STM JOURNALS

Research & Reviews: Journal of
Dairy Science & Technology

https://journals.stmjournals.com/rrjodst

ISSN: 2319-3409 (Online)
ISSN: 2349-3704 (Print)
Volume 14, Issue 2, 2025
May–August
DOI (Journal): 10.37591/RRJoDST

Review RRJoDST

Evaluation of Raw Cow Milk Preservation Using Extracted Mango Seed Kernels and Rosemary Leaves Powder, Ethiopia

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Abstract

The study aimed to evaluate the raw cow milk preservation using extracted mango seed kernels and rosemary leaves powder. Fresh cow milk samples collected aseptically from Debre Birhan University farm. The fresh cow milk treated at different concentrations (0.5, 1.0 and 1.5%) of ethanol extract of mango seed kernel, rosemary leaves and equal mix of both extracts with 100 ml of milk then store at room temperature. Mango seed kernels and rosemary leaves powder extract had total phenolic content (TPC) value of 97.4 and 49.2 mg GAE/g, respectively. Similarly, Mango seed kernels and rosemary leaves powder extracts had the total flavonoid content (TFC) value of 36.7 and 34.4 mg CE/g, respectively. The parameters considered for this study were titratable acidity, pH, Total Aerobic Mesophilic Bactria Count, Total Coliform Count and Total Yeast and Mold Count after 0, 24, 48 and 72hrs. The results of the current study were showed the values of titratable acidity and pH recorded at 1.5% mango kernel extract up to 48 hours were 0.17 and 6.52, respectively. The lowest TAMBC and TCC of 7.96 and 4.30 logcfu/ml respectively were observed at 1.5% mango kernel extract at 48 hrs. The result showed that titratable acidity, pH, TAMBC and TCC significant differences ($P < 0.05$) using MSKE with 1.5% until 48hours. In conclusion, ethanol extract mango kernel was higher antimicrobial activity than rosemary leaves without sensory and health effect on consumers. Further study by increasing proportion up to 2% of mango seed kernels extracts effect on physical and microbial effect of milk is essential, but rosemary extract used above 1.5%, that poor sensory on milk and health impact on consumers effects.

Keywords: Milk preservation, mango seed kernel extracts, rosemary extracts, Microbial Analysis, Yeast

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Received Date: March 17, 2025
Accepted Date: April 24, 2025
Published Date: April 26, 2025

Citation: Zinaw Zewdie, Abebe Bereda, Abebe Agonafir, Maradona Berhanu, Mekasha Seid. Evaluation of Raw Cow Milk Preservation Using Extracted Mango Seed Kernels and Rosemary Leaves Powder, Ethiopia. Research & Reviews: Journal of Dairy Science & Technology. 2025; 14(2): 20–28p.

INTRODUCTION

Milk is globally used for commercial purposes can be produced mainly from mammalian species namely cattle, buffalo, goat, sheep, and camel. However, the majority (83.3%) commercial milk is produced cattle followed by buffalo (15%), goat (4%), sheep (1.1%), and camel (0.2%) [1]. Milk has complex chemical composition with high-water activity and nutritional value that can serve as an excellent medium for the growth and multiplication of microorganisms [2]. The pathogenic microorganisms which can potentially cause food-borne diseases originate from different sources, such as air, milking equipment, feed, soil, cow, urine, feces and grass [3]. Milk must be stored in refrigerator and transported in cold chain from the moment of milking until it reaches to consumers' hand [4].

In Ethiopia, milking is practiced almost twice a day and mixing of evening and next morning fresh milk [1]. The time taken to deliver raw milk to collection center is longer due to a lack of transport and market chain [2]. Earlier research conducted in Ethiopia revealed that the microbial quality of milk is not within the acceptable limits of 5 log cfu/ml [5]. The major problems of fresh milk marketing in rural areas where 98% milk is produced, are long distance from producer to collection center, the volume of milk per single milking, storage and transportation container, and milking practice of producers, such factors to facilitate raw milk spoilage before reach to the processing plant [6]. The fresh raw milk becomes high in microbial load before it reaches to processing plant, even if processed products are also low quality based on the initial number and types of spoilage microbes in raw milk [3]. The problems are more common in developing counties, like Ethiopia, where shortage of cooling facility and other necessary infrastructures is prevalent.

Many plants and extracted products have antioxidant and anti-microbial properties and as a result addition of plant products are widely reported in dairy products [4]. However, the efficiency of those plant products varies; for instance, mango seed kernels have the highest antioxidant activity of many fruits because of the highest polyphenolic concentration. Gebeyew et al. (2016) and Legesse et al. (2015) [6, 7] reported that milk treated with methanolic mango seed kernel extract (MSKE) the pH remained (6.6-6.1) higher than that of control sample (6.6-4.7) after incubation time of up to 8 hours at 25°C. The authors also indicated that, the total bacterial count and coliforms growth was completely inhibited. Rosemary extracts used as ghee preserve for long time by retard the microbial growth and oxidative degradation safe for human consumption [3]. Ethanolic extracts of rosemary were found to have greater antioxidant activity than synthetic antioxidant [8]. Rosemary (Azmerino) extract site at cooled storage for successful function of antioxidant and antimicrobial compounds for 90 days after extraction. SAS Institute Inc. (2008) [9] reported that raw cow milk treated with ethanolic rosemary extract at 0.003% or 300 ppm showed lower total bacterial and coliform counts after 6 hours at 25°C. The current study was designed to achieve the following objectives [10].

General Objective

The main goal of this study was to determine anti-microbiological activity of ethanol extracted mango seed kernels and rosemary leaves powder on fresh cow milk.

Specific Objectives

- To assess the microbial quality of raw milk preserved using ethanol extract of mango seed kernels and dry rosemary leaves powder.
- To evaluate the antioxidant activity and phenolic content of mango seed kernels and rosemary leaves powder extracts.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at Debre Berhan University, College of Engineering (Chemical Engineering Laboratory, College of Agriculture and Natural Resource Sciences, Department of Animal Sciences, Dairy Microbiology Laboratory.

Plant Sources, Preparation and Determination of Phytochemicals

The dried sliced kernel and rosemary was grinded using coffee grinder until too fine powder (Nadja et al., 2019). Fifteen-gram powdered rosemary leaves, and mango seed kernel powder were extracted independently with 1:10 (w/v) of absolute ethanol (96%) at specific temperature (78°C) using soxhlet apparatus. The percentage yield after soxhlet extraction calculated using the formula (Mustefa, 2015). Quantitative determination of phytochemicals – Based on the analysis method – 0.4 ml of each extract was mixed with 2 ml of 10 % Folin-Ciocalteau reagent and 1.6 ml of 7.5% Na_2CO_3 and left at room temperature for 30 min. Antioxidant activity of MSKE and RE measured as per recommendation.

Determination of Physical Properties of Raw and Treated Milk

Milk sample was filtrated with muslin cloth and measured physical properties, and chemical composition was measured using Lactoscan. Titratable acidity was measured by 0.1 N NaOH using phenolphthalein as an indicator. Upon arrival at the laboratory, the pH values of the milk were measured using a pH meter.

Experimental Design

The experiments were homogenous and external factors that can affect the experiment can be controlled or conducted in a laboratory designed by complete random design (CRD) with two replications. The raw cow milk was divided into 10 equal parts: the first part was left without any treatment as a comparison sample(control), the other nine parts were treated at different concentrations (0.5, 1.0 and 1.5%) of ethanol extract of MSKE, RE and equal mix of the both extracts add to 100 ml of raw cow milk at room temperature To determine the individual and combined effects of both plant extracts on physical properties (pH, TA), aerobic mesophilic bacteria, coliform count, yeast and mold) at 0, 24, 48, and 72 hours of treated and control raw milk at room temperature.

MICROBIAL ANALYSIS (TAMBC, TCC and TYMC)

Total Aerobic Mesophilic Bacterial Count (TAMBC)

Milk samples were serially diluted by adding 1mL of the test portion into 9 mL of 0.1% sterile peptone water. Such dilutions were made so that plate counts range between 30 and 300 colonies. Finally, colony counts were read using a colony counter and bacterial colonies in each petri dish per ml milk were recorded and calculations were made using the following formula given by APHA (1992).

$$N = \sum C$$

$$(1 \times n_1) + (0.1 \times n_2) * d \quad (1)$$

where

N = number of colonies per ml milk sample.

$\sum C$ = sum of all colonies in plates counted.

n_1 = number of plates used in lowest dilution counted.

n_2 = number of plates used in highest dilution counted.

d = dilution factor of the lowest dilution used.

Total Coliform Count (TCC), Total Yeast and Mold Count (TYMC)

Samples of milk were serially diluted following similar methods as for total bacterial count, but dilutions were surface plated on Violet Red Bile Agar solution (VRBA) in sterile Petri dish. Total yeast and mold count (TYMC) the samples of milk serially diluted following similar methods as for total bacterial count but dilutions were surface plated on Potato Dextrose Agar (PDA).

Method of Data Analysis

The required data was processed and analysed using SAS, 2008 Version 9.1 with critical difference value at ($P < 0.05$). Also, Minitab software version 21 was used for main effect, interaction and regression procedure of independent and dependent variables. Calculated by the following model:

$$Y_{ijkl} = \mu + \alpha i + \beta j + \gamma k + (\alpha\beta)\, ij + (\alpha\gamma)\, ik + (\beta\gamma)\, jk + (\alpha\beta\gamma)\, ijk + eijkl \quad (2)$$

RESULTS AND DISCUSSION

Physicochemical Characteristics of Raw Cow Milk

The physicochemical characteristics of fresh milk samples results are indicated in Table 1. The mean percentage of moisture, fat, protein, lactose, salt and total solid of raw cow milk sample were 87.46%, 4.40%, 2.99%, 4.35%, 0.62% and 12.36%, respectively. On the other hands, the average pH and titratable acidity reported for raw milk collected for this study was 6.61 and 0.16, respectively. The protein content (2.99%) of raw milk in the current study was slightly lower than the minimum value of

milk protein (3.2%). The low protein content of the milk in the present study may be due to fats in the diet or raised in relation to milk fat percentage (https://www.ncbi.nlm.nih.gov/books/NBK218193/).

According to report that average fat content (4.40%) of raw milk samples in current study was to the similar value (4.25%). The minimum fat content of raw milk should not be less than 3.5%. However, in the current study the fat content observed (4.40%) was higher than the minimum fat content of raw milk set by ESA. The fat content of milk can be affected by factors, such as feed, parity, and stage of lactation. The average lactose content (4.35%) of raw milk samples in current study was higher than the value (3.79%). In Harar milk shed, Eastern Ethiopia. However, lactose content of raw milk samples in current study was lower than the value (4.69%), whereas, the lactose content of milk in the current study was the same as lactose content (4.35%) of Ethiopian standards.

The average salt content (0.62%) of the raw milk samples in current study was lower than the value (0.76%). However, similar salt content of milk (0.62%) was found in central highlands of Ethiopia. According to the ESA (2009) the total solids content of raw cow milk should not be less than 12.80% though slightly lower average total solids (12.36%) contents of raw milk was observed in the current study. The pH (6.61) value of raw milk samples in current study was higher than the value (6.32) in Shashamane. The pH (6.61) value of raw milk samples in current study was similar as raw milk pH values 6.60 to 6.80 average range as recommended by ESA (2009). The titratable acidity value (0.16%) of raw cow milk samples in current study was lower the value (0.18%) in central highlands of Ethiopia. The composition of milk depends upon the species, breed, season, feed & water, stage of lactation, age, time of milking, weather, etc.

Table 1. The physico-chemical values of fresh raw cow milk.

Variables	Batch 1	Batch 2	Overall Mean
Moisture content (%)	87.67	87.62	87.46
Fat (%)	4.19	4.61	4.40
Protein (%)	2.99	2.99	2.99
Lactose (%)	4.48	4.22	4.35
Salt (%)	0.67	0.56	0.615
Total solids (%)	12.33	12.38	12.36
pH	6.62	6.60	6.61
Titratable Acidity	0.16	0.16	0.16

Phytochemicals in Extracted Plants

The results of the current study showed that the number of extracts obtained using Soxhlet apparatus and ethanol solvent were 7.5 ml (12.5%) and 6.51 ml (10.85%) from 60 g of mango seed kernels and 60 g of rosemary leaves powder, respectively. Similarly, that ethanolic extract of mango seed kernel was 13.24%, which is greater than hexane extract in Nigeria. The total phenolic contents in ethanol extract for MSKE and RE were 97.4 and 49.2 mg GAE/g DW, respectively. Higher total phenolic content (80 mg GAE/g DW) of rosemary leaves than the current finding. This difference may be types of extraction methods, stage maturity of plants, time of extraction and temperature.

The amount of total flavonoids content (TFC) is expressed as mg of catechin equivalents per gram of dry weight (mg CE/g DW). The flavonoids content of ethanol extracts of mango seed kernels and rosemary leaves were 36.7 and 34.4mg CE/g DW, respectively (Table 2). In the current study the flavonoid content of mango seed kernels extract was almost equivalent to rosemary extract, while the previous researchers reported that ethanolic extract from mango seed kernels showed higher flavonoids contents (164.6 g QE/kg).

The DPPH activity of ethanol extracts of mango seed kernels and rosemary leaves were 81.32 and 62.51%, respectively. The antioxidant activity of methanolic extract from mango seeds kernels against

using DPPH was 83.56 mg AA/ml, which is agreed with this study. Mango seed kernels extract from single and mixed mango varieties did not show significant differences in the phytochemical content and biological activity. The antioxidant activity of mango seed kernel**s** and rosemary leaves might be due to the ability of phenolic compounds that donate hydrogen ions, which can prevent the oxidation and deterioration of food substances on course of storage. High antioxidant activity makes the plant a good organic preservative or additives to prevent deterioration of any food.

Table 2. Quantitative determination of phenolic, flavonoid content and antioxidant.

Compound	MSKE	RE
TPC (mg GAE/g dw)	97.4	49.2
TFC (mg CE/g dw)	36.7	34.4
DPPH % inhibition (50 µm)	81.32	62.51

Note: TPC = Total Phenolic Content, TFC = Total Flavonoid Content, DPPH = 2,2-Diphenyl-1-Picrylhydrazy

EFFECT OF PLANT EXTRACTS ON PHYSICAL PROPERTIES OF MILK

Titratable Acidity

The acidity of the treatments in control group (T_1) increased from 0.16 to 0.58% when the storage time increased from 0 to 72 hours at room temperature, respectively. The acidity of milk developed due to the breakdown of milk sugar (lactose) into lactic acid by the fermentative effect of acid producing bacteria. All treated milk samples showed significant difference ($P < 0.05$), compared with control up to 24 hours, except T_4 which had the most acceptable acidity (0.17%) found up to 48 hours. However, after 48 hours, in all the treatments milk became unacceptable with respect to acidity. Similarly, addition of 0.5% of water extract of betel leaves to the raw milk resulted in acidity acceptable up to 11 hours of storage. The titratable acidity (0.16) results obtained from the current study were lower than 0.25 reported in Debre Berhan. The production of acid in milk is normally termed souring and the sour taste of milk is due to lactic acid production. The titratable acidity of milk has long been recognized and employed as an indicator of quality. It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after the milk is drawn from the udder. Normal fresh milk should have an apparent acidity of 0.14 to 0.16 (Table 3).

Table 3. Determination of titratable acidity (TA%) of treated milk.

Time	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	P value
0	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	
24	0.32	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.16	0.16	
48	0.42	0.24	0.19	0.17	0.18	0.18	0.18	0.18	0.18	0.19	
72	0.58	0.28	0.172	0.18	0.19	0.192	0.24	0.22	0.19	0.19	
PE × C × ST										P = 0.006 < 0.05	

Note: T = Treatment (Appendix 4), PE – plant extract, C – Concentration, ST – storage time.

pH

The changes in pH of raw milk samples during storage at room temperature under different concentrations (0, 0.5, 1.0 and 1.5%) of ethanol extract of MSKE, RE and equal mix of the extracts as a natural preservative are given in Table 4. The pH of the control group (T_1) decreased from 6.61 to 4.02 at the end of 72hours storage period, while the remaining treatments (T_2 up to T_{10}) mixed by using different concentrations (0, 0.5, 1.0 and 1.5%) of ethanol extract of plants, the pH decreased slightly ($P < 0.05$), especially with 1.5% concentration of MSKE extract (T_4) from 6.61 to 6.52 at storage period 48 hours (Table 4). At 17 and 18 hours after preservation, adding 1.5% Tulsi and 1.5% Neem extract to milk resulted in a slower pH decrease in the treated sample than control in Egypt, respectively.

EFFECT OF PLANT EXTRACTS ON MICROBIAL QUALITY OF MILK

Microbiological analysis of cow milk treated with ethanol extract of mango seed kernels and dry rosemary leaves extraction was conducted to determine the effect on microbial quality of raw milk preservation at room temperature are presented.

Table 4. pH values of raw and plant extract treated milk during storage time.

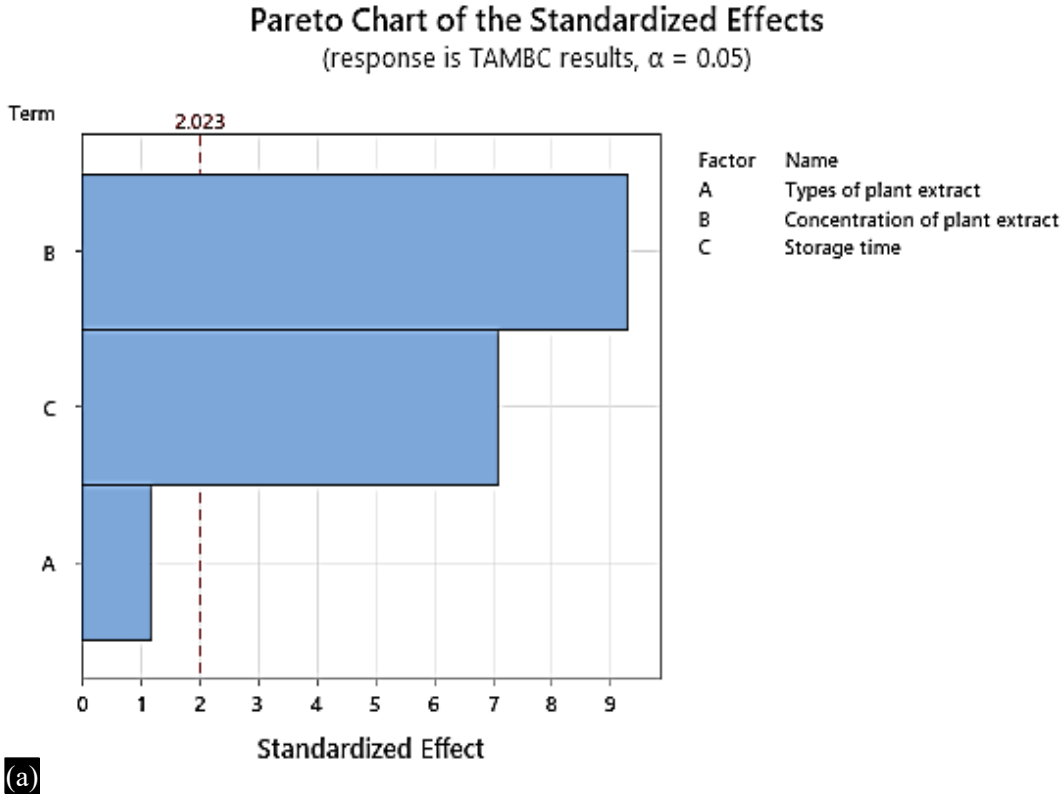
ST	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	P value
0	6.61	6.61	6.61	6.61	6.61	6.61	6.61	6.61	6.61	6.61	
24	5.04	6.22	6.52	6.59	6.01	6.45	6.54	5.84	5.91	6.51	
48	4.32	5.24	6.08	6.52	5.2	5.93	6.29	5.08	5.93	6.25	
72	4.02	4.42	5.54	6.38	4.01	5.34	5.54	4.4	5.34	6.19	
PE × C × ST										p = 0.002 < 0.05	

Note: ST = Storage Time in hours.

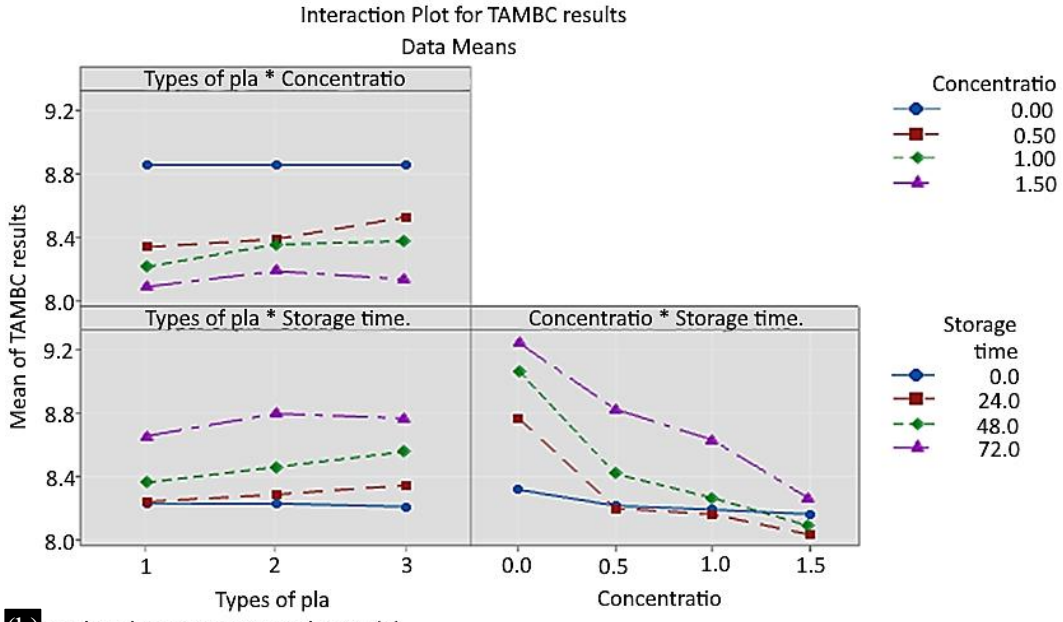
Total Aerobic Mesophilic Bacterial Count (TAMBC)

In the current study three factors (types of plant extract, level of addition and storage time) that effects on treatments by individual and their interaction were studied. In Minitab results (Figure 1(a)) the factor which above the red line are, considered, as critical factor of the treatments (i.e., the concentration of plant extract which represent letter B) was the highest impact of the treatments, storage time next to level of concentration, types of plant extract the last factors from both fine adjustment (B > C > A) (Figure 1(a)). Concentration was the main effects on TAMBC than storage time and types of plant extracts when increasing or decreasing. The concentration of plant extract was the main effects on TAMBC that increasing or decreasing of concentration of plants extract affect more than storage time and types of plant extract. Storage time also a critical factor next to concentration of plant extracts. However, the types of plant extract were least critical factors on TAMBC.

On two-way interaction from software results (Figure 1(b)), types of plant extracts (MSKE-1) with storage time meet at 24 hours the TAMBC become lower values. Next, the interaction between types of plant extracts (MSKE-1) and concentration of plant extracts at 1.5% were the best results of TAMBC. Then the interaction of concentration of plant extracts at 1.5% and storage time less than 48 hours were lower value of TAMBC. From the graph to conclude that MSKE had significant effect on decreasing TAMBC with 1.5% concentration and less than 48hours storage time.



(a)

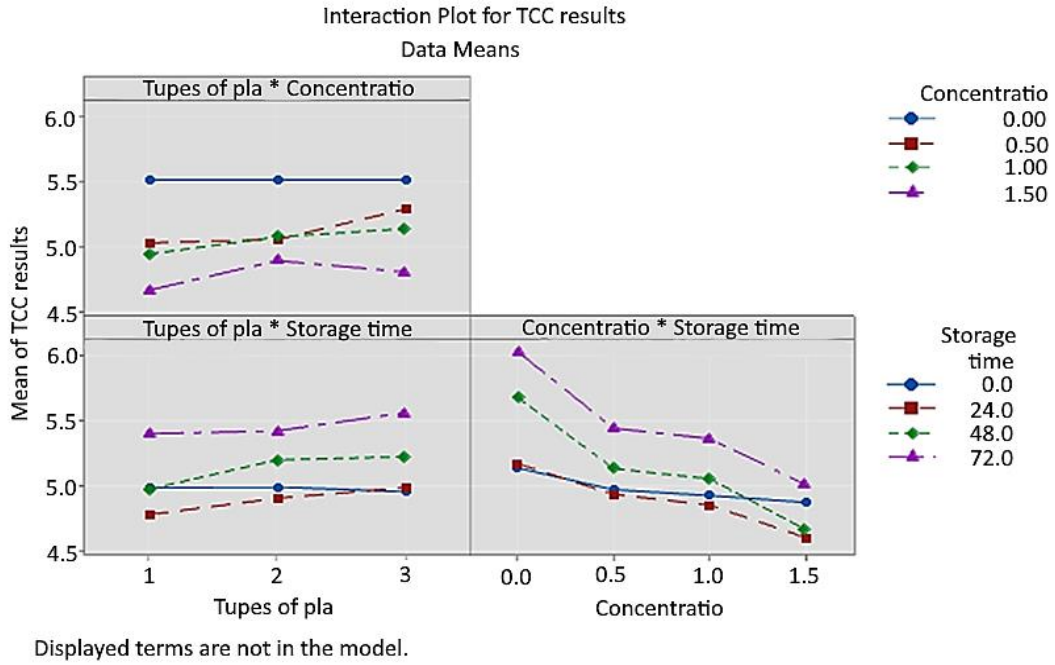


(b) Displayed terms are not in the model.
MSKE = 1, MIX = 2, RE

Figure 1. Single and interaction effect of independent and dependent variable (TMBC). (a) Pareto chart of main (Single) effect, (b) Interaction effect of plant extract, concentration and storage time with TAMBC.

Total Coliform Count (TCC)

In Minitab software results (Figure 2), types of plant extracts (MSKE) with storage time at 24hours the TCC become lower value. Next, the interaction between types of plant extracts (MSKE) and concentration of plant extracts at 1.5% gave the best results with respect to TCC. Then the interaction of concentration of plant extracts at 1.5% and storage time at 24 and 48 hours showed lower value of TCC. From the graph it can be inferred that types of plant and concentration level of plant extracts were directly related to TCC, but storage time was indirectly related to TCC of raw milk quality.



Displayed terms are not in the model.

Figure 2. Single and Interaction effect of independent and dependent variable (TCC).

Total Yeast and Mold Counts (TYMC)

Yeast and molds may be found as part of the normal flora of a food product on inadequately sanitized equipment or as airborne contaminants. Fungi are spoilage microorganisms that grow in foodstuffs during storage, reducing their nutritional value and sometimes producing mycotoxins as a result, it makes the food become unfit for consumption. Plant extracts can contribute to delay or prevent the formation of mycotoxins (Table 5).

Table 5. Yeast and mold count (YMC) of raw, and plant treated cow milk (log 10 CFU/ml).

ST	Cont.	MSKE (%)			Combined (%)			RE (%)			P Value
	T1	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*	*T9*	*T10*	
0	0.88	0.74	0.78	0.69	0.78	0.74	0.78	0.78	0.69	0.81	
24	1.20	0.98	0.74	0.74	0.78	0.78	0.65	0.98	1.23	0.88	
48	1.36	1.18	1.16	0.69	0.81	0.81	0.60	1.11	1.23	1.19	
72	1.78	1.31	1.22	0.93	1.38	1.27	1.08	1.33	1.41	1.49	
PE × C × ST											$p = 0.69 < 0.05$

This study indicated that there is no significant difference among treatments in yeast and mold count ($p > 0.05$). The mixed factorial treatment (types of plant extract, level of concentration of plant extract and storage time) showed non-significant results. Naturally, yeast and mold that can be grown wide range of pH, even if milk storage time increased. The results showed that the YMC in untreated raw cow milk increased from 0.875 to 1.78 in log 10 CFU/ml after 72 hours of preservation at room temperature. All yeast and mold count in this study were still lower than the acceptable limit. Mango peel, mango seed extracts with antioxidant, and anti-yeast properties tested against clinically pathogenic *(Candida species)* and food-spoilage yeasts. Based on different researchers, cinnamon, ginger, garlic and mustard has good antifungal activity for different dairy products and other foods.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Ethiopia has huge potential to produce more milk due to number of cattle; however, the productivity of dairy animals is quite lower than expected level. The problem is also aggravated by the poor quality of milk in the country due to poor hygienic practices of milk across the countryside. This may pose serious health problems besides huge economic loss of the country. Plants are used as common food additives worldwide to enhance the sensory qualities of foods and extend their shelf life by reducing or eliminating spoilages and pathogenic microorganisms. In current study, adding ethanol extracted mango seed kernels and rosemary dry leaves powder used as to improve the shelf life of raw cow milk at room temperature. About 1.5% mango seed kernels extract performed well on physical and microbial quality parameters of milk up to 48 hours. Hence, further study is required for reducing daily loss of several liters of milk by spoilage due to market problems. It is advisable for smallholder dairy farmers to keep evening milk until next morning and transported to the milk cooperatives by addition of mango seed kernels extracts.

Recommendations

- Further investigation on the effects of the extracts of various concentrations on both the *in-vitro* and *in-vivo* antimicrobial activity (single species of microbes) is necessary.
- Further research will require mixture of each plant extracts interaction (antagonistic or synergetic).
- Dairy processing plant owner special training and subside cost for milk supplies in each extract uses and their economic benefits.

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