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The Functionality of African *Streptococcus thermophilus* and *Streptococcus infantarius* SubSp. *infantarius* In Milk Fermentation

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Abstract

Fermentation serves a key role in inhibiting spoilage microorganism through acidification and production of antimicrobial compounds. The technological information on properties of *Streptococcus infantarius* sub sp. *infantarius* which is predominant in most African fermented is very little. This study was therefore carried out to determine the functionality of selected African *Streptococci* strains in fermented dairy products. Pasteurized milk samples from camels and cows were inoculated with different strains and a selected combination at a rate of 3 % v/v and incubated at different temperatures of 25°C, 30°C, 37°C, and 45°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. The different fermented milk samples were subsequently evaluated for consumer acceptability. Milk inoculated with both African type *Streptococcus thermophillus* (146A8.2)and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), and incubated for nine hours recorded the highest amount of titratable acidity of 0.97 for the camel milk and had the least pH value of 4.12 for cow milk compared to the other strains. The cow milk had the highest viscosity level of 59.64 cPs compared to camel milk which was 29.44 cPs. The levels of titratable acidity and viscosity depended on the strain and incubation temperature. The African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), isolated from fermented camel milk had good technological properties that are useful as starter culture for development of fermented milk products.

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Introduction

In Kenya, production of traditional fermented dairy products like *Suusac* and *Mursik* is carried out through spontaneous fermentation.[1] This technology has resulted in vast growth in the food industry because of low cost in energy, infrastructure, and

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the wide acceptance of traditionally fermented food products in Kenya.[1] Demand for fermented food products is rising due to the health benefits associated with it.[2] Several limitations such as low yields, inefficiency, and products with varying quality standards have been associated with spontaneous fermentation.[3] Traditional dairy 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.[4] 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.[5]

There is a need for the improvement and development of indigenous starter cultures under controlled conditions in order to exploit their Probiotic potential.[2] This will ensure production of safe food stuffs which are consistent in quality and that are widely accepted by the community.[3] There is a growing interest in research on potential starter microorganisms from various milk and milk products.[6,7] Lactic acid bacteria (LAB) are the main microorganisms involved in th e fermentation of various products.[3] Species such as *Lactococcus lactis, Lactobacillus* sp. *Streptococcus bovis/ Streptococcus equinus* complex (SBSEC), *Enterococcus* sp. and yeast are present in milk.[8] In Africa, species such as *Streptococcus thermophillus, Streptococcus salivarius, Streptococcus infantarius sub* sp. *infantarius, Streptococcus gallolyticus*, and *Streptococcus agalactiae* have been identified,[8] but only *Streptococcus thermophillus* has been approved for use in dairy processes. However, traditional Africa fermented dairy products (AFDPs) are dominated by *Streptococcus infantarius* sub sp. *infantarius* and not *Streptococcus thermophillus*. In Africa, dairy products are often consumed raw, as well as in the form of traditional AFDPs.[9]

Streptococcus infantarius sub sp. *infantarius*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 *Streptococcus infantarius* sub sp. *infantarius* 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.[10, 11, 12] Genomic analyses on *Streptococcus infantarius* sub sp. *infantarius* isolates have revealed an adaptation to lactose metabolism that is parallel to that of *Streptococcus thermophillus*. The common ancestor of *Streptococcus thermophillus* strain 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.[13, 14] In East Africa Camels were introduced around 2,500 years ago[15, 16] and the less genome decay in *Streptococcus infantarius* sub sp. *infantarius* CJ18 may be attributed to the start of fermentation of camel milk which came later.[11] The analysis of the African strain of *Streptococcus infantarius* sub sp. *infantarius* CJ[18] has also revealed more dairy adaptations like *Streptococcus thermophillus* to the dairy niche. *Streptococcus infantarius* sub sp. *infantarius* has been found to carry a partial additional gal-lac operon consisting of genes lacS and lacZ and exhibiting phenotypic lactose/galactose exchange as *Streptococcus* thermophilus.[11] *Streptococcus infantarius* sub sp. *infantarius* has not been classified 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.[11] Further research on the functional analysis of *Streptococcus infantarius* sub sp. *infantarius* 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 evaluate the technological functionality of African Dairy *Streptococcus thermophillus* (146A8.2), *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), and *Streptococcus infantarius* sub sp. *infantarius* CCUG(9381)) as starter culture in camel and cow milk fermentation.

Materials and Methods

Study Setting and Collection o0f Milk Samples

Raw camel milk was obtained from Isiolo County in Kenya, frozen and then transported in a cool box to pilot plant at the University of Nairobi where it was pasteurized. Raw cow milk was obtained from the university farm, Kanyariri, 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°C for 30 minutes, cooled

and dispensed into sterile 500 ml containers before inoculating with the culture strains (Figure 1).

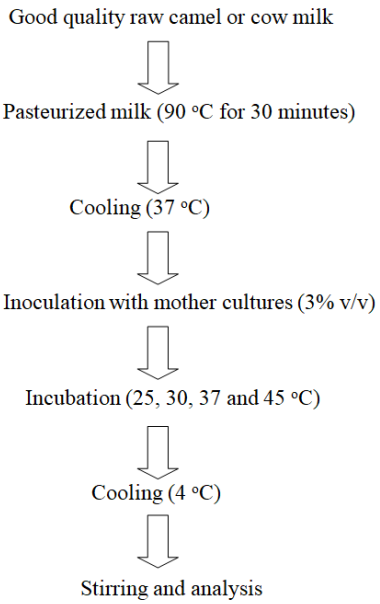

Fig. 1: Process flow chart of camel and cow milk fermentation trials Preparation of starter cultures

Preparation of Starter Cultures

Working cultures of African type *Streptococcus thermophillus* (146A8.2), *Streptococcus infantarius* sub sp. *infantarius* CJ 18(9377), *Streptococcus infantarius* sub sp. *infantarius* CCUG(9381) were prepared from pure isolates of frozen stocks after growth then transferred into fresh M17 broth (Oxoid, UK) twice at 37°C for 24 hours. Strains were selected based on the turbidity of the tubes, and 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.[17] 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, andxylose). Confirmed African type *Streptococcus thermophillus* (146A8.2), *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), *Streptococcus infantarius* sub sp. *infantarius* CCUG (9381) strain were then grown in MRS broth at 37ºC for 24 hours to make the working culture.[18]

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 was inoculated with each of the stock culture of African type *Streptococcus thermophillus* (146A8.2), *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), *Streptococcus infantarius* sub sp. *infantarius* CCUG(9381), at a rate of 3% v/v and shaken thoroughly to ensure proper mixing then incubated for 6 hours at 37°C temperatures. These inoculums were used for the starter culture fermentation trials.

Fermentation Trials

Standardized inoculums of African type *Streptococcus thermophillus* (146A8.2), *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), *Streptococcus infantarius* sub sp. *infantarius* CCUG(9381), combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), were prepared by heating 500 ml fresh raw camel and cow milk to 90°C for 30 minutes. Then cooled and inoculated with 3% v/v of the mother culture after that, each treatment was incubated at 25°C, 30°C, 37°C and 45°C for up to 9 hours.

Determination of pH of Milk

The pH was determined by using an electronic digital pH meter (Orion Research Inc., Cambridge, MA, USA). After each usage the pH meter was calibrated using a Buffer solution of pH 7 and pH of 4. The concentration of hydrogen ions present in the milk samples was measured after 3, 6 and 9 hours of incubation.

Determination of Acidity

Titratable acidity of raw milk and during fermentation was determined in triplicate, according to the AOAC (2000)[19] method number. 947.05. Nine mL of the milk samples was pipetted in a 250ml flask followed byaddition 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 camel and cow milk. The acidity was then calculated using the equation below and expressed in terms of lactic acid:

% Acidity (as lactic acid) = Volume of 0.1 N NaOH used X 0.009 ...(1)

0.009 is the multiplication factor. (Lactic acid is an organic acid (CH_3-CHOH-COOH) and has a molecular weight of 90. Therefore, one ml of 0.1 NaOH corresponds to 0.009 g lactic acid:

(90×0.1)/1000=0.009 glacticacid) AOAC (2000)[19] method number. 947.05

Determination of Viscosity

Viscosity was determined following the ISO 2555:2018(E) method. 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.

Sensory Analysis

The descriptive sensory analysis was carried out as described by Ludwig *et al*.[20] Twelve trained panelists from The Department of Food Science, Nutrition and Technology at the University of Nairobi were used. Fermented 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 over all 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)

Statistical Analysis

Statistical analysis of the data for the effects of various starter cultures and temperatures on SH, pH, viscosity was performed by ANOVA using Genstat software 15th Edition. Sensory analysis was performed using SPSS 20.0 statistical software. The mean differences were analyzed using Tukey's multiple- range test at 5% significance levels.

Table 3: Titratable Acidity of Camel milk inoculated with different strain of *Streptococcus* and incubated at different temperature and time

Strain	Time (hours)	Temperature			
		25°C	30°C	37°C	45°C
CCUG	0	0.85±0.010^{kl}	0.84±0.028^{l}	0.86±0.031^{ijk}	0.88±0.010^{hi}
	3	0.87±0.006^{i}	0.85±0.010^{kl}	0.87±0.030^{ij}	0.89±0.010^{fh}
	6	0.88±0.010^{hi}	0.88±0.010^{hi}	0.91±0.020^{f}	0.89±0.010^{fh}
	9	0.89±0.010^{gh}	0.89±0.020^{gh}	0.92±0.010^{d}	0.91±0.010^{f}
CJ18	0	0.85±0.013^{l}	0.84±0.070^{l}	0.81±0.030^{m}	0.89±0.020^{gh}
	3	0.85±0.006^{jkl}	0.85±0.010^{kl}	0.85±0.030^{k}	0.89±0.015^{fh}
	6	0.87±0.058^{ij}	0.89±0.020^{gh}	0.89±0.020^{gh}	0.89±0.006^{fh}
	9	0.89±0.010^{gh}	0.90±0.020^{fg}	0.91±0.010^{ef}	0.96±0.010^{ab}
ST	0	0.85±0.034^{jkl}	0.81±0.014^{m}	0.80±0.006^{m}	0.88±0.020^{hi}
	3	0.89±0.010^{gh}	0.85±0.020^{kl}	0.89±0.010^{gh}	0.89±0.152^{fh}
	6	0.91±0.010^{ef}	0.89±0.010^{gh}	0.92±0.010^{de}	0.92±0.010^{de}
	9	0.91±0.010^{ef}	0.92±0.010^{de}	0.94±0.006^{cd}	0.95±0.010^{bc}
ST and CJ18	0	0.89±0.022^{ghi}	0.85±0.021^{kl}	0.81±0.020^{m}	0.86±0.020^{jk}
	3	0.90±0.010^{fg}	0.85±0.010^{kl}	0.86±0.020^{jk}	0.90±0.010^{fg}
	6	0.91±0.000^{ef}	0.88±0.010^{hi}	0.91±0.020^{ef}	0.92±0.020^{de}
	9	0.93±0.010^{d}	0.94±0.006^{cd}	0.96±0.010^{ab}	0.97±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.

Results

Titratable Acidity of Fermented Camel Milk

Development of titratable acidity at 25°C, 30°C, 37°C, and 45°C for the different strains at 3 hours' interval is summarized in Table 3. The mean ranged from 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 incubation time. Milk inoculated with both African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) and incubated for nine hours recorded the highest amount of titratable acidity while milk samples treated with *Streptococcus infantarius* sub sp. *infantarius* CCUG (9381) had the least. Increasing the incubation temperature and time resulted in increased levels of titratable acidity due to fermentative action of the strains. At 30°C, 37°C and at 45°C, the levels of titratable acidity in camel milk treated with a combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) had the highest amount of titratable acidity after nine hours of incubation. Titratable acidity for all the strains was highest at 45°C, with a combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) performingbetter. The acidity for African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) and the combination of African type *Streptococcus thermophillus*(146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) increased sharply as from 30°C.

Table 4: pH of Camel milk inoculated with different strain of *Streptococcus* and incubated at different temperature and time

Strain	Time (hours)	Temperature			
		25°C	30°C	37°C	45°C
CCUG	0	6.92 ± 0.030^{a}	6.96 ± 0.028^{a}	6.89 ± 0.010^{a}	5.12 ± 0.010^{lm}
	3	6.15 ± 0.020^{bcd}	6.97 ± 0.070^{a}	5.44 ± 0.020^{i}	4.82 ± 0.020^{no}
	6	5.99 ± 0.010^{de}	5.88 ± 0.020^{ef}	5.21 ± 0.010^{k}	4.32 ± 0.020^{p}
	9	5.85 ± 0.020^{f}	5.80 ± 0.010^{fg}	5.18 ± 0.010^{lm}	4.30 ± 0.020^{p}
CJ18	0	6.95 ± 0.022^{a}	6.09 ± 0.010^{b}	6.89 ± 0.020^{a}	6.98 ± 0.010^{a}
(9377)	3	6.27 ± 0.015^{b}	6.01 ± 0.010^{cde}	6.01 ± 0.010^{cde}	4.78 ± 0.020^{o}
	6	6.03 ± 0.020^{cde}	5.69 ± 0.010^{fgh}	5.51 ± 0.020^{i}	4.77 ± 0.020^{o}
	9	5.88 ± 0.020^{ef}	5.64 ± 0.020^{fgh}	5.15 ± 0.020^{lm}	4.74 ± 0.010^{o}
ST	0	6.90 ± 0.013^{a}	5.80 ± 0.010^{fg}	6.92 ± 0.010^{a}	6.90 ± 0.010^{a}
(146A8.2)	3	6.22 ± 0.010^{bc}	5.63 ± 0.010^{g}	5.16 ± 0.020^{lm}	4.26 ± 0.010^{p}
	6	5.87 ± 0.010^{ef}	5.42 ± 0.020^{ijk}	5.14 ± 0.010^{lm}	4.17 ± 0.030^{p}
	9	5.72 ± 0.010^{fgh}	5.51 ± 0.010^{h}	5.00 ± 0.020^{mn}	4.15 ± 0.010^{p}
ST and CJ18	0	6.94 ± 0.026^{a}	5.42 ± 0.010^{ij}	6.90 ± 0.020^{a}	6.91 ± 0.010^{a}
	3	6.02 ± 0.010^{cde}	5.54 ± 0.020^{ij}	6.07 ± 0.020^{bcde}	5.54 ± 0.020^{ij}
	6	5.71 ± 0.020^{fgh}	5.30 ± 0.200^{j}	5.42 ± 0.020^{ijk}	5.32 ± 0.010^{jl}
	9	5.45 ± 0.030^{ij}	5.25 ± 0.020^{k}	5.12 ± 0.010^{lm}	4.71 ± 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.

Ph Of Fermented Camel Milk

pH development at 25°C, 30°C, 37°C, and 45°C for the different strain at 3 hours' interval is summarized in Table 4. The pH values ranged from 5.880±0.020 to 4.150±0.010 depending on the strain and incubation temperature. pH was significantly ($p<0.05$) affected by incubation time, temperature and microbial strain. Increasing the incubation time significantly ($p<0.05$)

reduced the pH while increasing the temperature significantly ($p<0.05$) increased the rate of change in the pH. Milk samples treated with a combination of both ST and CJ18 and incubated for nine hours recorded the lowest levels of pH while milk samples treated with *Streptococcus infantarius* sub sp. *infantarius* CCUG (9381) had the least.

Viscosity of Fermented 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. Increasing the incubation temperature of the starter cultures significantly increased viscosity at $p<0.05$. The viscosity ranged from 18.6v to 29.44cPs depending on the strain and incubation temperature. Milk samples treated with a combination of ST and CJ18 strains was the most viscous across all the incubation temperatures. Interaction between the samples and incubation temperatures had a significant ($p<0.05$) effect on the viscosity at. All the milk samples had the highest viscosity at 45°C with sample treated with a combination of ST and CJ18 being the most viscous.

Table 5: Viscosity (cPs) of Camel milk inoculated with different strains of *Streptococcus* and Incubated at different temperature

Strain	Temperature			
	25°C	30°C	37°C	45°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			

*Values with different letters in superscripts are significantly different at $P<0.05$. Each value is mean ± standard deviation for triplicate experiments.

Table 6: Titratable Acidity of Cow milk inoculated with different strain of *Streptococcus* and incubated at different temperature and time

Microbial strain	Incubation temperature (°C)	Incubation period (hours)			
		25°C	30°C	37°C	45°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±0.0^{3d}	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±0.0^{3c}	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}
Average		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.

Titratable Acidity of Fermented Cow Milk

Development of acidity at 25°C, 30°C, 37°C and 45°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 7: pHof fermented cow milk inoculated with different strains of *Streptococcus* and incubated at different temperatures and time

Microbial strain	Incubation period (hours)	Incubation temperature (°C)			
		25°C	30°C	37°C	45°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}
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.

Ph of Fermented Cow 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 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. 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. The interaction between microbial strain and incubation temperature did not significantly (p<0.05) affect the pH.

Viscosity of Fermented Cow 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 8. Increasing the incubation temperature of the starter cultures significantly increased viscosity at p<0.05. The viscosity values ranged from 19.77 to 59.64 cPs depending on the strain and incubation temperature. Milk samples inoculated with a combination of ST and CJ18 strain was the most viscous across all the incubation temperatures. All the milk samples had the highest viscosity at 45°C with sample treated with a combination of ST and CJ18 strain being the most viscous.

Table 8: Viscosityof fermented cow milk inoculated with different strain of *Streptococcus* and incubated at different temperature

Sample	Incubation temperature(°C)			
	25°C	30°C	37°C	45°C
CCCUG	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.

Table 9: Sensory analysis of both fermented camel and cow milk

Microbial strain	Type of milk starter culture	
	Fermented camel milk at 45°C	Fermented cow milk at 45°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}
Average	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}
STandCJ18	3.83±0.58^{a}	3.83±1.03^{a}
Average	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}
STandCJ18	3.50±0.52^{b}	4.00±0.74^{b}
Average	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.

Sensory Analysis

There was no significant difference in the scores for mouthfeel, sourness, however, there was significant ($P<0.05$) differences in the general acceptance of the milk samples inoculated with different starter cultures (Table 9). The scores for general acceptance for samples with the CJ18, ST, and combination of ST and CJ18 strain were significantly higher than that of CCUG microbial strain at $p<0.05$. Camel milk treated with CJ18 and cow milk treated with ST was generally accepted by the team sensory evaluation team.

Discussion

The highest level of acidity (0.970) was found in camel milk inoculated with a combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), at 45°C while in cow milk the highestlevel of acidity (0.890) was found in samples inoculated with *Streptococcus infantarius* sub sp. *infantarius* CCUG (9381) at 25°C and 30°C. The differences in acidification of camel and cow milk can be associated withthe differences in chemical composition of both milk samples.[21] The results obtained from this study suggest the ability of different strains as pure cultures in growing milk. The physical and chemical changes observed during fermentation of the two milk samples is as a result of the fermentative activities of lactic acid bacteria used as starter culture[22]

Increased incubation time, and temperature resulted in reduced pH. The optimal fermentation temperature for the strain or their combination in both kinds of milk was at 45°C with a combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377), giving the best results. Pasteurization of milk with starter cultures results in a drop of pH through acidification.[22] From the results pH value of above 4 is an advantage to fermented dairy products because of longer fermentation process causes quality impairment for example in yogurt.[23] There was a sharper decline in pH for the cow milk than in camel milk which could be explained by differences in buffering capacity levels, the difference in proportions of proteins and the specific salts in both kinds of milk.[21] The sharper decline in pH levels can be attributed to fermentation activity of the different strains. The final pH for the different strains or their combinations reached a value less than 5 but greater than 4 which is beneficial to pastoral communities in ASALs, where there are poor infrastructures.[22] The African type *Streptococcus* strain which has been found to be predominant in *Suusac*, when incubated at temperatures of between 37°C and 45°C took a short time to reach maximum acidification and to get the acceptable pH between 4 and 5 that is desired in fermented dairy products. This could give the strains competitive advantage over other bacteria during fermentation and could explain the predominance not only in *Suusac* but *Gariss,* in initiating *spontaneous fermentations*.[13] The *Streptococcus infantarius* sub sp. *infantarius* strain are highly adapted and competitive in traditional fermented dairy products.[13]

The viscosity for the different strains or their combination was strongly dependent on the incubation temperature, with a temperature at 45°C being optimal for both kinds of milk. Combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) gave the best viscosity results. 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. The changes in viscosity observed was due to the growth and fermentative activities of the starter culture strains that was used.[24] 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 organoleptic properties of the products.[25]

Sensory analysis was done on different products to identify had the best technological properties in terms of pH, acidity, and viscosity. A combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) strain at 45°C gave the best results for both milk types and the control. The samples did not have any significant difference in terms of mouthfeel, sourness but there was significant differences in general acceptability due to the variability in different strains. Milk samples made using African type *Streptococcus thermophillus* (146A8.2) culture at

45°C had a more general appeal than others. This variation was in agreement with a study done on quality parameters of starter cultures.[26] The flavour and sensory scores were influenced by the use of starter cultures and temperature. The selected strain is thermophilic as they worked best from 37°C to 45°C and can be used as mixed strain for best results. A long fermentation time trial at low temperatures of 25°C and 30°C is important in order to complete acidification process. This is because the activity of the strains was slow at low temperatures.The results obtained in this study indicate that a combination of African type *Streptococcus thermophillus* (146A8.2) and *Streptococcus infantarius* sub sp. *infantarius* CJ 18 (9377) strain at 45°C gave the best product however; in terms of general acceptability African type *Streptococcus thermophillus*(146A8.2) had more general appeal. Differences in the product were due to differences in chemical composition of the milks samples and the strain.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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