. Those that are commonly identified in Africa include; *Streptococcus thermophillus (S.thermophillus), Streptococcus salivarius, Streptococcus infantarius subspecies infantarius (Sii), Streptococcus gallolyticus, and Streptococcus agalactiae* (Jans *et al*., 2017). Of these, only *S. thermophillus* is approved for its use in dairy processes, however, Africa fermented dairy products (AFDPs) are dominated by *Sii* rather than *S. thermophillus*. According to Jans *et al*., 2017, most dairy products in Africa is often consumed raw, as well as in the form of traditional AFDPs.

Sii is a member of the SBSEC complex which is mainly associated with pathogenic microorganisms and it is the predominant LAB in AFDPs. The role of *Sii* in milk fermentation was not well known until it was isolated as the predominant LAB in cow and camel fermented milk in Kenya, Somalia and Cote d'Ivoire (Jans *et al*., 2012; Jans *et al*., 2013; Wullschleger *et al*., 2013). Genomic analyses on *Sii* isolates have revealed an adaptation to lactose metabolism that is parallel to that of *S. thermophillus*. The common ancestor of *S. thermophillus* strains is believed to have lived between 3,000 - 30,000 years ago based on genome decay and this is approximately when human dairy activity started (Bolotin *et al*., 2004 and Fox, 1993). In East Africa Camels were introduced around 2,500 years ago (Epstein, 1971; Mikesell, 1955) and the

less genome decay in *CJ18* may be attributed to the start of fermentation of camel milk which came later (Jans *et al*., 2013).

The analysis of the African strain of *Sii* that is *CJ18* has also revealed more dairy adaptations like *S. thermophillus* to the dairy niche. *Sii* has been found to carry a partial additional gal-lac operon consisting of genes lacS and lacZ and exhibiting phenotypic lactose/galactose exchange as *S. thermophilus* (Jans *et al*., 2013). *Sii* has not been classified as GRAS (generally recognized as safe). Its occurrence in intestinal tracts of humans and animals, together with its presence in AFDPs requires research to identify its phylogeny and host associations and the ability to move in different ecological niches and hosts (Jans *et al*., 2013). Further research on the functional analysis of *Sii* is required to ensure innovations like the development of starter cultures with the optimization of the manufacturing processes are implemented based on facts from the findings. The objective of the study was to test the functionality of selected African *Streptococci* strains; African type *S. thermophillus*, *Sii CJ 18*, *Sii CCUG*, in the fermentation of camel and cow milk. The study aimed at establishing suitable technological properties for adapting in development of suitable African type starter cultures, for quality improvement and standardized production of the traditionally fermented dairy products.

4.2 Materials and Methods

4.2.1 Study setting and Milk samples

Raw camel milk was obtained from Isiolo County in Kenya, frozen and then transported in a cool box to pilot plant at University of Nairobi where it was pasteurized. Raw cow milk was obtained at Kanyariri farm at the University of Nairobi and transported to pilot plant for pasteurization. Processing quality was determined by checking acidity, clot on boiling, alcohol test, smell, and taste, then pasteurized at 90 ^{o}C for 30 minutes, cooled and dispensed into sterile 500 ml containers before inoculating with the cultures

4.2.2 Process flow for camel and cow milk fermentation

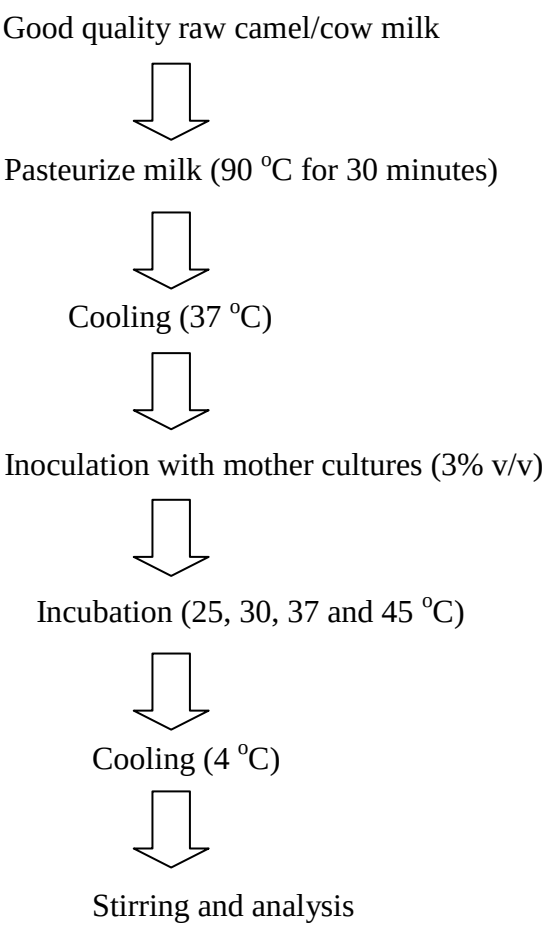

Figure 4. 1: Process flow chart of camel and cow milk fermentation

4.2.3 Preparation of starter cultures

Working cultures of African type *S. thermophillus, Sii CJ18, Sii CCUG* were prepared from pure isolates of frozen stocks obtained from Micalis Institute, France. The pure isolates were transferred into fresh MRS broth (Oxoid, UK) twice at 37 ^{o}C for 24 hours. Strains were selected based on the turbidity of the tubes, phenotypic characteristics including Gram staining, catalase reaction, cell morphology, arginine hydrolysis, and CO_2 production from glucose in modified MRS broth containing inverted Durham tubes (Sharpe, 1979).

The isolates were evaluated for acid production after fermentative growth on selected carbohydrates (maltose, lactose, fructose, galactose, raffinose, ribose, rhamnose, glucose, sucrose, arabinose, mannitol, mannose, melibiose, sorbitol, and xylose). Confirmed African type *S. thermophillus, Sii CJ18, Sii CCUG* strains were then grown in MRS broth at 37 ºC for 24 hours to make the working cultures (Milo and Phillips, 2015).

4.2.4 Preparation of inoculums

Skimmed milk powder was used to prepare the mother culture from the stock culture. This was done by reconstituting it to 10 % total solids then autoclaved and cooled to 37 °C. 250 ml of skimmed milk were inoculated with each of the stock culture (African type *S.thermophillus, Sii CJ18, Sii CCUG*) at the rate of 3 % v/v and shaken thoroughly to ensure proper mixing, then incubated for 6 hours at 37 ^{o}C temperatures. These inoculums were used for the starter culture fermentation trials.

4.2.5 Fermentation trials

Standardized inoculums of African type *S. thermophillus*, *Sii CJ18*, *Sii CCUG*, combination of African type *Streptococcus thermophillus* and *Sii CJ18*, were prepared by heating 500 ml fresh

raw camel and cow milk to 90 °C for 30 minutes, cooled and then inoculated with 3 % v/v of the mother culture and each inoculum incubated at temperatures of 25 °C, 30 °C, 37 °C and 45 °C for up to 9 hours.

The samples were analyzed for acidity, pH, viscosity and sensory analysis. Acidity and pH were analyzed after every 3 hours during incubation, while viscosity was done for the end products after refrigerating the samples. Sensory analysis on the potential cultures which showed a competitive advantage was done by 12 panelists for mouth feel, sourness and general acceptability

4.2.5.1 Determination of Acidity

Acidity of raw milk and during fermentation was determined in triplicate and according to the AOAC (2000) method number 947.05. Nine mL of the milk samples was pipetted in a flask followed by the addition of 3 drops of phenolphthalein indicator then titrated against 0.1N NaOH till a light pink color appeared. The titer value was recorded to determine the acidity of raw/fermented camel milk. The acidity was then calculated using the equation below and expressed in terms of lactic acid:

Equation 1: Acidity calculation

% Acidity (as lactic acid) = Volume of 0.1N NaOH used X 0.09.....1

0.09 is the multiplication factor.

(Lactic acid is an organic acid (CH_3-CHOH-COOH) and has a molecular weight of 90. So one ml of 0.1 NaOH corresponds to 0.09 g lactic acid)

AOAC (2000) method number 947.05

4.2.5.2 Determination of pH

ISO 26323:2009(en) method was used. This was done using an electronic digital pH meter (Orion Research Inc., Cambridge, MA, USA) which was calibrated using a Buffer solution of pH 4 and 7. Samples of the camel milk undergoing fermentation trials were taken and analyzed for pH. Readings were taken after immersing the pH meter electrodes into the samples and steady values displayed.

4.2.5.3 Determination of viscosity

ISO 2555:2018(E) method was used. Apparent viscosity was measured using a viscometer (Model uon-pp-004) and results expressed in cPs. Viscosity measurements were performed after the fermentation processes for each of the sample, and were done in triplicate.

4.2.5.4 Sensory analysis

The descriptive sensory analysis was carried out as described by Lawless and Heymann, 2010. Twelve trained panelists from The Department of Food Science, Nutrition and Technology at the University of Nairobi were used. Cow and Camel milk Samples were subjected to sensory analysis. The samples were coded and the panelists were advised to taste the coded samples without swallowing then rinse their mouth with warm water then rate as per the given scores for each attribute. Attributes analyzed were mouth feel (oral consistency, oral viscosity, oral presence of lumps), Sourness and overall acceptance with a 5 hedonic scale (like a lot-5, like a little 4, neither like nor dislike-3, dislike a little-2, dislike a lot- 1).

4.2.6 Statistical analysis

Data obtained from the analysis was subjected to ANOVA using Genstat software 15th Edition.

4.3 Results

4.3.1 Fermentation of camel milk

4.3.1.1 Titratable Acidity of camel milk

Development of acidity at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C, and 45 ^{o}C for the different strains at 3 hours interval was as summarized in Table. The mean ranges were between 0.89±0.010 to 0.97±0.010 depending on the incubation temperature and sample strain. The titratable acidity increased with increased incubation temperature and time. Milk treated with both *ST* and *CJ18* and incubated for nine hours recorded the highest levels of titratable acidity which increased with increase in incubation temperature and time.

Increasing the incubation temperature significantly ($p<0.05$) increased the titratable acidity due to fermentative action of the strains. Increasing the fermentation time also increased the titratable acidity. Titratable acidity for all the strains was highest at 45 ^{o}C, with *CJ18* performing better than the other strains followed by a combination of *ST* and *CJ18*.The acidity for *CJ18,ST* and the combination of *ST* and *CJ18* increased sharply as from 30 ^{o}C.

Table 4.1: Titratable Acidity of Camel milk inoculated with different strain of *Streptococcus* and incubated at different temperatures and time

Strain	Time (hours)	Temperature			
		25 °C	30 °C	37 °C	45 °C
CCUG	0	0.848±0.010^{kl}	0.840±0.028^{l}	0.863±0.031^{ijk}	0.880±0.010^{hi}
	3	0.867±0.006^{i}	0.850±0.010^{kl}	0.870±0.030^{ij}	0.890±0.010^{fh}
	6	0.880±0.010^{hi}	0.880±0.010^{hi}	0.910±0.020^{f}	0.890±0.010^{fh}
	9	0.890±0.010^{gh}	0.890±0.020^{gh}	0.920±0.010^{d}	0.910±0.010^{f}
CJ18	0	0.845±0.013^{l}	0.840±0.070^{l}	0.810±0.030^{m}	0.890±0.020^{gh}
	3	0.853±0.006^{jkl}	0.850±0.010^{kl}	0.850±0.030^{k}	0.893±0.015^{fh}
	6	0.867±0.058^{ij}	0.890±0.020^{gh}	0.890±0.020^{gh}	0.893±0.006^{fh}
	9	0.890±0.010^{gh}	0.900±0.020^{fg}	0.910±0.010^{ef}	0.960±0.010^{ab}
ST	0	0.855±0.034^{jkl}	0.810±0.014^{m}	0.803±0.006^{m}	0.880±0.020^{hi}
	3	0.890±0.010^{gh}	0.850±0.020^{kl}	0.890±0.010^{gh}	0.893±0.152^{fh}
	6	0.910±0.010^{ef}	0.890±0.010^{gh}	0.920±0.010^{de}	0.920±0.010^{de}
	9	0.910±0.010^{ef}	0.920±0.010^{de}	0.937±0.006^{cd}	0.950±0.010^{bc}
ST and CJ18	0	0.888±0.022^{ghi}	0.850±0.021^{kl}	0.810±0.020^{m}	0.860±0.020^{jk}
	3	0.900±0.010^{fg}	0.850±0.010^{kl}	0.860±0.020^{jk}	0.900±0.010^{fg}
	6	0.910±0.000^{ef}	0.880±0.010^{hi}	0.910±0.020^{ef}	0.920±0.020^{de}
	9	0.930±0.010^{d}	0.937±0.006^{cd}	0.960±0.010^{ab}	0.970±0.010^{a}
LSD		0.017			

*Values with different letters in superscripts are significantly different at P<0.05.

*Each value is mean ± standard deviation for triplicate experiments.

4.3.1.2 pH of camel milk

pH development at 25 °C, 30 °C, 37 °C, and 45 °C for the different strains at 3 hours interval is summarized in Table 4. The average pH ranged from 5.880±0.020 to 4.150±0.010 depending on the strain and incubation temperature after fermentation. pH was significantly affected by incubation time, temperature and microbial strain at $p<0.05$. The interaction between microbial strain and incubation temperature did not significantly affect the pH at $p>0.05$

Table 4.2: pH of Camel milk inoculated with different strains of *Streptococcus* and incubated at different temperatures and time

Strain	Time (hours)	Temperature			
		25 °C	30 °C	37 °C	45 °C
CCUG	0	6.923±0.030^{a}	6.960±0.028^{a}	6.890±0.010^{a}	5.120±0.010^{lm}
	3	6.150±0.020^{bcd}	6.975±0.070^{a}	5.440±0.020^{i}	4.820±0.020^{no}
	6	5.990±0.010^{de}	5.880±0.020^{ef}	5.210±0.010^{k}	4.320±0.020^{p}
	9	5.850±0.020^{f}	5.800±0.010^{fg}	5.180±0.010^{lm}	4.300±0.020^{p}
CJ18	0	6.950±0.022^{a}	6.090±0.010^{b}	6.890±0.020^{a}	6.980±0.010^{a}
	3	6.267±0.015^{b}	6.010±0.010^{cde}	6.010±0.010^{cde}	4.780±0.020^{o}
	6	6.030±0.020^{cde}	5.690±0.010^{fgh}	5.510±0.020^{i}	4.770±0.020^{o}
	9	5.880±0.020^{ef}	5.640±0.020^{fgh}	5.150±0.020^{lm}	4.740±0.010^{o}
ST	0	6.905±0.013^{a}	5.800±0.010^{fg}	6.920±0.010^{a}	6.900±0.010^{a}
	3	6.220±0.010^{bc}	5.630±0.010^{g}	5.160±0.020^{lm}	4.260±0.010^{p}
	6	5.870±0.010^{ef}	5.420±0.020^{ijk}	5.140±0.010^{lm}	4.170±0.030^{p}
	9	5.720±0.010^{fgh}	5.510±0.010^{h}	5.000±0.020^{mn}	4.150±0.010^{p}
ST and CJ18	0	6.938±0.026^{a}	5.420±0.010^{ij}	6.900±0.020^{a}	6.910±0.010^{a}
	3	6.020±0.010^{cde}	5.540±0.020^{ij}	6.070±0.020^{bcde}	5.540±0.020^{ij}
	6	5.710±0.020^{fgh}	5.300±0.200^{j}	5.420±0.020^{ijk}	5.320±0.010^{jl}
	9	5.450±0.030^{ij}	5.250±0.020^{k}	5.120±0.010^{lm}	4.710±0.010^{o}
LSD		0.21			

*Values with different letters in superscripts are significantly different at P<0.05.

*Each value is mean ± standard deviation for triplicate experiments**.**

4.3.1.3 Viscosity of camel milk

Viscosity after 9 hours of fermentation at 25 °C, 30 °C, 37 °C, and 45 °C for the different strains is summarized in Table 5. The viscosity ranged from 18.6±0.755 to 29.44±0.906 cPs depending on the strain and incubation temperature. Increasing the incubation temperature of the starter cultures significantly increased viscosity at p<0.05. *ST* and *CJ18* sample was the most viscous across all the incubation temperatures at p<0.05. Interaction between the samples and incubation

temperatures had a significant effect on the viscosity at $p<0.05$. All the samples had the highest viscosity at 45 ^{o}C with sample 4 *of ST and CJ18* being the most viscous ($p<0.05$).

Table 4. 3: Viscosity of Camel milk inoculated with different strains of *Streptococcus* and Incubated at different temperatures

Strain	**Temperature**			
	25 ^{o}C	**30 ^{o}C**	**37 ^{o}C**	**45 ^{o}C**
CCUG	19.7 ± 0.796^{efg}	20.13 ± 0.304^{efg}	21.08 ± 1.152^{de}	27.06 ± 1.907^{b}
CJ18	18.6 ± 0.755^{g}	20.68 ± 1.637^{ef}	22.8 ± 1.46^{d}	25.4 ± 1.48^{c}
ST	18.8 ± 1.143^{fg}	20.06 ± 0.122^{efg}	21.1 ± 0.872^{de}	22.88 ± 1.013^{d}
ST and CJ18	20.29 ± 1.206^{efg}	21.31 ± 1.107^{de}	21.53 ± 0.79^{de}	29.44 ± 0.906^{a}
LSD	1.882			

*Viscosity values expressed in cPs (units)

*Values with different letters in superscripts are significantly different at $P<0.05$.

* Each value is mean ± standard deviation for triplicate experiments.

4.3.2 Fermentation of cow milk

4.3.2.1 Titratable Acidity of cow milk

Development of acidity at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C and 45 ^{o}C for the different strains at 3 hours interval was as summarized in Table 6. The average was from 0.42±0.03 to 0.83±0.03 depending on the strain and incubation time. The incubation temperature, time and their interaction were significant factors that affected the titratable acidity of the starter culture at $p<0.05$. Increasing the incubation temperature significantly ($p<0.05$) increased the titratable acidity due to fermentative action of the strains. Increasing the fermentation time also increased the titratable acidity.

Table 4. 4: Titratable Acidity of Cow milk inoculated with different strains of *Streptococcus* and incubated at different temperatures and time

Microbial strain	Incubation temperature (^{o}C)	Incubation period (hours)			
		25 ^{o}C	30 ^{o}C	37 ^{o}C	45 ^{o}C
CCUG	0	0.18±0.02^{a}	0.17±0.01^{a}	0.17±0.01^{a}	0.17±0.01^{a}
	3	0.22±0.01^{b}	0.28±0.02^{b}	0.28±0.02^{b}	0.36±0.02^{b}
	6	0.42±0.02^{d}	0.50±0.02^{d}	0.55±0.03^{d}	0.71±0.03^{d}
	9	0.42±0.03^{d}	0.52±0.01^{d}	0.66±0.02^{e}	0.82±0.03^{e}
CJ18	0	0.16±0.02^{a}	0.18±0.01^{a}	0.18±0.01^{a}	0.17±0.01^{a}
	3	0.29±0.03^{c}	0.36±0.02^{c}	0.41±0.03^{c}	0.46±0.02^{c}
	6	0.52±0.02	0.41±0.02	0.72±0.04^{f}	0.80±0.02^{e}
	9	0.56±0.02	0.73±0.03	0.85±0.03^{h}	0.82±0.09^{e}
ST	0	0.17±0.01^{a}	0.17±0.01^{a}	0.17±0.01^{a}	0.17±0.01^{a}
	3	0.27±0.03^{c}	0.35±0.03^{c}	0.41±0.02^{c}	0.46±0.01^{c}
	6	0.48±0.01^{e}	0.50±0.02^{d}	0.67±0.02^{e}	0.72±0.04^{d}
	9	0.53±0.01^{ef}	0.56±0.02^{e}	0.76±0.02	0.83±0.05^{e}
ST and *CJ18*	0	0.17±0.01^{a}	0.17±0.01^{a}	0.17±0.01^{a}	0.17±0.01^{a}
	3	0.28±0.03^{c}	0.36±0.02^{c}	0.41±0.01^{c}	0.47±0.02^{c}
	6	0.50±0.02^{e}	0.49±0.03^{d}	0.66±0.02^{e}	0.72±0.04^{d}
	9	0.55±0.01^{f}	0.61±0.03^{f}	0.80±0.03^{g}	0.83±0.03^{e}
Mean		0.36±0.15^{A}	0.40±0.17^{B}	0.49±0.24^{C}	0.54±0.27^{D}

*Mean ± SD with different lowercase letters along a column and uppercase letters across a row are significantly different at $p<0.05$.

*Each value is mean ± standard deviation for triplicate experiments.

4.3.2.2 pH of cow milk

pH development at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C, and 45 ^{o}C for the different strains at 3 hours interval is summarized in Table 7. The average pH after fermentation ranged from 5.20±0.02 to 4.12±0.02 depending on the strain and incubation temperature. At initial stages of incubation with different temperature exposures, milk treated with all the strains had high pH levels, however, the levels

of pH reduced with increased incubation time. In general, as the temperature of incubation increased, the average pH level of milk reduced

Table 4.5: pH of Cow milk inoculated with different strains of Streptococcus and incubated at different temperatures and time

Microbial strain	Incubation period (hours)	Incubation temperature (^{o}C)			
		25 ^{o}C	30 ^{o}C	37 ^{o}C	45 ^{o}C
CCUG	0	6.72±0.04^{a}	6.78±0.03^{a}	6.79±0.02^{a}	6.78±0.04^{a}
	3	6.30±0.01^{b}	6.24±0.04^{b}	5.84±0.05^{b}	5.73±0.02^{b}
	6	5.61±0.02^{c}	5.38±0.0^{c}	5.01±0.03^{c}	4.73±0.04^{c}
	9	5.20±0.02^{d}	4.88±0.03^{d}	4.77±0.04^{c}	4.24±0.02^{c}
CJ18	0	6.75±0.04^{a}	6.71±0.02^{a}	6.81±0.04^{a}	6.75±0.02^{a}
	3	5.84±0.06^{c}	6.01±0.06^{b}	5.29±0.03^{c}	5.31±0.03^{b}
	6	5.25±0.04^{d}	5.22±0.04^{c}	4.71±0.03^{d}	4.20±0.02^{d}
	9	5.07±0.08^{d}	4.75±0.03^{d}	4.18±0.03^{e}	4.07±0.06^{d}
ST	0	6.74±0.03^{a}	6.78±0.03^{a}	6.77±0.03^{a}	6.81±0.03^{a}
	3	5.89±0.07^{b}	6.07±0.06^{b}	5.38±0.03^{c}	5.39±0.03^{b}
	6	5.46±0.04^{c}	5.33±0.04^{c}	4.82±0.04^{d}	4.48±0.04^{c}
	9	5.13±0.04^{d}	4.96±0.03^{d}	4.30±0.05^{e}	4.29±0.03^{c}
ST and *CJ18*	0	6.76±0.05^{a}	6.71±0.03^{a}	6.74±0.04^{a}	6.79±0.04^{a}
	3	5.86±0.06^{c}	6.03±0.06^{b}	5.29±0.01^{c}	5.41±0.04^{b}
	6	5.38±0.06^{c}	5.26±0.02^{c}	4.72±0.03^{d}	4.33±0.03^{c}
	9	5.05±0.08^{d}	4.86±0.06^{d}	4.22±0.04^{e}	4.12±0.02^{c}
Mean		5.81±0.64^{a}	5.75±0.74^{a}	5.35±0.93^{b}	5.22±1.04^{b}
LSD (P≤0.05)		0.34	0.41	0.51	0.57

*Mean ± SD with different lowercase letters along a column and uppercase letters across a row are significantly different at p<0.05.

*Each value is mean ± standard deviation for triplicate experiments.

4.3.2.3 Viscosity of cow milk

Viscosity after 9 hours of fermentation at 25 ^{o}C, 30 ^{o}C, 37 ^{o}C, and 45 ^{o}C for the different strains is summarized in Table 8. The viscosity ranged from 19.77±0.37 to 59.64±0.49 cPs depending on the strain and incubation temperature. Increasing the incubation temperature of the starter cultures significantly increased viscosity at $p<0.05$. *ST* and *CJ18* sample was the most viscous across all the incubation temperatures at $p<0.05$. Interaction between the samples and incubation temperatures had a significant effect on the viscosity at $p<0.05$. All the samples had the highest viscosity at 45 ^{o}C with sample 4 of *ST* and *CJ18* being the most viscous ($p<0.05$). Viscosity increased with increase in temperature.

Table 4.6: Viscosity of Cow milk inoculated with different strain of *Streptococcus* and Incubated at different temperatures

Sample	**Incubation temperature (^{o}C)**			
	25 ^{o}C	**30 ^{o}C**	**37 ^{o}C**	**45 ^{o}C**
CCUG	25.01±0.58^{c}	29.59±0.64^{b}	41.99±0.70^{c}	49.13±0.70^{c}
CJ18	21.90±0.53^{b}	27.57±0.35^{a}	41.10±0.67^{b}	47.42±0.57^{b}
ST	19.77±0.37^{a}	26.35±0.44^{a}	37.88±0.50^{a}	43.50±0.23^{a}
ST and CJ18	27.05±1.84^{d}	32.05±1.62^{c}	50.43±3.75^{c}	59.64±0.49^{d}
Average	23.43±3.05^{A}	28.89±2.39^{B}	42.85±5.12^{C}	49.92±6.25^{D}

*Mean ± SD with different lowercase letters along a column and uppercase letters across a row are significantly different at $p<0.05$.

*Each value is mean ± standard deviation for triplicate experiments.

4.3.3 Sensory evaluation of fermented camel and cow milk products inoculated with *CJ18* and *ST*

The scores for general acceptance of the starter culture samples was significantly influenced by the microbial strain used in inoculation at $p<0.05$, as shown in Table 9. However, there was no significant differences for scores in mouth feel and sourness for both camel and cow milk. The scores for sourness, mouthfeel and general acceptance for samples with the *CJ18, ST* and *CJ18* and *ST* strains were significantly higher than that of *CCUG* strain at $p<0.05$. In terms of general acceptance, there was no significant difference between the two types of milk; however, camel samples with *CJ18* were generally accepted more when compared with others including a combination of *ST and CJ18.* For cow milk, samples with *ST* were accepted more with an average acceptability of 4.25 while samples with *CCUG* were least accepted.

Table 4.7: Summary of sensory evaluation scores of camel and cow milk fermented at 45 ^{o}C.

Microbial strain	Type of milk starter culture	
	Camel milk at 45 ^{o}C	Cow milk at 45 ^{o}C
	Mouthfeel	
CCUG	2.83±0.83^{a}	2.42±0.90^{a}
CJ18	3.42±0.51^{a}	3.42±0.67^{a}
ST	3.75±0.75^{a}	4.25±0.75^{a}
ST and CJ18	3.83±0.83^{a}	4.17±0.58^{a}
Mean	3.46±0.82^{A}	3.56±1.03^{A}
	Sourness	
CCUG	2.83±1.03^{a}	3.00±0.74^{a}
CJ18	4.17±0.58^{a}	4.08±0.51^{a}
ST	3.67±0.65^{a}	4.08±0.90^{a}
ST and CJ18	3.83±0.58^{a}	3.83±1.03^{a}
Mean	3.63±0.87^{A}	3.75±0.91^{A}
	General Acceptance	
CCUG	2.92±0.67^{a}	2.75±0.62^{a}
CJ18	4.17±0.58^{c}	3.75±0.45^{b}
ST	3.92±0.79^{bc}	4.25±0.75^{b}
ST and CJ18	3.50±0.52^{b}	4.00±0.74^{b}
Mean	3.63±0.79^{A}	3.69±0.85^{A}

*Mean ± SD with different lowercase letters along a column and uppercase letters across a row are significantly different at $p<0.05$.

4.3.4 Correlation among pH, Titratable acidity and sensory attributes of milk

Table 10 presents the correlation coefficients between pH, Titratable acidity, viscosity and sensory characteristics, of milk stored at 45 ^{o}C for one day. Mouth feel and sourness were positively correlated ($P\leq0.05$, 0.5774, 0.6906) with overall acceptability of milk samples (Table 10). There was significant negative correlation between pH and titratable acidity ($p\leq0.05$, -0.9815). On the other hand, there was a negative correlation between pH and mouthfeel, sourness and general acceptance of milk

Table 4.8: Correlation coefficients among pH, Titratable acidity and sensory attributes of milk

	pH	Titratable Acidity	Viscosity	Mouthfeel	Sourness	General Acceptance
pH	-					
Titratable Acidity	-0.9815*	-				
Viscosity	-0.1924	0.1876	-			
Mouthfeel	-0.1954	0.2165	0.0684	-		
Sourness	-0.1745	0.1788	0.2948	0.3085	-	
General Acceptance	-0.1353	0.1677	0.1802	0.5774*	0.6906*	-

4.4 Discussion

The highest acidity in camel milk of 0.970±0.010 was by mixed strains of *ST* and *CJ18* at 45 ^{o}C while the lowest mean acidity of 0.890±0.01 was by *CCUG* at 25 ^{o}C and 30 ^{o}C. The strains worked best at 45 ^{o}C. The lowest acidity of 0.42±0.03 in cow milk was by *CCUG* strain at 25 ^{o}C after 9 hours of incubation while the highest was 0.85±0.03 by *CJ18* at 37 ^{o}C after incubating for 9 hours. The differences in acidification of camel and cow milk could have been as a result of the composition of both kinds of milk (Sawaya *et al*., 1984).

pH was significantly affected by incubation time, temperature and microbial strain at $p<0.05$. Increasing the incubation time and temperature significantly reduced the pH. This was due to the fermentation activity of the different strains. The final pH for the different strains or their combinations after incubating cow milk at the selected temperatures except at 25 ^{o}C reached a value less than 5 but greater than 4.This is beneficial to pastoral communities in ASALs, where there are poor infrastructure and no refrigeration facilities (Musinga *et al*.,2008). The pH of above 4 is an advantage to fermented Dairy products because continued fermentation causes quality impairment for example in yoghurt (Antunes *et al*., 2005). Camel milk reached a final pH

of less than 5 but greater than 4 at 45 ^{o}C. The optimal fermentation temperature for the strains or their combination in both kinds of milk was at 45 ^{o}C with a combination of *ST* and *CJ18* giving the best results. There was a sharper decline in pH for the cow milk than in camel milk which could be explained by differences in buffering levels, the difference in proportions of proteins and the specific salts in both kinds of milk (Sawaya *et al*., 1984).

The viscosity for the different strains or their combination was strongly dependent on the incubation temperature, with 45 ^{o}C being optimal for both kinds of milk. Combination of *ST* and *CJ18* gave the best results at 45 ^{o}C. The viscosity of cow milk was almost twice that of camel milk and this is as a result of the different proteins composition in each of the milk. Sensory Analysis was done on the end product which had the best technological properties in terms of pH, acidity, and viscosity. Combination of *ST* and *CJ18* strains at 45 ^{o}C gave the best results for both kinds of milk and the samples were subjected to sensory evaluation, with the fermented cow milk taken as the control. The samples did not have any significant difference in mouthfeel, sourness and general acceptability but these parameters varied from strain to strain. This variation was in agreement with a study done on quality parameters of starter cultures (Xanthopoulos *et al*., 2001).

The African type *Streptococcus* strains which have been found to be predominant in *Suusac*, when incubated at temperatures of between 37 ^{o}C and 45 ^{o}C took a short time to reach maximum acidification and to get the acceptable pH of less than 5 but greater than 4, that is desired in fermented dairy products**.** This could give the strains competitive advantage against other bacteria during fermentation and could explain the predominance not only in *Suusac* but *Gariss*, in initiating spontaneous fermentations (Jans *et al*., 2013b). The *Sii* strains are highly adapted

and competitive in traditional fermented dairy products (Jans *et al*., 2013a) .This explains their ability to ferment both camel and cow milk as indicated by the development of acidity, pH decline and change in viscosity in different incubation temperatures. The changes in pH, acidity, and viscosity observed was due to the growth and fermentative activities of the starter culture strains used (Panesar, 2011). Milk pasteurization and use of pure starter culture strains during fermentation resulted into faster development of acidity and a decline in pH as there was no microbial competition for nutrients. The proteolytic activity during fermentation which involved the utilization of casein led to the development of organoleptic properties of the products (Holzapfel, 2002). The selected strains are thermophilic as they worked best from 37 ^{o}C to 45 ^{o}C and can be used as mixed strains for best results.

4.5 Conclusion

The selected African *Streptococci* strains isolated from fermented camel milk showed important properties, like the drop in pH, acidification and viscosity changes which are useful for starter culture development for fermented dairy products. They are potential starter cultures for the dairy industry as they can ferment milk to various products. At optimal temperatures of 37 ^{o}C to 45 ^{o}C the mixed strains of *ST* and *CJ18* can make good yoghurt. They have very good technological properties which can be utilized to innovate and develop new products that can lead to industrial growth. If adopted, the products can retain the traditional qualities while enhancing their safety and nutritional benefits. Their historical background and biotechnological properties can be utilized in the formulation of African specific starter cultures for fermented camel milk products. The huge potential can be utilized by the camel milk industry to make

fermented products which will retain the exclusive organoleptic properties while enhancing their quality.

4.6 Recommendations

Survival and strength tests of the starter cultures under different storage conditions should be carried out. Viable bacterial counts should be done to check on the dynamic changes in growth and physiological state of the strains

CHAPTER FIVE: GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Traditionally fermented camel milk products from North Eastern Kenya were found to harbor pathogens which cause human diseases. The presence of various life-threatening pathogens in the *Suusac* samples was a strong indication that the products are not safe for human consumption despite the fact that, they have medicinal properties. There is a need to shift from the artisanal production process of using raw milk, to milk pasteurization and then fermentation in order to get safe quality products.

African *Streptococci* strains which have been found to be predominant in most African fermented dairy products can be utilized in fermentation. This will help maintain the health benefits, cultural background and general acceptance of the fermented products. *Streptococcus thermophillus, Streptococcus infantarius* subspecies *CJ18* and *CCUG* are the strains which there functionality in both cow and camel milk fermentation was investigated in this research. Practical use of working cultures of the above strains was studied at different temperatures for both single and mixed strains in pasteurized milk.

Use of combined strains of African *Streptococcus thermophillus* and *Streptococcus infantarius* subspecies *CJ18* at the ratio 1:1 gave the more superior product at 45 ^{o}C which was generally accepted. When the pH decline and acidification profiles are considered, the mixed strains are suitable for starter culture development in both camel and cow milk fermentation. More studies on sensory evaluation should be carried out at the community level in order to get more views on new product development and the possibility for the use of the starter culture in *Suusac*

production. More research on the safety of *Sii CJ18* and classification of the strains as Food additive should be fast-tracked.

5.2 Recommendations

i. Stringent measures along the value chain should be observed to safeguard the health of consumers and strengthen *Suusac* business by the informal producers and vendors

ii. Further study to determine the toxins produced by the *S. aureus* and *E. coli* present in the *Suusac* should be carried out.

iii. Infrastructure should be improved including the provision of clean potable water, refrigeration facilities, transport, and storage systems. Quality control checks and Veterinary services should also be put in place and implemented.

iv. There is need to create awareness on good hygienic practices along the *Suusac* production and market chain in order in order to prevent any harm which can have negative consequences to public health and the economy.

v. Survival and strength tests of the starter cultures under different storage conditions should be done. Viable bacterial counts should be done to check on the dynamic changes in growth and physiological state of the strains.

vi. Long fermentation time trials at low temperatures of 25^{o}C and 30 ^{o}C should also be carried out to complete the acidification.

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APPENDIX

1. SENSORY ANALYSIS SHEET

SN.....

Name..... **Date.....**

In front of you are coded fermented milk samples. Taste the samples and rate as per the given scores for each given attribute. Don't swallow and rinse your mouth thoroughly after tasting

Food characteristic: Appearance (color, visual consistency, visual presence of lumps) **Texture** (oral consistency, oral viscocity, oral presence of lumps) **Acidity, Overall acceptance.**

Score values like a lot-5, like a little 4, neither like nor dislike-3, dislike a little-2, dislike a lot 1.

Samples	**Appearance**	**Texture**	**Acidity**	**Overall acceptance**
Sample 1				
Sample 2				
Sample 3				
Sample 4				

2. CONFIRMATORY RESULTS

Pathogen / test	Properties
Staphylococcus aureus	
Catalase test	Positive (+ve)
Lipase test	Positive (+ve)
Glucose fermentation	Positive (+ve)
Escherichia coli	
Indole test	Positive (+ve)
Methyl red test	Positive (+ve)
Voges-Proskauer test	Negative (-ve)
Citrate test	Negative (-ve)
Shigella spp	
Oxidase test	Negative (-ve)
Urea agar test	Negative (-ve)
Methyl red test	Positive (+ve)
Voges-Proskauer test	Negative (-ve)
Citrate test	Negative (-ve)
Klebsiella spp	
Indole test	Negative (-ve)
Methyl red test	Negative (-ve)
Voges-Proskauer test	Positive (+ve)
Citrate test	Positive (+ve)