2022 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 12, Issue 6: 372-378; November 25, 2022

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2022.49

ANALYSIS OF PHYSICAL AND MICROBIOLOGICAL QUALITY OF RAW CAMEL MILK IN THE SOMALI REGIONAL STATE OF ETHIOPIA

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Supporting Information

RESEARCH ARTICLE

PII: S222877012200049-12

Received: September 18, 2022

Revised: November 21, 2022

Accepted: November 23, 2022

ABSTRACT: The objective of this study was to determine the physical and microbial quality of raw camel milk along the milk market chain a total of forty-two raw milk samples were taken from milk producers (21 samples) and milk collection centers (21 samples). Each sample was analyzed for physical and microbial quality including temperature, pH, titratable acidity, specific gravity, and clot on boiling, the overall mean and standard deviation values were 27.93 ^{o}C, 6.29, 1.030 g/cm^{3}, 0.95%, and 88.1% respectively. Microbial quality and safety attributes that include total bacteria count, coliform count, and yeast and mold counts were analyzed. The overall mean $\log_{10}$ counts per ml and standard deviation values for each total bacterial count, coliform count, and microbial analysis were 7.48 $\log_{10}$ CFU/ml, 5.85 $\log_{10}$ CFU/ml, and 4.78 $\log_{10}$ cfu/ml, respectively. The total bacterial count, coliform count, yeast, and mold counts were calculated and show that the milk collection center samples were significantly higher than milk samples obtained from household producers. This study indicated that the quality of camel milk in the study area had low quality and this could cause public risks through the consumption of raw camel milk produced and sold under the present production and handling conditions along the chain. Thus, these calls for strict hygienic measures to improve the quality and safety of camel milk produced and marketed in the study area.

Keywords: Camel milk, Hygiene, Microbial quality, Raw milk, Somali Regional State.

INTRODUCTION

The global population of domesticated large camelids (*Camelus dromedaries* and *Camelus bactrianus*) is estimated to be about 28 million (Faye, 2015). More than 80% of the camel population inhabits Africa with 60% in the Eastern African countries which are important exporters of dromedary camels to the Arabian Peninsula and Egypt (Faye, 2015). The camel population in Ethiopia is estimated at 4.8 million (Behnke, 2010). Milk is susceptible to contamination with pathogenic microorganisms from the time it is milked until it reaches the consumer. The hygienic quality of milk and dairy products is dependent upon the quality of the raw milk and the conditions under which the milk is produced (Carloni et al., 2016; Kaskous, 2019; Ayuob et al., 2020).

Microbial contamination in milk may cause milk-borne diseases in humans (Berhe et al., 2020; Kakati et al., 2021), while others are known to cause milk spoilage (Fusco et al., 2020). Sources of microbial contamination in milk include primary microbial contamination that comes from the infection or mastitis in lactating animals. The secondary causes of microbial contamination occur along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment, and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation, and storage. Poor or improper handling of milk can exert both public health and economic constraints thus requiring hygienic vigilance throughout the milk value chain (Swai and Karimuribo, 2011).

In the Gursum district Somali region of the study areas, camel milk is produced in traditional ways and camels are milked by hand. Then the milk is handled under poor hygienic conditions and transported a long distance (up to Jigjiga town) where it is sold on the street (open market) or distributed to retailers. Thus, milk transported and handled under such conditions may have poor quality and contain pathogenic microorganisms of public health concern. Therefore, studying the quality and safety of raw camel milk along the milk marketing chain, i.e., from the production site until it reaches the milk collection centers site is very important in that the results generated will be used to devise appropriate intervention strategies aimed at improving the quality and safety of camel milk produced in the study area.

MATERIALS AND METHODS

Description of the study area

Somali Region is the second largest region in Ethiopia. It borders Djibouti in the north, Somalia in the east and northeast, and Kenya in the south. In the west, it borders Oromiya Region, and in the north-west Afar Region. The specific survey area of the study was Gursum Woreda, Fafan zone, Somali region.

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Methods of data collection

The study conducted among producers and milk collection centers involved experimental work on the physical and microbiological qualities of raw camel milk samples in the study area. All of procedures are in according to animal welfare rules and hygiene consideration according to ethical rules of Department of Animal and Range Science of Haramaya University and Food Science and Nutrition Research Directorate.

Milk sample collection

A total of three potential Kebeles, namely Bombass, Tikdam, and Kobijaro, were selected purposively from 18 Kebeles in the Gursum Somali district due to their high potential in camel milk production and the common practice of marketing camel milk. Using simple random sampling (lottery method), a total of 42 milk samples were collected for physical and microbial quality analysis (14 samples from each Kebele). In each Kebele, 7 samples from households and 7 samples from milk collection centers were collected. Approximately 15 ml of morning raw milk samples were collected using sterile bottles from each household and milk collection center for a period of three months. All the samples were then transported to the laboratory of dairy microbiology at Jigjiga University using an insulated icebox without delay.

Physical analysis of milk

Titratable acidity

The percentage acidity of the milk was measured by titrating ten milliliters of raw milk sample with 0.1 NNaOH to the phenolphthalein endpoint as described in (O'Connor, 1994). Ten ml of raw milk sample was pipetted into a beaker, and then 3 to 5 drops of 1% phenolphthalein indicator were added into the milk. The samples were titrated with 0.1 N NaOH until the faint pink color persisted. The titratable acidity was calculated using the following formula:

PH

The pH of milk was recorded using a digital pH meter. The instrument was at first calibrated using a buffer of pH 7.0 and 4.0 before measuring the pH of milk samples.

Temperature

The temperature of the milk samples was measured using an electronic thermometer.

Specific gravity

Measurement of milk density was done by using a lactometer. A fresh milk sample was first filled sufficiently into a glass cylinder (100 ml capacity) (O'Mahony, 1988). The following formula was used to calculate the milk-specific gravity.

Specific gravity = $\frac{L}{1000} + 1$

Where, L corrected the lactometer reading at a given temperature i.e. for every degree above 15.56 C^0, 0.2 degrees were added, but for every degree below 15.56 C^0, 0.2 degrees was subtracted from the lactometer reading.

Clot on boiling test

Five ml of milk was placed in a test tube and held in an alcohol flame for five minutes. Then the test tube was carefully removed from the flame and examined for precipitate (O'Mahony, 1988).

Microbiological analysis

For the microbial quality tests, Standard plate count, Coliform Count, and Yeast and Mould count were used. Serial dilutions of milk samples were plated on plate count agar, violate red bile agar (HIMEDIA), and potato dextrose agar (MICOGEN) for standard plate count, coliform count, and yeast and mold count, respectively. Media used for the determination of standard plate count, Yeast, and Mould count, and those used for serial dilution (peptone water) were sterilized by autoclaving at 121 ^oC for 15 minutes, while the media used for determination of CC (violet red bile agar) were sterilized by boiling on a hot plate. All plates used for the enumeration of total bacteria and coliform bacteria were incubated for 48 and 24 hrs at 30 ^oC and 37 ^oC, respectively. In contrast, those used for the enumeration of yeasts and molds were incubated at 22-25 C^o for 5 days (Richardson, 1985).

Standard plate count

The total bacterial count was done using the pour plate method. Standard Plate Count Agar was used to determine the extent of microbial contamination of milk before any processing was done. One milliliter (1 ml) of milk sample was serially diluted up to six dilutions using six test tubes each containing 9 ml of peptone water (ratio of 1:10). Sterile duplicate glass Petri dishes were labelled corresponding to each dilution. One ml of the dilutions was then aseptically withdrawn using a sterile 1 ml Pasteur pipette and delivered into a sterile Petri dish. This was repeated till all the dilutions were pipetted into their corresponding plates up to 10^{-6}. This was followed by pouring about 15 ml of standard plate count agar which had been autoclaved at 121 °C for 15 min, cooled, and tempered in a water bath at 50 °C. The sample and the agar were gently swirled by an alternate clock and anti-clockwise rotations and left to solidify on the bench for about 30 min. The plates were inverted and incubated at 30°C for 48 h. After incubation, the plates inoculated with the dilution yielding between 30 and 300 colonies were counted. Colony counts were made using a colony counter (Richardson, 1985).

Estimation of the CFU/ml

The average colony count from the duplicate plates was used to estimate the total number of colony-forming units per ml (CFU/ml) using the following formula:

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$$N = \frac{\sum c}{[(1xn_1) + (0.1xn_2)]d}$$

Where, N = number of colonies per milliliter or gram; ΣC = sum of all colonies on all plates counted; n1 = number of plates in lower dilution counted; n2 = number of plates in the next highest dilution counted however, plates from two consecutive decimal dilutions yield colony counts of 30 to 300, the counts for each dilution was computed (APHA, 1992).

Total coliform counts

One ml of milk sample was added into a sterile test tube having 9 ml of peptone water. After mixing, the sample was serially diluted up to 10^{-5} and duplicate samples (0.1 ml) were spread plated on solidified Violet Red Bile Agar and evenly spread. The plates were then incubated at 32 ^{0}C for 24 hours. Finally, colony counts were made using a colony counter. Typical dark-red colonies were considered as Coli form colonies (Richardson, 1985). The total coliform counts were also expressed in CFUs/ml computed using the first formula shown above for total bacterial count.

Yeast and mold counts

For the enumeration of yeasts and molds potato dextrose agar spread plates were used. Here, the plates were, however, incubated at 22-25 C^o for 5 days. Either the first or the second formula was used for computing the CFU/ml of yeasts and molds as indicated for the determination of the TBC. The following models will be used to analyze the physical and microbiological quality of milk: 1, Model. $Y_{ij} = \mu + \beta_j + e_{ij}$

Where, μ =is the overall mean, Y_{ij} = refers to individual observation, β_i is the source effect i^{th} (i=1,2), e_{ij} is the error term.

Statistical analysis

Data recorded during sampling and laboratory findings were entered and stored in a separate Microsoft Excel spreadsheet. Mean and standard deviations were calculated for the physical quality of raw milk. Analyses of variance were performed. Microbial counts of raw milk samples were first transformed to logarithmic values (log_{10}) before statistical analysis by using general linear model procedures of SAS version 8.2 (SAS, 2001). For each experiment mean comparisons were done separately using the least significant difference for variables whose F-values showed significant differences at a 5 % significance level.

RESULTS AND DISCUSSION

Physical quality of raw camel milk

Specific gravity: the value of specific gravity of milk samples collected from the household and the milk collection centers were in the range of 1.028 g/cm^3 to 1.032 g/cm^3, respectively. The current results also show a significant difference (P<0.05) in density values between milk samples obtained from households (1.028g/cm^3) to milk collection centers (1.032 g/cm^3) (Table 1) the density of milk among others is commonly used for quality test mainly to check for the addition of water to milk or removal of cream addition of water to milk reduces milk density, while removal of cream increases it (O'Connor, 1994).

Table 1 - Physical properties (means ± SE) of camel milk produced and marketed (n=42)

Parameter	Household (n=21)	Milk collection center (n=21)	Overall mean (SE)
Specific gravity	1.028±0.00045^a	1.032±0.00045^b	1.03±0.00045^{ab}
Temperature	24.10±0.93^b	31.9±0.93^a	28±0.93^b
pH value	6.5±0.088^b	5.9±0.088^b	6.26±0.48^{ab}
TA (%LA)	0.83±0.043^a	1.07±0.043^a	0.95±0.043^a

Means are significantly (P<0.05) different; n=Number of samples; pH=, Hydrogen ion concentration; N=numbers; TA=titratable acidity; LA= lactic acid: $^{a, b, ab,}$ means in the same column with different letters show significant differences (p<0.05) among household and milk collection centers.

As indicated in Table 1, showing that the mean ± SE value of the physical quality of raw camel milk in the Gursum district. The temperature of milk samples collected from households and milk collection centers in the Gursum district were 24.10 and 31.9, respectively. The temperature of the milk samples collected at the household was significantly (P<0.05) lower than the temperature collected from milk collection centers in the Gursum district. The temperature difference of the milk along the chain might be because of the environment and exposure to sunlight. The pH of the milk samples progressively decreased from the time milk is collected from the household until it reaches the milk collection centers while the acidity follows the reverse trend. The pH of the milk samples collected from milk collection centers in the Gursum district was significantly (P<0.05) lower than the pH of milk samples collected from the household were 5.9, and 6.5 respectively with an average mean and standard division of 6.26±0.48. Milk pH indicates milk hygiene and it

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attributed to differences in the levels of hygiene of milking equipment; animal milkers wash water and the environment. Moreover, it might be due to the differences in milk holding time and temperature during storage and transportation. The overall value of coliform counts observed in the current study was much higher when compared with recommended values given by the American Public Health Association and EU (100 cfu).

Yeast and mold count

Although an increase in yeast and mold count was observed along the chain as the milk was transported from the producer households to the milk collection center markets in the Gursum district, there was a significant difference ($P>0.05$) in yeast and microbial count between the milk samples collected from milk collection centers (5.3 $\log_{10}$ CFU/ml) and the household producer (4.2 $\log_{10}$ CFU/ml). As seen from figure 3 the overall mean yeast and microbial count (4.7 $\log_{10}$ CFU/ml) observed in this study was greater than the yeast and microbial count (1.9 $\log_{10}$ CFU/ml) reported by (El-Ziney and Al-Turki, 2007) for camel milk. The presence of yeasts and molds in milk samples collected from the household and milk collection centers is higher than the acceptance levels of yeast and molds. This might be due to improper sanitary conditions in the milking area, as well as poor personal hygiene of milkers and milk sellers.

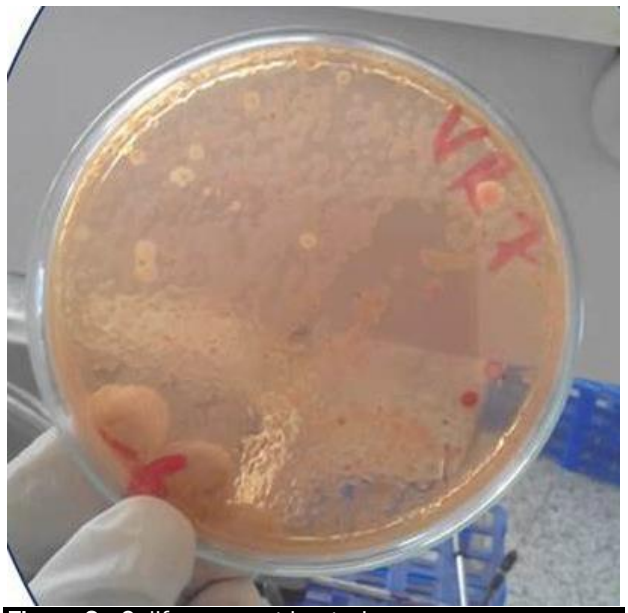

Figure 2 - Coliform count bacteria.

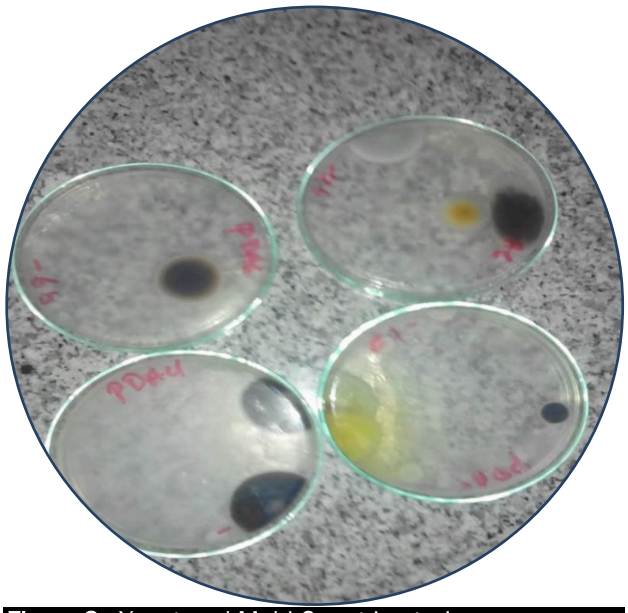

Figure 3 - Yeast and Mold Count bacteria.

DISCUSSION

The increase of standard plate count, coliform count and yeast and mold count thorough the market milk chain of raw camel milk could be associated with contaminated transporting and storage containers, water and the dust soil. Transferring of milk from container to the next during bulking towards the market makes milk sweep over wide container surfaces, thus collecting the microorganisms from container surfaces (Mwangi et al., 2016). Yeasts and Moulds are considered to be spoilage organisms. Some yeasts and moulds are a public health concern due to their production of mycotoxins, which are not destroyed during food processing or cooking (Adugna et al., 2013). Except for the tetra table acidity of milk samples obtained from milk collection centers, the majority of the physical quality of milk obtained from milk producers' samples was within the standards.

As the milk was carried from the homes to the terminal market of milk collection sites, this rise in overall bacterial counts may have happened throughout the chain. These depended on a number of factors, including the milk itself, infection of the camels' udders, the milking staff, other factors like transportation, and the type and hygienic state of the containers. This shows that raw milk supplied by retailers often had poor sanitary quality, which might most likely be attributed to inadequate milk handling during transit and storage. High total bacterial counts in raw milk primarily are a reflection of the unsanitary conditions the milk was handled in, including storage temperature, time since milking, and the health of the milking animals (Knight et al., 2016). Lack of knowledge about clean milk production, the lack of a separate area for milking, the use of non-boiled water for cleaning milking equipment and storage containers, the use of plastic containers (since plastic containers scratch easily and provide hiding places for bacteria during cleaning), and milk residues on equipment surfaces all have the potential to significantly contribute to the contamination of milk in this study. In general, greater bacterial counts could be a sign of udder disease, dirty milk handling, or unsuitable storage conditions. High levels of coliforms in milk are an indicator of poor hygiene standards utilized in milk production and show that the milk has been contaminated with fecal debris. This could be linked to inadequate udder preparation before to milking, poor milk handling techniques, and the use of subpar, unboiled water to clean milking implements (Martin et al., 2016).

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CONCLUSIONS

In the present study, there was a significant variation between the physical quality of the milk samples collected from milk producers and milk collection centers. The majority of the Physical quality of milk obtained from the milk producers' samples was within the standards except, for the tetra table acidity of the milk samples obtained from the milk collection centers. The microbial qualities of the milk obtained in the current study were poor, as judged by the high values of standard plate count; coliform count, and yeast and mold count which were significantly higher than the international standards safe for human consumption. These microbial loads may be due to poor hygienic standards during milking, and milk handling.

DECLARATIONS

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Conflict of interest
The authors declare that they have no competing interests.

Author collaboration
All authors were contributed equally.

Acknowledgments
I would like to express my gratitude, appreciation and acknowledge Mahamed Dol for his technical assistance during this study.

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