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Review

Spontaneously fermented kenyan milk products: A review of the current state and future perspectives

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Many spontaneously fermented milk products are produced in Kenya, where they are integral to human diet and play a central role in enhancing food security and income generation. Some of these products have demonstrated therapeutic and probiotic effects although recent reports have linked some to death, biotoxin infections, and esophageal cancer. These products are mostly processed from poor quality raw materials under unhygienic conditions resulting to inconsistent product quality and limited shelf-lives. Though very popular, research on their processing technologies is low. This review provides a comprehensive summary of the most common spontaneously fermented milk products from Kenya including *Mursik*, *Kule naoto*, *Amabere amaruranu* and *Suusa*. Their production challenges and future perspectives are highlighted; emphasizing the need for application of high throughput biotechnologies in their study. Available literature on their microbiology, biochemistry, and chemical composition is summarized. Moreover, knowledge on the value of clean starting raw material, fermentation parameters definition, and employment of standard equipment are discussed.

Key words: Starter culture, probiotics, lactic acid bacteria, fermented milk, high throughput biotechnology, spontaneous fermentation, *Kule naoto*, *Mursik*, *Amabere amaruranu*

INTRODUCTION

Fermentation of food is one of the oldest methods of food processing and preservation that is entrenched in traditional cultures and village life (Pederson, 1971; Campbell-Platt, 1994). It has evolved into a method of preserving foods during times of scarcity, imparting appropriate aroma and flavors to foods, decreasing product toxicity,and generating product diversity in diets including staple foodstuffs such as milk, tubers, cereals, and fish (Belton and Taylor, 2004; Chelule, Mokoena and Gqaleni, 2010). Additionally, fermentation reduces the bulk of material to be transported, it improves the nutritive value and appearance of food, and it reduces the energy required for cooking (Holzapfel, 2002). Traditionally, fermented products have a special part in social

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functions such as circumcision,marriage,victory, naming, and rain making ceremonies (Hounhouigan, 1994; Muigei et al., 2013). Currently, fermented foods contribute 20 - 40% of the global food supply and approximately one third of the food consumed by man is fermented food. This renders fermented foods and beverages a substantial component of the global diet (Campbell-Platt, 1994; Chilton et al., 2015).

Several traditional fermented products have been documented in different African countries including non-alcoholic beverages, alcoholic beverages, breads, pancakes, porridges, cheeses, and milks. Some of these products including fermented cereals, tubers, and roots are dietary staples in Africa hence; they are important for food security (Marshall and Mejia, 2012). In Kenya, fermented foods and beverages are produced at household/ village level using traditional fermentation technology from various raw materials such as cereals, milk, honey, sugarcane, bananas, and coconut sap, among others (Nout, 1981; Mathara et al., 2004; Kunyanga et al., 2009; Nyambane et al., 2014). Milk fermentation is a common method for preserving milk and many communities ferment milk to produce desired products. In some communities, fermented milk plays a crucial role in the nutrition of vulnerable groups of population such as infants, pregnant women, young children, and the elderly (Chilton et al., 2015). Examples of fermented milk in Kenya include the *Mursik* from the Kalenjin community (Muigei et al., 2013), *Amabere amaruranu* from the Kisii community (Nyambane et al., 2014), *Kule naoto* from the Maasai community (Mathara, 1999), and *Suusa* from the North Eastern pastoralist communities (Lore et al., 2005). The fermentation processes for the production of these fermented products are typically uncontrolled and are dependent on microorganisms from the environment.

These household fermentation technologies have not been upgraded into industrial scale in order to meet the growing demand for traditional fermented products by the urban and immigrant population. The household production of these products is laborious and time-consuming for the urban/ immigrant population and the raw materials could be absent; hence the need for production of these products efficiently with assured safety, quality, packaged for extended shelf life, broader acceptance, and in a ready-to-use/ easy-to cook form from high qulity raw materials (Nout and Sarkar, 1999). This can be realized only if the traditional fermentation technologies are characterized to identify starter cultures that could be used to give products with consistent and improved quality and assured safety together with fermentations taking place in reactors under controlled conditions, and the need for high quality raw materials emphasized (Marshall and Mejia, 2012). Fermented foods and the microorganisms that contribute to the fermentation process have also been associated with many beneficial effects on human health and food preservation, however they are underexploited. Although there have been studies characterizing the microorganisms in some of the Kenyan spontaneously fermented products (Lore et al., 2005; Mathara et al., 2004; Nieminen et al., 2013; Nyambane et al., 2014), there has been no technology in Kenya that has been developed to modernize the production of the traditional fermented products using starter cultures under controlled conditions or exploiting their probiotic and antimicrobial potential (FAO, 2010; Reid et al., 2014). This is partly because most studies intended to characterize traditional fermentations employs the classical microbiological culturing techniques, which are inadequate in identifying and characterizing microbial consortia. The more powerful high throughput biotechnologies have not been employed in identifying starter cultures/probiotics and other fermentation aspects have not been characterized; thus there is limited progress in upgrading the traditional fermentation technologies. Consequently, modern socio-economic changes may cause some traditional technologies for the production of fermented foods to be lost together with the associated microorganisms (Akabanda et al., 2013; van Hijum et al., 2013; Reid et al., 2014).

This review aims to list and recap the production processes of common Kenyan traditional spontaneously fermented milk products and to highlight, where available, some of the microbiological and biochemical properties of the fermented milk products, challenges, and suggest areas of research/ innovation for the advancement of these traditional technologies for industrialization, culture preservation, and for satisfaction of the growing demand for the products.

FERMENTED MILK PRODUCTS IN KENYA

Traditional spontaneously fermented milks are produced in Kenya and they have been consumed for many years due to the belief that they promote good health (Mathara et al., 2008). These products are mostly produced in rural areas and their preparation methods have some variations e.g. smoking/ nonsmoking, boiling/ not boiling milk, and use of back slopping/ non usage. These variations results to textural and flavor differences and microbial compositions. The most common fermented milk products in Kenya are discussed below.

Mursik

Mursik is the spontaneously fermented bovine milk product which is mainly produced by the Kalenjin community in Kenya, whom milk is a staple diet. The milk is prepared in gourds (*sotet*), where the milk is left to

ferment for 3–5 days or more dependent on sensory preferences. The Kalenjins have preferred this milk and use it to mark special occasions such as symbolizing success in negotiating marriages and weddings and success in athletics. *Mursik* is also consumed by breast feeding mothers and initiates. It is believed that one is strengthened and the immune system against common diseases is boosted by consuming *Mursik* (Muigei et al., 2013; Mathara, 1999).

Mursik production

The production of *Mursik* starts with the preparation of the gourd. First, the top of the gourd is cut and the seeds are removed. It is then cleaned using a cured wood stick (*Sosiot*), obtained from branches of a palm tree. The gourd is then left outside for a few hours to dry. After drying, the preserving tree (*Senna didymobotrya*) sticks are burnt and the burning embers are added into the gourd but taking care not to burn the gourd. The gourd is considered ready when the inside is evenly covered with fine dust. The purpose of using the burnt stick is to improve the flavor of *Mursik*, pasteurize the gourd, and the coloring of *Mursik* (Mathara et al., 1995). After milking, the milk is boiled and cooled down. The cooled milk is poured into the prepared *sotet* then covered tightly with a lid. The milk is stored in a cool and dry place for about one week to allow it to ferment. Upon fermentation, the gourd is shaken to ensure that the *Mursik* has a smooth uniform consistency. It is commonly consumed after meals or sometimes served along with other staple foods such as hot *Ugali* (Mathara et al., 1995).

Microbiology of Mursik

Studies on *Mursik* have shown that the fermentation is carried out by lactic acid bacteria (LAB) (Mathara et al., 1995). Nieminen et al. (2013) analyzed *Mursik* samples that were fermented using small batches of *Mursik* collected from Kenya. The total microorganisms at 48 and 72 h of fermentation were 10.42±0.21 and 9.54±0.43 $\log_{10}$ cfu/mL, respectively. The pH of the fermented milks was 3.46±0.04 at 14 days. The dominant bacteria (determined by 16S rDNA sequencing) were *Lactobacilli* (8 species), which were present in 6 out of 8 samples that were analyzed. Eight *Lactobacillus* species were identified and the *Lactobacillus kefiri* was more frequent in the samples (6 out of 8 samples) with a mean of 37% of the colonies. In 5 of the samples, *L. kefiri* was found in combination with *Candida krusei*. The other common *Lactobacillus* species were *L. casei*, *L. paracasei* and *L. rhamnosus*. Other species were less prevalent (mean 27%, range <1–93%, of total colonies). Among these, *Bacillus* spp. were most prevalent (present in 5/8 samples). Yeasts were present in all the samples of *Mursik* and their proportion in the samples varied between 7 and 90%. Three *Candida* species (*C. krusei, C. sphaerica* and *C. kefyr*) and one *Saccharomyces* species (*S. fermentati*) were detected in the samples, with *C. krusei* being the most common (identified in 5/8 samples). *Candida kefyr* and *C. sphaerica* were present in 3/8 samples whereas, *S. fermentati* was found in only one sample (1/8) (Nieminen et al., 2013).

Muigei et al. (2013) attempted to identify exopolysaccharide-producing LAB in *Mursik* obtained from Nakuru County, Kenya. Among the LAB, the *Lactococcus* spp. was more dominant than the *Lactobacillus* and *Leuconostoc* spp. The most prevalent *Lactococci* was *Lactococcus lactis* subspp. *lactis*. In another study (Digo, 2015), it was found that the proportion of LAB in *Mursik* samples collected from Bomet County, Kenya was 82% of the total microorganisms. The coliform counts were 6.98% and the yeasts and molds count were 11.02% prevalent. The dominant LAB were *Lactobacillus* (56.10%) while the other LAB (*Lactococcus*, *Enterococcus* and *Leuconostoc*) consisted 25.9% of the total microorganisms. The *Lactobacillus* species identified were *L. plantarum*, *L. fermentum*, *L. brevis* and *L. casei* (Digo, 2015).

From these reports, it is clear that *Mursik* has not been clearly characterized. Whereas Nieminen et al. (2013) recorded *L. kefiri* as the dominant LAB; Digo (2015) did not record it. The differences could be ascribed to the broad variation of *Mursik* due to household differences in preparation of *Mursik* and/or due to geographical differences. Moreover, Nieminen et al. (2013) used a back-sloping method to produce *Mursik* using small batches of *Mursik* that had stayed at room temperature for 4 weeks. These differences could also explain why Digo (2015) detected coliforms that were not identified by Nieminen et al. (2013). If *Mursik* is left at room temperature, the LAB could produce excess lactic acid that will inhibit coliforms and other microorganisms; however this needs to be confirmed.

Although *Mursik* is a popular product that can be found in urban areas, its consumption has been linked to the occurrence of esophageal cancer and deaths (Patel et al., 2013; Daily Nation, 2015). The consumption of *Mursik* as a risk factor to the occurrence of esophageal cancer has been attributed to the polycyclic aromatic hydrocarbons (PAHs), which are carcinogens that originate from charcoal powder added to the *Mursik*. *Mursik* has also been shown to contain high levels of acetaldehyde that may contribute to esophageal carcinogenesis (Patel et al., 2013; Nieminen et al., 2013). Acetaldehyde is a mutagenic carcinogen and was detected in *Mursik* at levels (>1800 µg/L) that are 4 times times higher those in commercial yogurt.Ethanol levels>100 mmol/Lwerefound in *Mursik* (Niemeinen et al., 2013).

The microbes in *Mursik*, particularly *C. kefyl* have been

demonstrated to produce high acetaldehyde in fermented milk when added together with LAB starter cultures (Gadaga et al., 2001). The high acetaldehyde levels were found to correlate positively with ethanol levels. Many microbes such as *Candida* and *Streptococcus* are capable of producing acetaldehyde from ethanol. In *Mursik*, *L. kefiri* was in combination with *C. krusei* and this combination had high acetaldehyde and ethanol levels. Although *C. krusei* is a poor producer of acetaldehyde from ethanol in pure cultures, its existence with other microorganisms in *Mursik* can enhance its production potential (Nieminen et al., 2013).

It is therefore pertinent that standardization of the *Mursik* fermentation process using starter cultures that will reduce contamination and acetaldehyde and ethanol production will be a great milestone towards assuring safety and quality of *Mursik*. To achieve that, there needs to be molecular characterization of the microorganisms in *Mursik*, trials to select the best combination of microorganisms (starter cultures/probiotics), a protocol giving a safe and acceptable product, sensory evaluation for acceptability of developed products needs to be done, and safety and quality standards developed.

Amabere amaruranu

Amabere amaruranu is the product of spontaneous fermentation of bovine milk produced by the Kisii community in Kenya. Mostly, the milk to be fermented is boiled, cooled and added to the gourd (*ekerandi*) for fermentation. The fermentation is spontaneous, however normally back-sloping is used where a small batch of previous successfully fermented milk (*enduranerio*) is added to fresh milk to initiate the fermentation (Nyambane et al., 2014). The milk is valued for provision of nutrition to children and the elderly. It is normally consumed along with *ugali* prepared from maize or millet flour. Sometimes, the milk is mixed with blood to prepare a fermented product called *omokoora*. In the preparation of *omokoora*, blood is first boiled and once it thickens, it is cooled, broken into small pieces then added to the gourd. It ferments for 2–3 days, then it can be consumed similar to *Amabere amaruranu*. Addition of blood can affect microbial metabolism since blood is rich in iron; a cofactor for numerous cellular processes (Nieminen et al., 2013). Both products can be consumed for extended periods whereby after consumption, backslopping starter culture (*enduranerio*) is left in the gourd, then fresh milk is added and the cycle can be conducted several times. This ensures that the milk does not sour excessively and the backslopping maintains the quality of the milk. In recent times, many people use different containers including plastics for fermenting the milk.

There has been little research on the microbiological and biochemical attributes of the *Amabere amaruranu*. However, in a pioneering study of *Amabere amaruranu*, Nyambane, Thari, Wangoh & Njage (2014) analyzed the microbiological composition of the product. The total viable counts were high ($\log_{10}$ 8.06 ± 0.59 – 8.24 ±0.40 cfu/ml). Coliforms, yeasts and molds were analyzed in addition to the LAB, which carries out the fermentation. The LAB were identified to be members of the genera *Lactobacillus* (45%), *Streptococcus* (25%), and *Leuconostoc* (20%). Of the LAB, the most dominant was *Streptococcus thermophiles,* which comprised of 25% of all LAB isolated and was in all the analyzed samples. *Lactobacillus bulgaricus* subsp. *bulgaricus* made up 15% of the total number of LAB isolates. This organism, together with *Streptococcus thermophilus* are normally used as starter cultures for yoghurt production. *Leuconostoc mesenteroides* subsp. *mesenteroides* consisted of 20% of all the LAB isolates. Another highly prevalent *Lactobacillus* in *Amabere amaruranu* was *L. plantarum,* which comprised 20% of the isolates. A 5% of the isolates were identified as *Lactobacillus helveticus.*

Yeasts were isolated from *Amabere amaruranu* but no mold was detected. The yeast species that were identified belonged to the genera *Saccharomyces* (25%), *Candida* (20%), and *Trichosporon* (15%). However, the Analytical Profile Index (API) system used was unable to identify 40% of the yeast isolates. Yeasts have been detected in many spontaneously fermented types of milk. Therefore, their role in the fermentation needs to be studied.

The study by Nyambane et al. (2014) identified most of the microorganisms in *Amabere amaruranu* particularly the bacteria. The identified microorganisms need to be studied further for their properties and their interactions in the design of starter cultures for controlled production of *Amabere amaruranu*. The bacteria in *Amabere amaruranu* have been demonstrated to exhibit probiotic potential. For example, Boyiri (2014) used 16S rRNA gene sequencing to identify a *Lactobacillus rhamnosus* strain in *Amabere amaruranu* that was bile stable, nonmucinolytic and had antibacterial activity. The strain also stimulated increase in MUC4 and MUC3 expression in colon cells. Kotala and Onyango (2015) have also demonstrated that the cell extract of *L. rhamnosus* from *Amabere amaruranu* downregulates the expression of numerous adipogenic-related transcription factors. At high dose levels, the cell extracts were found to down regulate peroxisome proliferator-activated receptor-γ, sterol regulatory element-binding protein 1, and adipose triglyceride lipase. These results demonstrated that the cell extracts from *L. rhamnosus* from *Amabere amaruranu* could be employed for anti-obesity management regimes; hence the probiotic potential of *L. rhamnosus*. Moreover, Mokua (2004) demonstrated the antibacterial effect of *Amabere amaruranu* on *E. coli*. This clearly indicates that the microorganisms in *Amabere amaruranu* have probiotic potential that is yet to be

exploited in product development. The role of yeasts in the fermentation also needs to be characterized. The API system that was employed failed to identify some yeast hence; combination with advanced tools such as molecular diagnostic tools can achieve complete profiling of the microbial diversity of *Amabere amaruranu* for starter culture and probiotic design. The physical fermentation aspects such as temperature, aeration, agitation rates and chemical composition needs to be defined so as to produce a product that can be scaled-up for industrial production.

Kule naoto

Kule naoto is the traditional fermented bovine milk product of the Maasai pastoralist community in Kenya. In its production, raw milk is filled into treated gourds made from the hollowed out dried fruit of the plant *Lagenaria siceraria*, then spontaneously fermented. The milk and the gourd treatment involves the addition of fresh cow's blood and rubbing the gourd's interior with a burnt stick from the tree; *Olea africana,* locally known as *Enkidogoe* (Onyango et al., 2014). The milk-blood mixture is then spontaneously fermented at ambient temperature for up to 5 days (Mathara, 1999). After fermentation, the product is gently shaken before consumption and averagely; an individual drinks 2–3 liters of *kule naoto* per day. *Kule naoto* is preferred by the Maasai community because of its natural taste and aroma. Moreover, the Maasai's believe that it has therapeutic value for treatment/ prevention of ills such as diarrhea and constipation (Mathara, 1999).

Although the milk might contain some probiotic microflora that confers such benefits, studies on the microbiology and biochemical aspects of *kule naoto* are limited and currently the product is prepared in the traditional way by spontaneous fermentation. This gives products of inconsistent quality and safety is not assured. However, some efforts have been made to study the microbiology of *kule naoto*. Mathara et al. (2004) identified microorganisms in the product, whose pH was found to range at 4.17–5.19. The microbial levels were in the level of 6.1 to 9.2 $\log_{10}$ cfu/ml of which the LAB dominated. The *Lactobacilli* and the *Lactococci* species were the dominant LAB (10^7–10^9 cfu/ml), whereas the *Enterococci* were less dominant (3.3–9.9 cfu/ml). However, at this concentration, the *Enterococci* may play a crucial role in fermentation of the milk. Other bacteria detected were *Enterobacteriaceae* group as well as the yeasts and moulds. In samples where *Enterobacteriaceae* were detected, yeasts were absent, which suggests a possible interaction between bacteria and yeasts in the production of kule naoto (Mathara et al., 2004). Overall, more than 500 strains were detected in *kule naoto* and all the LAB isolates were Gram-positive of which 55% belonged to the genus *Lactobacillus*, 25% to *Enterococcus*, 14% to *Lactococcus*, and 6% were *Leuconostoc*. Approximately 60% of the Lactobacilli strains were *Lactobacillus plantarum*. Other *Lactobacillus* strains identified were; *L. fermentum*, *L. casei*, *L. rhamnosus*, and *L. acidophilus*. For the *Enterococci*, *Enterococcus faecium* was the dominant species. *Lactococcus lactis* were also detected and the *Leuconostoc* strains were identified as *Leuconostoc mesenteroides* subsp. *dextranicum*. In the studies, most of the *kule naoto* samples had pH below 4.5. However, samples that had pH higher than 4.5 harbored the *Enterobacteriaceae* and yeasts, suggesting a potential health risk.

The main strain in *Kule naoto*, *L. plantarum* could be responsible for the characteristics of the product. However, the other *Lactobacilli* that were present such as *L. rhamnosus*, *L. fermentum*, and *L. acidophilus* and other present microorganisms such as Leuconostoc and yeasts could also contribute to the quality/ probiotic potential of the product. For instance, Mathara et al. (2008) demonstrated the probiotic potential of the *Lactobacillus* spp. in *Kule naoto*. The *L. acidophilus* had resistance to gastric juice and bile, while some other strains exhibited bile salt hydrolase activity, assimilated cholesterol *in vitro*, and had up to 70% adherence to HT29 MTX cells. The *L. fermentum* had almost 100% survival under simulated stomach acidic conditions and physiological salt concentrations of bile salts and had over 80% hydrophobicity values. Most strains of the *L. casei* and *L. acidophilus* had aggregation abilities above 50% (Mathara et al., 2008). These studies demonstrated that most *Lactobacillus* species found in *kule naoto* are probiotic and thus; needs full characterization for their application in the food industry. The technological features of some of the LAB from *kule naoto* have also been studied (Patrignani et al., 2006). These include growth kinetics and survival at 4°C. From the study, optimum conditions were detected, which could enhance sensory properties of the fermented product.

In efforts to upgrade the production of this fermented milk product using starter cultures, the interactions between the bacteria and yeasts should be elucidated and process parameters fully defined so as to develop a product as similar as the traditionally produced *kule naoto* under controlled conditions. Pasteurization of the milk should be practiced to minimize contaminations/ zoonoses. Further characterizations of the micro-organisms using advanced molecular techniques are necessary since culturing techniques have been demonstrated to be insufficient in mapping a microbial consortium (van Hijum et al., 2013). Moreover, the claims made regarding the health benefits of consuming *kule naoto* needs to be substantiated and studied using animal models for the product to benefit the masses.

Suusa (*Suusac*)

Suusa is the spontaneously fermented raw camel milk product that is common among the North Eastern pastoralists of Kenya. Camel milk is generally opaque white, and normally has a sweet, sharp taste though sometimes it tastes salty (Yagil, 1982). *Suusa* is white in color, has low viscosity, a distinct smoky flavor, and astringent taste (Lore et al., 2005). The composition of camel milk is relatively similar to milk of other domestic animals. However, camel milk is rich in vitamin C and the enzyme lysozyme (Wilson, 1984; Yagil, 1982). It therefore forms an important source of vitamin C in the diets of North Eastern Kenya pastoralists, who inhabit arid and semi-arid regions where fruits and vegetables are scarce. The lysozyme is good in preservation of the milk by inhibiting Gram-positive organisms.

There have been some studies documenting the traditional fermented camel milk (*Suusa*) in Kenya (Njage and Wangoh, 2008). The fermented camel milk has been given different names all over the world. It is called *Kefir* in the Middle East; *Lehben* in Egypt, Israel and Syria; *Yoghurt* in Bulgaria; and *Chal* or *Shubat* in Russia (Mohamed, 1993; Yagil, 1982). *Suusa* in Kenya is prepared by leaving fresh camel milk at room temperature (25–30°C) for 1–2 days for souring spontaneously. Mathara (1999) has documented the traditional production of *suusa* in Isiolo District, Kenya; the camels are milked directly into a gourd that has been cleaned, smoothed and treated with smoke. The smoking of the gourd is done using smouldering twigs of the acacia tree (*Acacia seyal*) after rinsing the gourd with water. The hot smoking chips are put into the gourd then the gourd is closed for a few minutes. Once the gourd cools, the charcoal chips are removed. The smoking is believed to improve the keeping quality of *suusa* and gives it its characteristic flavor and aroma, and improves the color. After the milking, there are no any heat treatments and the milk is bulked into larger containers, which are closed and left for two to three days for the milk to ferment spontaneously. After the fermentation, the top fatty layer is removed and the product is ready for consumption for up to a week at room temperature (26–29°C).

The microorganisms that participate in the spontaneous fermentation of traditionally produced fermented milk are mixed, undefined or partially defined. Some earlier studies had demonstrated the presence of various LAB, yeasts and molds (Oberman, 1985). Lore et al. (2005) studied the microbial counts and pH in traditional and laboratory-produced *suusa*. The total microbial counts and coliforms were similar. However, there were higher LAB, yeasts, and molds numbers in laboratory-produced than traditional *suusa*. The pH of *suusa* ranged between 3.6–4.4 and the lactic acid ranged from 0.52–0.71% (Njage and Wangoh, 2008). Among the dominant bacteria in *suusa*, *L. mesenteroides* subsp. *mesenteroides* consist of 24% and *L. plantarum* (16%). Other bacteria that have been reported in *suusa* are *Lactobacillus curvatus, L. salivarious, L. raffinolactis,* and *Streptococcus infantarious* subspp*. infantarious,* a pathogenic microbe. About 30 yeasts were isolated from *suusa. Candida crusei* (50%) were the dominant and others were *Geotrichum penicillatum* and *Rhodotorula mucilaginosa. C. krusei* has been recruited as an adjunct starter culture along with dairy starter cultures to maintain the activity of LAB and, as such increasing their longevity (Frazier and Westhoff, 2001). Jay (1992) also observed that *C. krusei* plays a crucial role in flavor development during fermentation of cacao beans, owing to its proteolytic activity. *Candida krusei* has been identified in other spontaneously fermented products such as *busaa* and *Amabere amaruranu* and *Mursik* (Nout, 1981; Nyambane et al., 2014). It could be possible that it plays a role in flavor development in *suusa* and the other traditional spontaneously fermented products, hence having a synergistic association with LAB. The presence of *G. penicillatum* and *R. mucilaginosa* could be linked to flavor and aroma development.

The limitation of *suusa* production similar to other spontaneously fermented milks is that products with wide variations in quality are produced due to the uncontrolled nature of spontaneous fermentation. Moreover, little is known about the nature and the interactions of the microorganisms contributing to the fermentation of *suusa* (FAO, 1990). As mentioned, *C. crusei* and *Saccharomyces cerevisiae* are detected in most spontaneously fermented foods. Their interactions with LAB needs to be studied as it will be vital in starter culture design. The presence of *S. infantarious* subspp*. infantarious,* a pathogenic microbe in *suusa* renders it unsuitable for consumption as it could be a vehicle for other pathogenic agents. Moreover, the pathogenic bacteria, *Salmonella enterica* has been found in raw camel milk (Matofari et al., 2007). Therefore, boiling/pasteurization are necessary before fermenting the milk to produce *suusa*. The boiling of milk will require the use of starter cultures in subsequent fermentation; hence detailed microbial studies are necessary to design starter cultures for *suusa* production.

SUMMARY OF THE PRODUCTION AND MICROBIOLOGY OF KENYAN FERMENTED MILK PRODUCTS

The fermentation process for the production of all milk products in Kenya occurs spontaneously in gourds. In the production of *Amabere amaruranu*, back-sloping is often used while for the other products,fermentation occurs without back-sloping, which could affect the microbial dynamics in the products. In modern production techniques, milk is fermented using starter cultures,which

produces consistent products that are safe and have extended shelf-lives in comparison to the spontaneously fermented milk products. Whereas *Mursik*, *Amabere amaruranu* are boiled before fermentation, *kule naoto*, and *suusa* are fermented raw, which could compromise their safety. Other practices such as smoking of the fermentation containers are carried for *suusa*, *Mursik*, and *kule naoto.* Addition of blood is also another practice especially to *Amabere amaruranu* and *kule naoto*. The fermentation period of the products ranges from 2 to 7 days.

In the production of these fermented milk products, Lactic acid bacteria (LAB) are the predominant fermenting microflora. In the production of *Mursik*, *Amabere amaruranu* and *kule naoto*, *Lactobacilli* are the predominant microorganisms. For *suusa*, *Leuconostoc* are the predominant but the *Lactobacillus* are also present. The LAB are known to be safe in their fermentation processes hence they have been employed for food preservation and flavor development (Khalid, 2011). As a result of their safety, there is need for their application in food biopreservation. In all the milk products, yeasts are found to be present. In particular the *C. crusei* and some *Saccharomyces* species are present in the milk along with LAB. Their role in the fermentation need to be characterized so that they could be employed as starter cultures.

CHALLENGES OF CURRENT METHODS (STARTER CULTURE DEVELOPMENT, PROCESSING AND CONSUMPTION) AND FUTURE PERSPECTIVES

In Kenya, as already discussed, all the traditional fermented foods and beverages produced at the household and village level using spontaneous fermentation are a staple diet for many communities and a cultural heritage (Franz et al., 2014; Mokoena et al., 2016). The fermentations draw substrates from a diversity of locally available raw materials including cereals, milk, coconut, honey, fruits, and vegetables. The technologies for their production have vast potential for stimulating development in the food industry owing to their low cost, tractability, little energy and infrastructural requirements, and the broad acceptance of the traditional fermented foods in Kenya (Nout and Motarjemi, 1997; Tamang and Samuel, 2010). The rising awareness of the health benefits of fermented foods, urbanization, and migrations of people has seen the rise in demand of these foods (Franz et al., 2014). It is apparent that with the increased demand, there is need for improvement in quality and safety of the products.

The rising demand for the traditional fermented milk products requires modernization of the technologies to assure safety, offer products with consistent quality and broadly accepted by the community. The spontaneous fermentation has limitations including inefficiency, low product yields, and variable product quality (Marshall and Mejia, 2012; Chilton et al., 2015). Moreover, there are safety concerns relating to pathogenic bacteria or chemical intoxicants produced by contaminating microorganisms as demonstrated by the deaths and risks of oesophageal cancer reported by the consumption of *Mursik* in Kenya (Patel et al., 2013; Wakhisi et al., 2005; Nieminen et al., 2013; Daily Nation, 2015). Although back-sloping process that makes use of samples of a previous successful batch of a fermented product as inoculants has been practiced in the production of African fermented food products such as the *Amabere amaruranu* giving products of relative consistency, safety of the products is not guaranteed.

With regard to the fermentation technologies developed in more advanced countries, it is evident that the identification and full description of microorganisms and the insights on their interactions within the fermentation ecosystems of *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* is required for the design of starter cultures to facilitate production under controlled conditions and tap into their unexploited probiotic potential (Nout, 2009; Franz et al., 2014; Holzapfel, 2002). There are numerous research reports showing the microorganisms that are associated with some of these Kenyan traditional fermented milk products (Nyambane et al., 2014; Digo, 2015; Nieminen et al., 2013; Patrignani et al., 2006; Mathara, 1999; Lore et al., 2005). Most of these studies have applied the classical microbiological techniques involving culturing (culture-dependent techniques) to identify microorganisms. However, cultivability of the microbes in the laboratory using synthetic media is most often inaccurately presumed and the absence of prior knowledge of their presence, selectivity of culture media and microbial interdependence, can result to utterly lopsided conclusions and misleading information (van-Hijum et al., 2013). Hardly any of the studies has corroborated culture-dependent techniques with the modern high throughput biotechnologies (culture-independent techniques) in studying the microbial consortia for designing starter cultures or isolating probiotic microorganisms, and none of these products uses a defined culture or is being produced at industrial level (Reid et al., 2014; Marshall and Mejia, 2012). The design and consequential improvement of starter cultures and exploitation of the probiotic potential could be the impetus for transforming the traditional fermentation technologies into a science, which can further spur innovation in equipment design for controlled processing of these products.

The application of high throughput biotechnologies for the characterization of microorganisms in fermented food products started when Polymerase Chain Reaction (PCR) was employed to conduct community profiling of microorganisms in traditional fermented food products

using the sequencing of the 16S rRNA genes and Denaturing Gradient Gel Electrophoresis (DGGE) (Tamang et al., 2016; van-Hijum et al., 2013). The application of 16S rRNA sequencing has demonstrated its superiority over culturing techniques with regard to microbial abundance and detection of specific microbes (van-Hijum et al., 2013; Tamang, 2014; Jianzhonga et al., 2009). Sequencing approaches for the portions of 16S or 18S rRNA genes are mainly useful to discern different genera or at best species within the fermentation. The 16S rRNA gene is highly conserved such that primers can be developed that can be used for almost all bacteria (Mayo et al., 2014). However, the gene also contains hypervariable regions whose variability is species-specific. Therefore, an amplification of the of the variable 16S rRNA gene from all microorganisms whose DNA is extracted *in situ* followed by sequencing can allow production of a fingerprint, which corresponds to microbial identity in the food. After identification, comparisons can be done to publicly available data bases hence; help in the isolation of starter cultures and probiotic microflora (Ercolini, 2013). For example, the Polymerase Chain Reaction- Denaturing Gradient Gel Electrophoresis (PCR-DGGE) approach has been used to identify microbial species occurring in natural whey cultures used as starter for water buffalo Mozarella cheese (Ercolini et al., 2001). In this study, both thermophilic and mesophilic LAB were identified by sequencing the V3 region of the 16S rRNA gene from the DGGE fragments on natural whey culture profiles. Other fermented products that have been characterized by sequencing techniques include *pulque*, a traditional Mexican alcoholic beverage from Maguey (Escalante et al., 2008*)*, *kimchi,* a naturally fermented vegetable product of Korea (Jung et al., 2011; Park et al., 2012), *narezushi*, a fermented salted fish and cooked rice of Japan (Kiyohara et al., 2012), *doenjang*, soy bean paste (Nam et al., 2012[a]), *kochujang*, a traditional Korean fermented food that is made with red pepper, glutinous rice, salt, and soybean (Nam et al., 2012[b]), seafood, and rice bran among others. Some of the other molecular diagnostic/ typing techniques that have been applied in the study of microorganisms in food and could be useful in the study of *Mursik*, *Amabere amaruranu*, *kule naoto* and *suusa* are listed in Table 1. The sequencing studies including the Next Generation Sequencing (NGS) can enrich our biodiversity knowledge in the traditionally fermented foods, which can be important also in selecting probiotic microorganisms besides the design of starter cultures. RAPD, Random amplification of polymorphic DNA; rep-PCR, repetitive extragenic palindromic sequence-based PCR; AFLP, Amplified fragment length polymorphism; DGGE of 16S rRNA, denaturing gradient gel electrophoresis of 16S rRNA; ARDRA, Amplified ribosomal DNA restriction analysis; MLSA, Multilocus sequence analysis.

Already in developed countries, starter culture design and bioreactor technology improvement for controlled fermentation processes have led to the development of high value-added products such as enzymes, microbial cultures, and functional food ingredients, which in most cases are imported to Kenya and other developing countries (Marshall and Mejia, 2012). Moreover, the starter cultures used for the synthesis of products such as yogurt, cheeses, and alcoholic beverages are imported from developed countries. There is a growing consumer interest in attaining wellness through diet, necessitating the need for incorporation of the probiotic strains into diets and the African traditional fermented products offer a vast source of those microorganisms, yet these opportunities have not been explored (Franz et al., 2014; Reid et al., 2014). As demonstrated, *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* are dominated by LAB. Most LAB have the 'generally regarded as safe' (GRAS) status and some are probiotics such as the *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Streptococcus*, and *Leuconostoc* (Masood et al., 2011). It is therefore important to note that *Mursik*, *Amabere amaruranu*, *kule naoto*, and *suusa* have probiotic strains and efforts to purify, characterize, and incorporating them into foods could contribute to the wellbeing of Kenyans, particularly the vulnerable groups.

The application of high throughput biotechnologies for starter culture design can allow the tailoring of starter cultures to yield products with specific flavors and/or textures. For example, in Thailand, Random amplified polymorphic DNA (RAPD) techniques have been recruited in the development of defined starter cultures for flavor development for the production of fermented pork sausage, *nham*, which is now produced commercially (Valyasevi and Rolle, 2002). The successful application of starter cultures and improved bioreactor technology is highlighted by the fermented soy sauce production. The production of soy sauce has transited from a craft to a technology-based production system in Thailand (Valyasevi and Rolle, 2002). The process takes a shorter time and uses the *koji* culture *Aspergillus oryzae* for proteolysis of the soy proteins in the initial phase and *Saccharomyces rouxii* in the second phase (Moromi fermentation) (Beuchat, 1995; Valyasevi and Rolle, 2002). All the processes are controlled and the physical parameters (temperature and humidity) are controlled in the fermenters. All these developments have enhanced product safety and consistency and have ultimately led to economic gain for the soy sauce industry and greater value added to the product in terms of quality and safety. The production of *Som Fug*, a traditional fermented fish paste has also employed starter cultures.

In recent times, the genetic characterization of micro-organisms has advanced at a rapid pace with exponential growth in the collection of genome sequence information, high-throughput analysis of expressed products, that is,

Table 1. Some of the techniques appropriate for studying indigenous fermentations.

Name	Description	Reference
RAPD	A typing method based on the genomic DNA fragment profiles amplified randomly by PCR, and is commonly used for disintegration of LAB strains from fermented foods.	Coppola et al. (2006); Chao et al. (2008).
rep-PCR	A technique permits typing at subspecies level and reveals significant genotypic differences among strains of the same bacterial species from fermented food samples.	Tamang et al. (2008)
AFLP	A technique based on the selective amplification and Separation of genomic restriction fragments.	Tanigawa and Watanabe (2011)
Illumina	Sequencing technique generating millions of short reads from a single lane up to 100 bp.	http://www.illumina.com
Roche 454	Sequencing technique generating approximately one million longer reads (450–700 bp) from a sequencing plate.	http://www.my454.com
DGGE of 16S rRNA	Allows ‘‘community fingerprinting’’ by PCR amplified DNA of 16S or 26S rRNA from mixed microbial communities to visualize variations in microbial diversity and give an estimate of richness/abundance of predominant microbial members.	Alegría et al. (2011)
ARDRA	An adaptation of the restriction fragment length polymorphism that creates ‘fingerprints’ from the 16S rRNA gene that can be analyzed on agarose gel.	Jeyaram et al. (2010)
MLSA	Uses housekeeping genes as molecular markers alternative to the 16S rRNA genes for LAB species identification	Tanigawa and Watanabe (2011)
Metagenomics	Application of sequencing method(s) to DNA obtained directly from a given environmental sample. The sequencing reads can either be used or assembled into contigs for determining prevalence of open reading frames specifying molecular functions.	Bigot et al. (2015)

transcripts and proteins and the application of bioinformatics which allows high throughput comparative genomic approaches that provide insights for further functional studies (Ercolini, 2013; Alkema et al., 2015). Genome sequence information, coupled with the support of highly advanced molecular techniques, have allowed scientists to establish mechanisms of various host-defensive pathogen counter-defensive strategies and have provided industry with tools for developing strategies to design healthy and safe food by optimizing the effect of probiotic bacteria, the design of starter culture bacteria and functional properties for use in food processing. Characterization of the genomes of lactic acid probiotics has, for example, shed light on the interaction of pathogens with LAB (de Vos, 2001).

There has been a slow adoption of microbial starter cultures and application of high throughput biotechnologies in Kenya (Reid et al., 2014; Franz et al., 2014). This can be due to high poverty levels, whereby the price of the food is more relevant than the quality and safety. Studies aimed at upgrading the traditional fermentation technologies are few and the funded studies so far aim at identifying microorganisms and improving hygienic conditions of the fermentation processes and not process development.

However, with the demand of the products rising due to improved living standards and potential for export, exploitation of the vast microbial diversity in *Mursik*, *Amabere amaruranu*, *kule naoto* and *suusa* to optimize the current products or to create new ones, use of hygienic conditions, and development of fermentation processes and shelf-life extension of the products is essential. Moreover, co-creation processes where product end users and stakeholders are engaged to discuss the challenges and share the goal of valorization of the spontaneously fermented products will be key tothe broadening the acceptance of the developed products. This is because the stakeholders’ experiences, ideals and senses of value,which will allow the generation of products based on a common ground, will enable rapid adoption of newly developed products.

To achieve this, there needs to be an enabling environment. There needs to be collaborative research efforts between Kenyan research institutions, their counterparts in the developed countries and stakeholders in Kenya including farmers, consumers and regulatory bodies. The research institutions also need to link with the industry in the application of the developed technologies. The Kenyan government needs to come up with policy regarding traditional fermented foods in order to protect consumers and organize the stakeholders to promote fermented foods. The government should invest in manpower and research funds that can develop bioprocesses for the production of traditional fermented foods/ beverages. Moreover, food biotechnological information sharing is necessary in guiding research agenda. Programs should be set to scale research into business ventures. Since Kenya has laws protecting

intellectual property, development of traditional fermentation technologies can be protected. The information from scientists and industry therefore need to be transparent to gain consumer confidence. As the fermented foods have potential to contribute to food security, scientists in Kenya need to rise to the occasion and give direction in this arena. First, they need to apply the recent high throughput biotechnologies in characterizing the products for up-gradation. By doing so, they will contribute to process development, knowledge creation, industrialization, and curation of indigenous knowledge and it remains a challenge.

Conflict of Interests

The authors have not declared any conflict of interests.

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