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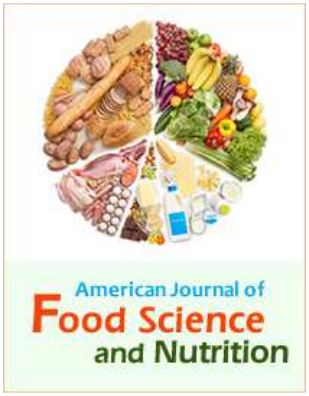

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Evaluation of physico-chemical, microbial and organoleptic properties of cow milk in smoked container with olive (*Olea africana*) and added leaves of Koseret (*Lippia adoensis*)

Tadewos Hadero, Abadi Gebre Mezgebe*, Henok Kurabachew

Hawassa University, College of Agriculture, School of Nutrition, Food Science and Technology, P.O.Box: 05, Hawassa, Ethiopia

Email address
abadigbr46@gmail.com (A. G. Mezgebe), abadig@hu.edu.et (A. G. Mezgebe)

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Abstract
Milk is perishable product which needs special attention in handling and storage. Milk handling container smoking and addition of leaves are common practices. The effects of the practices on the properties of raw cow milk are not evaluated. Hence, the aim of this study was to evaluate the effect of smoking milk handing pots with *Olea africana* and addition of leaves of *Lippia adoensis* on physico-chemical, microbial and sensory properties of milk. Milk samples were collected and treated in laboratory. The experiment had four treatments; milk sample kept in neither smoked pot nor leaves added(T_1), milk sample kept in smoked pot(T_2), milk sample with added leaves(T_3), milk sample with added leaves in smoked pot(T_4). Physico-chemical properties (pH, titratable acidity, alcohol and specific gravity), microbial properties (standard plate count, total coliform count and methylene blue reduction tests) and sensory properties (flavor, mouth feel, aroma, appearance and overall acceptability) were determined. Results indicated that the treatments significantly affected the properties of the milk. Highest pH and specific gravity was recorded for T_2(6.80±0.01) and (1.0324±0.00), respectively. Maximum plate and coliform count was recorded for T_1(2.47 and 0.54 log10 cfu/ml), respectively. Longest methylen blued reduction time was for T_3(7:15 hours). Better score of mouth feel, aroma and overall acceptability was for T_3(4.20±0.71), T_2 (3.90±0.76) and T_2(4.20±0.71), respectively. Thus, the use of *Olea africana* and *Lippia adoensis* improved the physico-chemical, microbial and sensory properties of the milk to some extent.

1. Introduction

Milk is an ideal medium for most of microorganisms specifically bacteria, and this is the reason why milk spoils quickly. Fresh and clean milk is excellent and healthy food stuff; however, it is dangerous for the consumer if it is contaminated. Since milk is perishable commodity with high water content, its handling demands special consideration to ensure safety and acceptability for

consumption. If the hygienic standard of production, handling, processing, storage and transportation is poor, quality of milk and its products will also be poor (Mennane *et al*., 2007). As indicated by Omar *et al*. (2012) in recent years, natural antimicrobial compounds, especially those extracted from plants has been used for the preservation of milk and milk products to increase shelf life and improve organoleptic property. Many investigators used natural flavoring additives in dairy products such as black cumin, cardamom and fenugreek. Smoking is commonly applied to give pleasant flavor and aroma to the milk and milk products (Lemma *et al*., 2005); moreover, smoking can retard the growth of different microorganisms. Similarly, different researchers' report shows that milk and milk products handling equipments are usually smoked using wood splinters of olive (*Olea africana)* to bring desirable aroma to the dairy products. Smoking was also found to lower the microbial load of raw milk (Almaz *et al*., 2001).

As reported by Lemma *et al*. (2005), Alganesh *et al*. (2007), Rahel (2008), Adebabay (2009), Abebe (2011), Asrat (2011) and Kassahun (2013) some plant species commonly used in different parts of Ethiopia such as East Shoa, East Wollega, Wolaita, Awi, Gurage, Boditi and Ada'a, respectively, includes Olive (*Olea africana)*, Eucalyptus *(Eucalyptus globulus),* Koseret (*Lippia adoensis),* Rue *(Ruta chalepensis),* Basil *(Ocimum basilicum),* Thyme (*Thymus vulgaris)* and others. Haile *et al*. (2012) reported that communities practices of cleaning and smoking of dairy products handling equipment with different plants to add flavor and/ or to increase the shelf life of products with different plants.

All the mentioned researchers assessed the community's traditions and practices of cleaning and smoking milk handling utensils with different plant species and adding different plant species into raw milk and milk products. However, none of these studies conducted controlled laboratory experiment by treating according to communities traditional practices. Therefore, the purpose of this study was to examine the effect of communities traditional practices of raw milk handling on physico-chemical, microbial and sensory properties in order to develop information.

Definitions of Terms Used. (a) Koseret/ Lippia adoensis Hochst. Ex Walp**:** make up the family *Verbenaceae* and the representative genus is *Lippia.* Leaves aromatic, covered with soft hairs. It is commonly used to clean milking pots and milk handling containers and also for flavoring milk and butter (Reinhard and Admasu, 1994). (b) *Olive/ Olea europaea* var. Africana: make up the family *Oleaceae* and the representative genus is *Olea.* Indigenous *Oleaceae* African wild olive, widely distributed in dry forest and forest margins, often with *Juniperus procera*, in East Africa and Ethiopia. It is commonly used to milk flavoring (smoking wood), tooth brushes (twigs), and so on (Azene *et al*., 1993). (c) Phytonutrients or phytochemicals: are naturally occurring "plant-based" nutrients or chemical compounds. It is a class of nutrient that is thought to have health-protecting properties (Deutsch-Mozian, 2003).

2. Materials and Methods

2.1. Sample Collection and Preparation

Before milking, the udder of the cow and the surroundings were washed with tap water and detergent then dried with towel. The hands of personnel were disinfected with 75% alcohol solution and then washed with tap water and detergent then dried with towel. This is used to keep cows and personnels cleanness, *i.e*., to prevent unhygienic conditions which contributes to suppress the effect of treatments. The total of 5 liter raw cow milk samples were collected during morning lactation period directly from the udder of the cows using screw-cap sterilized sampling bottles from local dairy farmers, Hawassa, Ethiopia. Hawassa is geographically located with latitude and longitude of 07° 03' N and 38° 28' E, respectively and an elevation of 1750 masl. The milk sample was kept in icebox at a temperature between 0°C and 4°C (to control growth of microorganisms and some chemical deterioration by cooling during transportation) and it was transported to the laboratory after milking within 30 minutes.

2.2. Experimental Set-up

The whole experimental work was conducted at the laboratory of School of Nutrition, Food Science and Technology, College of Agriculture, Hawassa University. The collected raw milk was mixed thoroughly to have representative sample. Small and equal size of 4 Earthenware, chips of *Olea africana* and leaves of *Lippia adoensis* were obtained from the local market (Hawassa, Ethiopia). All pots were cleaned and filled up with tap water, then kept for 72 hours at room temperature to be aged. After discarding tap water, the pots were dried at room temperature for 12 hours in order to reduce the moisture content (Indigenous method). From the dried pots, 2 of the pots, *i.e*., pot 1 and 2 were washed with only boiled tap water, while the rest 2 pots, *i.e*., pot 3 and 4 were washed with both boiled tap water and leaves of *Lippia adoensis*, and dried again at room temperature. The two pots (2 and 4) were smoked by using Smoke-out Machine (RO/EV-R700-980-1300, England, UK) with smoking chips of *Olea africana* (pots were inverted over the smoking chips) for 30 minutes. After preparation, the pots were covered with Enset or False banana leaves (washed with tap water) and kept at room temperature until (for 15 minutes) the representative sample was made and poured (Indigenous method). Each pot was filled up with 500 ml representative milk sample. Leaves of *Lippia adoensis* (5 gram) was added to the milk samples of pot 3 and 4 (Indigenous method).

Finally, the milk samples were covered with Enset leaves and stored at 0-6°C in refrigerator (used to hinder external or environmental factors contributing to the growth of microorganisms and chemical deterioration, which helped to give focus on only primary factors effect known as treatments) for 7 hours (to keep morning milk as raw as possible until lunch time) to test the raw milk property before consumption or further processing as raw as possible. Then, the laboratory analysis was done within 8 hours of sampling from the sources.

2.3. Physico-chemical Property Determination

2.3.1. pH

The pH of the milk samples was determined using a digital pH meter (InoLab D-82362, Weliheim, Germany). The pH meter was first calibrated against standard buffer solutions of pH 7 (Super Enterprise 039039, Ambala Cantt, India). The buffer solution was used with a pH as close as possible to that of the test solution. Sample of 10 ml was poured into a beaker and the electrode was allowed to dip into the sample and reading was recorded. Finally, the electrode was rinsed with distilled water between tests and kept in distilled water after use (Mendi, 2000; AOAC, 2006). The test was done in duplicate and the mean values were taken as a single data.

2.3.2. Titratable acidity

Titratable acidity was determined by titrimetric method as described by AOAC (2006) and FAO (2009) with little modification. The indicator solution of 0.5% phenolphthalein was made by mixing 1 gm 0.5% phenolphthalein indicator powder (EMD Chemicals Inc. VW3342, Gibbstown, USA) and 100 ml alcohol (75%) and 0.1N NaOH solution was prepared by mixing 2 gm NaOH powder (UN-Chem. 1310-73-2, Geel, Belgium) with 500 ml distilled water according to the manufacturers guideline. Milk sample of 10 ml was pipetted into a beaker then 4 ml of 0.5% phenolphthalein indicator solution was added into the sample. After addition of the indicator solution, the sample was titrated with 0.1N NaOH solution until definite pink color persisted. The titration was made in duplicate and the mean values taken for the report as a single data. The titratable acidity of milk was expressed as percentage lactic acid and calculated by using equation 3.1.

$$\%\ \text{Lactic acid} = \frac{\text{ml of 0.1N NaOH x 0.009}}{\text{ml of sample}} \text{ x } 100 \qquad (3.1).$$

2.3.3. Alcohol test

Alcohol solution (75%) was prepared by mixing 79 ml of 95% alcohol (Fine Chemical General Trading 026029, Addis Ababa, Ethiopia) with 21 ml distilled water as indicated in manufacturer's manual. A milk sample of 5 ml was placed in a screw cap test tube and an equal volume of 75% alcohol (ethanol) solution was added. The test tube screw-cap was slowly tightened and the sample was mixed by inverting the test tube several times for about 3 minutes. After mixing, the tube was examined to determine whether the milk sample was coagulated or not (Adnan, 2009; FAO, 2009). The test was done in duplicate and the mean values were taken as a single data for reporting.

2.3.4. Specific gravity test

Specific gravity was determined by using digital Gibertini-DensiMat and Alcomat-2 (Gibertini electronical SRL, Millano, Italy) (FAO, 2009) with little modification. According to the manufacturer's guideline the digital Gibertini was calibrated by transferring 70 ml of 97% alcohol (Fine Chemical General Trading 026029, Addis Ababa, Ethiopia) into 100 ml graduated cylinder then both the electrode and the temperature analyzer were inserted into the cylinder. Finally, the Gibertini was checked for calibration with 97% alcohol at room temperature. The temperature analyzer and graduated cylinder were rinsed with distilled water. First, the milk sample temperature was ensured to about 20 °C and 70 ml of the sample was placed into graduated cylinder and the electrodes of Gibertini and temperature analyzer were inserted into the sample. Then, the reading of specific gravity was recorded. The test was made in duplicate and the result was reported as single mean value. The electrode, temperature analyzer and cylinder were rinsed with distilled water between tests and the electrode was put in electrode box after use.

2.4. Microbial Property

2.4.1. Aseptic techniques

Laboratory equipment such as petri-dishes, pipettes and pipettors were sterilized at 180 °C for 2 hours in hot air oven and different bottles and test tubes were sterilized by autoclaving at 121°C for 15 minutes. The diluent (peptone water) (HiMedia laboratory Pvt Ltd. M028S, Maharashtra, India) used was sterilized by autoclaving at 121°C for 15 minutes. Similarly, Plate count agar (PCA) (Titan Biotech LTD 331058, Washington DC, USA) used for determination of total viable microorganisms of milk and Violet red bile glucose agar (VRBG) (HiMedia Laboratory Pvt Ltd. MH581, Maharashtra, India) used for enumeration of *Enterobacteriaceae* the representatives of coliforms were sterilized by autoclaving at 121°C for 15 minutes.

2.4.2. Dilutions

Serial dilutions of milk sample used for determination of standard plate count (SPC) and total coliform count (TCC) were prepared according to Roberts and Greenwood (2003) and Michael and Joseph (2004). The sample was mixed by shaking the bottle 25 times with an arc of 30 cm for 7 sec. Using aseptic technique (reducing contamination by heating laboratory gas burner in working place, using fume hood, sterilized sampling bottles, sterilized screw-cap bottles, sterilized test tubes, sterilized mechanical pipettors and using fresh sterile pipette for each dilution) the initial dilution was made by transferring 10 ml of the sample into dilution tube of 90 ml sterilized peptone water 'blank' then mixed by rotating quickly to create a vortex by using

vortex mixer (Vortex, Qilinbeier Scientific Instruments, China, Beijing). The first dilution was labeled as 10^{-1}. Immediately after the first dilution was shaken, 1 ml was transferred aseptically into dilution tube of 9 ml sterilized peptone water 'blank'. The second dilution was labeled as 10^{-2}. The third dilution was prepared after shaking the second dilution and transferring aseptically 1 ml of it into dilution tube of 9 ml sterilized peptone water 'blank' and labeled as 10^{-3}. Third dilution was shaken and 1 ml was transferred aseptically into dilution tube of 9 ml sterilized peptone water 'blank' and then fourth dilution was prepared and labeled as 10^{-4}. Fifth dilution (10^{-5}) was made after shaking the fourth dilution, 1 ml of fourth dilution was transferred aseptically into dilution tube of 9 ml sterilized peptone water 'blank'.

2.4.3. Standard plate count (SPC)

The bottom of petri-dishes was labeled with wax pencil with the respective dilution, date and researcher's name. Dilution was shaken again and 1ml was transferred aseptically into sterilized and labeled petri-dish. Molten plate count agar of 10 ml was poured to the petri-dish in opposite direction to the sample. The sample and the agar were immediately mixed gently moving the plate on a flat surface in a figure-eight motion lasting 8 sec. The plate was allowed to solidify. Then the plate was inverted and incubated at the temperature of 32°C for 48 hours. Finally, at the end of the incubation period, all of the petri-dishes containing between 30 and 300 colonies (this range is considered as statistically significant) were selected and counted by using Quebec colony counter (Yerco Colony Counter-Electronic, New York Scientific Instruments) (Roberts and Greenwood, 2003; Michael and Joseph, 2004). The test was done in duplicate and the mean values were taken as single data. The counted colonies were recorded and used in the equation 3.2, in order to report computed count as SPC per ml of diluted sample.

$$\text{SPC (cfu/ml)} = \frac{\text{Average number of colonies from duplicate plates}}{\text{Dilution factor x Volume plated}} \quad (3.2).$$

2.4.4. Total coliform count (TCC)

Coliform count was determined according to Roberts and Greenwood (2003) and Michael and Joseph (2004). Labeling of petri-dishes was done with wax pencil with the respective dilution, date and researcher's name. Dilution was shaken again and 1 ml was transferred aseptically into sterilized and labeled petri- dish. The molten violet red bile glucose agar of 14 ml was poured aseptically to petri-dish in opposite direction to the sample. The sample and the agar were mixed thoroughly during pouring. After hardening, 8 ml of the same agar medium was overlaid. The plate was allowed to solidify. Then the plate was inverted and incubated at the temperature of 32°C for 24 hours. Finally, to report the TCC per ml, all countable plates between 15 and 250 colonies (this range is considered as statistically significant) characterized as dark red colonies with typical appearance were selected and counted by using Quebec colony counter. The test was made in duplicate and the report was made by taking the mean values as single data. The counted colonies were calculated using equation 3.3 to report the TCC.

$$\text{Coliform colonies/ ml} = \frac{\text{Colonies counted}}{\text{Sample size (ml)}} \quad (3.3).$$

2.4.5. Methylene blue reduction test (MBRT)

Methylene blue solution was made by mixing 1 gm methylene blue thiocyanate powder (Volu-Sol Inc. KKM/664.1850, Utah, USA) with 200 ml distilled water. Solution of 1ml was transferred to sterilized screw-cap test tube and 10 ml of the sample was added after thorough mixing of the sample. The test tube was tighten and slowly inverted 3 times, to mix the sample and solution thoroughly. The tubes were placed in the water bath and, when the temperature reaches 36°C, the tubes were removed from the water bath and inverted several times (for 1 minutes) to assure uniform mixing again. The test was done in duplicate and the mean values were taken as single data. The sample was examined for color change at every interval of 30 minutes after start time. Any mixtures that have changed from pale blue-green to at least 80% white (80% loss of blue color) were considered to have reached the end-point result and the time of color disappearance was recorded. The time taken to decolorize milk samples and the relation to the milk grade is indicated in Table 1 (Douglas, 2009; FAO, 2009).

Table 1. *Decolonization time and milk grading*

Time (hour)	Milk quality				
	Very bad	Bad	Poor	Fair	Good
< ½	X				
½ to 1		X			
1 to 2			X		
2 to 4½				X	
> 4½					X

2.5. Sensory Evaluation

Panelists were selected from the same study area on their willingness to participate on platform sensory evaluation. The panel was consisted of 30 members with similar age group ranging from 23 to 30 years. The panellists were requested to evaluate the sample and indicate the degree of liking for the product and characteristics of the product. They were given a hedonic ballot/score sheet and coded samples of 50 ml tempered to 20°C to allow flavors to become more volatile to assess the sensory attributes (flavor, mouth feel, aroma, appearance and overall acceptability). The test was done in duplicate and the mean values were taken as single data. The panellists were allowed to score the sample based on 5 point hedonic scale, because the panellists are not trained to assess the samples by 7 or 9 point hedonic scale (5=like extremely, 4=like

moderately , 3=neither like nor dislike, 2=dislike moderately or 1=dislike extremely (Stephanie *et al*., 2009).

2.6. Data Analysis

The data of physico-chemical parameters (pH, titratable acidity and specific gravity with the exception of alcohol test, which was observed visually) and the microbial quality (SPC, TCC and MBRT) were analyzed using one-way Analysis of Variance (ANOVA) model of Duncan's Multiple Range test. The microbial counts were first transformed into log10, and then analyzed. General linear model (GLM) Duncan's Multiple Range test was used to analyze the sensory characteristics (flavor, mouth feel, aroma, appearance and overall acceptability) of cow milk in which the effect of panelists were blocked. All comparison was made at significance level of 5% using Statistical Analysis System (SAS) for windows version 9.1.3.

3. Results and Discussions

3.1. Effect of *Olea africana* and *Lippia adoensis* on physico-chemical property of cow milk

Table 2 shows the effect of the treatments on the physico-chemical properties. All the samples analyzed had a pH value between 6.55 and 6.80, and were significantly ($p \leq 0.05$) different from each other. The titratable acidity or percentage lactic acid of the samples was not affected by all treatments, *i.e*., there was no significant difference between each other. The results of alcohol test indicated that all the milk samples were negative to the test (no clot formation or coagulation). The specific gravity of T_2 (1.0324 ± 0.0001) (milk sample in smoked pot) was significant ($p \leq 0.05$) higher than T_3 (1.0316 ± 0.0004) (milk sample with added leaves), while the others have intermediate values with no significant difference (Table 2).

Table 2. *Effect of different treatments on the pH, titratable acidity, alcohol test and specific gravity of cow milk.*

Treatments	Physico-chemical parameters			
	pH	Titratable acidity	Alcohol test	Specific gravity
T_1	6.64 ± 0.06^{b}	0.22 ± 0.01^{a}	Negative	1.0321 ± 0.0001^{ab}
T_2	6.80 ± 0.01^{a}	0.21 ± 0.03^{a}	Negative	1.0324 ± 0.0001^{a}
T_3	6.55 ± 0.07^{c}	0.22 ± 0.01^{a}	Negative	1.0316 ± 0.0004^{b}
T_4	6.74 ± 0.01^{ab}	0.21 ± 0.01^{a}	Negative	1.0320 ± 0.0001^{ab}

Mean ± Standard deviation values within columns bearing with the same superscripts are not significantly different at $p \leq 0.05$
Where, T_1: Control milk sample kept in neither smoked nor leaves added pot 1; T_2: Milk sample kept in smoked pot 2;
T_3: Milk sample with added leaves kept in pot 3; T_4: Milk sample with added leaves kept in smoked pot 4.

pH test measures total acidity of milk and used for screening the milk quality. As bacterial count of milk increases, its quality deteriorates, *i.e*., its pH will fall (Adnan, 2009). As shown in Table 2, the pH of milk sample in smoked pot (T_2) (6.80 ± 0.01) was significantly ($p \leq 0.05$) higher than T_1 (6.64 ± 0.06) (control milk sample) and T_3 (6.55 ± 0.07) (milk sample with added leaves). This might be due to the effect of smoking of milk handling pot with smoking chips of *Olea africana*. Similar finding was reported by Hellen and Eyassu (2007) that the milk sample in smoked containers had high pH value. The pH measure of T_3 was significantly ($p \leq 0.05$) the least of all samples. Its pH value decreased probably due to the effect of phytonutrients found in leaves of Lippia *adoensis*. This agreed with the finding of Kasali *et al*. (2004) that showed some phytonutrients of plants decreased pH. The synergetic effect of both treatments (smoking milk and addition of leaves) made the pH value of T_4 (6.74 ± 0.01) in between T_2 and T_1. Similar result was reported by Tsioulpas *et al*. (2007) and Adnan (2009) stated that fresh cow milk had a pH value between 6.4 and 6.8 and is therefore, slightly acidic.

Titratable acidity test determines lactic acid content. It is also used for screening and to monitor storage conditions, accordingly determines suitability of milk for processing (FAO, 2009). The present study showed that titratable acidity of milk sample did not show significant variation amongst the treatments (Table 2). Moreover, the overall percentage lactic acid was in the range of 0.21 to 0.22. Similar report was presented by Hellen and Eyassu (2007).

Alcohol test is used for screening and rapid assessment of milk quality whether the milk sugar or lactose was fermented or not. If the milk is of good quality, there will be no coagulation while addition of alcohol. If the milk has become acidic (pH below 6.4) it will flocculate (Tsioulpas *et al*., 2007). The result of alcohol test (Table 2) showed that the entire milk samples were negative to the alcohol test during addition of alcohol and there was no coagulation means the tested samples were at good milk status. The treatments had no significant effect on alcohol test. This was consistent with Horne (2003) and Adnan (2009).

Specific gravity is the relation between the mass of a given volume of any substance (milk) and that of an equal volume of water at the same temperature. Its test determines level of solids, *i.e*., it is used as main indicator for fraudulent behavior by the milk suppliers (added water, or removal of fat) (FAO, 2009). All treatments had significant effect on specific gravity; however, the entire milk samples were in acceptable range from 1.0316 and

1.0324 at room temperature (Table 2). This implies that the treatments, *i.e*., smoking milk handling pots and addition of leaves into milk sample not changed the level of solids of milk sample. This was in the acceptable range of cow milk specific gravity (1.030 - 1.035) (Li-Qiang *et al*., 2009). Hence, the milk samples were not outside the normal range, this implies the samples were unadulterated by either smoking the pots or by adding leaves.

3.2. Effect of *Olea africana* and *Lippia adoensis* on the Microbial Property of Cow Milk

Results in Table 3 shows, the main effect of the treatments on microbial properties. The bacterial count of the whole sample was affected significantly ($p \leq 0.05$) by the treatments. The bacterial count of control milk sample (T_1) was 3.47 log10 cfu/ml was the highest of all milk samples. The total coliform count of milk sample was in the range of 0.00–0.54 log10 cfu/ml. The recorded value for T_1(0.54 ± 0.09) and T_2(0.39 ± 0.13) were different from T_3 and T_4. The MBRT for T_3 (7:15 hours) (milk sample with added leaves) was the highest of all milk samples.

Table 3. *Effect of different treatments on the SPC, TCC and MBRT of cow milk.*

Treatments	SPC (log10 cfu/ml)	TCC (log10 cfu/ml)	MBRT (hours)
T_1	3.47 ± 0.01^a	0.54 ± 0.09^a	$4:45 \pm 0.00^c$
T_2	3.42 ± 0.01^b	0.39 ± 0.13^a	$6:15 \pm 0.00^b$
T_3	3.37 ± 0.01^c	0.00 ± 0.00^b	$7:15 \pm 0.00^a$
T_4	3.39 ± 0.01^c	0.00 ± 0.00^b	$6:15 \pm 0.00^b$

Mean ± Standard deviation values within columns bearing with the same superscripts are not significantly different at $p \leq 0.05$

3.2.1. Standard Plate Count

Standard plate count also referred to as total bacterial count or total viable count 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 bacterial counts of 3.37 to 3.47 log10 cfu/ml of this study was lower than the findings of Fekadu (1994) (6 to 8.8 log10 cfu/ml) and Alganesh *et al*. (2007) (7.60 log10 cfu/ml) of cow milk produced in South region and East Wollega, respectively. The bacterial counts of control milk sample T_1 (3.47 log10 cfu/ml) significantly ($p \leq 0.05$) the highest of all samples (Table 2). This is probably due to the treatments effect. Similar report was made by Mogessie and Fekadu (1993), Almaz (2001), and Hellen and Eyassu (2007), stated that the disinfecting of the milk handling containers with smoking wood chips and treating milk with different plants reduces microbial load. The bacterial counts of T_3 (3.37±0.01) (milk sample with added leaves) and T_4 (3.39±0.01) (milk sample in smoked pot and with added leaves) were lower than T_1 and T_2. This could be due to the addition of *Lippia adoensis,* and synergetic effect of treatments. The overall bacterial counts of this study was at the food grade level of bacterial count (< 4.3 log10 cfu/ml) according to Braunig and Hall (2005), which is satisfactory for consumption and processing.

3.2.2. Total Coliform Count

A coliform count on fresh product is used as an initial screening tool for raw milk, *i.e.*, it is a good indication of whether sanitary methods were used in processing and handling, and they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also can cause rapid spoilage of milk because they are able to ferment lactose with the production of acid and gas, and are able to degrade milk proteins (Chizart *et al*., 2008; Douglas, 2009). The TCC in control milk sample T_1 (0.54 log10 cfu/ml) and milk sample in smoked pot (T_2) (0.39 log10 cfu/ml) had no significant difference, while there was no coliforms detected in T_3 (milk sample with added leaves) and T_4 (milk sample in smoked pot and with added leaves) (Table 3). The factors which contributed to the present study findings were addition of leaves and synergetic effect of the treatments. The overall coliform counts were within coliform limits of food grade (1.0 log10 cfu/ml) for sensitive foods such as milk as presented by James (2000) and Chizart *et al*. (2008). The TCC obtained in the current study is lower than the findings of Mogessie and Fekadu (1993) and Haile *et al*. (2012) and Alganesh *et al*. (2007).

3.2.3. Methylene Blue Reduction Test

The length of time milk takes to decolorize methylene blue indicate the degree of microbial content and hence of its hygienic quality. It is generally assumed that the greater the number of bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk. The time taken for the reduction of methylene blue is inversely proportional to the number of viable bacteria. The shorter the methylene blue reduction time poorer is the quality of the milk (Douglas, 2009; FAO, 2009). The results of MBRT indicated that the color disappearance time of milk samples were in the range of 4:45 and 7:15 hours (Table 2). All milk samples color disappearance took long time with the exception of T_2 and T_4. The color disappearance time of T_3 (milk sample with added leaves) was the highest of all samples. This might be due to the effect of antimicrobial activities of some phytonutrients found in *Lippia adoensis*. T_1 (control milk sample) (4:45 hours) was the shortest of all samples. Similar report was made by Abd *et al*. (2002) and El-Tantawy *et al*. (2006) that antimicrobial activities of herbs and spices can retard microbial growth in dairy products. The present study result showed all milk samples were at the standard level of good milk. This was in accordance with Douglas (2009) and FAO (2009) reports.

3.3. Effect of *Olea africana* and *Lippia adoensis* on the Sensory Characteristics of Cow Milk

Results in Table 4 shows the effect of *Olea africana* and *Lippia adoensis* on sensory characteristics of milk samples. The analysis of data showed that all treatments had significant effect on sensory attributes, namely; mouth feel, aroma and overall acceptability; however, there was no significant effect on flavor and appearance. The value of mouth feel recorded for milk sample with added leaves (T_3) (4.20 ± 0.71) and control milk sample (T_1) (3.83 ± 0.83) were different from each other but had no difference with T_2 (4.07 ± 0.91) (milk sample in smoked pot) and T_4 (4.07 ± 0.94) (milk sample in smoked pot and with added leaves). The highest score for aroma was recorded in T_2 (3.90 ± 0.76), while the lowest one was for the T_3 (3.57 ± 1.00). However, the value of T_1 (3.77 ± 0.82) and T_4 (3.83 ± 0.87) were positioned in between T_2 and T_3. The overall acceptability score of T_2 (4.20 ± 0.71) was significantly ($p \leq 0.05$) higher than T_1 (3.90 ± 0.71). The score of T_3 (4.00 ± 0.74) and T4 (4.00 ± 0.91) were positioned between T_1 and T_2.

Table 4. *Effect of different treatments on the sensory characteristics of cow milk.*

Treatments	Sensory attributes (hedonic scale)				
	Flavor	Mouth feel	Aroma	Appearance	Overall acceptability
T_1	3.90 ± 0.89^{a}	3.83 ± 0.83^{b}	3.77 ± 0.82^{ab}	3.83 ± 0.83^{a}	3.90 ± 0.71^{b}
T_2	3.90 ± 0.96^{a}	4.07 ± 0.91^{ab}	3.90 ± 0.76^{a}	4.00 ± 0.83^{a}	4.20 ± 0.71^{a}
T_3	3.93 ± 0.98^{a}	4.20 ± 0.71^{a}	3.57 ± 1.00^{b}	3.77 ± 0.77^{a}	4.00 ± 0.74^{ab}
T_4	3.97 ± 0.85^{a}	4.07 ± 0.94^{ab}	3.83 ± 0.87^{ab}	4.00 ± 0.83^{a}	4.00 ± 0.91^{ab}

Mean ± Standard deviation values within columns bearing with the same superscripts are not significantly different at $p \leq 0.05$

Milk of a good quality is with a slightly sweet taste, very little odor and a smooth, rich feel in the mouth that leaves only a clean and pleasing sensation (Drake, 2004). Sensory characteristics of mouth feel, aroma and overall acceptability of milk sample improved as a result of the treatments (Table 4). Mouth feel of T_3 (milk sample with added leaves) was accepted more than T_1 (control milk sample). Aromatically, T_2 (milk sample in smoked pot) was more accepted than T_3. T_2 (4.20 ± 0.71) was accepted in overall, while T_3 (4.00 ± 0.74) and T_4 (4.00 ± 0.91) (milk sample in smoked pot and with added leaves) overall accepted moderately, but T_1 (3.90 ± 0.71) neither liked nor disliked. These changes can be attributed to the treatments and it might be due to the presence of some active ingredients in plants used for treatments. The report of this study was consistent with Peter and Babu (2004). The report stated that herbs and spices added into milk and milk products impart flavor and odor. Similarly, Muchuweti *et al*. (2007) reported that plant products herbs and spices that can be added to the food to provide additional aroma, taste, flavor and color.

4. Conclusions

The physico-chemical parameters such as pH and specific gravity, microbial properties (Total bacterial, coliform counts and methylene blue reduction time) and Sensory Properties (Mouthfeel, aroma, and overall aacceptability) of cow milk were affected by smoking milk handling pots with chips of *Olea africana* and addition of leaves of *Lippia adoensis*. However, the percentage lactic acid, alcohol test, flavor and appearance were not affected by all treatments. The overall values of all parameters were within acceptable limit set for raw milk. Generally, it can be concluded that the communities traditional practices of raw milk handling methods such as milk handling pot smoking with smoking chips of *Olea africana* and addition of leaves of *Lippia adoensis* into milk improved some parameters of physico-chemical property, reduced microbial load and improved organoleptic properties of raw milk to some extent. It requires more elaborative studies in identification and specific characterization of microorganisms influenced by the treatments, some other physico-chemical factors, the active phyto-nutrients playing the role in relation with shelf-life of milk and milk products.

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Declaration of Interest

The authors have read and understood the American Journal of Food Science and Nutrition, American Association for Science and Technology's Policy on declaration and they have no interest to declare.

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