

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

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Milk container sanitation regime using wood smoke: Perceived roles and effects on milk quality in Borana, Ethiopia

By

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A Thesis Submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Public Health

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
ILRI	International Livestock Research Institute
LAB	Lactic Acid Bacteria
NAFDAC	National Agency of Food and Drug Administration Con
PH	Potential of Hydrogen
TBC	Total bacterial count
TA	Titribel acidity
Cfu/ml	Colony form unit per milliliter
FGD	Focus group discussion
IDI	In-depth interviews

ABSTRACT

Smoking of containers by using specific wood is widely practiced in many rural parts of the Eastern Africa and constitutes traditional dairy utensils cleaning and sanitation procedures. However, studies assessing the detail procedures and further validating such practices for possible integration into milk quality improvement are quite limited. Specifically, the effect of container smoking on the microbial load of fermented milk over a given duration is not known. Therefore, the present study was designed with two main objectives: (1) to explore the details milk container smoking focusing the detail procedure of smoking, types of plants used and the perceived roles the practice on the quality of traditional fermented milk in typical pastoral area of Borana in Ethiopia, and (2) to assess the effect of smoking on the total bacteria count (TBC), pH and titratable acidity (TA) of whole milk fermented under laboratory condition by simulating the traditional practices. Qualitative approaches to assess the practices of milk container smoking and its perceived benefits towards quality of milk and milk products in Borana pastoral area of southern Ethiopia were used. The data collection involved 40 individual interviews and four focus group discussions (FGD) the women keeping livestock and involved in milk production and processing. The main focus of the qualitative data collection includes: (1) the details of the types of milk containers used in the area, (2) smoking processes of the containers, (3) types of plants used for smoking of the containers, and (4) perceived role of smoking with regard to quality and safety of milk or milk products. The containers were smoked by simulating the local practice identified during the qualitative investigation. In a laboratory experiment, the effect of sanitizing milk containers with smoke from three tree species (*Olea europaea subsp. africana*, *Faurea speciosa* and *Terminalia brownii*) using two milk containers (traditional vs. stainless steel) were assessed. The result of the qualitative study shows that various traditional milk containers made from diverse materials with different shape and sizes were used by Borana pastoralists. The smoking procedure as described and demonstrated by the women had different steps and varied according to the types of milk containers. The TBC, pH and TA of the fermented milk did not show statistically significant difference among the different milk container types and the sanitizing procedures. Comparison of TBC, pH and TA of the fermented milk product with the raw milk samples showed significant increment of TBC and increment in the pH and the TA. Finally, the experiment involving container sanitation using smoking has no appreciable effects in reducing microbial load in fermented milk. Though the smoking had no significant effect in reducing the bacterial load compared to raw milk, strong preference of the pastoralists for milk stored in smoked containers can warrant continues use of the procedure for improvement of the organoleptic profile of the milk.

Keywords: traditional practice, milk hygiene, container smoking, traditional yoghurt, milk fermentation, Borana, pastoral area, Ethiopia

1. INTRODUCTION

In developing countries adoption of good milk hygienic practices and safety measures, for example, in smallholder dairy production systems are quite minimal and not encouraging (Kumar *et al.*, 2011, 2016). As a result, in many parts of the world, food preservations including milk are largely dependent on traditional techniques and are the only available and accessible options for use by local communities (Grace, 2015). In such cases, though still not standardized and with a lot of limitations, the traditional food processing techniques can play significant positive roles in reducing microbial growth and further protecting against spoilage and associated health risks (Mensah, 2002; Dwyer and Bermudez, 2004; Pittia and Antonello, 2015). For example, a synthesis report some years back in sub-Saharan Africa on informal markets of animal source foods showed that microbial hazards are not directly translated into health risks due to various local risk mitigation strategies practiced by communities in food handling and processing (Grace and Roesel, 2014). The common traditional food preservations in various parts of the world are often dependent on simple infrastructure and include techniques such as fermentation, smoking, drying, salting and heating, used alone or in combinations (Jans *et al.*, 2016). In this regard, it is better to use existing food systems as springboard towards the successful implementation of food safety interventions (Grace, 2015) in which the local food handling and processing practices should be understood through further studies.

Microbial contamination of milk or milk products can be related to the health and hygiene of the milking animal, the environment in which the animal is housed and milked, the hygiene of the person milking the animal and further handling the milk and the procedures of cleaning and sanitizing the milking and storage containers (Getachew, 2003; FAO and IDF, 2011). Milk handlers hand hygiene and effective of cleaning/disinfection milk containers very important in keeping quality and safety of milk. Improperly cleaned and sanitized milk container can be one of the major sources of contamination in dairy production and processing (Bonfoh *et al.*, 2006). There exist standard means of cleaning milk containers involving use of good quality water and detergents of proper concentration. The normal steps cleaning and sanitizing milk contact surface including containers involve removal of visible dirt, use of alkaline detergent in warm water, rinsing in acidic solution and finally drying (Schmidt, 1997; Jones, 2009; FAO and IDF, 2011). However, smallholder or extensive livestock farming communities may not have standard dairy

utensils and access to good quality water sources intended (Gran et al., 2002; Amenu et al., 2014). In such circumstances, rural farming communities may utilize other cleaning and sanitizing strategy for traditional milking and milk storage utensils, for example by utilizing smoke of different woods. Constituting traditional dairy utensils cleaning procedures, smoking of containers by using smoldering wood of specific trees is widely practiced in many rural parts of Eastern Africa, especially in the dry areas (Gonfa et al., 2001; Wanjala et al., 2016). The perceived role of the smoking milk containers is to increase the shelf life and add a pleasing flavor and taste to the milk and milk products (Mekonnen and Lemma, 2011; Wanjala *et al.*, 2016; Amenu *et al.*, 2019).

In Ethiopia, milk is converted into different traditional products mostly through natural fermentation of whole milk. These fermented milk products are very popular and widely consumed next to raw milk throughout the country with variation in the preparation depending on localities (Gonfa et al., 2001). In most parts of Ethiopia spontaneous fermentation of raw milk is completed and the product becomes ready for consumption in less than 3 days (Gonfa et al., 2001). On the other hand, in Borana pastoral areas, naturally fermented sour milk, known as *ititu* (traditional yoghurt), is prepared by putting raw fresh milk into smoked container and keeping it for 2-3 days at warm place and by subsequent removal of the whey (the fluid part) and replenishing with fresh milk. The process to get the final ready-to-eat product can take up to 7-30 days (Amenu *et al.*, 2019).

Apart from the wide use of various plants in traditional dairy processing in many parts of eastern African countries including Ethiopia, studies validating such practices for possible integration into milk quality improvement are quite limited (Mekonnen and Lemma, 2011). A previous laboratory-based experiment in Ethiopia showed that smoking of milk storage container has been found beneficial in improving microbiological and organoleptic quality of traditionally fermented milk product kept over 36 hours period and sequentially sampled (Ashenafi, 1996). A similar laboratory-based study in Kenya also reported smoking as an effective method for disinfection of milk-handling containers and reduced bacteria load of raw milk bulked in the smoked container (Wanjala *et al.*, 2016). These two earlier studies cited here were focusing on assessing the impacts of smoking containers on microbiological quality of milk or fermented milk after storing for a short duration. On the other hands, study on the effects of container

smoking using different trees on milk fermented for long duration, like the practice in case of Borana, is not available and needs further investigation in this regard. Specifically, the effect of container smoking on the microbial load of the milk fermented over several weeks is not known.

Therefore, the present study was designed with two main objectives:

- 1) to explore the details milk container smoking focusing the detail procedure of smoking, types of plants used and the perceived roles the practice on the quality of traditional fermented milk and

- 2) To assess the effect of smoking on the TBC and milk quality by simulating of the preparation of traditional fermented whole milk.

2. LITERATURE REVIEW

2.1. Importance of Milk in Human Health

Milk is known as the most nutritious food due to its rich nutrient content. It is the most complete food product of animal origin providing more essential nutrients (protein, energy, vitamins and minerals) in significant amounts than any other single food. The nutritional value of milk as a whole is greater than the value of its individual nutrients because of its unique nutritional balance (Dror and Allen, 2011; de la Fuente and Juárez, 2015). In addition, it contains immunoglobulin, which protects the newly born against a number of diseases. Milk and milk products have an immune enhancing property as well, particularly for the benefit of immune suppressed people. In addition, milk contains various properties, which make it easy to convert into different milk products or to use it as an ingredient for other food items. Various human cultures have their own traditional ways of using milk and preparing different milk products (Getachew, 2003).

2.2. Microbiological quality and safety of milk

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production, it is impossible to avoid contamination of milk with microorganisms. Therefore, the microbial content of milk is a major feature in determining its quality (Food Standards Australia New Zealand, 2009). The safety of raw milk like any other food is of a worldwide concern where various efforts are directed, since it is associated with food-borne diseases. Despite milk and milk products are considered as a complete diet in human nutrition, ensuring high hygienic quality of the products is very critical, as milk is a suitable substrate for microbial growth and development because of its fluid and semi-fluid nature and chemical composition (Oliver et al., 2009).

Hence, for fulfilling consumer's demand, quality milk production is necessary. Quality milk means, the milk which is free from pathogenic bacteria and harmful toxic substances, free from sediment and extraneous substances, of good flavor, with normal composition, adequate in keeping quality and low in bacterial counts (Valeeva and Huirne, 2008).

2.3. Fermentation of milk and its significance

Fermented milk was normally produced by inoculating fresh milk with part of previous fermented batch. This traditional method is still used in some part of societies. Fermented milk is common all over Africa for example, sethemi is a South African fermented milk prepared by naturally fermenting milk (Kebede et al., 2007). Kule naoto is traditionally fermented milk by the Maasai of Kenya (Patrignani et al., 2006; Mathara et al., 2008). It is spontaneously fermented from raw milk in traditionally prepared calabashes. The calabashes are first treated with burnt smoky twigs of a traditional plant. The milk is further pre-treated by adding fresh cow's blood, before initiating the fermentation process. *Lactococcus* and *Lactobacillus* spp were the main isolated bacteria from the fermented product. Suusac, is another Kenyan traditional fermented camel milk product (Mathara et al., 2008; Elhadi et al., 2015). This is usually prepared and consumed by the Somali people in the semi-arid areas of northern Kenya. It is prepared by spontaneously fermenting unboil milk in calabashes treated with smoky, burnt traditional plant twigs. Amasi, is a traditional fermented milk consumed both in South Africa and Zimbabwe (Gadaga et al., 1999; Mufandaedza et al., 2006).

Current industrial dairy production process used modern techniques of milk fermentation. In such dairy industries, starter cultures with known characteristics are widely used. . Uses of modern techniques have advantages over the traditional methods for the production of consistent fermented milk products with best qualities. Fermented food products play a significant socio-economic role in the developing world. The importance of traditional fermented foods has been reviewed. These products also contribute to the protein requirements of the indigenous consumers. Lowering the pH of food products through fermentation is a form of food preservation. This is a self-limiting in process in that further reduction of pH may be inimical to organisms. As a result, the pH normally stays just below five. Other benefits of fermentation include improvement of food quality through food digestibility to increase essential amino acids, vitamins and protein (Sahlin, 1999).

Fermentation is also known to soften food texture and alter its composition in such a way that it will require minimal energy both in cooking and preservation process. Thus, less fuel will be used for cooking and eliminates the need of preservation as fermentation increases the shelf life

of food. These advantages make fermentation a highly desirable technique in rural communities of the third world where resources for cooking and preservation are scarce (Chelule et al., 2010).

2.4. Traditional fermented dairy products in Ethiopia

These fermented products have different vernacular names such as ititu, ergo, meomata or geinto among the Oromo, Amhara, Wolayta or Sidama people, respectively. The fermentation process is usually carried out using natural wild microorganisms, without using defined starter cultures to initiate fermentation process. In most cases, fermentation of dairy products carried out through the propagation of the initial microorganism, with serious of microbial succession governed by chemical changes and ambient temperatures in the fermenting milk. Traditional dairy fermentation process is conducted with no attempt to control the fermentation processes. In this case, fresh milk is left either at room temperature or kept in a moderate warmer place to facilitate fermentation process. In rural areas, especially among the pastoralists, raw milk is mostly kept in properly smoked container and fermented milk from a previous fermentation uses as source of inoculums. Lactic acid bacteria (**LAB**) from the inner walls of the container also become established and serve as starter culture. Incubation temperature does have significant role in the lowlands and the quality and taste of the fermented product may be more or less uniform. The fermented dairy product may also be served as raw material for the production of traditional butter (qibe) and butter milk (arrera). The butter milk can be further be processed into Ayib or traditional cottage cheese and whey (aguat) (Ashenafi, 2011).

2.5. Yoghurt

Yogurt is a widely consumed as functional food due to its good taste and nutritional properties (rich in potassium, calcium, protein and vitamin B) and excellent vehicle to deliver probiotics to consumers (Reid et al., 2003). Regular consumption of yogurt is thought to be beneficial in the strengthening of the immune system, improvement in lactose digestion, blood glucose management (Yadav et al., 2007) and the reduction of constipation, diarrhea, colon cancer, inflammatory bowel disease and allergies (Adolfsson et al., 2004). .The beneficial health effects of yogurt have partly linked to the proteolysis products, produced during fermentation and storage in particular, a group of peptides can lower the blood pressure in hypertensive patients (Caplice and Fitzgerald, 1999; FitzGerald et al., 2018)

Yoghurt is produced by the controlled fermentation of milk by lactic acid producing bacteria. Two species are commonly used in the commercial production, which are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These two species of bacteria have now been established as the yoghurt starter cultures. Any sort of milk may be used to make yoghurt, but modern production is dominated by cow milk (Quigley et al., 2013).

Yoghurt is made by inoculating certain bacteria (starter culture), usually *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, into milk. After inoculation, the milk is incubated at approximately $110^{\circ}\text{F} \pm 5^{\circ}\text{F}$ until firm; the milk is coagulated by bacteria-produced lactic acid (Jones, 2009) (Heaton and Jones, 2008). The presence of coliforms in these yoghurt brands is of serious public concern because of its health implication on the consumers of these brands of yoghurts (Mbaeyi-Nwaoha and Egbuche, 2012). It had reported based on the standard stipulated by the National Agency of Food and Drug Administration Control (NAFDAC) that *E. coli* and coliforms generally must not be detectable in any 100 ml of yoghurt sample. Yoghurt contains all the protein, fat, calcium and vitamins of the original milk, but contain a higher percentage of lactic acid than other fermented milk and it is rich in vitamin B complex. The high water activity of milk, moderate pH and ample supply of nutrients make it an excellent medium for microbial growth (Swai and Schoonman, 2011).

2.6. Milk handling practices

Hygienic handling practice of the milk with respect to quality has received a great concern around the world. This is especially true in developing countries where production of milk and various milk products usually takes place under unsanitary conditions and poor production practices. It was also reported that dairy production has a great contribution in improving human nutrition, particularly women and children (Ahmed and Ehui, 2004). Production of milk for consumers requires good hygienic practices such as clean milking utensils, washing milker's hands, washing the udder and use of individual towels during milking and handling, before delivery to consumers or processors (Getachew, 2003). The unsafe handling practice results in the higher bacterial count, which in turn may cause spoilage of the milk and poor yields of its products (Oliver et al., 2005).

Hygienic milk production and handling practice are important in order to deliver safe milk to the consumers. Thus, the basic considerations during milk handling practices are: hygienic milking practices, cleanliness of milking and processing utensils, condition of storages, and manner of transport.

3. MATERIALS AND METHODS

The present study has two parts: (1) qualitative assessment of the practices and roles of container smoking on the quality and safety of milk or milk products, and (2) a laboratory-based investigation of the effects of container smoking on the microbial load, pH and TA of traditional fermented milk, traditional yoghurt (*ititu*).

3.1. Qualitative investigation of the practices and perceived role of container smoking in milk quality and safety

This was the first part of the present study carried out in four village administrations of the Yabello district of Borana Zone, Oromia Regional State in southern Ethiopia, as part of a research project aimed assessing milk handling and processing practices, milk consumption behavior, and microbiological assessments of ready-to-consume milk or milk products and associated health risks. Details of the description of the study area and methodological approaches had been presented elsewhere (Amenu et al., 2019). Generally, the climatic condition of Borana pastoral area is semi-arid to arid with a bimodal rainfall distribution (the long rainy season extending from March to May and the short rainy season from September to November). Four village administrations in Yabello district (Dharito, Elweya, Surupha and Did Yabello) were included in the current study. The field data collection was carried out in July 2015 using mix of qualitative methods which include in-depth interviews, focus group discussions and observations. The main topics covered in the qualitative study were very similar in both interviews and focus group discussion and included: (1) types of milking and milk storage containers, (2) details on smoking processes of the containers, (3) plants used for smoking and (3) perceived role of smoking with regard to quality and safety of milk or milk products. A total of 40 in-depth interviews (involving 10 women in each village) were carried out using a pre-tested questions guide. The women involved in the in-depth interviews were identified by driving in the villages and stopping at a cluster of households locally called *olla* and 1-2 women were interviewed from there. Follow up of the interviews, 6-8 women in each of the four villages were selected with the assistance of pastoral development agents with which focus group discussion was held. In addition to the interviews and discussions, observations were made by visiting different informal raw milk and dairy products open markets in which informal discussions with

sellers were carried out. The interviews and discussions were documented using field note and audio recording which later transcribed verbatim by translating into English.

For the qualitative data analysis, the topics covered in the discussions and the interviews were used in identifying themes. Further, the themes were coded using free software QDA Miner Lite v1.4.3, Provalis Research (Péladeau, 2014) and the thematic analysis made.

3.2. Effect of container smoking on the microbiological load of traditional yoghurt (fermented milk curd)

A laboratory-based experiment was carried out to assess the effect of milk container smoking using different trees on the microbial load of fermented milk prepared in the containers following a similar procedure identified by the qualitative investigation presented in the previous section of this paper. The experiment was carried out in the Veterinary Public Health Lab of the College of Veterinary Medicine and Agriculture of Addis Ababa University (CVMA-AAU), situated in Bishoftu town of Oromia Regional State some 45 km southeast of Addis Ababa. Fresh cow milk used for the preparation of the traditional yoghurt was obtained from milk collection centers in the town. Two types of containers (traditional and stainless steel) were used for the preparation of yoghurt. Five traditional containers called locally *gorfa* already in use were purchased from Borana pastoral women and brought to Bishoftu after which smoked before use. The stainless steel containers (n=6) with a capacity of 3 liters were purchased from local market in Addis Ababa and smoked similar to the traditional container. The woods of used for smoking were collected from the bushes of the different villages of Borana with the help of local pastoralists. The trees considered in the experiment were those commonly utilized for the smoking in the area and identified during the qualitative investigation described in the previous section.

The three plant species used for the experiment included: *ejersa* (*Olea europaea subsp. cuspidata*), *daanse* (*Faurea speciosa*) and *birreessa* (*Terminalia brownie*). The traditional yoghurt was prepared by using containers sanitized the following procedures:

- (1) stainless steel container sterilized in autoclave (S1)
- (2) stainless container washed with tap water (S2)

(3) traditional containers smoked by putting burning wood chips inside the container and swirling for several times, and repeating this for several time (three treatments corresponding to the trees used for smoking) (T1 to 3)

(4) stainless steel containers smoked following same procedure with that of traditional containers (similarly three treatments) (S3 to S5).

The containers were smoked by simulating the local practice identified during the qualitative investigation described in the first part of this paper. Raw milk used for the preparation of the traditional yoghurt was obtained from the local milk producers of Bishoftu town sold at the level of milk collection centers. Each of the containers was sanitized by simulating the cleaning procedure practiced by Borana pastoralists and described in the previous section in this paper.

The cleaning was done before noon on the days of starting the experiment. Raw milk was purchased from the collection centers and transported to laboratory where thoroughly mixed and a liter of the milk distributed to each container and allowed to ferment by keeping at room temperature for about 24 hours. After that the whey part was removed by pouring slowly and carefully and sample of the fermented milk was collected from each of the container for the determination of bacterial load, pH and TA. After the sample collection, raw milk was added to it and kept for another 24 hours and same procedures carried (i.e., whey removed, specimens collected and replenished with raw milk). Lastly, new raw milk was added to the fermented milk and kept again for the final 24 hours and same procedures carried out. Raw milk added on the first and subsequent days was also determined for bacterial load, pH and TA similar to the fermented milk.

Standard protocol (ISO 4833-2, 2013) was followed for the determination total aerobic bacteria count (TBC) of raw fresh milk or fermented milk curd by using pour plate method on plate count agar (PCA) (Oxoid, UK). The samples were diluted (ten-fold up to nine dilutions) using sterile saline solution in a tube (i.e. 1 ml of well mixed sample was transferred from the specimen into 9 ml of diluents and repeated till the last tube). After that, 1 ml of the diluted sample from each level of dilutions was transferred to a sterile Petri dish and about 12 ml to 15 ml molten PCA kept at 44 °C to 47 °C poured into and carefully mixed by rotating the Petri dishes and allowed the mixture to solidify by keeping on a horizontal table surface. After that the plates were

incubated for 24 hours in an incubator at 30 °C and if possible to get, the plates having about 15-300 colony forming units (CFU) were selected and counted. However, in some cases when the number of bacteria counts was more than about 300 CFU, from a half or a quarter the Petri dishes was counted and extrapolated. To get the final result, the count was multiplied by reciprocal of the dilution factors to get the number of CFU per ml of milk or the traditional yoghurt. In addition to the bacterial load, pH and titratable acidity of the milk and yoghurt were determined. The pH was determined using digital pH meter. The titratable acidity was determined by adding continuously 0.1N NaOH solution into 10 ml of milk or yogurt and monitored with phenolphthalein indicator until faint pink color developed showing end of the titration (Fabro et al., 2010).

3.3. Data management and analysis

All the data generated through lab experiment were stored in Microsoft Excel and finally imported to Stata version 12 for analysis. The TBC of raw milk or fermented milk was converted to logarithm and mean and standard deviation calculated. One-way analysis of variance (ANOVA) was used to compare the mean of TBC logarithmic values, pH and TA of the fermented milk prepared in containers sanitized by different procedures. Pair-wise of comparison the different mean values was made using Tukey's adjustment method.

4. RESULTS

4.1. Qualitative investigation on perceived roles of the smoking of milk containers

4.1.1. Traditional milk containers in Borana

Traditional milk containers made from diverse materials were used by the pastoralists for milking, milk storage, processing and transport. Mostly the use of the containers for milking was specific to the types of livestock species. In this respect, *okolee*, a bucket made from fresh skin of giraffe or thick strong part of cattle skin (e.g. skin on back) was used for milking cows. *Okolee* had different sizes and the pastoralists consider the container a culturally important item milking utensil. Culturally milk from cows only milked into *okolee* and use of other container for milking cows not welcomed by the pastoralists and the practice considered something out of culture. *Kokii*, a small metallic cup of about 200 ml capacity, was widely mentioned to be used for milking goats. The container used for camel milking was *dhammeela* or *welkii* (deep carved wooden bowl). Unlike strict cultural preference of *okolee* for cows, any wide-mouth container (e.g. plastic container from which commercial vegetable butter used and termed *ananas* by the pastoralists) can be used for milking camels. From the description of the pastoral women, *qodaa* refers to any lidded traditional container used for storage and transport of milk and milk products. The commonly stated traditional containers used for milk storage were *gorfa* and *cicoo*, lidded containers made by weaving plant fibers and both having pear shaped. The only difference was *gorfa* decorated with cowries and mostly used to store milk for natural fermentation to take place at home without ritual function. *Cicoo* commonly used for carrying milk to ritual places and it was also mentioned that it is used to carry milk to market for sale. Container made from gourd was also used to carry milk to market. Our investigation indicated that different containers which were in earlier times perceived to be non-cultural by the pastoralists are nowadays being utilized for milk storage and transport. For example, it was observed that plastic jerry-can was one of the most common containers to carry milk to market places. In addition, used plastic water bottles and stainless steel containers were also used by milk buyers on market. The main reason for resorting to jerry-can was ease of handling when taking milk to market place as revealed in the following quotes.

“In the past qodaa [locally made container] was best. Nowadays, jerry-can is also good, when you wash and fumigate. Ititu is also prepared in jerry-can. Both are okay, it is up to the individual to keep the hygiene of the containers. Jerry-can is convenient to carry. You can put 5 or 6 jerry-cans in a sack and easily carry to market. Traditional containers cannot be carried like that. You can only carry one or two on your back (FGD 1)”.

“Jerry-can can be kept by individual. It can be equivalent with gorfa. What reduces hygiene is due to lack of smoking. Qodaa is not easy to carry. Qodaa is convenient when women prepare milk at home. Jerry-can is convenient to transport” (FGD 1)”.

4.1.2. Milk containers cleaning and sanitation in Borana

In Borana milk containers cleaning and purification procedure, *qoraasuu*, involved smoking of the containers by using the burning branches of different plants. The procedure had different steps and varied based on the types of containers. For *okolee*, first the container washed with water and then continuously rubbed with one end of freshly burning pieces of wood several times with handling the other end of the wood. When the burning wood dies due to vigorous rubbing with the internal surface of milk container, it was put back again into fire and the rubbing continue with freshly burning wood again. After such vigorous rubbing for about 4-5 minutes, the fine powdered wood charcoal removed from the inside of the container by cleansing the inner surface with, *ossoo*, a piece of textile or hair from tail-end of cattle. Some respondents mentioned that the container is cleansed with water after the rubbing. Finally, the container is fumigated with the same or different burning wood by inverting the container and putting the smoking wood under it for some minutes. Observation of the overall smoking process showed that proper hygienic procedures were not followed with potential recontamination after cleaning. For example, when a volunteering pastoral woman was demonstrating the overall process of cleaning, it was observed that after smoking of the container and cleansing with water she inverted the milk container on soil and then the rim of the container found apparently soiled.

Cleaning of *gorfa* or *cicoo* carried out following similar cleansing and smoking procedure but without aggressive rubbing. First, the container cleansed with water and pieces of the burning aromatic wood added into *gorfa/cicoo* and then lid closed. After putting the burning wood, the

container turned rhythmically back and forth until the burning wood completely extinguished. After that the wood was taken from the container and put back into the fire. In next round, the freshly burning wood put back into the container and same rhythmic movement performed. The whole smoking procedure was repeated several times. Finally, the inside wall of the container cleansed with *sossoo*. Narrow-mouthed containers such as plastic jerry-can was fumigated after washing with water and by inserting thin piece of freshly burning wood into the container and waiting until the burning wood extinguishes and this done several times.

4.1.3. Plants used for milk container smoking

The result of the qualitative investigation showed that most commonly mentioned plants used for the smoking and fumigation of milk containers were: ejersa (*Olea europaea subsp. cuspidata*), birreessa (*Terminalia brownie*), daanse (*Faurea speciosa*), Baddana okolee (*Balanites aegyptiaca*), rukeessa (*Combretum molle*) and xaaxessaa (*Premna resinosa*). In some cases, the following plants in local names were mentioned which include: Burquqee (red tree), *iddii*, *dabaqqa*, *karroo*, *gadda*, *dhumayya* and *fakkantee*.

Some of the plants were used for smoking specific milk containers. For example, *baddana okolee* was only used for smoking *okolee* (milking container). In terms of availability of the trees high variation was reported among the different villages. As reported during the group discussions, *baddana okolee* was found to be available compared with other trees. The following quotes describe the differences in the availability of different trees.

“It [baddana okolee] is available everywhere. The whole hilly area is the tree. For not easily available [trees], you collect from far place and keep at home using only for smoking of [milk] utensils. Not used for cooking fire and kept away from children and only used for [milk container] smoking (FGD 4)”.

“The trees are not uniformly distributed in different villages. All of the trees are not found in every village. You use what is available in your surroundings (FGD 2)”.

4.1.4. Roles of container smoking

One of the main reasons of smoking milk containers was imparting good aroma and health benefits. It was emphasized that all containers having contact with milk should be smoked in order to get maximal benefits. The participants of the study indicated that when container is not properly smoked, the milk or milk products will have extreme sour taste and bad aroma.

“It [smoking] makes milk good. If not smoked, the milk will have bad aroma. If you add milk after smoking, it has good aroma (IDI 8)”.

“It makes the food [milk products] good. If the pot [you use for preparation of food] is not well cleaned, the food cannot be good. [The same is true], if okolee [container used for milking] is not smoked, the milk cannot be good. If you smoke okolee but not qoda [milk storage container], it has no value [with regard to milk quality] (IDI 7)”.

“Milk becomes extremely sour when there is no smoking of okolee (IDI 11)”.

The other reason mentioned was increased shelf life of milk and milk products with good consistency when stored in smoked containers as indicated in below quotes.

“If [the container] is not smoked, [the fermented milk] will have extreme sour taste even within one week. If the container is not smoked, the milk shows spoilage with appearance of precipitation (FGD 4)”.

“If you smoke the container well, you can accumulate [milk to ferment] for one month and even two months (IDI 11)”.

4.2. Experimental effects of container smoking on the microbial load, pH and titratable acidity of fermented milk

Quality parameters (TBC, pH and TA) considered in the present study for the assessment of the fermented milk did not show statistically significant difference among the different milk container types (traditional versus stainless steel) and the sanitizing procedures. Lack of differences in the quality parameters was found across the days of fermentation (Tables 1-3)

Table 1: Effects of various containers sanitation procedure on TBC (logCFU/ml), pH and titratable acidity (TA) of fermented milk on third day of fermentation (number of replicates=4)

Containers	Container sanitizing regime	TBC (logCFU/ml)		pH		TA	
		Mean	Sd	Mean	Sd	Mean	sd
Stainless steel							
S1	Sterilized in autoclave	8.62	1.40	4.74	0.30	0.22	0.04
S2	Washed with tap water	8.84	1.40	4.79	0.39	0.20	0.03
S3	Smoked with ejersa	9.91	0.97	4.88	0.19	0.21	0.04
S4	Smoked with birressa	8.69	2.00	4.82	0.23	0.19	0.05
S5	Smoked with daanse	9.22	2.01	4.72	0.23	0.22	0.06
	Overall stainless steel container	9.05	1.51	4.79	0.26	0.21	0.04
Traditional container							
T1	Smoked with <i>ejersa</i>	9.38	1.14	4.89	0.23	0.27	0.07
T2	Smoked with <i>birressa</i>	9.60	1.03	4.87	0.27	0.25	0.05
T3	Smoked with <i>daanse</i>	8.47	1.95	4.77	0.20	0.27	0.05
	Overall traditional container	9.15	1.40	4.84	0.22	0.26	0.05

Note: Mean values for the three quality parameters of the fermented milk product were not different among the different container sanitizing regimes

Table 2: Effects of various containers sanitation procedure on TBC (logCFU/ml), pH and titratable acidity (TA) of fermented milk on fifth day fermentation (number of replicates=4)

Containers	Container sanitizing regime	TBC (logCFU/ml)		pH		TA	
		Mean	Sd	Mean	Sd	Mean	Sd
Stainless steel							
S1	Sterilized in autoclave	9.19	1.71	4.83	0.01	0.21	0.03
S2	Washed with tap water	8.71	1.35	4.86	0.02	0.22	0.02
S3	Smoked with <i>ejersa</i>	10.36	0.96	4.77	0.09	0.24	0.05
S4	Smoked with <i>birressa</i>	8.27	1.63	4.79	0.18	0.20	0.26
S5	Smoked with <i>daanse</i>	8.96	1.51	4.69	0.14	0.25	0.07
	Overall stainless steel container	9.10	1.48	4.78	0.12	0.23	0.04
Traditional container							
T1	Smoked with <i>ejersa</i>	9.51	1.18	4.76	0.13	0.29	0.09
T2	Smoked with <i>birressa</i>	9.75	1.10	4.80	0.23	0.25	0.05
T3	Smoked with <i>daanse</i>	10.44	0.80	4.60	0.11	0.27	0.05
	Overall traditional container	9.90	1.03	4.72	0.17	0.27	0.06

Note: Mean values for the three quality parameters of the fermented milk product were not different among the different container sanitizing regimes

Table 3: Effects of various containers sanitation procedure on TBC (logCFU/ml), pH and titratable acidity (TA) of fermented milk on seventh (final) day of fermentation (number of replicates=4)

Containers	Container sanitizing regime	TBC (logCFU/ml)		pH		TA	
		Mean	sd	Mean	sd	Mean	sd
Stainless steel	Sterilized in autoclave	8.572	1.03	4.813	0.04	0.24	0.04
	Washed with tap water	10.02	1.89	4.82	0.02	0.24	0.05
	Smoked with <i>ejersa</i>	8.693	1.12	4.763	0.15	0.30	0.07
	Smoked with <i>birressa</i>	9.073	2.35	4.743	0.18	0.26	0.06
	Smoked with <i>daanse</i>	7.896	0.79	4.673	0.22	0.30	0.05
	Overall stainless steel container	8.85	1.55	4.76	0.14	0.27	0.06
Traditional container							
	Smoked with <i>ejersa</i>	8.89	2.24	4.66	0.15	0.29	0.09
	Smoked with <i>birressa</i>	8.96	1.69	4.72	0.21	0.28	0.06
	Smoked with <i>daanse</i>	9.14	1.51	4.57	0.10	0.30	0.06
	Overall traditional container	9.00	1.67	4.65	0.16	0.29	0.07

Note: Mean values for the three quality parameters of the fermented milk product were not different among the different container sanitizing regimes

Comparison of the mean value of TBC and TA of the fermented milk product with the raw milk samples showed significant increment. On the other hand, the mean value of pH of the fermented milk significantly decreased compared to the raw milk. TA of the seventh day fermented milk was significantly higher than the the third day fermented product (Table 4).

Table 4: Effects of the duration of fermentation on TBC, pH and titratable acidity (TA) of the fermented milk

Product	TBC (logCFU/ml)		pH		TA	
	Mean	Sd	Mean	Sd	Mean	sd
Raw milk	7.59 ^a	1.82	6.68 ^a	0.13	0.12 ^a	0.03
Fermented milk on third day	9.09 ^b	1.45	4.81 ^b	0.24	0.23 ^b	0.05
Fermented milk on fifth day	9.40 ^b	1.37	4.76 ^b	0.14	0.25 ^{bc}	0.05
Fermented milk on seventh day	8.91 ^b	1.57	4.72 ^b	0.15	0.27 ^c	0.06

Note: mean values with same superscript letter in the same column were not statistically significant from each other

5. DISCUSSION

This study used experimental research methods to assess the effect of smoking on TBC (logCFU/ml) count, pH and TA of traditional fermented whole milk. In addition, qualitative assessment method has been done. The result of the qualitative study shows that various traditional milk containers made from diverse materials with different shape and sizes were used by Borana pastoralists, which similar finding with Tollossa et al. (2014) that stated in Borana pastoralist community, fresh milk is stored in, gorfa ,okolee ,cicuu, aluminum cans, and plastic jerry-cans. According to the assessment by the authors, which are smoked for preservation except the equipment's made up of plastics to enhance, taste and odour of the product and to disinfects the vessels. On the other hand, it was stated that the traditional containers are very difficult to clean and keep clean. The milk containers cleaning and sanitation in Borana have its own procedure. The smoking procedure as described and demonstrated by the women had different steps and varied according to the types of the milk containers. For bucket-shaped container, the inner surface of the container was vigorously rubbed with burning pieces of wood for about 3-5 minutes. Thereafter, remnants of wood charcoal removed from the inside of the container pieces of textile or hair from the tail end of cattle. The bucket-shaped container was also fumigated by inverting the container and putting the smoking wood under it for some minutes either separately or in combination with earlier rubbing of the inner wall. In case of lidded container, the freshly burning aromatic wood is put in the container and the lid closed after which the container moved rhythmically back and forth until the burning wood completely extinguished. The same wood is taken out and put on fire and again the burning wood taken back to the container. The smoking procedure (putting and taking out the burning wood) is repeated several times and finally, the inside wall of the container is cleansed with piece of cloth.

According to the investigation showed that most commonly mentioned plants used for the smoking and fumigation of milk containers were: ejersa (*Olea europaea* subsp. *cuspidata*), birreessa (*Terminalia brownie*), daanse (*Faurea speciosa*), Baddana okolee (*Balanites aegyptiaca*), rukeessa (*Combretum molle*) and xaaxessaa (*Premna resinosa*) which is as similar reported by plants species that are frequently used for smoking milk vessels in Borana zone are *Balanites aegyptica* (Badena okole), *Terminalia brownii* (Biressa), *Olea africana* (Ejersa), *Premna resinosa* (Tetessa) and *Combretum mole* (Rukesa).

Experimental effects of container smoking on the microbial load, pH and titratable acidity of fermented milk were performed at laboratory. According to the result the TBC, pH and TA of the fermented milk did not show statistically significant difference among the different milk container types and the sanitizing procedures.

Comparison of TBC of the fermented milk product with the raw milk samples showed significant increment. Total bacterial count (TBC) is an indicator of the general hygienic condition during milk production, transportation and storage, and ultimately quality. It is used to measure total bacterial numbers (Franciosi et al., 2009). The TBC counts of 7.59 to 9.40 log₁₀ cfu/ml (Table 4). This amount of colony count at the food grade level of bacterial count (< 4.3 log₁₀ cfu/ml) is not satisfactory for consumption and processing (Elmoslemany et al., 2009).

On the other hand, the mean value of pH decreased of the fermented milk significantly compared to the raw milk. PH test measures total acidity of milk and used for screening the milk quality. All the samples analyzed had a pH value between 6.68 and 4.72, and were significantly ($p \leq 0.05$) different from each other (Table 4). As bacterial count of milk increases, its quality deteriorates, i.e., its pH will fall (Tamime, 2009). Lower pH mean values of fermented milk on seventh day (4.72) are obtained compared to raw milk (6.68). As milk begins to sour, its pH levels fall sharply making it even more acidic. Lactobacilli bacteria convert the sugars in milk into acids, thereby reducing the pH of milk. Completely sour milk has a pH of about 4.4 (Goff, 2010). Tamime (2009) also stated that fresh cow milk had a pH value between 6.4 and 6.8 and is therefore, slightly acidic.

TA or percentage lactic acid test determines lactic acid content. It is also used for screening and to monitor storage conditions, accordingly determines suitability of milk for processing (FAO and IDF, 2011). The present study showed that treatable acidity of milk was also affected by duration of fermentation .TA of the seventh day-fermented milk was significantly higher than the third day fermented product (Table 4). Moreover, the overall percentage lactic acid was in the range of 0.12-0.27.

6. CONCLUSION AND RECOMMENDATIONS

The present study assessed the traditional practices of sanitizing milk containers using smoke of different trees by utilizing different qualitative research methods (in-depth interviews and focus group discussion). Following the qualitative survey, the effects of the smoke on the quality of traditional fermented milk was assessed based on laboratory experiment. The qualitative study revealed detail procedures of smoking milk container in Borana pastoral area of southern Ethiopia. Utensils made from different materials were used for milking, storage or transport after smoking the containers with different plants. The pastoralists had strong preference for milk or milk products stored in the smoked container by stating the good aroma the smoked imparts on food and health benefits of it. Our study shows, in contrast to similar previous studies, smoking cannot be a guarantee for the microbiological quality and safety, studies towards controlling the natural fermentation. Smoking cannot be a sustainable option for food safety given that availability of the plants is also not sustainable. Works towards hygienic production and consumption practices should be promoted instead of relying on the smoking containers as food safety procedure especially when milk is fermented for long period of time. Further work should focus on how to modify microbiota-fermenting milk either based on local bacterial strains or commercial starter cultures.

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ANNEXES

Annex: I: Pictorial representation of the study (experiments and field investigation)



Wood used for smoking: *danse* (*Faurea speciosa*)



Wood used for smoking: *ejersa* (*Olea europaea* subsp. *cuspidata*)



Wood used for smoking: *Biressa* (*Terminalia brownii*)



Wood collection in Borana



Used traditional container purchased from Borana



Smoking of milk containers (field investigation)



In-depth interviews with women (fieldwork)



Focus group discussion



In-depth interviews



Cleaning milk container by simulating traditional practices



Laboratory prepared traditional yoghurt (ititu)