EVALUATION OF RAW COW MILK PRESERVATION USING EXTRACTEDMANGO SEED KERNELS AND ROSEMARY LEAVESPOWDER, ETHIOPIA

A Thesis Submitted to the Department of Animal Sciences, College of Agriculture and Natural Resource Sciences, College of Graduate Studies

DEBRE BERHAN UNIVERSITY

MSc. Thesis

Zinaw Zewdie

December,2023

DebreBerhan, Ethiopia

EVALUATION OF RAW COW MILK PRESERVATION USING EXTRACTED MANGO SEED KERNELSAND ROSEMARY LEAVES POWDER, ETHIOPIA

A Thesis Submitted to the Department of Animal Sciences, College of Agriculture and Natural Resource Sciences, College of Graduate Studies

DEBRE BERHAN UNIVERSITY

In Partial Fulfillment of the Requirements for Degree of Master of Science (in Dairy Science and Technology)

ZinawZewdie

Major Advisor: AbebeBereda (PhD.)

Co- advisor: Abebe Agonafir (DVM, MSc.)

December, 2023

DebreBerhan, Ethiopia

COLLEGE OF GRADUATE STUDIES

COLLEGE OF AGRICULTURE AND NATURAL RESOURCE SCIENCES

DEBRE BERHAN UNIVERSITY

APPROVAL SHEET – I

This is to certify that the thesis entitled. Evaluation of Raw Cow Milk Preservation Using ExtractedMango Seed Kernels and Rosemary LeavesPowderat DebreBerhanUnivesity , Ethiopia submitted in partial fulfilment of the requirements for the degree of Masters of Science in Dairy Science and Technologyof the Graduate Program of the Department of Animal Science, College of Agriculture and Natural Resource Sciences, DebreBerhan University and is a record of original research carried out by ZinawZewdie, PGR/ 112/13 under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during this investigation have duly acknowledged. Therefore, I recommend that it accepted as fulfilling the thesis requirements.

AbebeBereda(PhD.) _____ _____

Name of Major Advisor Signature Date

Abebe Agonafir (DVM, MSc) _____ _____

Name of Co-Advisor Signature Date

COLLEGE OF GRADUATE STUDIES

COLLEGE OF AGRICULTURE AND NATURAL RESOURCE SCIENCES

DEBRE BERHAN UNIVERSITY

APPROVAL SHEET – I I

Undersigned members of the board of the examiners of the final open defence by ZinawZewdie have read and evaluated his thesis entitled. Evaluation of Raw Cow Milk Preservation UsingExtracted Mango Seed Kernels and Rosemary Leaves Powder at DebreBerhanUnivesity, Ethiopia, and examined the candidate, therefore to certify that the thesis has been accepted in partial fulfilment of the requirements for the degree of Master of Science in Dairy Science and Technology.

_____	_____	_____
Name of Chairman	Signature	Date
_____	_____	_____
Name of Major Advisor	Signature	Date
_____	_____	_____
Name of Internal ExaminerSignature		Date
_____	_____	_____
Name of External Examiner	Signature	Date

Final approval and acceptance of the thesis are contingent upon the submission of the final copy of the thesis to the Council of Graduate Studies (CGS) through the department graduate Committee (DGC) of the candidate's major department.

STATEMENT OF THE AUTHOR

I declare that, this thesis is my genuine work, and that all sources of materials used for this thesis have been profoundly acknowledged. This thesis submitted in partial fulfilment of the requirements for a Master of Science (MSc.), in Dairy Science and Technology at DebreBerhan University and it deposited at the University library to available for users under the rule of the library. I intensely declare that this thesis not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Short excerpts from this thesis permitted without additional authorization as long as the source correctly cited. Requests for permission to use a lengthy quotation form or to reproduce this document in completely or in part may granted by when in his/her opinion the proposed use of the material is in the interest of scholarship, the head of the major department or the dean of the college of graduate studies. However, in all other cases, consent from the thesis' author and advisors is required.

Name: ZinawZewdie Signature: _____

Place: College of Agriculture and Natural Resource Sciences, DebreBerhan University.

Date of Submission: _____

ACKNOWLEDGEMENTS

Above all, I want to thank the Almighty and Compassionate God, Saint Mary, his mother, for making it possible for everything to accomplish.

Foremost, I would like to express my sincere gratitude to my advisors Dr. AbebeBereda from Federal Technical and Vocational Training Institute and Dr. AbebeAgonafir, DebreBerhan University for their unreserved advice with great affability, enthusiasm, and immense knowledge they provided me throughout my study.I benefited greatly from their direction, feedback, recommendations, and wise counsel during the entire research process as well as while I was writing my MSc. Thesis.

I would like to express my gratitude to Mrs. EmbetLegese (MSc.), department head of Animal Science, DebreBerhan University, College of Agriculture and Natural Resource for her facilitation and supporting the success of the study.

Last, but not least, I am grateful to my family for their unreserved encouragement, support, brainwave, and motivation during the study. I would like to extend my genuine gratitude to my best family Mrs. MuluneshDemssieGetaneh, Mr. AmezeneWorku, AsnakeHailu (Chemilcal Eng. MSc.) and Mr. Belay GirmaEshetie for all their diligent inspiration, backup, and assistance throughout the research period.

LIST OF ABBREVIATIONS

BHA	ButylatedHydroxyaniole
BHT	Butylated Hydroxytoluene
CE	Catein Equivalent
CFU	Colony Forming Unit
CSA	Central Statistical Agency
DPPH	1,1-diphenol-2-picrylhydrazyl
FAO	Food and Agriculture Organization of the United Nations
GAE	Gallic Acid Equivalent
MSKE	Mango Seed Kernels Extract
Ml	Milli Litter
LAB	Lactic Acid Bacteria
RE	Rosemary Extract
Spp.	Species
TAMBC	Total Aerobic Mesophilic Bacterial Count
TCC	Total Coliform Count
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
YMC	Yeast Mold Count

TABLE OF CONTENTS

Content Page

STATEMENT OF THE AUTHOR i

ACKNOWLEDGEMENTS ii

LIST OF ABBREVIATIONS iii

TABLE OF CONTENTS iv

LIST OF TABLES vi

List of Graphs in the Text vii

List of Graphs in the Appendix vii

ABSTRACT viii

1. INTRODUCTION 1

1.1. General Objective 3

1.1.1. Specific Objectives 3

2. LITERATURE REVIEW 4

2.1. Concept of Milk 4

2.2. Quality of Raw Milk 4

2.3. Source of Raw Cow Milk Contamination 4

2.3.1. Contamination of raw milk in the udder 5

2.3.2. Raw milk contamination after milking 5

2.4. Common Milk Spoilage Microorganisms 5

2.4.1. Effect of spoilage microorganisms on the dairy industry 6

2.4.2. Effect of Spoilage Microorganisms on Public Health 6

2.5. Raw Cow Milk Preservation Methods 6

2.5.1. Traditional raw cow milk preservation 7

2.5.2. Preservation of cow milk using herbal extract 7

3. MATERIALS AND METHODS 9

3.1. Description of the Study Area 9

3.2. Plant Sources and Preparation 9

3.3. Determination of Phytochemicals in Extracted Plants 10

3.3.1 Plant extraction and yield determination 10

3.3.2. Quantitative determination phytochemicals 10

3.3.3. Antioxidant activity 12

3.4. Determination of Physical Properties of Raw and Treated Milk....12
3.4.1. Determine the physico-chemical composition of fresh raw cow milk....12
3.4.2. Titratable acidity....12
3.4.3. pH....13
3.5. Experimental Design....13
3.6. Microbial Analysis....14
3.6.1. Total aerobic mesophilic bacterial count (TAMBC)....14
3.6.2 Total coliform count (TCC)....14
3.6.3. Total yeast and mould count (TYMC)....15
3.7. Method of Data Analysis....15
4. RESULTS AND DISCUSSION....16
4.1 Physicochemical Characteristics of Raw Cow Milk....16
4.2. Phytochemicals in Extracted Plants....17
4.3. Effect of Plant Extracts on Physical Properties of Milk....18
4.3.1. Titratable acidity....18
4.3.2. pH....20
4.4. Effect of Plant Extracts on Microbial Quality of Milk....22
4.4.1. Total Aerobic Mesophilic Bacterial Count (TAMBC)....22
4.4.2. Total Coliform Count (TCC)....25
4.4.3. Total Yeast and Mold Counts (TYMC)....28
5. CONCLUSION AND RECOMMENDATION....30
5.2. RECOMMENDATIONS....30
6. REFERENCES....31
7. APPENDIX....41

LIST OF TABLES

Table 1. The experimental layout 13
Table 2. The physico-chemical values of fresh raw cow milk. 17
Table 3. Quantitative determination of phenolic, flavonoid content and antioxidant 18
Table 4. Determination of titratable acidity (TA %) of treated milk. 19
Table 5. pH values of raw and plant extract treated milk during storage time 21
Table 6. Total Aerobic Mesophilic Bacterial Count (TAMBC) of treated cow milk..... 23
Table 7. Total Coliform Count (CC) of raw and plant treated cow milk (in log 10 CFU/ml). 26
Table 8.Yeast and Mold Count (YMC) of raw and plant treated cow milk (log 10 CFU/ml)…….28

List of Graphs in the Text

Graph 1. Main and Interaction effect of types plant extract, concentration level of plant extracts and storage time with TA……20

Graph-2) Interaction effect of plant extract, concentration level, storage time and pH…...…21

Graph 3. Main and Interaction effect of types plant extract, concentration level of plant extracts and storage time with TMBC……..24

Graph 4. Main and Interaction effect of types plant extract, concentration level of plant extracts and storage time with TCC……27

List of Graphs in the Appendix

Graph-1.Determination of MSKE (a) and RE(c) sample solution by calibration curve TPC (x-axis concentration and Y-axis Absorbance at 765nm.....41

Graph-2.Determination of MSKE (b) and RE (d) sample solution by calibration curve TFC (x-axis concentration and Y-axis Absorbance at 510nm) 43

ABSTRACT

The study aimed to evaluate the raw cow milk preservation using extracted mango seed kernels and rosemary (Azmerino) leaves powder, Ethiopia. Mango seed kernels were collected from local juice-making waste, while rosemary leaves were purchased from DebreBerhan market. The crude extracts were isolated from two plant species using Soxhlet extraction method and characterized for the presence of bio-active compounds quantitatively. Fresh raw milk samples collected aseptically from DebreBirhan University dairy farm.The raw cow milk was divided into 10 equal parts: the first part was left without any treatment to serve as a control group, whereas the remaining nine parts were treated at different concentrations (0.5, 1.0 and 1.5%) of ethanol extract of mango seed kerenls extract, rosemary leaves extract and equal mix of the both extracts mixed with 100 ml of milk at room temperature.The DPPH free radical scavenging assay was used to determine the antioxidant capacity of various extracts, and the absorbance was measured at 517 nm. Mango seed kernels powder extract had the highest total phenolic content (TPC) value of 97.4 mg GAE/g, while rosemary leaves powder extract had the lowest at 49.2 mg GAE/g.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 was titratable acidity, pH, Total Aerobic Mesophilic Bactria Count, Total Coliform Count and Total Yeast and Mold Count after 0, 24, 48 and 72 hours. The results of the current study 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 total aerobic mesophilic bacterial and total coliform counts of 7.96 and 4.30logcfu/ml respectively were observed at 1.5% mango kernel extract up to 48 hours. However, both bacterial counts were observed in the current study was beyond the acceptable consumption limit of Ethiopia standard agency (5.30 to 6.00 log cfu/ml), so the milk reaches to the collection centerbeteewn 24 and 48 hours. The result showed that are titratable acidity, pH, Total Aerobic Mesophilic Bactria Count and Coliform Count) significant differences ($P<0.05$). In conclusion, ethanol extract kernels were higher antimicrobial activity than rosemary leaves extract as result ethanol extracts performed well in milk preservation. Generally, Mango seed kernels powder extracts had the longest shelf life that compared from rosemary and mixed plant extracts tested for milk samples.Further study by increasing proportion up to 2% of mango seed kernels extracts effect on physical and microbial effect of milk is essential.

Keywords: *Milk preservation, Mango seed kernel extracts, Rosemary extracts*

1. 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%) (FAO, 2019). Milk is a complex chemical composition and with high-water activity and nutritional value that can be serving as an excellent medium for the growth and multiplication of microorganisms (ZegeyeshTaye*et al*., 2017). As a result, milk harbor many pathogens and spoilage microorganisms such as, *Pseudomonas* spp, *B. cereus, Clostridium spp., Salmonella* spp., *S. aureus, L. monocytogenes, Campylobacter, Enterobacter* and *E. coli,* whichmay be harmful for consumers. Those pathogenic microorganisms cause for food-borne diseases are originated from different sources such as air, milking equipment, feed, soil, cow, urine, feces and grass (Seema, 2015). Hence milk should protect from contaminants that will be downgrade the quality, safety and shelf life. Milk must be stored in refrigerator and transported in cold chain from the moment of milk until it reaches to consumers' hand (James*et al.,* 2020).

In Ethiopia, milking is practiced almost twice a day and mixing of evening and next morning fresh milk (ZelalemYilma*et al*., 2013). The time taken to deliver raw milk to collection center is longer due to a lack of transport and market chain (KassahunMelesse *et al.,* 2014).Milk delivery twice a day can become very time consuming for some farmers in the rural areas, also resulting in higher risks for deterioration of milk quality. Ideally, travel time to the milk collection center should not exceed one hour for a single trip (FAO, 2002).Moreover, unsuitable storage conditions, unclean udder/teats, poor quality water and dirty hands of milker's increase the total bacteria load of raw milk (GezuTadesse *et al.,* 2015). Earlier researches conducted in Ethiopia revealed that the microbial quality of milk is above the acceptable limits of 5log cfu/ml. For instance, the average AMBC reported that in raw milk in different parts of the country was within the range of 6 to 9.28log cfu/ml (AbebeBeredа*et al.,* 2012; Solomon Mosu*et al.,* 2013).

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 (TeshomeGemechu and TesfayeAmene, 2017). The quality of fresh raw milk becomes high microbial load before reach to processing plant, even if processed products are also low quality based on the initial number and types of spoilage microbes in raw milk (TeklemichaelTesfay*et al*., 2013). Spoilage milk the change of flavor as results of increasing the concentration of free fatty acids and amino acids. The shelf life of milk is limited to few days due to spoilage bacteria. The problems even more common indeveloping counties like Ethiopia, where shortage of cooling facility and other necessary infrastructures.

Milk marketing system in Ethiopia is mainly characterized by informal marketing system in which the majority of the raw milk produced is directly sold to consumers or middlemen that sell raw milk to consumers without passing through processing plants and in the absence of legal processes such as government tax and trade related regulations (BeleteAnteneh*et al*., 2010). Past studies also revealed that distance from point of milk production to milk market had significant impact on the decision to choose milk market channel (BerhanuKuma*et al*., 2013; ZegeyeshTaye*et al*., 2017).

Studies revealed that farm characteristics such as number of cows owned and herd size had significant impact on milk market channel choice decision (Berhanu et al., 2013; Mohammed Ayyano*et al*., 2020; TadeleMamoand Tewodros Tefera, 2013). Similarly, breed type was found to significantly influence milk market channel choice (MengistuKetema*et al*., 2016). Milk buyers' related factors such as purchase frequency and quantity purchased were found to significantly influence milk market channel choice (Berem*et al*., 2015). Previous studies also revealed that milk quality requirement significantly affect the choice of milk market channel (Innocent *et al*., 2018; Singh, 2018). Studies also indicated that milk selling price had a significant influence on milk market channel choice decision (Ishaq*et al*., 2017; Moturi*et al*., 2015; Vykhaneswari and Devi, 2019).

The success of a milk processing plant depends on its ability to source a predictable, sufficient supply of milk, and its ability to assure a sizable market. So many large processors operate at less than 50% capacity, because of sourcing constraints (MulugetaTesfaye*et al*., 2019). Large-scale processors are located near urban areas to facilitate market access and available services. Institutional buyers are very important to many processors (universities,

hospitals, schools or factories can provide a constant and assured customer base. The only option to expand market linkage between producer and processor is by appropriate preservation techniques. Consumers demand milk and milk products with fewer synthetic additives but increased safety and shelf-life. These demands have increased the importance of natural antimicrobials which prevent the growth of pathogenic and spoilage micro-organisms.

Many plants and extracted products have anti-oxidant and anti-microbial properties as a result addition of plant products are widely reported in dairy products (Coralie *et al.,* 2019). However, the strength of effects varied among plant; for instance, mango seed kernels have highest antioxidant activity of many fruits because of highest polyphenolic concentration (Rai *et al*., 2020). Abdalla *et al.* (2007) reported that, milk treated with methanol 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 up to 8 hours at 25^{o}C. The authors also indicated that, the total bacterial count and coliforms growth completely inhibited. In addition, Rosemary (*RosmarinusofficinalisL.*)/*Azmerino/Sigametbesha*/ extracts with ghee which preserve long time by retard the microbial growth and oxidative degradation safe for human consumption (Rahile*et al*., 2017). Ethanol extracts of rosemary found to have greater antioxidant activity than synthetic antioxidants (Renata *et al*, 2015; Raluca *et al*., 2018).Rosemary (*Azmerino*) extract is antioxidant and antimicrobial compound properties over 90 days. Sayed*et al* (2022) reported that raw cow milk treated with ethanolic rosemary extract at 0.003% or 300 ppm showed lower total bacterial and coliform counts reduces after 6 hours at 25^{o}C. The current study designed to achieve the following objectives;

1.1. 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.

1.1.1. Specific Objectives

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

2. LITERATURE REVIEW

2.1. Concept of Milk

Milk is a complex mixture of fat, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water (Hassan and Frank, 2011). It meets the nutritional requirements of human body preferable than any other single food. However, this unique composition and properties make milk an excellent medium for bacterial growth and source of bacterial infection (Claeys*et al*., 2013). Milk-borne pathogenic bacteria pose a serious threat to human health and economic loss (Silva *et al*., 2016).

2.2. Quality of Raw Milk

Quality milk is free from harmful microbes and toxic substances such as antimicrobials and chemical residues like acaricides, sediment and extraneous substances (Ngasala*et al.*, 2015). Thus, milk safety and quality are the combination of the physical, chemical and microbiological qualities of milk (Samarzija*et al.*, 2012). The acidity and pH of milk used as the best indicators of milk quality. Within a short time after milking, the "acidity increases and pH decreases" due to bacterial activity and the release of carbon dioxide (Hossain*et al.*, 2011). The degree of bacterial contamination and temperature are the principal factors, which influence acid formation and decrease of pH.

2.3. Source of Raw Cow Milk Contamination

Microorganisms, which are present in fresh milk, derived from two main sources, from raw milk itself and the exterior of the udder such as equipment, wash water, air, packing materials and personnel (GebeyewKefyalew e*t al.*, 2016).Therefore, poor bacteriological quality of milk results from sick or carrier animal, poor hygienic practices in farms, and poor handling and transportation of milk (TegegneAzage *et al.*, 2013). The quality of raw milk affects the microbiological quality of the finished product (AsaminewTassew and SeifuEyassu, 2010).

2.3.1. Contamination of raw milk in the udder

Bacteria that enter the milk directly from the animal can infect consumers of raw milk. Spoiled feed can introduce organisms into a cow's udder that released into the milk (MekibibBerhanu*et al.,* 2010). Many studies showed that antibiotic used for dairy herds leads to the development of antibiotic resistance and its cause public health problem (GebeyewKefyalew e*t al.,* 2016).

2.3.2. Raw milk contamination after milking

Milk can be contaminated with microorganisms from external various sources such as dung, water, soil, personnel, milking environment and types of equipment. (LegesseGaredew*et al.,* 2015). Most tropical countries suffer from problems of keeping raw milk for long periods due to high ambient temperatures, poor transportation facilities and high bacteria counts of raw milk (Aprodu*et al*. 2014). JermenMamo*et al* (2016) reported that Total Aerobic Mesophilic Count of raw cow milk samples from five districts of the North Shoa Zone Amhara Region is greater than the acceptable value for America, which is less than $3x10^5$ CFU/mL and Ethiopian $2x10^6$ CFU/mL. The microbial count of milk is over the permitted limits of 5log cfu/ml. Earlier research done in Ethiopia, the average aerobic mesophilic bacterial counts (AMBC) reported for raw milk in various regions of the country ranged from 6 to 9.28 log cfu/ml (Solomon Mosu*et al*., 2013).

2.4. Common Milk Spoilage Microorganisms

Milk spoilage marked by a change in flavour and an increase in the quantity of free fatty and amino acids (Seema, 2015). Many types of bacteria that use food as a carbon and energy source mediate chemical reactions that result in unpleasant sensory alterations in milk. The majority of these species are bacteria, with yeast and moulds making up the minority. Several bacteria, including Pseudomonas spp., Bacillus spp., Alcaligenes, Microbacterium, Clostridium, and Salmonella spp., cause milk spoilage.Several enterotoxins produced by Staphylococcus aureus and E. coli are known to cause human infections, intoxication, and raw milk spoiling (Higginbotham *et al*., 2014). The three human-pathogenic fungi *Candida*

spp., Aspergillus spp., and Alternaria species are found in milk and milk products (Elshafie and Camele, 2017).

2.4.1. Effect of spoilage microorganisms on the dairy industry

Even with contemporary dairy preservation procedures, the process of milk deterioration is complicated, and a lot of food is lost because of microbiological spoilage. Microbes are responsible for visible or non-visible defects in milk, such as off-odour and flavour, and lead to significant waste (Seema, 2015). Due to the highly perishable nature of milk and mishandling, the amount produced subjected to high post-harvest losses up to 40% have been reported in Ethiopia for milk and dairy products from milking to consumption (Habtamu Korma, 2018). Microbial spoilage is found to be one of the causes that offer the losses. Some modification to milk spoilage includes souring, gas production, proteolysis, ripening, change in milk fat, creation of alkalis, flavour defects, and colour defects (AmentieTadesse*et al*., 2016).

2.4.2. Effect of Spoilage Microorganisms on Public Health

Around 90% of all disease disorders associated with dairy consumption caused by pathogenic bacteria found in milk, which are a severe threat to human health. Both pathogen and spoilage were capable of more rapid growth than the indigenous microflora. *S. aureus* and *salmonella* have encountered in raw milk(Mohamed *et.al.* 2014).

2.5. Raw Cow Milk Preservation Methods

Naturally, milk is a highly perishable food that needs protection from spoilage during harvest, preparation, storage, and distribution to give it the desired shelf life (AmentieTadesse*et al*., 2016). The temperature of raw milk kept in the storage tank at the collection center maintained at 4 °C or below and delivered to the processing plant within 24 hours. Due to this reasons, raw milk preserved by several methods or processed in different products should be immediately after milking.Preservation of raw milk from spoilage by traditional like smoking, Natural/biopreservation/plant and their extracts and modern milk preservation like refrigeration, heat treatment, microfilitration, high pressere treatment and chemical

preservatives (TewodrosBimerow*et al*., 2016). Methods of preservationselect by cost, availability, and quality of product after preservation on human health effect.

2.5.1. Traditional raw cow milk preservation /Smoking/

According to TsegayLijalem and GebreegziabherZeru (2015), almost all milk producers in peri-urban and rural areas smoke milking and milk storage utensils to add flavour and aroma, extend shelf life, facilitate fermentation, and only slightly prevent bacterial development. It was typical for milk producers to wash their utensils after use, which had a detrimental impact on some cooperatives and made milk taste and smell bad. The most frequently used plant for smoking milk vessels were *Olea Africana, 'mitie', Clerodendrummyricoides, Terminalia brownie* and *Juniperousprocera* in south wollo (TewodrosAlemu and MulukenGirma, 2018). In addition, small pieces of plant materials may have left on the milk that makes it unattractive for consumers (BirhanuYeserah*et al*., 2020) and facilitate the fermentation of raw milk which breakdown of lactose by Lactic Acid Bacteria (LAB) before reaches to processing plant.

2.5.2. Natural preservation of cow milk using herbal extract

The refrigerated storage of raw milk controls efficiently the proliferation of mesophilic spoilage populations, while it favors simultaneously the growth of psychrotrophic flora. The psychrotrophic bacterial populations developing in milk are represented mainly by Gram-negative genera including Pseudomonas, Aeromonas, Achromobacter, Alcaligenes, Flavobacterium and Serratia and a minor number of Grampositive genera including Bacillus, Clostridium, Corynebacterium, Lactobacillus, Streptococcus and Microbacterium (Asma*et al*., 2022). Sayed*et al*.,(2022) reported that preservative effectiveness is highly related to the conditions of the foods, such as moisture content, pH and the oxidation-reduction potential of the food.

Herbal preservation produced from different plants and their products as excellent sources of phenolic compounds with potential antimicrobial activity next to nutritional sources (Samah and Ahmed, 2019). It is better than chemical preservatives based on nutritional quality and less harmful residual effects on human health. At present, there is a shift to natural

antioxidants due to serious health concerns posed by synthetic antioxidants; however, synthetic antioxidants tend to be cheaper compared to natural ones (Panghal*et al.*, 2018). According to Modi *et al*. (2017), the plant extracts in trace amount does not cause any change in the nutritional composition of the milk. Mango seed kernels and rosemary leaf powder ethanol extracts evaluated individually and combined to preserve raw cow milk at room temperature.

2.5.2.1. Preservation of cow milk by mango seed kernels extract

Mango seed kernel have high polyphenolic content and antioxidant activity than other fruit seeds like tamarind, avocado, and jackfruit, which is why it is used industrially as a functional food ingredient (Rai *et al*., 2020). Dorta*et al* (2012) reported that kernel extract (12% in yield) possessed high antioxidant activity. It protects vitamin C against destruction and is important for natural antioxidant and anti-microbial which is responsible for the removal of free radicals scavenging reactive oxygen and binding metal ions in raw milk. Ethanol is effective solvent for extracting mango seed kernel with high yield and polyphenol content without involving heating (Dent *et al*., 2013).

2.5.2.2. Preservation of cow milk by dry rosemary leaves extract

Rosemary (*Rosmarinus officinalis L*.) contains several phytochemicals, which well known to retard microbial growth and oxidative degradation (Embuscado, 2015; Mangang*et al.*, 2020). Ethanol extract's antioxidant and antimicrobial activities mainly rely on its phenolic acid, flavonoid, rosmarinic acids and deterrence contents (Jordan *et al.*, 2012). Hence, rosemary extract successfully used as an antioxidant to preserve the lipids in cow milk that been supplemented with fish oil (Qiu, *et al*., 2018). Rosemary Extract (RE) found to have effective free radical scavenging activity in cheese spread (Santos *et al*., 2012). Additionally, these extracts found to be more effective than commercial mixtures of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylatedhydroxyaniole BHA, and propyl gallate in stabilizing vegetable oils and animal fats.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study conducted at DebreBerhan University, College of Engineering (Chemical Engineering Laboratory, College of Agriculture and Natural Resource Sciences, Department of Animal Sciences, Dairy Microbiology Laboratory. DebreBerhan is located at 130 kilometers Northeast of Addis Ababa. DebreBerhanregio-politanthas a latitude and longitude of 9°41′N 39°32′E with an altitude of 2,840 meters (9320ft) above sea level (Molla A., 2013). It covers an area of 18,081.95 hectares and mean annual temperature and rainfall of the area are 6.6°C and 940 mm, respectively. Topography covers 86, 10 and 4% flat, plateau and mountainous terrains, respectively. Brown colored soil is the predominant soil type in the North Shewa Zone (Solomon Ayeleand DemelTeketay, 2018).

3.2. Plant Sources and Preparation

Mango seed kernels and rosemary leaves powder extracts used for raw cow milk preservation at room temperature. The world production of mango (*Mangiferaindica*) fruit is more than 35 million tons while, in Ethiopia, 97.32 thousand tons of mango fruits were produced in 2011 (FAO, 2012). As the seed kernel thrown away as waste and becomes a source of pollution and contains a kernel that makes up 45% to 75% of the entire seed (Hyldgaard*et al*., 2012). In current study, it is collected from different sources from local juice-making and freshly eaten waste from production site low land of Ethiopia (shewarobit and collection center of eat fruit). The kernels enclosed hard covers were separated mannualy. The sliced kernel was dried in the oven at 50^{o}C for 12 hours to constant weight to reduce moisture content. Then the dried sliced kernel was grinding by coffee grinder until to fine powder (Nadja*et al*., 2019). The extraction solvent was eliminated by vaccum evaporation using a rotary evaporator at 30^{o}C. The extracted sample stored in brown vials under nitrogen gas or refrigerator at (4^{o}C) in dark place until to use that prevent oxidation of phenolic compounds.

Similarly; Rosemary (*Rosmarinus officinalis Linn*) fresh leaves from highland part of Ethiopia are purchased in local market then washed and dried in 5 days in a shaded area at

ambient temperature and grinding (AbaynehKassahun*et al.*, 2019). About 15-gram powder rosemary leaves were extracted with 1:10 (w/v) of absolute ethanol (96%) at specific temperature (78^{o}C) using soxhle apparatus (Walid*et al*., 2022). The extraction solvent was eliminated by vaccum evaporation using a rotary evaporator at 30^{o}C. The extracted sample stored in brown vials under nitrogen gas or refrigerator at (4^{o}C) in dark place until to use that prevent oxidation of phenolic compounds.

3.3. Determination of Phytochemicals in Extracted Plants

3.3.1 Plant extraction and yield determination

The percentage yield after soxhlet extraction calculated using the formula below (Mustefa Kemal, 2015). The ethanol solvent extracts non-oil components and antimicrobial compounds due to the presence of OH bonds or polar solvent.

Percentage of extracted yield (%) = mass of extract (m) *100……equation (1)
Mass of sample (M)

m = mass of extract in gram, M = mass of sample powder in gram

3.3.2. Quantitative determination of phytochemicals

Based on the analysis method from Brand *et al*. (1995), 0.4 ml of each extract 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. The mixing solution`s absorbance was measured at 765 nm by UV-spectrophotometer and calculated as gallic acid equivalent. The total phenolic compound (TPC) calculated as the following equation. The lines of the calibration curves were governed by a linear equation of;

$$Y = mX + b \quad \text{equation (2)}$$

Where, y- is the absorbance, X- is the concentration of the standard in ppm, b- is the y-intercept and m- is the slope of the line. Then the concentrations of the sample (in ppm) were obtained from the equation of calibration curves as;

$$X = \frac{Y-b}{m}$$

Where the absorbance result in Appendix 1(a and c), that are important sources to calculate the total phenolic compound (TPC) of both mango seed kernels and rosemary leaves extracts.

TPC = (C * V)/M..... .....equation (3)

Where, C= X= concentration of GA obtained from calibration curve in mg/ml

V= the volume of the extract solutions used in mL

M = the weight of the extracts used in grams

Total flavonoids measurement of MSKE and RE measured as per recommendation of Zhishen*et al.* (1999). 0.25 ml extract, 1.25 ml distilled water and 0.075 ml of 5% $NaNO_2$ incubated for 6 min, and then added 0.15 ml of 10% $AlCl_3$. After 5 min, 0.5 ml of 1.0 M NaOH and 0.275 ml distilled, water added. The absorbance measured at 510 nm by UV-spectrophotometer (model:Beckman Coulter DU 720, USA). The results expressed as mg catechin equivalent (CE)/g seed. Catechin was chosen as a standard, and a standard curve was prepared. Total flavonoid compound (TFC) calculated as the following equation. The lines of the calibration curves were governed by a linear equation of;

Y = mX +b ---------- equation (4)

Where, y- is the absorbance, X- is the concentration of the standard in ppm, b- is the y-intercept and m- is the slope of the line. Then the concentrations of the sample (in ppm) were obtained from the equation of calibration curves as;

$$X = \frac{Y-b}{m}$$

Where the absorbance result in Appendix 1(b and d), that are important sources to calculate the total flavonoid content (TFC) of both mango seed kernels and rosemary leaves extracts.

TFC = (Q * V)/M .....equation (5)

Where,

Q= X= concentration of CE obtained from calibration curve in mg/ml

V= = is the volume of the extract solutions used in mL

M = the weight of the extracts used in grams

3.3.3. Antioxidant activity

Antioxidant activity of MSKE and RE measured as per recommendation of Raghavan *et al*., (2003). 0.2 ml of each sample's extract was combined with a 0.1 mM 1, 1-diphenol-2-picrylhydrazyl/DPPH/ solution in a volume of 2 mL then vortex and allowed to stand at the dark place for 30 mins. The blank was made by replacing the extracted solution with ethanol (0.1 mL). Using a UV- spectrophotometer the mixture absorbance measured against a blank at 517 nm. DPPH scavenging activity calculated based on the following equation:

DPPH Scavenging Capacity (%) = $\frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$…..... equation (6)

Where A_{sample} is the absorbance of the experimental sample when all reagents are present, and A_{blank} is the absorbance of control ethanol, which contains all reagents but not the testing compound.

3.4. Determination of Physical Properties of Raw and Treated Milk

3.4.1. Determine the physico-chemical composition of fresh raw cow milk

Three litter of fresh raw cow milk samples collected from the DebreBerhan University dairy farm at three rounds from cross breed cow. Milk sample was filtrated with muslin cloths and measured physical and chemical composition by lactoscan (Sadia*et al*., 2020).The physicochemical composition of fresh cow milk measured by using a lactoscan after calibration of standard making 3% acidic and 3% basic solution of lactoscan powder, and then it reads raw milk pH, density, freezing point, fat, protein, lactose, salt and total solids.

3.4.2. Titratable acidity

Titratable acidity measured by 0.1 N NaOH and phenolphthalein indicator (AOAC, 2005). Ten ml of milk pipetted into a beaker, and then 3-5 drops of 0.5% phenolphthalein indicator added. Then the sample was titrated with 0.1N NaOH until the pink colour persists. Acidity was expressed as a percentage of lactic acid (LA %) and it was determined as follows.

Lactic acid (%) = $\frac{\text{Vt ml 0.1 NaOH} \times 0.009 \times 100}{\text{Volume (ml) of raw or treated milk sample}}$ ……...equation (7)

3.4.3. pH

Upon arrival of the laboratory, the pH values of the milk were measured using a pH meter (3015 Janway). As per recommendation of manufacturer's instructions, the pH meter adjusted at 7.2. The milk samples pipetted into a beaker and then the pH was read and recorded of control and treated raw milk.

3.5. Experimental Design

The experiment was a factorial experiment 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, 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, titratable acidity/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.

Table 1. The experimental layout

Treatments	**Concentration(%)**	**Mix ratio (extract: milk)**	**Replications**
Control	0	100ml milk	2
Ethanol extracts mango seed kernels (MSKE)	0.5	0.5ml with 100ml milk	2
	1.0	1.0ml with 100ml milk	2
	1.5	1.5ml with 100ml milk	2
Ethanol extracts of dry rosemary leaves extracts (RE)	0.5	0.5ml with 100ml milk	2
	1.0	1.0ml with 100ml milk	2
	1.5	1.5ml with 100ml milk	2
Combination ratio of ethanol extracts of mango seed kernels and dry rosemary leaves powder	0.25/0.25	0.25ml MSKE and 0.25 RE to 100ml milk	2
	0.50/0.50	0.50ml MSKE and 0.50 RE to 100ml milk	2
	0.75/0.75	0.75ml MSKE and 0.75 RE to 100ml milk	2

MSKE= mango seed kernel extract, RE = rosemary leaves powder extract

3.6. Microbial Analysis

3.6.1. Total aerobic mesophilic bacterial count (TAMBC)

Milk samples serial diluted by adding 1mL of the test portion into 9 mL of 0.1% sterile peptone water. Dilution made so that plate counts range between 30 and 300 colonies. Appropriate dilutions were placed on Petri dishes and pour-plated with 10 to 15 mL plate count agar (about 45°C), allowed to solidify for 15 minutes, and incubated for 48 hours at 35°C. Finally, colony counts made using a colony counter and recording bacterial colonies in each petri dish the number of bacteria in 1 milliliter calculated by the following formula given by APHA, (1992).

$$N = \frac{\sum C}{(1 \times n_1) + (0.1 \times n_2) * d} \quad \text{.....equation (8)}$$

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

3.6.2 Total coliform count (TCC)

The coliform count made by mixing 1 ml of milk sample into 9 ml peptone water (1%). After mixing, the sample was serially diluted up to 10^{-4} in sterile test tubes having 9ml of peptone water and duplicate samples were pour plated using 15-20 ml Violet Red Bile Agar solution (VRBA) in sterile Petri dish. After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 35ºC for 48 hours. Results from only those plates, that counted between 15 and 150 colonies recorded. Typical dark red colonies were considered as coli form colonies in each petri dish the number of bacteria in 1 milliliter calculated by the above formula given by APHA, (1992)

3.6.3. Total yeast and mould count (TYMC)

Samples of milk serially diluted following similar methods as for total bacterial count but dilutions were surface plated on Potato Dextrose Agar (PDA). The dried plates then incubated at $25^{O}C$ for 3 to 5 days. Finally, blue-green colony counts less than 150 made using a colony counter and recording bacterial colonies in each petri dish the number of bacteria in 1 milliliter calculated by the above formula given by APHA, (1992).

3.7. Method of Data Analysis

The required data was collected, processed and analysed using SAS, 2008 Version 9.1 with critical difference value at ($P < 0.05$)and the mean separation by general linear model (GLM) Tukey test. Also, minitab software version 21 is used for main effect, interaction and regression procedure of independent and dependent variables. The experiment determined the reduction of spoilage microbes in raw cow milk by adding ethanolic extract of selected plant.A total microbial count was log-transformed (log10cfu/g) before statistical analysis. Calculated as using by the following model:

$Y_{ijkl} = \mu + \alpha i + \beta j + yk + (\alpha\beta)\, ij + (\alpha y)\, ik + (\beta y)\, jk + (\alpha\beta y)\, ijk + eijkl$……. Equation (9)

Where, μ is the overall mean, $\alpha\beta$, αy & βy are two-factor interaction effects for interactions MSKE/RE, MSKE/MIX and RE/MIX, respectively, $(\alpha\beta y)ijk$ are the three-factor interaction effects for the MSKE/RE/MIX interaction and eijkl is the error term (experimental error). Based on Minitab software analyzed result, the regression equation of (titratable acidity/TA/, pH, TAMBC, TCC and TYMC were used to estimate each presence to which the dependent variables explained by the independent variable (Appendix 2).

4. RESULTS AND DISCUSSION

4.1 Physicochemical Characteristics of Raw Cow Milk

The physicochemical characteristics of fresh milk samples results are indicated in Table 2. 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 reported in the current study is slightly lower than the minimum value of milk protein (3.2%) recommended by the ESA (2009). The low protein content of the milk in the present study could be due to the low protein contents of natural pasture, which is predominant feed resources dairy cattle feed in the area, and the lack of supplementary feeds with protein-rich concentrates.

The average fat content (4.40%) of raw milk samples in current study was similar value (4.25%) reported by TeshomeGemechu*et al.* (2015) in Shashamane. ESA, (2009) reported that the minimum fat content of raw milk should not be less than 3.5%. However, the current study note higher fat content (4.40%) than the minimum fat content of raw milk set by ESA. The fat content of milk was affected by factors such as feed, parity, and stage of lactation (LegesseGaredew*et al*., 2105). The average lactose content (4.35%) of raw milk samples in current study was higher than the value (3.79%) reported by EstifanosHawaz (2015) in Harar milk shed, Eastern Ethiopia. However, lactose content of raw milk samples in current study was lower than the value (4.69%) reported by TeshomeGemechu*et al*. (2015) in Shashamane. Whereas, the lactose content of milk in the current study was the same as lactose content (4.35%) of Ethiopian standards (ESA, 2009).

The average salt content (0.62%) of the raw milk samples in current study was lower than the value (0.76%) reported by TeshomeGemechu*et al*. (2015) in Shashamane, Ethiopia. However, similar salt content of milk (0.62%) was found in central highlands of Ethiopia (DesalegniGenzebu*et al.*, 2016). 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 reported in the current study.The pH (6.61) value of raw milk samples in current study was higher than the value (6.32) reported by TeshomeGemechu*et al*.

(2015) 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%) reported by DesalegniGenzebu (2016) in central highlands of Ethiopia.According to (ZerihunAsefa and Getenesh Teshome, 2019) the composition of milk depends upon the species, breed, season, feed & water, stage of lactation, age, time of milking, weather etc.

Table 2. 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

4.2. Phytochemicals in Extracted Plants

The results of the current study showed that the amount extracts obteind by using Soxhlet apparatus and ethanol solvent were 7.5ml (12.5%) and 6.51ml (10.85%) from 60 grams of mango seed kernels and 60 grams of rosemary leaves powder, respectively. Similarly, Asemave*et al*., (2020) reported 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. Rodriguez *et al*. (2012) reported that 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 3). 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) (Vega *et al.,* 2013).

The DPPH activity of ethanol extracts of mango seed kernels and rosemary leaveswere 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 (Thalia*et al*., 2018), which is agreed with this study. Mango seed kernels extracts from single and mixed mango varieties were not show significant differences in the phytochemical content and biological activity (Jasminder*et al*., 2010). The antioxidant activity of mango seed kernel**s** and rosemary leavesmight 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 as a good organic preservative or additives to prevent deterioration of any food (Sohaimy and Masry, 2014).

Table 3. 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(50μm)	81.32	62.51

TPC=Total Phenolic Content, TFC=Total Flavonoid Content,
DPPH=diphenol- picrylhydrazyl

4.3. Effect of Plant Extracts on Physical Properties of Milk

4.3.1. Titratable acidity

The acidity of the treatments in control group (T_1) was increasing from 0.16 to 0.58% when the storage time was increasing 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 were significance difference ($P<0.05$), compared with control up to 24 hours, except T_4 which was most

acceptable acidity (0.17%) found up to 48 hours. However, after 48 hours, all treatments showed less acceptable with regarding to milk 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 (Sivakumar and Dhanalakshmi, 2016). The titratable acidity (0.16) results obtained from the current study were lower than 0.25 reported in DebreBerhan(AlganeshTola, 2016).

Table 4. Determination of titratable acidity (TA %) of treated milk.

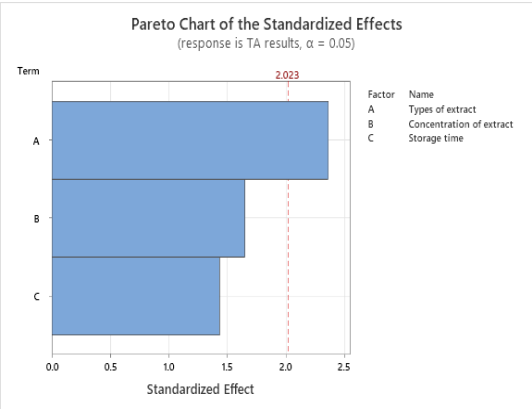
Time	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	P value
0	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	
24	0.32	0.17	0.17	0.16	0.16	0.16	0.16	0.17	0.16	0.16	
48	0.42	0.24	0.19	0.17	0.18	0.18	0.18	0.18	0.18	0.17	
72	0.58	0.28	0.172	0.18	0.19	0.192	0.24	0.22	0.19	0.19	
PE×C× ST											P=0.006<0.05

T= Treatment (Appendix 4),PE-plant extract, C-concetration, ST-storage time

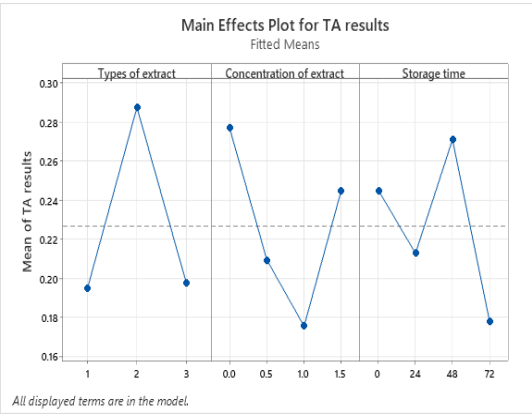
The production of acid in milk is normally termed souring and the sour taste of milk is due to lactic acid production (Julijana*et al.,* 2016). The titratable acidity of milk has long been recognized and employed as an indicator of quality. It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk drawn from the udder. Normal fresh milk should have an apparent acidity of 0.14 to 0.16 (Wanjala*et al*., 2017).

As indicate in graph 1b, inclusion of MSKE was the remarkable effect on decreasing TA. In addition, blending of MSKE and RE slowed decreasing TA next to MSKE. However, RE was gradually increasing (that means MSKE>MIX>RE). Similarly, when the concentration of plant extracts were increasing 0 to 1.0% the TA were rapidly increased unlike slow TA was observed when the concentration was increased from 1.0 to 1.5%. Finally, storage time increasing from 0 to 24hours the TA was slowly decreasing, while the reverse is true for storage time was increasing from 24 to 48hours. However, storage time increasing from 48 to 72 hours TA was rapidly increasing which indicates storage time and TA were direct relationship. On the other hands, when storage time is increasing the TA also increasing that have negative impact on milk quality.

Based on the regression equation (Appendix 2), the titratable acidity of fresh cow milk treated by MSKE and extract MIX decreased by 0.0317 and 0.0292 units, respectively. However, the milk samples that treated by RE was increase titratable acidity by 0.0608 units in plant extract types. Similarly, the titratable acidity of milk treated at 0.5 and 1.0% was decreasing by 0.00173 and 0.0513 units, respectively. Generally, types of plant extract, concentration level of plant extract and storage time on milk preservation at positive sign indicates titratable acidy increases and negative sign indicates acidity decreases on front numerical values. The titratable acidity test measures the acidity of the milk. TA is a more reliable indicator because; it is more sensitive to small changes in milk acidity.



a) Pareto chart of main effect



b) Main effect with each type, level and time

Graph 1. Main and interaction effects of types plant extract, concentration level of plant extracts and storage time with TA

4.3.2. 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 5. The pH of the control group (T_1) was decreasing from 6.61to 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% concentrate of MSKE extract (T_4) from 6.61 to 6.52 at storage period 48 hours (Table 5).

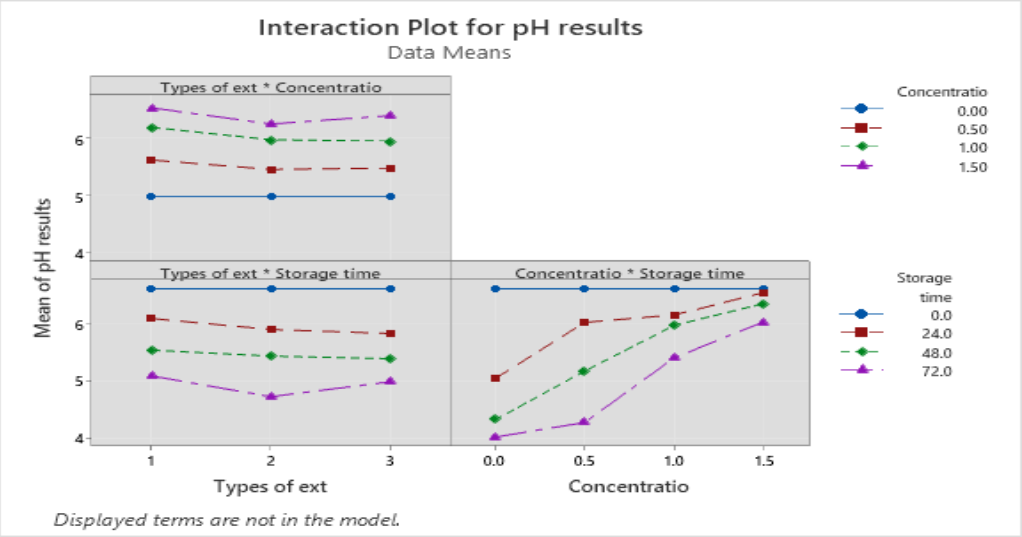
Sadia *et al*. (2020) reported that at 17 and 18 hours after preservation, adding 1.5% Tulsi and 1.5% Neem extract to milk resulted in a slower pH decrease in the treated sample than control in Egypt, respectively.

Table 5. 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		

ST= Storage Time in hours

On two-way interaction from software results (graph - 2), types of plant extracts (MSKE) with storage time at 24hours the pH become higher value next control at 0 hours. Then, the interaction between types of plant extracts (MSKE) and concentration of plant extracts at 1.5% were the higher results of pH. After that the interaction of concentration of plant extracts at 1.5% and storage time at 24hours were higher value of pH next to control at 0 hour. From the graph to conclude that types of plant and concentration level of plant extracts were directly related to pH, but storage time increased the pH of raw milk decreased.



Types of plant extracts (1= MSKE, 2=RE, 3=MIX)

Graph-2) Interaction effect of plant extract, concentration level, storage time and pH.

4.4. 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 in the Tables below.

4.4.1. Total Aerobic Mesophilic Bacterial Count (TAMBC)

The total aerobic mesophilic bacterial counts were determined by using ethanol extract of Mango seed kernels, rosemary and equal mix of both plants as a natural preservative of milk at room temperature (Table 6). The study showed significant differences among treatments on total aerobic mesophilic bacterial count ($p<0.05$). The highest total aerobic mesophilic bacterial count obtained in untreated milk samples (control) at 72 hours' storage, which was increasing from 8.32 to 9.25 (log cfu/ml) when the storage time increasing from 0 to 72 hours. Addition of 1.0, 1.5 and 1.5% were equal number of TAMBC ($8.05 \log_{10}$cfu/ml) results at 24hours storage of MSKE, RE and MIX, respectively. However, the TAMBC decreased from 8.17 to 7.96 in $\log_{10}$ CFU/ml of raw milk was recorded for 1.5% MSKE up to 48 hours of preservation at room temperature from others (Table 6). Though the TAMBC observed in the current study was beyond the acceptable consumption limit of Ethiopia standard agency (5.30 to 6.00 log cfu/ml), treating milk with the selected plant extracts has positive impact if the milk reaches to the collection center or consumption between 24 and 48 hours. The major reasons of reporting high TAMBC includinf the treated milk due to high microbial load observed in initial milk collected, experimental and working environmental error was the first estimated factors.

The untreated (control) sample when storage time increase from 0 up to 72hours the TAMBC also increased directly. Phenols and polyphenols are water-soluble compounds that can be easily mixed with milk. The TAMBC of treated samples showed a decreasing trend from 0 hours up to 24hours because of extracts that immediately effect on microbial inhibition or destruction at incubation time until 48 hours, but increasing again when the storage time extended to 48 and 72 hours, because of biological activity of plant phytochemical decrease. In contrast, Sadia *et al.* (2020) reported that preservation of raw milk by Tulsi and Neem

extract that the lowest number of total viable bacterial count recorded at 18hours storage in Bangladesh. Besides, adding 0.5% Tulsi leaf extract in raw milk remained acceptable up to 11 hours of storage at 37°C (Sivakumar, 2017). Similarly, addition of 3000-ppm rosemary, roselles and lemon ethanolic extracts to raw cow milk resulted in reduction of total bacterial count in Egypt (Sayed et al., 2022). Hac-Szymanczuk*et al*. (2017) the effect of rosemary on lipids oxidation and the microbial quality of chicken meat kept at -18 °C for four months.

Table 6. Total Aerobic Mesophilic Bacterial Count (TAMBC) of treated cow milk

ST	Cont	MSKE (%)			Combined (%)			RE (%)			P value
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	
0	8.32	8.22	8.21	8.17	8.23	8.21	8.17	8.21	8.16	8.17	
24	8.77	8.13	8.05	8.03	8.19	8.17	8.06	8.29	8.28	8.05	
48	9.08	8.20	8.23	7.96	8.30	8.28	8.20	8.76	8.30	8.11	
72	9.25	8.81	8.37	8.21	8.83	8.76	8.36	8.85	8.78	8.22	
PE× C× ST											p= 0.001<0.05

Cont= control

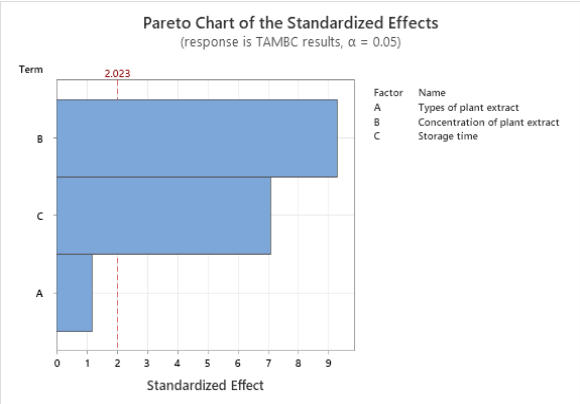
In the current study three factors (types of plant extract, level of concentration and storage time) that effects on treatments by individual and their interaction. In Minitab results (graph-3a) the factor which above the red line are, consider, as critical factor of the treatments (i.e. the concentration 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) (Graph 3a). 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.

In the results (graph 3b) MSKE was the main effect of decreasing TAMBC. But RE slow increasing TAMBC and MIX was gradually increasing next to RE (MSKE>RE>MIX). Similarly, concentration of plant extracts from 0 to 1.5% increasing TAMBC rapidly

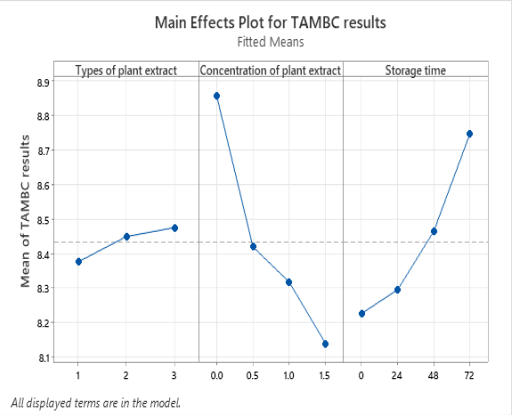
decreased. Finally, storage time from 0 to 48hours the TAMBC rapidly increased, but still now major area below the mean. Storage time from 48 to 72 hours TAMBC rapidly increased above the mean, which understands storage time and TAMBC were directly relationship when storage time increases TAMBC aslo increased, but TAMBC increased that negative impact on milk quality.

On two-way interaction from software results (graph -3c), types of plant extracts (MSKE) with storage time at 24hours the TAMBC become lower values. Next, the interaction between types of plant extracts (MSKE) 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 at 24 and 48 hours were lower value of TAMBC. From the graph to conclude that types of plant and concentration level of plant extracts were directly related to TAMBC, but storage time was indirectly related to TAMBC of raw milk quality.

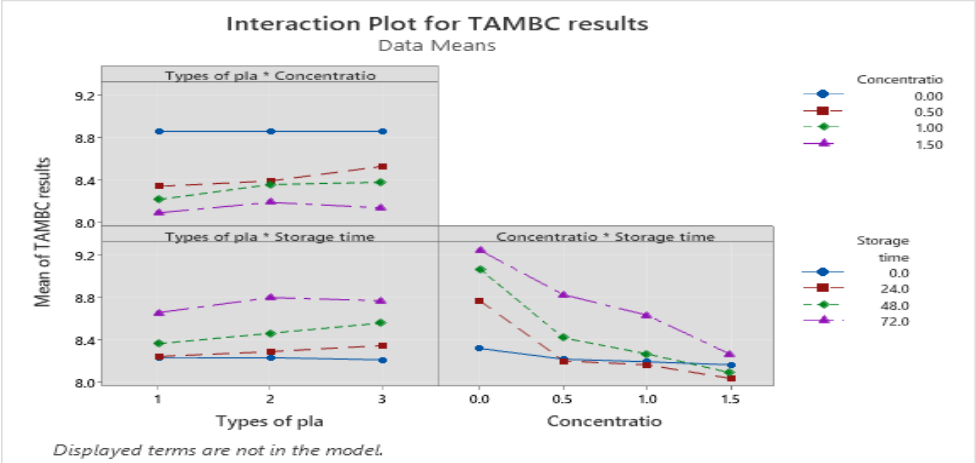
Based on the regression equation (Appendix 2), the TAMBC of fresh cow milk treated by MSKE, RE and MIX increased by 0.0542, 0.0367 and 0.0175 units, respectively. Similarly, the TAMBC of milk treated concentration level if plant extracts at 1.0 and 1.5% was decreased by 0.0097 and 0.1634 units, respectively. However, the TAMBC of milk treated concentration level of plant extracts at 0.0 and 0.5% was increased by 0.0411 and 0.1126 units, respectively. Finally, the TAMBC of treated milk storage time at 0, 24 and 48hours were decreased by 0.0899, 0.0097 and 0.0909 units, respectively. However, the TAMBC of treated milk storage time at 72hours was increased by 0.1902 units.



a) Pareto chart of main effect



b) Main effect with each types, level and time



c) Interaction effect of plant extract, concentration and storage time with TAMBC

Graph 3. Main and Interaction effect of types plant extract, concentration level of plant extracts and storage time with TMBC.

4.4.2. Total Coliform Count (TCC)

In current study revealed that there is a significant difference amongtreatments in coliform count ($p<0.05$). The highest total coliform counts obtained in untreated milk samples (control) and during storage it was increased from 5.14 to 6.04 (log cfu/ml) at room temperature (Table 7). The total coliform count of milk treated with 1.5% MSKE ethanolic extracts reduced from 4.88 to 4.30logcfu/ml up to 48hours at room temperature. Still the TCC not acceptable limit for consumption at 48 hours, then milk reaches to the collection center or consumption between 24 and 48 hours. In the current study TCC some errors when working laboratory and equipment were less effective. According to the ESA (2009), good quality milk should not contain a total coliform bacterial count more than 10^3cfu/ml. However, addition of 3000-ppm sumac, tamarind, rosemary, roselle and lemon ethanolic extracts to raw cow milk resulted in completely inhibited coliform growth (Sayed *et al*., 2022).

Coliforms are another bacterial group which affects milk quality that is associated with the level of hygiene during milking and handling (AbiotDeddefo*et al*., 2023). It includes all facultatively anaerobic, Gram-negative, non-spore-forming rods able to ferment lactose with the production of acid and gas at 35^{o}C within 48 hours. Coliform bacterial may be due to contamination of milk during milking poor milker's hygiene or facal contamination from the

udder and lower abdominal parts of the body of cows mainly from bedding materials as a result microorganisms gain access into milk during milking (FufaAbunna*et al.*, 2019).

Table 7. Total Coliform Count (CC) of raw and plant treated cow milk (in log 10 CFU/ml)

ST	Cont	MSKE (%)			Combined (%)			RE (%)			P value
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	
0	5.14	4.97	4.97	4.88	4.99	4.97	4.88	4.96	4.86	4.88	
24	5.18	4.80	4.61	4.55	4.93	4.88	4.65	5.09	5.08	4.62	
48	5.69	4.94	4.99	4.30	5.11	5.08	4.94	5.36	5.09	4.77	
72	6.04	5.42	5.21	4.96	5.19	5.37	5.11	5.74	5.52	4.97	
PE× C× ST											P=0.011<0.05

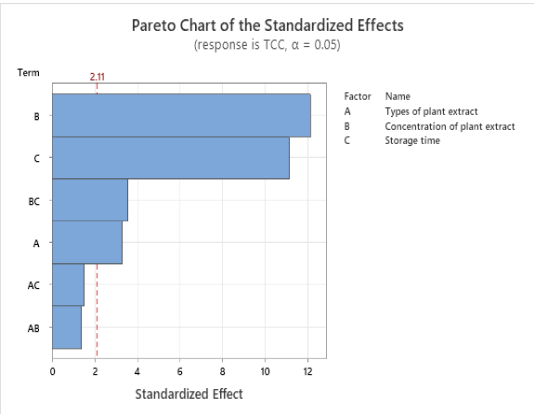
In Minitab software results (graph-4a) the factor which above the red line consider as critical factor of the treatments (i.e. the concentration of plant extract which represent letter B) was the highest impact of the treatments. The concentration of plant extract was the main effects on TCC that increasing or decreasing of concentration of plants extract affect more than storage time and types of plant extract. Storage time and types of plant extract also a critical factor next to concentration of plant extracts. Similarly, interaction between concentration of plant extract and storage time were critical on the experiment. Whereas, interaction between types of plant extract and storage time at the same time affects one another. Similarly, interaction between types of plant extract and storage time also the same time affects one another on the treatments (B>C>BC>A>AC>AB>A).

In the results (graph 4b) MSKE was the main effect of decreasing TCC. But RE rapidly increasing TCC and MIX was gradually decreasing TCC, but still above the mean. Both RE and MIX were almost least effect on treatments (MSKE >MIX>RE). Similarly, concentration of plant extracts from 0 to 1.5% increasing TAMBC rapidly decreased. However, the concentration of plant extracts decreasing from 0.5 to 15% was critical importance which the graph indicates below the mean. Finally, storage time increasing from 0 to 24hours the TCC decreasing and from 24 to 48hours TCC increased gradually, but still now both of them below the mean which means critical importance for treatments. Storage time increases from 48 to 72 hours the TCC rapidly increased above the mean, which understands storage time and

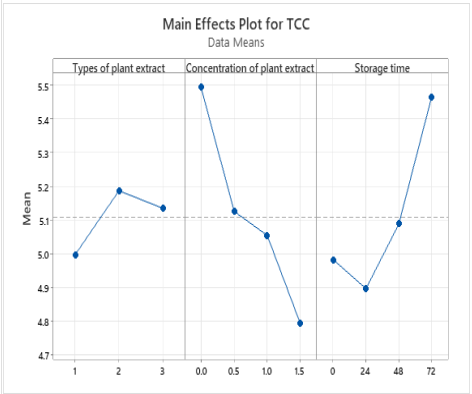
TCC were directly relationship when storage time increases TCC also increased, but TCC increased that negative impact on milk quality.

On two-way interaction from software results (graph -4c), 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% were the best results of TCC. Then the interaction of concentration of plant extracts at 1.5% and storage time at 24 and 48 hours were lower value of TCC. In graph to conclude 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.

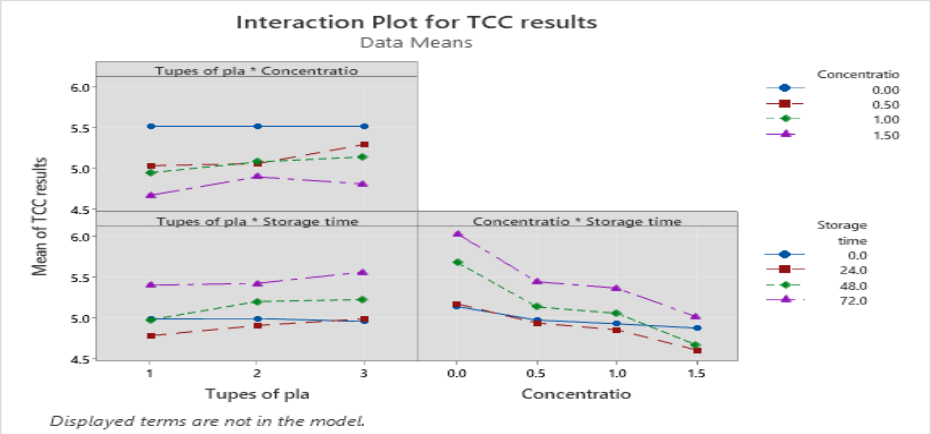
Based on regression equation results (Appendix 2), the TCC of fresh cow milk treated by RE and MIX increased by 0.0705 and 0.0187 units of plant extract types, respectively. However, the milk samples that treated by MSKE was increase TCC by 0.0892 units in plant extract types. Similarly, the raw milk treated at 0.0, 0.5 and 1.0% concentration of plant extracts were the TCC increased by 0.3764, 0.0092 and 0.0621units, respectively. Whreas, the milk samples treated at 1.5% concentration of plant extracts the TCC decreased by 0.3235units. Finally, the TCC of treated milk storage time at 0 and 24hours were decreased by 0.1340 and 0.2205 units, respectively. However, the TCC of treated milk storage time at 48 and 72hours was increased by 0.0046 and 0.3499 units, respectively.



a) Pareto chart of main effect



b) Main effect with each types, level and time



c) Interaction effect of plant extract, concentration level and storage time with TCC

Graph 4. Main and Interaction effect of types plant extract, concentration level of plant extracts and storage time with TCC.

4.4.3. 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 (Ritota and Manzi, 2020). Plant extracts can contribute to delay or prevent the formation of mycotoxins (Mahmoud *et al*., 2023).

Table 8. 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 amongtreatments in Yeast and mould 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. Yeast and mold in raw milk comes from soil, dust, unclean milk storage and milk leftover in storage for a long period of time. Inaddition, Yeast and Mold that growth wide range of pH when milk storage time increased and milk pH decreased that types of other microbes decreased. 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 counts in this study are still lower than the acceptable limit. Torkar and Vengest (2008) reported that Yeast and mold from udder milk sample not exceeded 2.1 and 1.7logcfu/ml, respectively. In contrast, Ahlam*et al.* (2017) reported that some yeast and mold isolated from raw cow milk samples were some *candida species, Rhodotorula species, Aspergillus species, AspergillusspceiesRhizopous species* and others identified in Egypt.

Maribel *et al.* (2022) reported that mango peel, mango seed extracts with antioxidant, and anti-yeast properties tested against clinically pathogenic *(Candida species)* and food-spoilage yeasts (*Dekkeraanomala, Hanseniasporauvarum, Zygosaccharomycesbailii, Zygosaccharomycesbisporus, and Zygosaccharomycesrouxii).* In current sutdy concluded that yeast and mould were fewer effects on raw milk until it reaches a processing plant when using clean milking equipment, personal hygiene and transportation hygienically. Those determine high prevalence of microorganisms among dairy animals may attribute to the lack of sanitary conditions that adapted from dairy farm until to market outlets. Based on different researchers, cinnamon, ginger, garlic and mustardhasagoodantifungalactivityfordifferentdairyproductsandotherfoods(Wang *et al*., 2020).

5. CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Ethiopia has huge potential to produce more milk due to number of cattle; however the productivity of dairysectors is very lower than expected level. The problem is also aggravated by the quality of milk in country is reported to be very low due to poor hygienic practices of milk across the country side. This may pose serious health problems besides huge economic loss of the country. Plants are used as food additives are common worldwide to enhance the sensory qualities of foods and extend their shelf life by reducing or eliminating spoilages and pathogens 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 is performed well on physical and microbial quality parameters of milk beteewn24 and 48 hours.Hence, further study will have requiredreducing daily loss of several liters of milk by spoilage due to market problems. It is adivisable for smallholder dairy farmers to keep evening milk until next morning and transported to the milk cooperatives by addition on mango seed kernels extracts.

5.2. 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) will be necessary.
- Further research will require mixture of each plant extracts interaction (antagonistic or synergetic).

6. REFERENCES

AbaynehKassahun, YosephShiferaw and Gezu Feleke.2019. Proximate analysis, physicochemical properties and chemical characterization of Rosmarinus officinalis L. oil, DebreBerhan University, DebreBerhan, Ethiopia. *Natural Volatiles and Essential Oils Vol. 6(2): 20-24*

Abdalla A. E., Darwish S. M., Ayad E. H. and El-Hamahmy R. M. 2007. Egyptian mango by-product Compositional quality of mango seed kernel. *International Journal ofFood chemistry.* Vol.103(4):1134-1140.

AbebeBereda, ZelalemYilma and AjebuNurfeta. 2012. Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. *Wudpecker Journal of Agricultural Research*, Vol.1(11): 459-465

AbiotDeddefo, GezahegneMamo, MindaAsfaw and KebedeAmenu. 2023. Factors affecting the microbiological quality and contamination of farm bulk milk by Staphylococcus aureus in dairy farms in Asella, Ethiopia. *BMC Microbiology23:65* *https://doi.org/10.1186/s12866-022-02746-0*

Ahlam E. L., Amr A. A., Hussin S. A. E. and Ibrahim E.K. 2017. Chemical and Microbiological Status of Raw Milk Sold at Local Markets, Department of Food Hygiene, Faculty of Veterinary Alexandria University. Egypt. *Alexandria Journal of Veterinary Sciences*, Vol. 55 (1): 125-132.

AlganeshTola. 2016. Assessment of Safety and Quality of Raw Whole Cow Milk Produced and Marketed by Smallholders in Central Highlands of Ethiopia. *Food Science and Quality. Vol.49: 63-71.*

AmentieTadesse, EshetuMitku, Mekasha Y, Kebede A. 2016. Milk post-harvest handle practice across the supply chain in Eastern Ethiopia. *Journal of Advanced Veterinary and Animal Research Vol.*3 (2):112-126.

AOAC. 2005. Official Methods of Analysis. Association of Official Analytical Chemist, 18th ed. Horwitz William Publication, Washington, DC. BIS, 1981. Handbook of food analysis, Vol. XI: Dairy products SP: 18 Bureau of Indian Standards. New Delhi.

APHA .1992. Standard Method for the Examination of Dairy Products. 16th Ed, American Public Health Association (APHA), Washington.

Aprodu J., Stanciuc N., Dumitrascu L., Râpeanu G., Sanciu S. 2014. Investigations towards understanding the thermal denaturation of lactoperoxidase. *International Dairy Journal***,** Vol.38: 47-54.

Asma S., Abdelhafid B., Khadidja B. and Saber B. 2022. Effect of Rosemary and Oregano Aqueous Extracts Supplementation on Microbial Growth during Refrigerated Storage of Milk, Laboratory of Natural Sciences and Materials, Algeria. *Agriculture Science* Vol. 88(1), 67-73

AsaminewTassew and SeifuEyassu. 2010. Microbial Quality of Raw Cow's Milk Collected from Farmers and Dairy Cooperatives in Bahir Dar Zuria and Mecha District, Ethiopia. *Agriculture and Biology Journal of North America*. 29-33.

Asemave K., Abakpa D. O. and Ligom. T. T. 2020. Extraction and Antibacterial Studies of Oil from three Cultivars of Mango Kernel obtained from Makurdi – Nigeria, Chemistry Department, Benue State University, Makurdi-Nigeria. *Progress in Chemical and Biochemical Research, Vol.*3 (1):74-80

Azza M. A., Walaa H. S., Mohamed B. H., Afaf S. F. and Saleh A. M. 2018. Phenolic-antioxidant capacity of mango seed kernels: therapeuticeffect against viper venoms, Molecular Biology Department, National Research Centre, Dokki, Cairo, Egypta. *Brazilian Journal of Pharmacognosy* Vol.28:594-601

BirhanuYeserah, AsaminewTassew and HailuMazengia. 2020. Handling Practices of Raw Cow's Milk and Major Constraints of Clean Milk Production in and around Bahir Dar City, Ethiopia. *Journal of Advances in Dairy Research Vol.8*:234

Brand W.W., Cuvelier, M.E. and Berset C. 1995. Use of total phenolic content and a free radical method to evaluate antioxidant activity. *Food Science and Technology* Vol.28: 25-30.

Claeys W, Cardoen S, Daube G, De Block J, Dewettinck K. and Dierick K. 2013. Raw or heated cow milk consumption: Review of risks and benefits. *Journal of Food Control.Vol.*31:251-262

Dent M, Dragovic-Uzelac V, Penic M, Bosiljkov T. and Levaj B. 2013. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (Salvia officinalis L.) extracts. *Food Technology and Biotechnology.* Vol.51 (1):84-91

DesalegniGenzebu, BerhanTamir and GebreyohanesBerhane. 2016. Physicochemical Properties of Raw Milk Collected from Different Value Chain Points in Central Ethiopia. *World Journal of Dairy & Food Sciences* Vol.11 (1): 88-94.

Dorta, E., Lobo, M. G. and Gonzalez M. 2012. Using dry treatments to stabilise mango peel and seed: Effect on antioxidant activity. *Food Science and Technology*, Vol.45(2), 261–268.

Elshafie H. Z. and Camele I. 2017. An overview of the biological effects of some Mediterranean essential oils on human health. *Bio Medical Research International*, 9268468. http://dx.doi. org/10.1155/2017/9268468. PMid: 29230418.

Embuscado M. E. 2015. Herbs and spices as antioxidants for food preservation. Hand book of Antioxidants for Food Preservation. Hand book of Antioxidants for Food Preservation. PP: 251-283.

ESA (Ethiopian Standard Agency). 2009. Unprocessed Whole/Raw Cow Milk Specification. 2nd Ed., Ethiopian Standard Agency (ESA) 3460.

EstifanosHawaz, Tarekeg G, YonasHailu, EyassuSeifu, Mengistu K. and Mohammed Ahmed. 2015. Physicochemical Properties and Microbial Quality of Raw Cow Milk Collected from Harar Milk shed, Eastern Ethiopia, *Journal Biological Chemistry Research*, 32(2): 606-616.

Fajriyati M., Sri I., Abigael T., Akhmad R. and Muhammad S. 2021. Mango seed kernel oil extraction with ethanol: optimization of mango seed kernel oil extraction yield and polyphenol. Department of Chemical Engineering, Gorontalo State University, Gorontalo, 96111, Indonesia, *Chemical Industry and Chemical Engineering Quarterly* Vol.27 (3): 207-214.

FAO (Food and Agriculture Organization of the United Nation). 2012. Annual production of mango fruits in Ethiopia, at the web page: Retrieved on March, 2012.

FAO (Food and Agriculture Organization of the United Nation). 2019. Dairy and dairy products, online library of organization for economic corporation and development books.

FufaAbunna, NigusTasew, FikruRagassa, DinkaAyana and KebedeAmenu. 2019. Handling Practices, Quality and Safety of Milk along the Dairy Value Chains in Selected Sub Cites of Addis Ababa, Ethiopia.*Biomedical Journal of Scientific & Technical Research* vol.13(1).

GebeyewKefalew, Amakelew Solomon, EshetuMitku and AnimutGetachew. 2016. Production, Processing and Handling of Cow Milk in DawaChefa District, Amhara Region, Ethiopia.*Journal Veterinary Science Technology* Vol.7:286. doi:10.4172/2157-7579.1000286.

GezuTadesse, HaftuKebede and SefaSefasalo. 2015. Production, processing and constraints of cow milk in and around Hosanna Town, Hadya Zone, Southern Ethiopia. Wachemo University, P. O. Box 667, Hossana, Ethiopia. *Global Journal of Dairy Farming and Milk Production.*

Habtamu Korma,AjebuNurfetaand Edessa Negera. 2018. Microbiological quality and safety of milk productionand marketing in Hawassa district, Ethiopia, Department of Biology, College of Natural and Computational Sciences, Hawassa University, Ethiopia. *African Journal of Microbiology Research Vol. 12(25):587-594.*

Hac-Szymanczuk E., Cegiełka A., Lipinska E., Piwowarek K. 2017. Application of Rosemary for the Prolongation of Microbial and Oxidative Stability in Mechanically Deboned Poultry Meat from Chickens. *Italy Journal Food Science*. Vol.29: 329-342.

Hassan A. N.and Frank J. F. 2011. Microorganisms Associated with Milk. *In Encyclopedia of Dairy Sciences (2nd edition.)* CA, USA, 2011:447–457.

Higginbotham K. L., Burris K. P., Zivanovic S., Davidson P. M. and Stewart, C. N. 2014. Antimicrobial Activity of Hibiscus sabdariffa Aqueous Extracts against Escherichia coli O157: H7 and Staphylococcus aureus in a Microbiological Medium and Milk of Various Fat Concentrations. *Journal of Food Protection* Vol.77(2): 262-268.

Hossain T. J., Alam M. K. and Sikdar D. 2011. Chemical and Microbiological Quality Assessment of Raw and Processed Liquid Milk Markets in Bangladesh. *Continental Journal of Food science and Technology Vol.*5(2): 6–17.

Hyldgaard M., Mygind T. and Meyer R.L. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, Vol.3(1): 1-24.

James O., Fortune A., Dominic A. and Lene J. 2020. Microbial Safety of Milk Production and Fermented Dairy Products in Africa, Department of Food Science and Technology, University of Energy and Natural Resources, BS-0061-2164 Sunyani, Ghana. *microorganisms MDPI. 8. 752. www.mdpi.com/journal/microorganims.*

Jasminder K., Xavier R., Marimuthu K., Khoo M.L., Rajasekaran A., Kathiresan S., and Sreeramaniam S. 2010. Preliminary investigation of the antibacterial activity of mango (*Mangiferaindica L. Anacardiaceae*) seed) kernel, Malaysia. *Asian Pacific Journal of Tropical Medicine* .707-710.

JermenMamo, BultiKumera and MengistuAsmamaw. 2016. Evaluation of microbiological quality of raw milk, homemade Ergo and homemade Ayib in North Shoa District, Amhara, Ethiopia.*Pakistan Journal of Food Sciences*. Vol.26: 83-91

Jordan M.J., Lax V., Rota M.C., Loran S., Sotomayor J. A. 2012. Relevance of carnosic acid, carnosol and rosmarinic acid concentrations in the in vitro antioxidant and antimicrobial activities of Rosmarinus officinalis (L.) methanolic extracts. *Journal Agriculture Food Chemistry* Vol. 60: 9603–9608.

Julijana T., Nikola G. and Borche M.2016. Examination of pH, Titratable Acidity and Antioxidant Activity in Fermented Milk. *Journal of Materials Science and Engineering*, Vol.6 (11-12), 326-333.

KassahunMelesse, BilatuAgza and AdeyMelesse. 2014. Milk marketing and post-harvest loss problem in Ada'a and Lume districts of east Shoa Zone, Central Ethiopia Debre Zeit Agricultural Research Center P. O. Box 32, Debre Zeit, Ethiopia. *Journal of Food Science* Vol. 3(4) :027-033

LegesseGaredew, DesalegneMengesha, AyalewBirhanu and Ali Mohammed. 2015. Diverse Gram-positive bacteria identified from raw and pasteurized cow milk consumed at Gondar town and its environs, Faculty of Veterinary Medicine, University of Gondar, P. 0. Box 196, Gondar, Ethiopia. *BMC Public Health*, 12:950.

Mahmoud A. E., Heba H. S., Rehab S. S.2023. Plant extracts and essential oils in the dairy industry: National Research Centre, Giza, Egypt. *Foods and Raw Materials*. 11(2):321–337. https://doi.org/10.21603/2308-4057-2023-2-579

Mangang I.B., Tiwari A., Rajamani M. and Manickam L. 2020. Comparative laboratory efficacy of novel botanical extracts against Triboliumcastaneum. *Journal of the Science of Food and Agriculture*, 100(4): 1541-1546.https://doi.org/10.1002/jsfa.10162.

Maribel G., Herlinda S., Elizabeth C., Tomas J. M., Maria G. L. and Citlali C. 2022. Coordinacion de Tecnologia de Alimentos de Origen Vegetal, Centro de

Investigacionen Alimentacion y Desarrollo, A.C. (CIAD), Hermosillo 83304, Sonora, Mexico. *Journal of Molecules Vol.*28 (1), 458.

MekibibBerhanu, FurgasaMulatu, AbunnaFufa, MegersaBekele and RegassaAlemayehu. 2010. Bovine Mastitis: Prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *Veterinary World*, 397-403.

Modi A. A., Hanan, H. A., Lojayn, S. T. and Amal B. S. 2017. 'Antioxidant properties of dairy products fortified with natural additives: A review'. *Journal of the Association of Arab Universities for Basic and Applied Sciences, Vol.24: 101-106.*

Mohamed E. A., Marcos Q., Karola B., Inmaculada F., Sonia C., Pillar C. and Jorge Barros-V. 2014. The Immunology of Mammary Gland of Dairy Ruminants between Healthy and Inflammatory Conditions, *Journal of Veterinary Medicine Article*.

Mustefa Kemal. 2015. Optimization of Process Parameters for the Extraction of Oil from Mango Seed Kernel (Mangiferaindica) for Cosmetics Application, MSc thesis. Institute of Technology, Addis Ababa University, Ethiopia

Nadja N. P. F., Ednardo R. F., Herbenson M. G., Davyd H. S., Edibergue O. d. S., Germana C. A., Danilo R. F., Lina R. S. A. and Pedro H. W.2019. Ethanolic extract of mango seed used in the feeding of broilers: effects on phenolic compounds, antioxidant activity, and meat quality. *Canadian Journal of Animal Science*. Vol.*2(1): 181-196.*

Ngasala J. U. B., Nonga H. E. and Mtambo M. M. A. 2015. Assessment of raw milk quality and stakeholder awareness on milk-borne health risks in Arusha City and Meru District, Tanzania. *Tropical Animal Health and Production Vol.47: 927–932.*

Panghal A., Yadav D. N., Khatkar B. S., Sharma H., Kumar V. and Chhikara N. 2018. Post-harvest malpractices in fresh fruits and vegetables: Food safety and health issues in India. *Nutrition & Food Science*, Vol.48 (4), 561–578.

Qiu X., Jacobsen C. and Sorensen A.D. M. 2018. The effect of rosemary (Rosmarinus officinalis L.) extract on the oxidative stability of lipids in cow and soya milk enriched with fish oil. *Food Chemistry*, Vol.263:119-126.

Raghavan G., Adarsh K. A., Sayyada K., Ajay K. S. R. and Shanta M .2003. Pharmacognostical Evaluation of an Antioxidant Plant Acorus calamus Linn Pharmacognosy & Ethnopharmacology Division, *National Botanical Research Institute*, Rana Pratap Marg, Lucknow-226 001, India

Rahila M. P., Surendra N. B. and Laxmana N. N. 2017. Rosemary (Rosmarinus officinalis Linn.) extract: A source of natural antioxidants for imparting autoxidative and thermal stability to ghee Southern Regional Station, *ICAR-National Dairy Research Institute,* Bengaluru, Karnataka, India.

Rai A. K., Das J.H., Dash S. R. and Behera S. 2020. A potential source of nutrition from waste, Krishi Vigyan Kendra, Malkangiri of Mango Seed, College of Agriculture, OUAT, Bhawanipatna. *Indian Farmer* Vol.7 (03): 244-248

Raluca N. D., Cornelia V., Elena S., Niela P., Traian Z., Liliana T. and Karol L. 2018. Evaluation of the Rosemary Extract Effect on the Properties of Polylactic Acid-Based Materials, Department of Physical Chemistry of Polymers, "PetruPoni" Institute of Macromolecular Chemistry, National Institute for Electrical Engineering (INCDIE ICPE CA), 313 SplaiulUnirii, P.O. Box 149, 030138. *Materials Journal of MDPI*, www.mdpi/ journal of/materials.

Renata J., ZvonimiraMedverec K., Jadranka F., Ksenij M., Snjezana K., Jasna M. 2015. Improved Properties and Microbiological Safety of Novel Cottage Cheese Containing Spice. *Food Technology and Biotechnology*. Vol.53 (4):454-462.

Ritota M. and Manzi P. 2020. Natural preservatives from plant in cheese making. *Animals.*;10(4). https://doi.org/10.3390/ ani10040749**.**

Rodriguez S., Visentin A., Maestri D. and Cocero M.J. 2012. Assisted extraction of rosemary antioxidants with green solvents, Universidad de Valladolid, Escuela de IngenieríasIndustriales, Spain. *Journal of Food Engineering* Vol.109 (1): 98-103

Sadia A. K., Zakiural I. M., Mohammed A. I., Mohammed S. R. S. and Harun R. M. 2020. Efficacy of raw cow milk preservation by aqueous extracts of tulsi (Ocimum sanctum) and neem (Azadirachtaindica).*Journal of Bangladesh Agricultural University* 18(2): 449–455 https://doi.org/10.5455/JBAU.78613

Samah M. E. and Ahmed M. Y. 2019. Potential application of herbs, spices and their effects in functional dairy products. *Heliyon 5, 01959, Journal of homepage: www.heliyon.com*

Samarzija D., Zamberlin S. and Pogacic T. 2012. Psychotrophic Bacteria and Milk and Dairy Products Quality. University of Zagreb, Faculty of Agriculture, Department of Dairy Science 25, 10000 Zagreb, Croatia. *Journal Agriculture Food Chemistry* Vol. 62(2): 77–95.

Santos R. D., Shetty K., Cecchini A. L. and Miglioranza H. D. S. 2012. Phenolic compounds and total antioxidant activity determination in rosemary and oregano extracts and its use in cheese spread. Semina: CienciasAgrarias, Vol.33, 655–666.

SAS (Statistical Analysis System). 2008. (Version 9.1) Informatica Power Exchange for SAS User Guide. USA.

Sayed E. H. A., Magdy R. S., Hanan A. R., Nahed S. M., Maha A. A., Nisreen K. A., Maha A. A., Haitham H. S., Mamdouh A. D. and Ehab K. E. 2022. Antimicrobial Activity of Some Plant Extracts and Their Applications in Homemade Tomato Paste and Pasteurized Cow Milk as Natural Preservatives, Zagazig University, Egypt.*Fermentation* Vol.8: 428. https://doi.org/10.3390/ fermentation8090428

Seema R. 2015. Food Spoilage: Microorganisms and their prevention Department of Botany and Microbiology, H. N. B. Garhwal University, Srinagar, Uttarakhand, India. *Pelagie Research Library*: ISSN: 2249-7412

Silva S.A.S.D., Kanugala K.A.N.P and Weerakkody N.S. 2016. Microbiological quality of raw milk and effect on quality by implementing good management practices. Procedia *Food Science*, Vol.6: 92–96.

Sivakumar G.M. and Dhanalakshmi B. 2016. Betel leaf extract as shelf life extender of raw milk. *International Journal of Science, Environment and Technology;* 5(5): 2832 – 2836.

Sivakumar G.M. 2017. Effect of tulsi leaf extract on physico-chemical and microbial quality of raw milk. *International Journal of Science, Environment and Technology*, Vol.6 (3): 1626-1631.

Sohaimy E.S.A. and Masry S.H.D. 2014. Phenolic Content, Antioxidant and Antimicrobial Activities of Egyptian and Chinese Propolis. *American-Eurasian Journal Agriculture & Environment Science, Vol.14 (10): 1116-1124.*

Solomon Ayeleand DemelTeketay .2018. Supply and Consumption of Household Energy in Central Ethiopia: The Case of DebreBerhan Town. *J. Fundam Renewable Energy Appl*. Vol.8 (5): 269. doi:10.4172/2090-4541.1000269.

Solomon Mosu, MulisaMegersa, YibeltalMuhie, DesalegnGebremedin and SimenewKeskes. 2013. Bacteriological Quality of Bovine Raw Milk at Selected Dairy Farms in Debre Zeit Town, Ethiopia. *Comprehensive Journal of Food Sciences and Technology Research. 1: 1-8.*

Takar K.G. and Vengust A. 2008. The presence of yeast, mold and aflatoxin M1 in raw milk and cheese, in Slovenia. *Food Control* 19, 570-577.

TegegneAzage, GebremedhinBerhanu, Hoekstra Dirk, Belay Berhanu and MekashaYoseph. 2013. Smallholder dairy production and marketing systems in Ethiopia: IPMS experiences and opportunities for marketoriented development Working Paper No.31. ILRI: Addis, Ababa, Ethiopia.

TeklemichaelTesfay, AmehaKebede and Eyassu Seifu.2013. Quality and safety of cow milk produced and marketed in Dire Dawa town, eastern Ethiopia,*International Journal of Science and Technology Sec. B. Vol.2: 01-05.*

TeshomeGemechu, FikaduBeyen and MitikuEshetu. 2015. Handling Practices and Microbial Quality of Raw Cow's Milk Produced and Marketed in Shashemene Town. *Journal of Food and Agricultural Science* Vol.2 (9): 153–162.

TeshomeGemechu and TesfayeAmene. 2017. Dairy cattle milk production, handling, processing, utilization and marketing system in Bench Maji Zone, Southwest Ethiopia, Mizan-Tepi University, P. O. Box 260, Mizan-Tepi, Ethiopia.*International Journal of Livestock Production. Vol.8: 158-167.*

Thalia B. A., Cristhian A. H., Brenda A. S., Reynaldo C. M. , Gustavo A. G., Filomena N., Mohammed W. S., Fernando A. J., Florinda F.and Javier V. F. 2018. Antioxidant and Antimicrobial Capacity of Phenolic Compounds of Mango (Mangiferaindica L.) Seed depending upon the Extraction Process in Mexco.*Journal of Medicinal Plants and By-products Vol (2): 209-219*

TsegayLijalem and GebreegziabherZereu. 2015. Hygienic Milk Handling and Processing at Farmer Level in Wolaita Zone, Southern Ethiopia.*Food Science and Quality ManagementISSN 2225-0557 (Online)*

Vega V. V., Silva-Espinoza B.A., Cruz-Valenzuela M.R., Bernal-Mercado A.T., Gonzalez-Aguilar G.A., Ruíz-Cruz S., Moctezuma E., Siddiqui M.W., Ayala-Zavala J.F. 2013. Antimicrobial and antioxidant properties of byproduct extracts of mango fruit. *Journal Applied Botanical Food Quality*, Vol.86, 205–211.

WalidYeddes, HammamiMajdi, HamzaGadhoumi, TaycirGratiAffes, Salma Nait Mohamed, WissemAidiWannes and Moufida Saidani-Tounsi.2022. Optimizing ethanol extraction of rosemary leaves and biological evaluation. Laboratory of aromatic and medicinal

plants in BorjCedria Biotechnology Center, Tunisia. *Journal of Exploratory Research in Pharmacology*, Vol. 7(2) 85–94.

Wang X., Shen Y., Thakur K., Han J., Zhang J. and Hu F. 2020. Antibacterial Activity and Mechanism of Ginger Essential Oil against Escherichia coli and Staphylococcus aureus. School of Food and Biological Engineering, Hefei UniversityofTechnology,Hefei230601,China;18158951863@163.com, 2019111359@mail.hf.*Molecules*.

Wanjala G.W., Mathooko F.M, Kutima, P.M and Mathara, J.M. 2017.Microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi region. *African Journal of Food, Agriculture, Nutrition and Development***,** Vol.17(1), 11518-11532.

ZegeyeshTaye, DegyeGoshu and EndriasGeta. 2017. Determinants of milk market outlet choices in Soddozuria district, Wolaita zone of Southern Ethiopia. p. 19-30. *In Proc.* of the National Conference on Agricultural Value Chains for Food Security and Pro poor Development. Haramaya University, Ethiopia.

ZelalemYilma. 2013. Quality factors that affect Ethiopian formal milk business; experience from selected dairy potential areas. Addis Ababa, Ethiopia. *Netherlands Development Organization (SNV).*

ZerihunAsefa and Getenesh Teshome. 2019. Physical properties and chemical composition of raw cow milk in milk shades around Addis Ababa, Ethiopia. *Journal of Natural Science Research.* Vol.9, No.19

Zhishen J., Mengcheng T. and Jianming W. 1999. The determination of flavonoid con-tents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*. Vol. 64:555–559.

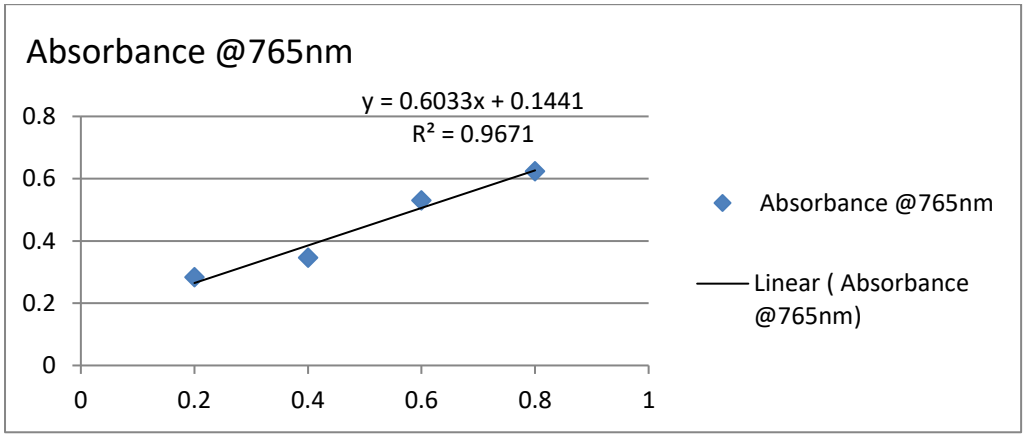
7. APPENDIX

Appendix 1a. Determination of concentration of unknown solution by calibration curve TPC of MSKE

sno.	Concentration GA in µg/ml	Absorbance known at 765nm
1	0.2	0.2832
2	0.4	0.3458
3	0.6	0.5297
4	0.8	0.6241

Sample	Concentration	Absorbance unknown at 765nm
T1	0.4	0.42355
T2	0.4	0.429

a1	y=0.6033x+0.1441	TPC= (C* V)/m
	x= y-0.1441/0.6033	C=0.472&0.4632 mg/ml
		v=volume of extract solution in
	x= 0.429-0.1441/0.6033	ml=0.4
	x=0.2849/0.6033	m= mass of extract in gram=0.00192g
	0.4720mg/ml=c	TPC=mg GAE/g
a2	y=0.6033x+0.1441	TPC_1= 0.4632*0.4/0.00192=96.5
	x= y-0.1441/0.6033	TPC_2=0.472/0.00192=98.3
		Average TPC value= 97.4 mg
	x= 0.42355-0.1441/0.6033	GAE/g
		C=con. of GA obtained from calibration curve
	x=0.27945/0.6033	in mg/ml
	0.4632mg/ml=c	



a) Calibration curve total phenolic content (TPC) of MSKE

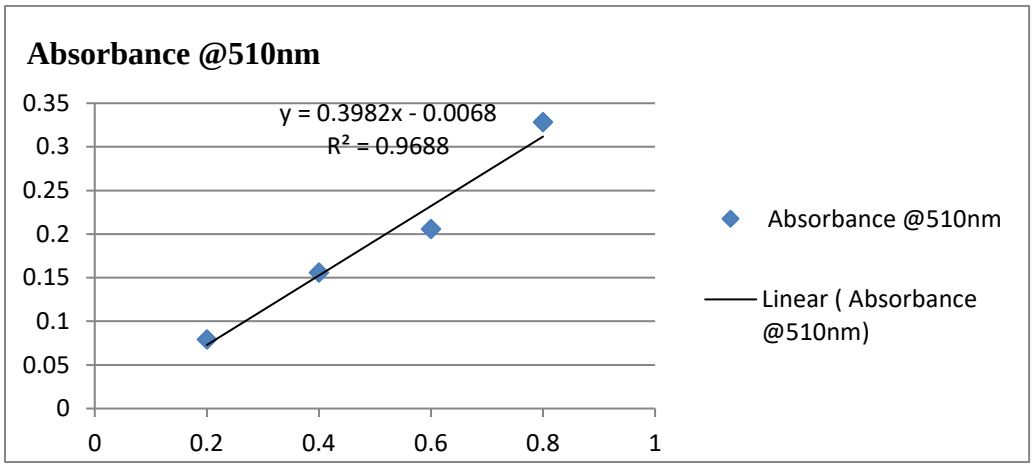
Appendix 1b. Determination of concentration of unknown solution by calibration curve TFC of MSKE

sno.	Concentration in µg/ml	Absorbance at 510nm
1	0.2	0.0793
2	0.4	0.156
3	0.6	0.2057
4	0.8	0.3282

Sample	Concentration	Absorbance at 510nm
T1	0.25	0.0584
T2	0.25	0.0683

b1	y=0.3982x-0.0068	TFC= (C*V)/m
	x= y+0.0068/0.3982	C=0.1637&0.1886mg/ml
	x= y+0.0068/0.3982	V=volume of extract solution in ml=0.25
	x=0.06518534/0.3982	m= mass of extract in gram=0.0012
	0.1637=c	TFC=mg CE/g
b2	y=0.3982x-0.0068	TFC= 0.1637*0.25/0.0012=34.1
	x= y+0.0068/0.3982	0.1886*0.25/0.0012=39.3
	x= 0.0683+0.0068/0.3982	Average TFC value= 36.7 mg CE/g

x=0.0751/0.3982 C=con. of CE obtained from calibration curve in mg/ml



b) Calibration curve total flavonoid content (TFC) of MSKE

Appendix 1c. Determination of concentration of unknown solution by calibration curve TPC of RE

sno.	Concentration GA in µg/ml	Absorbance known at 765nm
1	0.2	0.1652
2	0.4	0.35974
3	0.6	0.4931
4	0.8	0.6268

Sample	Concentration	Absorbance unknown at 765nm
T1	0.4	0.2072
T2	0.4	0.2689

c1	y=0.7591x+0.0317	TPC= (C*V)/m
	x= y-0.0317/0.7591	C=0.2312&0.3125 mg/ml
	x= 0.2072-0.0317/0.7591	V=volume of extract solution in ml=0.4
	x=0.1755/0.7591	m= mass of extract in gram=0.00221
	0.2312mg/ml=C	TPC=mg GAE/g
c2	y=0.7591x+0.0317	TPC= 0.2312*0.4/0.00221=41.84

x= y-0.0317/0.7591

x= 0.2689-0.0317/0.7591

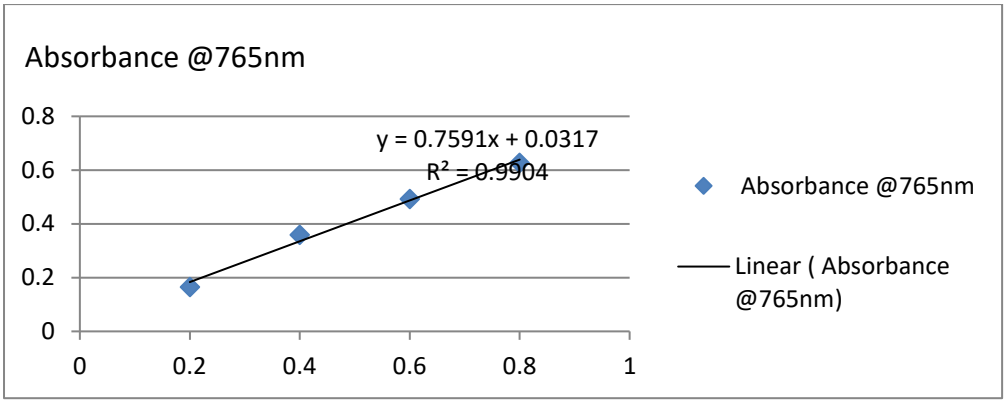
x=C= 0.2372/0.7591

0.3125mg/ml

0.3125*0.4/0.00221=56.56

Average TPC value= 49.2 mg GAE/g

C=con. of GA obtained from calibration curve in mg/ml



c) Calibration curve total phenolic content (TPC) of RE

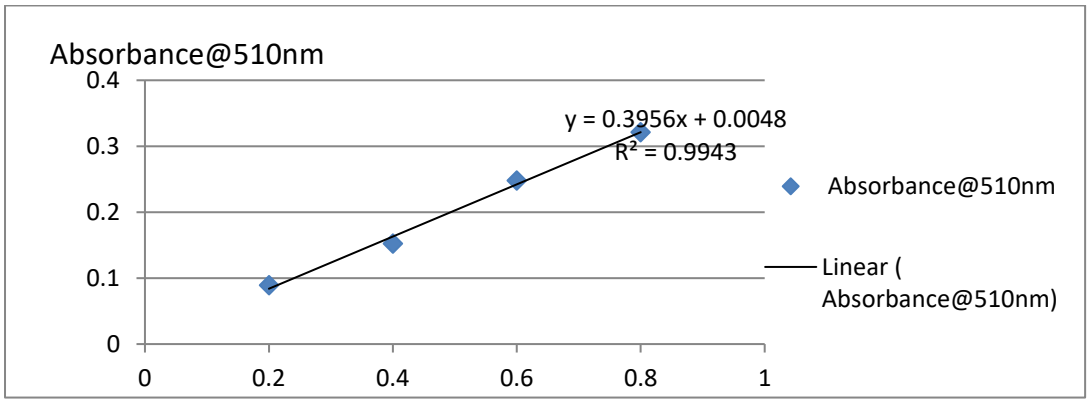
Appendix 1d. Determination of concentration of unknown solution by calibration curve TFC of RE

sno.	Concentration in µg/ml	Absorbance known at 510nm
1	0.2	0.0893
2	0.4	0.15215
3	0.6	0.2479
4	0.8	0.32111

Sample	Concentration	Absorbance unknown at 510nm
T1	0.25	0.092
T2	0.25	0.0694

d1	y=0.3956x+0.0048	TFC= (C*V)/m
	x= y-0.0048/0.3956	C =0.22&0.1632mg/ml
	x= 0.092-0.0048/0.3956	V=volume of extract solution in

		ml=0.25
	x=0.087032/0.3956	m= mass of extract in gram=0.0014
	X=0.22=C	TFC=mg CE/g
d2	y=0.3956x+0.0048	TFC= 0.22*0.25/0.0014=39.29
	x= y-0.0048/0.3956	0.1632*0.25/0.0014=29.51
		Average TFC value= 34.4 TFC=mg
	x= 0.0694-0.0048/0.3956	CE/g
		C=con. of CE obtained from calibration curve in
	x=0.0646/0.3956	mg/ml
	x=C=0.1632	



d) Calibration curve total flavonoid content (TFC) of RE

Appendix 1e. MSKE/Absorbance measurement data for calculation of scavenging % (in DPPH assay)

sno.	Con. AA in µg/ml	control	Sample	RSC %
1	50	0.42	0.09114	78.3
2	50	0.42	0.0657	84.34

RE/Absorbance measurement data for calculation of scavenging % (in DPPH assay)

sno.	Con. AA in µg/ml	control	Sample	RSC %
1	50	0.34	0.12053	64.55
2	50	0.34	0.1344	60.47

DPPH Scavenging Capacity (%) = $\frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$…..... equation

% = (Ab-As/Ab)*100

= (0.42-0.09114/0.42)*100

=78.3% MSKE

% = (Ab-As/Ab)*100

= (0.34-0.12053/0.34)*100

=64.55% RE

Appendix .2 Regression equation of (TA, pH, TAMBC, TCC and YMC) estimates each extent to which the dependent variables or criterion variables changed by independent variables (predictor variables) by one unit.

$TA = 02267-0.0317MSKE +0.0608RE -0.0292MIX +0.0503L_0 -0.0173L_1 – 0.0513L_2 +0.0183L_3 + 0.0183H_0 – 0.0140H_1 + 0.0443H_2 – 0.0487H_3$

Where, i. PE- Types of plant extract (MSKE, RE, MIX)

ii. L- level of concentration plant extract (L0=0%, L1= 0.5%, L2=1%, L3=1.5%)

iii. H- storage time (H0= 0hours, H1= 24hours, H2=48hours, H3=72hours)

$pH = 5.736 – 0.017MSKE – 0.081RE + 0.098MIX – 0.082L_0\ 0.009L_1 -0.029L_2 +0.103L_3 + 0.252T_0 – 0.207T_{24} + 0.011T_{48} – 0.056T_{72}$

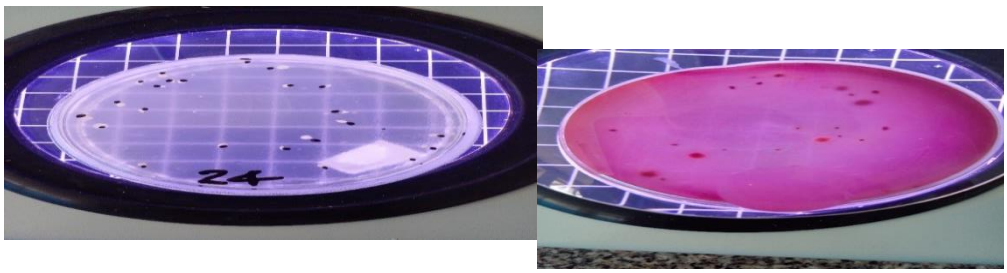
$TAMBC = 8.3049 + 0.0542MSKE + 0.0367RE + 0.0175MIX + 0.0411L_0 + 0.1126L_1 + 0.0097L_2 - 0.1634L_3 - 0.0899H_0 – 0.0097H_1 - 0.0907H_2 + 0.1902H_3$

$TCC= 5.1165 -0.0892MSKE +0.0705RE +0.0187Mix +0.3764\ L_0+0.0092\ L_1+0.0621\ L_2 - 0.3235\ L_3-0.1340H_0 - 0.2205\ H_1+0.0046H_2+0.3499\ H_3$

Appendix 3. Powder preparation and extraction procudere and Microbial colony counting

i. Powder preparation and extraction procudere

ii. Microbial counting or colony counting



Appendix 4. Treatement symbol on plant extract on treat milk sample.

Symbol	Experimenet
T1	Control
T2	Mango seed kernel extract 0.5%
T3	Mango seed kernel extract 1.0%
T4	Mango seed kernel extract 1.5%
T5	Mango seed kernel and Rosemary extract 0.25/0.25%
T6	Mango seed kernel and Rosemary extract 0.5/0.5%
T7	Mango seed kernel and Rosemary extract 0.75/0.75%
T8	Rosemary extract 0.5%
T9	Rosemary extract 1.0%
T10	Rosemary extract 1.5%

BIOGRAPHICAL SKETCH

The author was born on August 20, 1990 G.C, to his father MrZewdieTeketelew and his mother MrsKetemashMasresha in ShoaRobit Town, North Shoa Zone, and Amhara National Regional State, Ethiopia. He attended his elementary School (grades 1-8 at ShoaRobit Town 06 Kobo Elementary School from 1997-2004 G.C, Secondary School at ShoaRobit Town General and Secondary School (grades 9-12) from 2005-2008 G.C. Then enrolled at Samara University in October 2009 and earned a BSc in Animal Science in 2011 G.C.

He began working as an animal production expert in TarmaberWereda Agricultural Office in July 2013 G.C. he joined DebreBerhan University, College of Natural Resoure, Departmet of Animal Science to pursue his MSc in Dairy Science and Technology, in October 2021 G.C.