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Identification and Evaluation of Preservative Plants on traditionally fermented Cow milk

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Abstract

The majority of communities in different regions of Ethiopia are relying on a variety of plants to improve the quality of their dairy products. However, this cultural perception was scientifically not well strengthened. Therefore, the objectives of the study were to identify milk preservative plants and evaluate the effects of preservative plants on fermented milk redox potential, Potential hydrogen (pH), and sensory analysis. The survey study was conducted on purposefully selected 80 households in the Haramaya district. However, the laboratory study was conducted on four top-ranked plants for the preparation of fermented milk samples at ambient temperature following similar techniques and procedures observed at households. All the collected data were analysed by Statistical Analysis software (SAS). In the study area, five plants in the families of *Oleaceae, Celastraceae, Lamiaceae*, and *Rutaceae* were identified and used by the majority of respondents with perceptions of enhancing the flavour of their products. The Analysis of variance (ANOVA) result of redox potential was proved the cultural perception of the majority of respondents that they were used both *Olea Africana* and *Catha edulis* in substitute to each other for the same purpose. The pH of all treatments was continuously decreased and the milk samples treated by *Olea Africana* and *Catha edulis* were recorded the lowest values at the end. The observed pH results have disproved the communities cultural perceptions that they believed smoking increased the shelf life and extended the fermentation time whereas the Hedonic scores of panelists proved the local perceptions of respondents that they were mainly intended to make their products much more acceptable and preferable by its flavour to the consumers.

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Introduction

Plants have tremendous importance in many rural African communities as medicines, flavour enhancers, nutritional additives, and appetizers. Thus, these plants are alternative milk preservation methods that enhance shelf life, organoleptic property, and storage stability of milk and milk products.[1,2] Among the many preservative plant species, Olive (*Olea Africana*), Eucalyptus (*Eucalyptus globulus*), Koseret (*Lippiaadoensis*), Rue (*Ruta chalepensis*), Basil (*Ocimumbasilicum*), and Thyme (*Thymus vulgaris*) are commonly used in different parts of Ethiopia for their good flavor and aroma, and increasing the shelf life of the products.[3]

These preservative plants are applied to milk and milk products handling utensils mainly through smoking or cleaning or by using both techniques together. Smoking milk utensils are commonly applied to give a pleasant flavor and aroma to the milk and milk products.[4] Similarly, many researchers reported that handling equipment is 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 and retard the growth of different microorganisms.[5] Furthermore, there was a report as communities are practicing hygienic clean and smoking of milk utensils with different plants to add flavor and/ or increase the shelf life.[6] Thus, several researchers have conducted a survey study in different parts of the country and identified certain preservative plants, and reported their effects on the final products organoleptic, compositional, microbiological, and possible health risks. However, the effects on the milk fermentation process, Redox potential, pH, and sensory analysis were scientifically not well studied and strengthened. Hence, it was clear that most researchers were emphasized identification and documentation with little evaluation of milk physical, chemical, organoleptic properties, microbial loads, fermentation time, and shelf life. Therefore; the objectives of the study were to identify milk preservative plants and evaluate the effects of preservative plants on the milk fermentation process, redox potential, pH, and sensory analysis.

Materials and Methods

Study Area

The study was conducted in the eastern Hararghe zone, Haramaya district, Oromia regional state, which is located 510 Km east of Addis Ababa. The altitude of the area ranges from 1400 to 2340 meters above sea level. It receives an annual rainfall of 900 to 1600 mm with the mean minimum and maximum annual temperatures of 5 and 38°C, respectively (Haramaya district Agricultural office,2019).

Sample Size and Sampling Methods

With the help of governmental bodies of Haramaya district, four Kebelle's such as Damotaa, Ifa Oromiya, Haqaa, and Fendisha were purposefully selected by their known traditional milk processing potential. Besides, a total of eighty households (twenty from each Kebele) were selected by multi-stage sampling techniques which were snowball (directed by first selected household) and purposefully (some selected by their long year's experiences of using traditional methods of milk processing and preservation) and then interviewed.

Survey and Data Collection

A semi-structured questionnaire was used to interview and collect data on the traditional procedures and usage of plants in the milk processing and preservation system. Moreover, observations by demonstration were made to share the household's indigenous milk processing knowledge along with the interview. The interview information included local names of plants, used parts of plants, condition of plant part used (fresh/ dried), methods of plant utilization (smoking, rubbing milk utensils, pouring on milk and others), fermentation/preservation period, the effect on milk products (change milk flavor, aroma, color, and others), the effect on milk shelf life, and noticeable effects on consumer preferences.

Plant Identification and Laboratory Evaluation In Milk Processing and Preservation

The top-ranked plants by respondents based on their use in traditional milk processing or fermentation in the study area were coded by their vernacular names and brought to Haramaya University Herbarium where they were scientifically identified and authenticated by identification specialists. After identification, four frequently used and easily available plants to the households were brought to Haramaya University Dairy Technology laboratory for evaluation on milk samples fermentation process, redox potential, pH, and sensory analysis.

Preparation of Equipment and Fermented Milk Samples

Fresh cow milk from the pooled container was collected from Haramaya University Dairy Farm and taken to the University Dairy Technology Laboratory. Then, four selected plants such as *Olea Africana*, *Catha edulis*, *Ocimum hardiensem*, and *Thymus serrulatus* were used in the preparation of milk fermentation following similar techniques of applications and procedures that observed at households during the survey. Thus, all beakers (500 ml capacity) used for milk fermentation were rinsed thoroughly with distilled water before used on the sample. Then after washed and dried, some internal walls of beakers were scrubbed/rubbed with leaves and twigs of *Ocimum hardiensem* and *Thymus serrulatus* whereas other beakers were fumigated inverting them over the smoking chips of *Olea Africana* and *Catha edulis* stem for 20 minutes, then rubbed by cheesecloth. This procedure of scrubbing and fumigation/smoking of beaker was repeated four times every five-minute intervals. Finally, all beakers were coded and immediately filled with 400ml of fresh cow milk samples in two duplicates, and then Calibrated and disinfected *iCinac* (Alliance Instruments, Frepillon, France) probes were inserted into each beaker consisted milk sample and sealed, then sample were left to ferment at ambient temperature until the desired acidity of the fermented samples are attained that is the ideal pH of yogurt (4.6 or lower).[7] At this point, the acid gel is definitively formed, gelation is completed, and aggregation of casein micelles is irreversible.

Oxidation-Reduction Potential, pH and Sensory Evaluation

Redox potential (Eh) and pH measurements were automatically recorded every minute by the *iCinac* instrument continuously,[8] then average values were calculated every 12 hrs intervals. However, the sensory attributes of each fermented samples were evaluated after the fermentations were ceased. It was evaluated by thirty profesFsional panelists who were purposefully selected by their related specialization to food and animal sciences. The products were evaluated for the perception of the basic attributes such as color, taste, flavor, and overall acceptance based on a 1-5 hedonic scale where 1 represented “dislike extremely” and 5 represented “like extremely. Each prepared sample was coded and presented in duplicate to each panelist then, mean values were taken. Each panelist was presented with a glass of water to rinse their mouths after each testing session to prevent the carryover effect.

Experimental Design and Statistical Analysis

The four selected plants (treatments) were randomly assigned to the beakers and each beaker coded by the respective plant names. Thus, a completely randomized design was employed and the data were analyzed using Statistical Analysis software version 9.4. When the analysis of variance indicates the existence of a significant difference between treatment means, Duncan’s multiple test method was used to locate the treatment means that are significantly different when the p-value was <0.05. The following model was used for the analysis.

$$Y_{ij} = \mu + T_i + e_{ij}$$

where,

Y_{ij}= individual observations,
μ= Overall mean,
ti= Treatment effect (plant type),
e_{ij}= random error

Results and Discussion

Identification of Preservative Plants and Indigenous knowledge in Milk Processing

Five preservative plants frequently used in traditional milk fermentation were identified and botanically categorized into the families of Oleaceae, Celastraceae, *Lamiaceae*, and *Rutaceae* (Table 1). Among the identified plants, *Olea Africana* (*Ejerssa* or *Woira*) was the most frequently used as reported by the majority of respondents. The wider use of this plant in traditional milk handling by different groups of societies in different parts of Ethiopia and other African countries were correspondingly noted by many scholars.[2, 3, 9] Such widespread use of *Olea Africana* by different groups of societies in many parts of the country could be to a certain extent attributed to its efficacy in traditional milk utilization. Khat (*Catha edulis*) as an important plant in traditional milk handling was newly identified and 31.25% of respondents used the plant to replace *Olea Africana*. This was because of an expensiveness and inaccessibility of *Olea Africana* in their vicinity provided that they perceived Khat has a similar purpose as Olea Africana. Moreover, the use of *Lamiaceae* (*Ocimum*

hardiensem and *Thymus serrulatus*) and *Rutaceae* (*Ruta chalepensis*) for traditional milk handling and processing was reported in different regions of the country by many scholars.[2] The nature of these preservative plants was categorized under trees, shrubs, and herbs (Table 1). Likewise, the dominance of these shrubs, trees, and herbs was extensively reported in earlier studies for traditional milk handling, fermentation, and processing and as well for the preparation of several ethnobotanical medicines.[2, 9, 10, 11] Thus, variation in the dominance of growth forms of preservative plants could be attributed to the wide agro-ecological diversity and specific indigenous knowledge of different communities.

Table 1: The most frequently used preservative plants in traditional milk fermentation

Name of the plants			Family	Plant	Parts type	Form used	Preference rank (%) (n=80)
Afaan Oromo	Amharic	Scientific					
Ejerssa	Woira	*Olea Africana*	Oleaceae	Tree	Stem	Dry & fresh	40.00 (1st)
Muka Jima	Khat	*Catha edulis*	Celastraceae	Tree	Stem	Dry	31.25 (2nd)
Suke	Kesie	*Ocimum hardiensem*	Lamiaceae	Shrub	Leaf	Dry & fresh	10.00 (3rd)
Xoosiynoo	Tosigne	*Thymus serrulatus*	Lamiaceae	Shrub	Leaf	Dry & fresh	10.00 (3rd)
Xalatam	Tenadam	*Ruta chalepensis*	Rutaceae	Herb	Leaf & stem	Fresh	8.75 (4th)

n= Number of respondents

All respondents agreed on the use of stem and/ or leaf parts of the identified plants. The uses of leaves in preparation of remedies and traditional milk handling and processing were widely reported in previous studies because of there high content of many metabolites.[12] This finding was also in line with earlier studies that noted the stem part was 100% used from all the smoking plants for preserving the milk and on contrary, they have noted the use of root parts from *Olea Africana* for the same purpose.[2] This extensive use of stem and/ or leave parts of plants, threatens survival and continuity of valuable preservative plants. The majority of identified preservative plants in this study were used in the form of fresh and dry except the *Catha edulis* and *Ruta chalepensis* which were only used in the form of dry and fresh, respectively (Table 1).

Table 2: The preservative plants application techniques and respondents local perceptions

Plant type	Application techniques	Local perceptions (%)					n
		Color	Flavour	Taste	Shelf life	Thickness	80
Olea Africana	Smoking utensils	10.0	60.0	5.0	20.0	5.0	80
Catha edulis	Smoking utensils	11 .0	59.5	5.0	19.5	5.0	80
Ocimum hardiensem	Rinsing utensils	0.0	80.0	10.0	10.0	0.0	80
Thymus serrulatus	Rinsing utensils	5.0	80.0	10.0	5.0	0.0	80
Ruta chalepensis	Soaking in milk	0.0	90.0	5.0	5.0	0.0	80

n= number of respondents

Preservative Plants Application Techniques and Respondents Local Perceptions

In the study area, three application techniques of preservative plants to milk handling and processing were reported by the respondents (Table 2). The uses of the varied techniques were determined by the availability and types of preservative plants in their vicinity. *Olea Africana* and *Catha edulis* were

applied to milk utensils and storage vessels through smoking before milk fermentation with perceptions of enhancing color, flavour, taste, shelf life, and thickness of the products. This result agreed with the result noted that smoking of milk and milk equipment by different kinds of plant materials is one of the cultural practices to increase shelf life and to add flavour to the product.[3] Similarly, many communities practiced smoking of dairy products handling equipment with different plants to add flavour and/ or to increase the shelf life of products with different plants.[6] Moreover, many other scholars in earlier studies conducted in different regions of the country were frequently reported as smoking of milking and storage vessels by different aroma produced plants such as *Olea Africana*, *Juniperous procera, Acacia nilotica, Cordiaglarfa, Cordia ovalis, Deinbollo kilimandshorica, Syzygium guinecnse*, and *Heeria reticulala* extended the fermentation time besides its provision of good flavour.[2,9,13]

Ocimum hardiensem and *Thymus serrulatus* were applied thorough rinsing with water and then rubbing the leaves of the plants against the internal wall of the milk utensils and storage vessels before milk fermentation with perceptions of enhancing flavour, taste, and shelf life of the products. The result is in agreement with the observations made in some parts of Ethiopia, that was milk utensil is and storage vessels were scrubbed with leaves of preservative plants before being filled with milk.[2, 12] Similarly, many communities practiced cleaning dairy products handling equipment with different plants to add flavour and/ or to increase the shelf life of products with different plants.[6] Likewise, the use of *Sida cuneifolia, Cucumis prophetarus,* and *Ocimum hardiense* leaves to clean the milk utensils and storage vessels for traditional milk handling and processing was noted in the Eastern Showa zone of the Oromia region.[14] It had also similarly reported to the current result that 73.4% of respondents in northwestern Ethiopia were used leaves of *Combretum molle, Ocimum suave*, and B*uddleja polystachia* with water to clean the equipment for milk handling and processing.[9] The third technique applied to traditional milk handling was by washing utensils and plants (*Ruta chalepensis*) and then appending the plant into the milk-filled to milk utensils and storage vessels then left to ferment with the plant. This was mainly to impart good flavour (90%) and hard to enhance taste and the shelf life of the milk and milk products (Table 2).

The majority of respondents used all the identified preservative plants in traditional milk handling with perceptions of enhancing the flavour of their products (Table 2) which make the products much more acceptable and preferable by the consumers but few respondents intended to enhance color, taste, thickness and shelf life of products. Consistent with this result, many researchers reported that milk utensils and storage vessels were traditionally treated by various preservative plant species to bring desirable flavor and aroma to the dairy products.[3] Likewise, the use of plants to enhance the shelf life, organoleptic property, and storage stability of milk and milk products were noted in previous studies.[1,2] The perceived impact on improving the sensory attributes of the products may be attributed due to the presence of different types of phytochemicals in preservative plants which should be scientifically further strengthened.

Effects of Preservative Plants on Redox Potential, PH, and Sensory Analysis

Oxidation-Reduction Potential (E_h)

The E_h measured for fermented samples were given in Table 3. There was a strongly significant difference among the treatments (P<0.0001). However, there was no significant difference between the E_h values of *Olea Africana* and *Catha edulis* throughout the fermentation period. This is in agreement with the majority of respondent's cultural perception that they could use both *Olea Africana* and *Catha edulis* in substitute to each other in smoking milk utensils and storage vessels to ensure similar overall acceptance of the fermented products. *Ocimum hardiensem* plant was recorded the highest E_h values throughout the fermentation period than other treatments. Besides, the use of this plant was highly oxidized which is indicated by positive mV values. This was in agreement with the ideas of panelists who observed that the uses of *Ocimum hardiensem* and *Thymus serrulatus* were resulted in more flavour defects of fermented samples and were not appreciated. The prone of milk to undergo oxidation might also be due to its high content of milk fat (4.95 %) used in the experiment. The E_h of fermented milk samples were consistently decreased and this agrees with results obtained by other authors who attributed

the decrease in E_h and it could have been caused by the uptake of oxidizing compounds such as oxygen and the production of metabolites with reducing characteristics in the milk.[15] A similar finding in this regard was also indicated that the decrease in redox potential was highest at the time of oxygen depletion, followed by a more gradual decrease to the final redox values, indicating that both oxygen and biological factors are involved in the reduction process.[15] The negative mill volt values of E_h indicated the reduced states of a chemical/ biochemical system in fermented milk samples. The negative values of E_h proved the respondents' cultural perceptions that they were perceived as a majority of plant types applied to traditional milk handling and used to add flavour characteristics to the products as long as they decreased. Besides, the negative value of E_h is an indicator of the establishment of the conditions required for the formation of aroma compounds and it is essential for the satisfactory development of the characteristic flavour of certain fermented dairy products.[16]

Table 3: Effects of preservative plants on Eh (mill volt-mV) of fermented milk samples

Treatments	Fermentation Time (Hours)				
	Fresh (0)	**12**	**24**	**36**	**48**
Control	52.23^{c}	-225.09^{c}	-312.57^{b}	-355.82^{b}	-449.13^{c}
Olea Africana	19.25^{d}	-134.40^{b}	-312.88^{b}	-344.29^{b}	-379.07^{b}
Catha edulis	11.38^{d}	-157.36^{b}	-310.23^{b}	-342.79^{b}	-370.16^{b}
Ocimum hardiensem	267.73^{a}	-76.21^{a}	-161.14^{a}	-276.29^{a}	-257.94^{a}
Thymus serrulatus	76.48^{b}	-295.93^{d}	-405.94^{c}	-476.09^{c}	-457.18^{c}
P-Value	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Significance level	***	***	***	***	***

^{abc}Means with the same letter within a column are not significantly different. Significance level (SL) when the *p*-value <0.05; ***= SL when p<0.0001.

Table 4: Effects of preservative plants on pH of fermented milk samples

Treatments	Fermentation Time (Hours)				
	0	**12**	**24**	**36**	**48**
Control	6.61^{a}	6.40^{a}	5.50^{a}	4.82^{a}	4.50^{a}
Olea Africana	6.55^{a}	6.50^{a}	5.78^{a}	4.90^{a}	4.48^{a}
Catha edulis	6.57^{a}	6.50^{a}	5.74^{a}	4.88^{a}	4.48^{a}
Ocimum hardiensem	6.60^{a}	6.45^{a}	5.50^{a}	4.76^{a}	4.54^{a}
Thymus serrulatus	6.61^{a}	6.39^{a}	5.46^{a}	4.74^{a}	4.49^{a}
P-Value	0.5579	0.8483	0.8123	0.9957	0.9945
Significance level	NS	NS	NS	NS	NS

^{abc}Means with the same letter within a column are not significantly different. NS-non-signficant; Significance level when the *p*-value <0.05.

pH

The pH decreased continuously throughout the fermentation time and drop to the range of 4.48 to 4.54. This is according to the general pathway for fermentation of milk, which involves the production of lactic acid from lactose in the milk by lactobacillus that lowers the pH. This result was following the pH of milk fermentation that usually drops to 4.3-4.5[17]

or the ideal pH of the finished product of yogurt (4.6 or lower).[7] This implied an increase in acid production and this, in turn, inhibits the growth of food spoilage and pathogenic organisms which can cause food spoilage, food poisoning, and disease. There was no significant difference in pH among milk samples fermented using milk utensils and vessels treated by the identified preservative plants as indicated in Table 4, while individually, each type of yogurt sample has a slight difference. The fermented milk within milk utensils and vessels smoked by the *Olea Africana* and *Catha edulis* had recorded the lowest pH (4.48) values at the end of fermentation in comparison to the other fermented milk samples with other treatments and the control. This indicated that smoking milk utensils and storage vessels with *Olea Africana* and *Catha edulis* were more determined the quality of fermented products than others fermented by milk utensils treated by rubbing *Ocimum hardiensem* and *Thymus serrulatus*. Thus, this study disproved the respondent's cultural perceptions that they were believed smoking milk utensils and storage vessels increased shelf life and extended the fermentation time of products. This cultural perception was verified by many scholars in different regions of Ethiopia and other African countries that smoking is applied to disinfect the vessels, thereby reducing the microbial load and therefore extended the shelf life of the product as compared to unsmoked containers.[2, 3, 6, 9, 13, 14] The pH recorded for *Ocimum hardiensem* at the end of fermentation was slightly higher than for other treatments. This may be attributed to the phytochemicals in the plant leaves, which may have lowered the proliferation of microorganisms thus reduced the production of acid, thus milk utensils treated by this plant relatively need more time to fully fermented to the desired acidity that is the ideal pH of yogurt (4.6 or lower). Accordingly, this plant could relatively be used for the preservation of milk and milk products or increase shelf life.

Table 5: Effects of preservative plants on sensory properties of fermented milk samples

Treatments	Sensory attributes (hedonic scale)			
Treatment	**Color**	**Taste**	**Flavour**	**Overall acceptance**
Control	4.00^{ab}	3.83^{ab}	3.58^{bc}	3.58^{b}
Olea Africana	4.67^{a}	4.50^{a}	4.83^{a}	4.77^{a}
Catha edulis	4.58^{a}	4.42^{ab}	4.42^{ab}	4.50^{a}
Ocimum hardiensem	3.58^{bc}	3.50^{b}	3.83^{bc}	3.42^{b}
Thymus serrulatus	3.08^{c}	3.75^{ab}	3.33^{c}	3.42^{b}
P-value	0.0023	0.00976	0.0089	0.0037
Significance level	**	**	**	**

^{abc}Means with the same letter within a column are not significantly different. Significance level (SL) when the *p*-value <0.05; **= SL when p<0.01. 1-5 hedonic scale where 1- dislike extremely, 2-dislike moderately, 3-Neuteral, 4-like very much and 5 -like extremely.

Sensory Evaluation (Hedonic Test)

The results confirmed by ANOVA analysis for sensory characteristics of the fermented products were presented in Table 5. The ANOVA results of the sensory evaluation were indicated a moderate significant difference (P<0.01) in the appearance/ color, taste, flavour, and overall acceptance of the samples. The Hedonic scores of color, flavour, and overall acceptance were significantly higher for samples made using fumigation of milk equipment by burning wooden chips of *Olea Africana* and *Catha edulis* than samples made using rinsing milk equipment by other plant types. The imparting of special taste and odor to milk and milk products by fumigation of milk equipment by using burning wooden chips of specific trees and shrubs correspondingly reported in the previous study.[10] However, the use of *Thymus serrulatus* leaves with water to clean the milk containers was resulted in more flavor and color defects of fermented samples

and were not appreciated by panelists. As compared to control, milk equipment treated by *Olea Africana* and *Catha edulis* were added more flavour to the products. Generally, the Hedonic scores of panelists proved the local perceptions of respondents that they were mainly intended to make their products much more acceptable and preferable by their favour to the consumers.

Conclusion

In the study area, plants such as *Catha edulis*, *Olea Africana*, *Ocimum hardiensem*, *Thymus serrulatus* and *Ruta chalepensis* were identified. The majority of respondents were used the above identified plants to enhance the flavour of their milk products. The results of Eh proved the respondent's cultural perception that they could use both *Olea Africana* and *Catha edulis* in substitute to each other for the same purpose. The pH results were decreased continuously throughout the fermentation time and drop to the range of 4.48 to 4.54 within 48 hours due to the preservative plants used. The fermented milk in utensils and vessels smoked by the *Olea Africana* and *Catha edulis* had recorded the lowest pH values at the end of fermentation in comparison to the other treatments. This study disproved the respondent's cultural perceptions that they believed smoking milk utensils and storage vessels increased the shelf life and extended the fermentation time of products. The Hedonic scores of color, flavour, and overall acceptance were significantly higher for samples made using fumigation by burning wooden chips of *Olea Africana* and *Catha edulis* of milk equipment than samples made using rinsing milk equipment by other plants. Generally, the Hedonic scores of panelists proved the local perceptions of respondents that they mainly intended to make their products much more acceptable and preferable by their favour to the consumers. Further studies should be conducted on phytochemicals of these preservative plants which are attributed to modify the Eh, pH, and sensory attributes of the dairy product and their roles should be further scientifically strengthened.

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Conflict of Interest

The authors do not have any conflict of interest.

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