**SCHOOL OF GRADUATE STUDIES**

# MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITY ANALYSIS AND MARKETING OF RAW COW MILK IN SELECTED AREA OF GURAGE ZONE, CENTRAL ETHIOPIA REGION

**MSc THESIS**

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# Microbiological and Physicochemical Quality Analysis and Marketing of Raw Cow Milk in Selected Area of Gurage Zone, Central Ethiopia Region

**A Thesis Submitted to School of Graduate Studies, in Partial Fulfillment of the Requirements for the Degree of Master of Animal Production and Technology**

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# LIST OF ABBREVIATIONS

ADMY ..... Average Daily Milk Yield

AOAC ..... Association of Official Analytical Chemists

APHA ..... American Public Health Associations

BGLS ..... Briliant green lactose bile broth

CC ..... Coliform Count

CFUS ..... Colony-Forming Units

CSA ..... Central Statistics Agency's

EAC ..... East African Community

ESA ..... Ethiopian Standard Agency

E,coli ..... Escherichia coli

EMB ..... Eosin methylene blue agar

FAO ..... Food and Agricultural Organizations

FSA ..... Food Standard Agency

GDP ..... Gross Development Program

GLM ..... General Linear Model

GZPEDD ..... Gurage zone planning and economic development department

IDF ..... International Dairy Federation

LPC ..... Lab Pasteurization Count

LSD ..... List Significance Difference

M.A.S.L ..... Meters Above Sea Level

MR ..... Methyline red test

S,aureus ..... Staphylococcus aureus

SAS ..... Statistical Analysis Software

SCC ..... Somatic Cell Count

SG ..... Specific Gravity

SNF ..... Solid Non Fat

SPC ..... Standard Plate Count

TA ..... Titratable acidity

TS ..... Total Solid

TSI ..... Triple sugar iron

VP ..... Vogous proscours test

WHO ..... World Health Organization

XLD ..... Xylose lysine Deoxycholate agar

YMC ..... Yeast and Mould Count

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# ABSTRACT

Raw cow milk is crucial for nutrition and income in Ethiopia's, particularly in Gurage Zone. In this zone, smallholder dairy farming plays a vital role in rural livelihoods, and consuming unpasteurized raw cow milk alongside "kocho" is a common practice. However, traditional milk handling methods, coupled with inadequate hygiene awareness and insufficient sanitation measures during milking, storage and transportation, contribute to a decline in milk quality and pose health risks. Conversely, there is no comprehensive study exists in Wolkite, Gummer and Ennor, leaving a critical knowledge gap. This study was aimed to assess milk handling practices, physicochemical properties, microbial quality analysis, identification and prevalence of pathogenic bacteria and marketing of row cow milk in selected areas of Gurage zone. For survey part, data were collected using semi-structured questionnaire from a total of 338 respondents (producer and vendor) that were selected from three study sites. In addition, a total of 60 milk samples were collected for physicochemical and microbial quality analysis.

Findings revealed that small-scale milking was predominantly managed by women (95.86%), with most households owning 1–3 cows. Commonly cows fed natural grass, crop residues and *Enset*, with milking performed twice daily (73.37%) but wolkite 100% achieving in barns (94.08%). Hygiene gaps were identified, including minimal detergent use and reliance on plastic and clay containers for milk storage. Traditional cleaning and smoking of milking utensils commonly used local plants like *Weyira* (*Olea africana*), *Tid* (*Juniperus procera*) in Gummer district highland area used Kosso (*Hagenia abyssinica*) significantly reduce spoilage and extend milk shelf life. Most milk is sold informally; Gummer and Ennor process it into other products, while Wolkite mainly sells raw cow milk.Physicochemical analysis indicated deviations from Ethiopian and EU standards, Vendor milk exhibited lower pH (5.40–6.50), higher acidity (0.18–0.27%) and inconsistent specific gravity (1.0123–1.031), suggesting spoilage and possible adulteration. Chemical composition analysis indicated higher total solids, protein and ash content in producers' milk compared to vendor milk, reflecting significant quality loss. Microbial analysis revealed alarming contamination, with TPC (6.23–6.66 log cfu/ml), YMC(3.15–4.12 log cfu/ml), CC (4.95–5.77 log cfu/ml exceeding the acceptable safety limits, highlighting significant hygiene concerns. Pathogenic bacteria like *E. coli, S. aureus* and *salmonella* were detected at worrying rates, indicating poor hygiene and fecal exposure. Gummer had the best microbial quality, while Ennor Nad Wolkite showed the highest contamination risk, This study underscores the urgent need to enhance milk hygiene, handling practices and infrastructure to ensure safety, higher-quality milk production in the Gurage Zone.



***Keywords****: Handling practices, Microbial quality, Pathogenic bacteria, Physicochemical properties, Raw milk.*

# 1. INTRODUCTION

## 1.1Background of the Study

Ethiopia has one of the largest livestock populations in sub-Saharan Africa, ranking ninth globally. It has about 70 million cattle, 43.9 million sheep and 52.4 million goats. The dairy herd includes 7.56 million cows, with 15.04 million milking cows producing 4.69 billion liters of milk annually (CSA, 2021; Fereja *et al*., 2023).

Ethiopia's livestock sector is vital to agriculture and rural livelihoods, It is an economically important farm commodity and investment option for smallholder farmers in developing countries (Haile et al., 2012). Livestock sector contributing 40% of agricultural GDP, 18.7% of national GDP and 16.5% of foreign exchange income. It provides over 50% of household income ( (Eshetie *et al.,* 2018; Azage, 2018; Adugna & Eshetu, 2021; Fereja *et al.,* 2023).

According to Misganaw *et al*. (2017) and CSA (2017), Ethiopia has the largest cattle population in Africa, but its milk yield is still much lower than that of many other nations. This is due to a number of issues, including poor genetic potential; approximately 98.2% of Ethiopia's total cattle are local breeds, with the remaining 1.62% and 0.18% being hybrid and exotic breeds, respectively; inadequate feeding; and traditional management practices. The average daily milk yield (ADMY) performance of indigenous cows is 1.85 liters/day and ranges from 1.24 liters in the rural lowland agro-pastoral system of Mieso to 2.31 liters in the rural Fogera highland dairy production system (Azage *et al.,* 2013). For hybrid cows, milk production per day is 8 to 10 liters (Tadesse *et al.,* 2015). In addition to this, annual per capita milk consumption remains low at 19 liters, rising to 52 liters in Addis Ababa, far below the WHO-recommended 200 liters (Azage, 2018; Eshetie *et al*., 2018; Adugna & Eshetu, 2021; Fereja *et al.,* 2023).

Milk is a vital nutrient source for humans and animals (Adugna & Eshetu, 2021). It consists of water (87.4%), total solids (12.6%), fat (3.6–7%), protein (3.4%), lactose (4.9%), and minerals (0.7%), varying by breed, genetics, diet, environment , milking interval, lactation period, age, disease, and the overall milking process (Abera & Angaw, 2015; Eissa, 2019). Milk is also prone to microbial contamination from infected animals, unsanitary milking practices, and poor handling, transport, and storage conditions (Parekh, 2008; Adugna & Eshetu, 2021).

Milk quality encompasses a combination of chemical, physical, bacteriological and aesthetic characteristics that enhance the product's acceptability. In contrast, milk safety refers to the absence of pathogenic organisms and other contaminants that may pose health risks to consumers(Merwan *et al*., 2018).

Milk quality and safety depend on its physico-chemical composition and microbiological profile (Merwan *et al.*, 2018). Due to bacterial presence, milk undergoes various quality tests, including standard plate count (SPC), total coliform count (TCC) and yeast and mold count (YMC) (Getabalew Mebrate & Etagegneh B, 2020). Further analysis detects harmful pathogens like *E. coli*, *Staphylococcus aureus* and *Salmonella*, which pose risks to humans and cows itself (Merwan *et al.*, 2018).

Ethiopia's milk marketing system includes both formal and informal channels, with the informal sector being dominant. Producers sell fresh milk directly to consumers with minimal government intervention. Despite pasteurization regulations, much of the milk is sold raw without proper hygiene. About 95% of milk is marketed informally, while only 5% is sold as liquid milk due to limited infrastructure. Traditional dairy processing, especially soured butter, remains prevalent and most milk is consumed at the household level (Diriba *et al.*, 2024).

## 1.2 Statement of Problem

Raw cow milk is a critical source of nutrition and income for many households in Ethiopia, particularly in Gurage Zone, Central Ethiopia, where smallholder dairy farming is a cornerstone of rural livelihoods; in adition to that, it is common habit of consuming unpasteurized raw cow milk alongside "kocho" (staple food derived from Enset) in Gurage zone, of the study areas. Despite its importance; in Gurage Zone, traditional handling practices, coupled with limited hygiene awareness and measure, limited infrastructure and inadequate hygiene measures during milking, storage and transportation are suspected to contribute degrade milk quality. These practices lead to physicochemical deterioration and fostering microbial contamination, including the presence of pathogenic bacteria like *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*. As a result, these challenges pose serious public health risks and significant economic burdens. Despite these concerns, no comprehensive study has assessed these issues in Gurage Zone, specifically in Wolkite, Gummer and Ennor, leaving a critical gap in evidence-based interventions

Studies across Ethiopia have shown that milk handling practices often fall below hygiene standards due to inadequate pre- and post-milking treatments, making milk highly susceptible to microbial contamination (Tsedey & Asrat, 2015; Fufa *et al.,* 2019). This contamination leads to foodborne illnesses and poses significant health risks to consumers. In fact, milk-borne pathogenic bacteria account for nearly 90% of all dairy-related diseases (Ryser, 1998). Meanwhile, the demand for milk in Ethiopia is rising due to population growth, increasing incomes and urbanization. Ensuring the quality production and supply of microbiologically safe milk is essential to protect public health and meet growing consumer needs (Zelalem, 2010).

From this point of view; handling practices in Gurage Zone face broader Ethiopian challenges, prior studies in Ezha and Cheha districts offering alarming insights. In Ezha district, 96.7% of farmers lack designated milking areas and over 50% clean barns infrequently, yielding total plate counts (TPC) of 9.82 log cfu/ml (Bereda *et al*., 2012). Cheha district reports similarly high microbial loads (5.67 log cfu/ml TPC) and coliform counts (CC) of 4.41 log cfu/ml, linked to poor udder hygiene and warm storage (Babege *et al.*, 2020). Nationally, Keba *et al.* (2020) found pathogens in 6% of raw milk samples, yet

specific data on Wolkite, Gummer and Ennor remain absent. Given the local tradition of consuming raw milk with kocho without quality control or boiling, these microbial risks and pathogen presence, pose significant foodborne illness.

Without such information, efforts to improve milk safety and quality remain inadequate. Therefore, this study seeks to evaluate and document the handling practices, physicochemical properties, microbial quality, identification of pathogenic bacteria and assessing marketing daynamics of raw cow milk in selected areas of Gurage Zone (Wolkite, Gummer and Ennor). The findings will provide essential data for policymakers, dairy stakeholders, quality control and market linkage authorities and public health authorities to enhance milk safety, strengthen quality control systems and ensure better consumer protection.

## 1.3 General Objective of the Study

- To investigate handling practices, physicochemical and microbial quality analysis and assessment of marketing of raw cow milk in selected areas of Gurage zone.

### 1.3.1 Specific Objectives

- To assess milk handling practices and marketing of raw cow milk in the study areas.
- To evaluate physico-chemical and microbiological quality of raw cow milk in the study areas
- To examine and identify constraints of marketing of milk in the study areas.
- To isolate major bacterial species contaminating raw cow milk.

## 1.4 Research Questions

1. How was hygienic milk handling practiced and marketed in the study area?
2. What were the major constraints of milk marketing in the area?
3. Did the milk produced from the study area meet physicochemical and microbiological quality standards?
4. Was there a major bacterial species contaminating raw cow milk in the study sites?

# 2. LITRATURE REVIEW

## 2.1Milk Production in Ethiopia

Ethiopia has the largest herd in Africa, estimated at 70 million heads of cattle, of which about 7.56 million are dairy herds, and 15.04 million are milking cows (CSA, 2021). Despite the large number of livestock, the milk production is very low, and the annual per capita milk consumption is also very low, estimated at 19 liters/person, although the increased milk consumption in Addis Ababa has brought it to about 52 L/person/year (Azagé, 2018). This figure is extremely low compared to the per capita consumption of 200 liters recommended by the World Health Organization (WHO); despite this, the country's milk production in general has increased in the past 10 years from about 3.07 billion liters in 2015 to about 3.89 billion liters in 2020. This uptrend is mainly related to an increase in the number of cows (Tadesse A., 2021 ; Gebreyohanes, G., Yilma, Z., Moyo, S., and Mwai, O. A., 2021).

The national average daily milk yield and lactation period of local cow are 1.482 L and 7 months, respectively (CSA, 2020 ) , and for hybrid cow's milk production per day is 8 to 10 liters (Tadesse *et al*., 2015).

**Table 1: Ethiopian Cattle Population, Female Herd Proportion, Milking Cows, and Annual Milk Production**

| Year | Cattle population (million) | | | Femal ecattle (%) | dairy cows (%) | Milking cows (%) | milk yield/yr(million litres) |
|---|---|---|---|---|---|---|---|
| | T | M | F | | | | |
| 2014/2015 | 56.71 | 25.26 | 31.44 | 55.45 | 6.50 | 20.66 | 3.07 |
| 2015/2016 | 57.83 | 25.81 | 32.02 | 55.38 | 6.74 | 21.05 | 3.06 |
| 2016/2017 | 59.49 | 26.48 | 33.01 | 55.49 | 7.16 | 21.68 | 3.10 |
| 2017/2018 | 60.39 | 27.37 | 33.02 | 54.68 | 6.66 | 20.17 | 3.10 |
| 2018/2019 | 61.51 | 27.27 | 34.24 | 55.67 | 7.09 | 20.71 | 3.30 |
| Average | 59.19 | 26.44 | 32.75 | 55.33 | 6.83 | 20.85 | 3.13 |

(Tadesse A, 2021; Gebreyohanes, G., Yilma, Z., Moyo, S. and Mwai, O. A. 2021).

## 2.2 Dairy Production Systems in Ethiopia

There are different classifications of the Ethiopian dairy system by different researchers, but according to the Dairy Development and Policy Inventory, the dairy systems in Ethiopia can be classified as pastoral (traditional pastoral livestock farming), agro-pastoral (traditional lowland mixed livestock farming), mixed crop livestock system (traditional highland mixed farming), urban and peri-urban (the emerging smallholder dairy farming), and commercial (specialized commercial intensive dairy farming) (Abasimal, 2021). Pastoralist and smallholder farmers produce 98% of the country's total milk production (Abasimal, 2021).

## 2.3 Composition of Milk

Milk is an opaque, yellowish-white liquid secreted by the mammary glands of all mammals. It is the main source of nutrition and the sole food for the offspring of mammals before they can eat and digest other foods. Milk is a high nutrient containing macronutrients and micronutrients, as well as a large number of active compounds that play important roles in nutrition and health protection (Abera & Angaw, 2015).

The main component of milk is water (87-88%); the remainder is total milk solids. This chemical composition is not fixed; average milk composition percentages vary by animal species and breed, season, feed, lactation stage, health, and physiological status of a particular animal. Sometimes the composition can even change from day to day depending on the feed and climate, but during milking the first drop of milk differs from the last (Abera & Angaw, 2015; O'connor, 1994; Eissa, W.E.M., 2019; Ahmed *et al.*, 2021). In addition, milk is an excellent source of high quality protein, vitamins, and minerals such as calcium and phosphorus. Fresh milk has a pleasant, mild sweetness and is practically odorless.

**Table 2: Approximate Compositional Quality Measures of Milk.**

| Components | Average content(%) | Ranges |
|---|---|---|
| **Water** | 87.2 | 85.3-88.7 |
| **Lactose** | 4.5 | 3.8-5.3 |
| **Fat** | 4.0 | 2.5-5.5 |
| **Protein** | 3.4 | 2.3-4.4 |
| **Mineral Substance** | 0.9 | 0.57-0.83 |

( McDonald *et al.,* 1995, Atkins, 2005, ) (Ahmed *et al*., 2022)

## 2.4 Hygienic Quality of Milk and Microbial Contamination

### 2.4.1 Hygienic Practices in Milk Production

The careful observance of hygienic production standards is necessary to guarantee milk quality and customer safety. Barns, access lanes and milking parlor cleanliness are all part of facility hygiene, which is a basic component of hygienic milk production and quality control. Good facility hygiene has a direct impact on cow cleanliness, especially on the udder and teats, which in turn affects the incidence of mastitis and microbial contamination of milk through external teat surfaces. Regularly clearing barn waste, changing bedding materials frequently, keeping milking parlors clean and easily accessible, providing enough cubicle space for each cow and designating non-crossing walking lanes are all important ways to preserve hygiene (Ahmed *et al.*, 2022).

Depending on the production method, resources available, awareness level and established procedures, hygienic conditions in milk production can vary. In smallholder contexts, milking hygiene practices are usually limited to washing the udder before extraction and/or letting the calf nurse for a few minutes before milking (Muleta, 2016).

One of the most crucial goals of milk production nowadays is sustaining a high degree of sanitation. The economics of production are directly impacted by the degree of hygiene. consequence and dairy companies are implementing this by gradually increasing their raw

milk quality standards. But more significantly, customers are worried about the circumstances of production and the safety of dairy products. Therefore, it is crucial to make sure that healthy animals can produce high-quality raw milk under hygienic conditions and that controls are put in place to safeguard human health. In order to produce clean milk, good hygiene practices are crucial. Clean milk possesses the qualities listed below. low bacterial count, a nice creamy color and scent, and no offensive odors, No dirt and extraneous matter and no residues of antibiotics, sanitizers, or pesticides (Ahmed *et al.*, 2022).

### 2.4.2 Hygienic Practice During Milking

The farm should be the first to have an effective sanitary procedure for milking. The milking process and the farm environment can add microbiological risks to the milk. The creatures themselves. It is important to adhere to correct animal husbandry procedures and take precautions to ensure the milking animals remain healthy. Furthermore, during primary production, unacceptable levels of contamination with chemical residues and other contaminants may result from poor agricultural, animal feeding and veterinary practices, as well as from improper milking techniques, poor general hygiene of milking staff and inadequate equipment(Ahmed *et al*., 2022).

A key component of producing safe and appropriate milk and milk products is using effective handling techniques during milking. Not keeping up sufficient it has been demonstrated that poor sanitation practices can lead to milk becoming contaminated with harmful or undesired microorganisms or physical or chemical dangers (Anwer *et al.*, 2018). Unintentional exposure to milk by bacteria can result in health issues for consumers or defective products. Bacterial contamination of milk can all be minimized by starting the manufacturing process with raw milk of good hygienic quality(Ahmed *et al.*, 2022).

There are very few germs in milk that comes from a healthy udder. Nevertheless, milk is a highly perishable commodity and provides microbes with an excellent medium. As a liquid, it is prone to bacterial invasion and contamination. Most bacteria in milk come from external sources such as air, dirt, dung, hair, and other foreign substances. In other words,

the primary contamination of milk occurs during milking. It is possible to milk animals in a hygienic manner, resulting in raw milk containing only 500 to 1,000 bacteria per milliliter. Typically, the total bacterial count after milking may rise to 50,000 per milliliter, but in cases of poor hygiene, the count can reach several million bacteria per milliliter. This indicates poor hygienic practices during milking and milk handling, or milk from a diseased animal, such as one with mastitis (Pandey and Voskuil, 2011; Ahmed *et al.*, 2022). Poor hygiene introduces additional bacteria, causing the milk to spoil rapidly. To keep raw milk fresh for longer, proper hygiene practices during milking and handling are essential(Ahmed *et al.*, 2022).

**Table 3: Milk Quality Standard in Ethiopia and Europe.**

| Milk | Ethiopian standard | | EU legislation | |
|---|---|---|---|---|
| | **TBC** | **Requirement** | **Parameters** | **Requirements** |
| **Raw cow milk** | Aerobic mesophilic bacteria | <1,000,000 | Plate count (cfu) at 30°C | ≤100,000 |
| | Coliforms | <50,000 | SCC | ≤400,000 |
| | SCC | ≤400,000 | Antibiotic residues | Does not exceed any maximum permitted value |

Where, TBC = Total bacterial count; EU = European union; SCC = somatic cell count

(Teshome and Ketema, 2014; Alganesh, 2017; Ahmed *et al.*, 2022, Mahari, 2016).

All cases of dairy illness continued to be of bacterial pathogens that have been involved in communicable diseases associated with the consumption of milk, including *Escherichia coli, Staphylococcus aureus, Salmonella spp., Listeria monocytogenes, Campylobacter spp., Yersinia, and Clostridium botulinum* (Bereda *et al.*, 2014).

This problem can be severe in countries like Ethiopia, where most of the milk produced is marketed to consumers without being pasteurized and where there is no functional official quality control standard (Bereda *et al.*, 2014).

**Table 4: Overview of Pathogens Most Commonly Associated with Outbreaks in Milk and Dairy Products**

| Microorganisms' | Products | Incidence |
|---|---|---|
| **Escherichia coli** | Milk | 95.5 |
| **Campylobacter** | Dairy products, Milk | 15.3 |
| **Clostridium** | Dairy products | 4.8 |
| **Cryptosporidium** | Dairy products | 1 .4 |
| **Salmonella** | Dairy products, Milk | 40.6 |
| **Staphylococcusaureus** | Dairy products | 0.4 |
| **Shigella** | milk, sour cream, Dairy products | 60.4 |
| **Corynebacterium** | Raw cows'milk | 0.1 |
| **Streptococcus** | Cheese, raw cows' milk | 0.1 |
| **Campylobacter** | Raw milk | 66.9 |
| **Cryptosporidium** | Raw milk | 0.5 |
| **Escherichia coli** | Raw milk, raw milk cheese | 15.0 |
| **Listeria Monocytogenes** | Raw milk, raw milk cheese | 2.6 |
| **Yersinia** | Raw milk | 0.2 |

Asia 2007–2010 ; (Safe Food International, 2011); Mesfin Zewdu, 2015

## 2.5 Measures of Milk Quality

### 2.5.1 Physicochemical Quality Analysis of Raw Cow Milk

Some of the Physico-chemical analysis included the estimation of PH, SG, TA, TS, SNF, Ash and protein.

2.5.1.1 Physical quality

PH value

The acidity of milk can be determined by measuring its pH, or hydrogen ion concentration. The pH of normal cow milk falls between 6.6 and 6.8. Even though milk normally has an acidity range of 0.1 to 0.16%, the development of acidity can cause the pH value to drop below 6.6. This is mostly caused by the phosphates, citrates, and carbon dioxide that are present in milk. Mastitis is the primary cause of a pH value higher than 6.8 (Podorozhniaya, I. and S. Vetokhin, 2016).

Specific gravity (SG)

One important quality indication that is frequently used to identify adulteration in milk is its specific gravity. The specific gravity rises as cream is removed and falls when water is added. It is the mass of a substance or solution divided by the mass of an equivalent amount of water. The proportion of milk's components affects its specific gravity. While removing fat and lowering the temperature raise the specific gravity of milk, adding water and cream lowers it (Abdelhakam, M.A., *et al*., 2017). Milk typically has a specific gravity of 1.027 to 1.035 g/ml, in accordance with Ethiopian standards (Gurmessa, T., E. Mitiku, and R. Alemayehu, 2015).

Titratable acidity(TA) (% lactic acid)

It is a gauge of milk's bacterial activity and freshness. Lactic acid generation is the cause of the sour taste of milk, which is commonly referred to as souring. According to Ahmedsham, M., N. Amza, and M. Tamiru (2018), it was also a significant signal of the growth of acid-producing bacteria from lactose fermentation in the milk and a measure of the milk's freshness. Normal fresh milk has a titratable acidity of 0.1 to 0.17%, in accordance with Ethiopian standards (Gurmessa, T., E. Mitiku, and R, 2015). A general measure of milk's age and handling quality is the proportion of acid in dairy products. Depending on factors like species, breed, individuality, lactation stage, udder physiological condition, etc., the natural acidity of each milk varies significantly (Mehta, B.M., 2015). Long-term storage of the milk causes the bacteria to grow, use the lactose, and turn it into lactic acid, which lowers the pH and increases the milk's acidity. This is known as actual or developed acidity. Titratable acidity is the total of produced acidity and natural acidity (Burke, N., *et al*., 2018). Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity (Dey, S. and M. Karim, 2013).

2.5.1.2 Chemical compositions of milk

In general, milk has a high nutritional value and it is a good diet for children. On average, cow's liquid milk has 87.4% water, 3.7 % fat ,8.9% SNF, 3.4% protein ,4.8% lactose, 0.7% minerals (Eissa, W.E.M., 2019).

Protein

Among the most complicated organic compounds are proteins, which comprise the elements C, H, O, N, S, and occasionally P. All of the essential amino acids and other components that our systems are unable to produce are present in the high-quality proteins found in milk. It is crucial to keep in mind that all living tissue is composed of proteins. The complex milk protein has a good proportion of all amino acids necessary for growth and maintenance, and its composition is similar to that of egg protein (Wolfe, R.R., *et al*., 2018).

Total solid / TS /

Since total solids in raw cow milk determine the amounts of proteins, lipids, carbs, vitamins, and minerals, they are a crucial indicator of nutritional value. It is the whole residue that remains after all of the water in milk has evaporated, and it contains solids rather than fat, protein, lactose, and mineral matter. Its components are found in milk as a mechanical combination. TS contents = SNF (%) + Fat (%) is the mathematical expression for it (Ismail, H.A. and H. Hamdon, 2017).

Solid not fat / SNF /

The substances in milk other than water and butter fats are termed as solid not fat. It is the residual component that left after the complete evaporation of water from the milk. Mathematically it can be expressed as: SNF % = TS (%) –Fat (%) (Ismail, H.A. and H. Hamdon, 2017. Milk differs in composition due to different factors like species of animal, variety, individuality, lactation's stage, incidence of milking, age, feed, disease, and administration of hormones and drugs (Boro, P., *et al.*, 2016). Milk can be obtained from different species like goats, sheep, camels, cows, etc. and the composition of the milk that is obtained from these different species varies as the species vary (Boro, P., *et al.*, 2016).

### 2.5.2 Bacteriological Quality Analysis of Raw Cow Milk

When taken from a healthy udder, milk contains very little bacteria. It selects a variety of bacteria from the moment it leaves the cow's nipples until it is consumed or further processed. Microorganisms can enter milk through cows air, feed, milk handling equipment, and milking machines, and once they enter the milk, their numbers increase rapidly (Tollessa, 2016; Ahmed *et al*., 2022).The microbiological content of milk indicates the level of hygiene during milking, including the cleanliness of the milking equipment, proper storage and transport, and the safety of individual udders. Commonly used microbiological quality tests for milk and milk products include determination of somatic cell count(SCC), standard plate count ( SPC), YMC and coliform count (CC) (Gemechu, 2016; Tamirat, 2018; Fufa *et al*., 2019).

2.5.2.1 Methylene blue reduction test

The methylene blue reduction test is predicated on the notion that milk dyed with methylene blue loses its color at different rates. The loss of oxygen from the milk and the production of reducing chemicals as a result of bacterial metabolism are the causes of this color shift (Anwer *et al.*, 2018). The bacterial population in a milk sample can be estimated using the blue-colored reagent methylene blue. After adding a known dilution of the methylene blue solution, the milk is periodically checked to see if the blue hue disappears. The quantity and kind of bacteria in the milk determine how long it takes for the color to fade. Prior to pasteurization, this test is typically used to grade the quality of raw milk. On the basis of this test, the test tube was placed in a water bath immediately for incubation so that the temperature was maintained at 37°C. The initial time was noted down, and the test tube was again examined after half an hour; then subsequent readings were taken at hourly intervals, and results were interpreted as follows (Hawaz *et al.*, 2015; Yonas Hailu, 2015; Mahari and Yemane, 2016; Sewgil *et al.*, 2018; Hussien *et al.*, 2021).

Sorting cow milk samples according to the guidelines provided by (Hawaz *et al*. 2015; Yonas Hailu, 2015; Sewgil *et al*., 2018; Hussien *et al.*, 2021).
Excellent, no decolorization within 8 hours; Good, decolorized in at least 6 hours but less than 8 hours; Fair, decolorized in at least two hours but not more than six; Poor, decolorized in less than two hours.

2.5.2.2 Standard plate count (SPC)

The standard plate count (SPC), which counts all aerobic bacteria in raw milk at the moment of pick-up, is one of the most popular microbiological quality tests for milk and milk products. Naturally, milk gathered or handled in an unsanitary way will have more bacteria than really clean milk. Milk grading is based on the conventional plate count (Mahari and Yemane, 2016; Mahari, 2016). To encourage bacterial growth, milk samples are plated in a typical plate count agar medium and then incubated for 48 hours at 32°C. Bacteria can grow into visible colonies that are subsequently counted, or they can form into single bacteria or close clusters (like chains or clumps). All bacterial plate counts are expressed as the number of colony forming units (CFU) per milliliter (ml) (Mahari, 2016; Ahmed *et al.*, 2022).

Aseptically collected milk from healthy cows usually has SPC values below 1,000 cfu/ml. Increased numbers suggest that the milk may include contaminated bacteria from a variety of sources. Plate count requirements have been set in order to ensure proper factory cleanliness and product safety. The plate count approach has been used as a helpful adjunct to assist sanitarians in addressing sanitation concerns and improving the quality of milk (Mahari, 2016; Ahmed *et al.*, 2022). Its usefulness is restricted, though, as it does not reveal the quality of microbial populations with respect to pathogens and non-pathogens. The most reliable and instructive technique for determining the bacteriological quality of milk is the standard plate count (Kurwijilla *et al.*, 1992; Mahari, 2016).

**Table 5: Shows the System for Classifying Raw Milk According to its SPC.**

| Bacteria Count/ml | Grade |
|---|---|
| Not Exceeding 200,000 | Very Good |
| 200,000-1,000,000 | Good |
| 1,000,000-5,000,000 | Fair |
| >5,000,000 | Poor |

Where, SPC = standard plate count

(kurwijila *et al*., 1992; Mahari, 2016)

2.5.2.3 Yeast and Mold Count ( YMC)

Yeast and mold contamination of food products including milk plays an important role in spoilage and shortened shelf life (Gamal *et al.*, 2015).

2.5.2.4 Coliform count(CC)

According to the level of hygiene during and after handling, coliforms are another type of bacteria that have an impact on milk quality (Tollessa, 2016). ESA (2009) states that a total coliform count of more than 0-1000 CFU/ml is not acceptable for high-quality milk. However, many studies have shown that this coliform is beyond the standard. For example, Tsedey and Asrat (2015) reported that the coliform counts for raw milk collected from producers were 4.00 log10cfu/ml and 4.29 log10cfu/ml for producers and consumer, respectively. Similarly, Habtamu et al. (2018) reported that the mean CC of the sample of households, dairy farms and pasteurized milk was 5.58, 6.63, 7.24 Log10Cfu/ml, respectively. Similarly, Fufa *et al*. (2019) found the total CC of milk from dairy farms, suppliers and restaurants to be5.91, 5.77 and 2.17 log10cfu/ml, respectively. The researchers suggested that the higher total coliform count could be due to contamination of milk during milking, poor milkman hygiene, fecal contamination of the udder and lower abdomen. Bovine bodies mainly from the bedding material cause microorganisms to enter the milk during milking. The presence of coliforms represents a safety risk and therefore,

these numbers should correspond to the minimum recommended in dairy products (Alganesh,2016; Fufa *et al*., 2019).

### 2.5.3 Isolation and Identification of Pathogenic Bacteria from Raw Milk

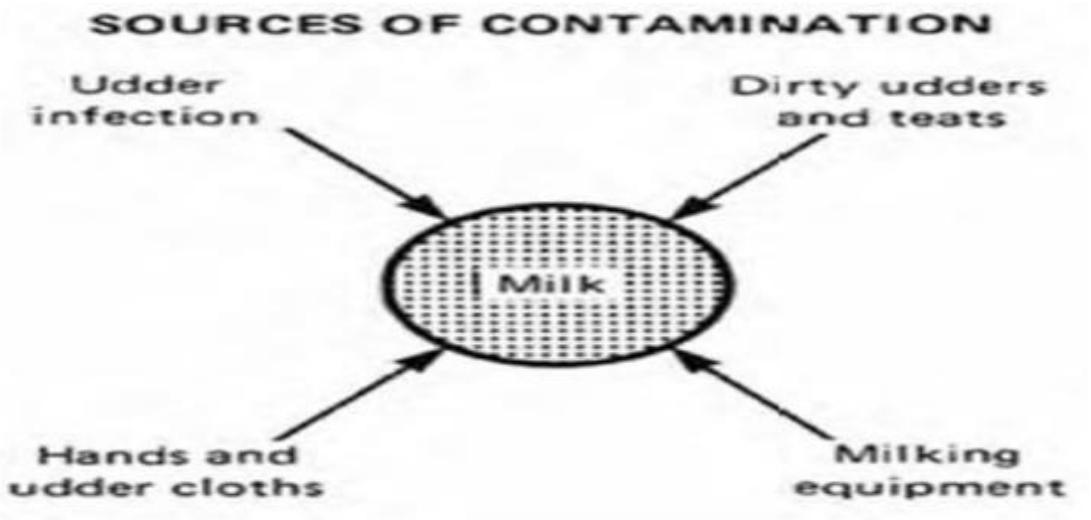
*Escherichia coli, Vibrio cholerae, Shigella spp., Staphylococcus aureus, Yersinia spp., Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis, Salmonella spp., Brucella abortus, Campylobacter jejuni,* and *Bacillus cereus* are among the pathogenic microorganisms that have been detected in raw milk and naturally fermented raw milk (Gran *et al.,* 2003; Herreros *et al.,* 2005; Mufandaedza *et al.,* 2006; Teshome Gemechu, 2016). *E. coli, Staphylococcus aureus, Salmonella,* and *Streptococcus species* are the most significant pathogenic bacteria found in raw milk samples gathered from various regions of Ethiopia (Godefay and Molla, 2000; Molalign *et al.,* 2011; Tollessa *et al.,* 2012; Shunda *et al.,* 2013; Teklemichael *et al.,* 2013; Tadesse and Bacha, 2014; and Teshome Gemechu, 2016). Nevertheless, the presence of these pathogenic microorganisms in milk products may be hazardous to the public's health.

A number of food-borne illnesses may occur due to consumption of raw milk or dairy products vended on the street since the chance of contamination of dairy products sold on the street is very high. Poor sanitation of the vending environment, poor hygiene of the vendors, improper (inadequate) pasteurization or boiling, poor handling and absence of cooling facilities may cause contamination of dairy products by pathogenic microorganisms (Aberra, 2010; Debebe, 2010, Teshome gemechu, 2016) .

## 2.6 Sources of Microbial Contamination of Milk

A healthy udder produces milk with very few microorganisms. Milk is a perishable good, though. Since it is a liquid, it is highly vulnerable to bacterial invasion and contamination, making it the perfect habitat for microbes. The air, dirt, feces, hair, and other external materials are the source of nearly all of the germs found in milk. Stated differently, the process of milking is the primary source of bacterial contamination. It is feasible to milk animals so cleanly that there are only 500–1000 bacteria per milliliter of fresh milk. Although the total number of bacteria after sampling often approaches 50,000 bacteria/ml, it can reach several million bacteria/ml (Rodrigues *et al.*, 2005; Ahmed *et al.*, 2022). This indicates very poor standards of hygiene in milking and when handling milk or milk from diseased animals, for instance. mastitis. Any stage of the milk production process can result in contamination. The operator of the food business (milk producer) is in charge of identifying these issues and putting control measures in place to prevent contamination of the milk. Animal droppings, particularly from soiled teats, udders, and tails; bacteria from improper milking techniques, unclean hands, and improperly cleaned and disinfected equipment (including bulk milk tanks); failure to clean and disinfect teats prior to milking; foreign objects, particularly from malfunctioning parts of milking machines and tanks, dust, bedding materials, manure, insects, and animal hair; chemicals, metals, organics, etc. from veterinary drug residues; and the use of non-food-grade equipment are the main sources of contamination (Bekuma, 2018).

The milking environment, the cow it self, milking staff, milking equipment, milk transportation, feed, and water are the typical risk factors for microbial contamination of milk (Bekuma, 2018).




Source: ( national mastitis council, 2005 , Mesfin Zewdu,2015)

**Figure 1:** Major Sources of Contamination of Milk

## 2.7 Impacts of Public Health

The economic and nutritional value of milk and dairy products in developing countries is evident. However, as the industry grows and becomes more market oriented, focus needs to be placed on the potential risks associated with the production and consumption of dairy products. In developed countries, up to 30% of the population is affected by foodborne illness each year, imposing a major burden on public health and the economy. America's food supply system is among the safest in the world, but there are still an estimated 76 million cases of foodborne illness each year, causing 5,000 deaths and 325,000 hospitalizations (WHO, 2007; Mesfin Zewdu, 2015). The major pathogens alone cause $35 billion in medical costs and lost productivity each year. Information on the impact of foodborne diseases in developing countries is limited due to a lack of reporting systems and poor health care infrastructure. Nevertheless, the burden of food-borne illnesses in developing regions is estimated to be high due to the high incidence of diarrheal diseases. In 2005, 1.8 million deaths in children under the age of 5 worldwide were attributed to diarrheal diseases, and a large proportion were due to contaminated food and drinking water. Of the 1.8 million deaths, 78% (1.46 million) occurred in Africa and Southeast Asia (Boschi-Pinto *et al.,* 2008; Mesfin Zewdu, 2015). Diarrheal diseases in developing countries are a major public health problem, not only because of their direct cause of disease and morbidity, but also because of their role in infant and young child malnutrition (WHO, 2007). When a child has diarrheal disease, the inability to absorb nutrients undermines the nutritional benefits of a diet of adequate quantity and quality and can exacerbate malnutrition.

Milk and dairy products are a potential source of transmission for many foodborne pathogens due to their neutral pH and rich nutrient composition (LeJeune and Rajala Schultz, 2009; Pal and Jahdav, 2013; Mesfin Zewdu, 2015). Milk-borne outbreaks in the US and other developed countries have been drastically reduced over the years due to the close attention paid to quality control, including the widespread use of pasteurization, the guidelines set out in the HAACP procedures. In 1938, dairy-related outbreaks accounted for 25% of all outbreaks in the US due to contaminated food and water. Currently, milk-borne outbreaks account for less than 1% of all foodborne outbreaks in the United States. The majority of documented dairy-related outbreaks have been due to unpasteurized dairy products. Between 2000 and 2006, 40 outbreaks in the United States were traced to raw milk, compared to only 4 outbreaks from pasteurized milk (Oliver *et al.*, 2009).

In developing countries, over 80% of milk consumed is unregulated, and in Ethiopia less than 1% of milk consumed is pasteurized (FAO, 2009; Mesfin Zewdu, 2015). Again, there is limited information on the impact of milk-borne diseases in these regions, but due to the large amount of unregulated milk consumed and the risks of consuming unpasteurized dairy, the impact is likely to be large. Milk can be contaminated with bacteria of both human and animal origin at any stage of the process from production to consumption. Pathogenic organisms can be shed into the milk of an infected animal (before harvest) or contamination can occur at the time of collection, processing, distribution and storage (after harvest) (LeJeune and Rajala Schultz, 2009). As the dairy industry in developing countries moves towards a more market-oriented system, food safety becomes extremely important. When mass contamination occurs, outbreaks affect more people and cause greater economic impact. Emphasis must be placed on food safety standards and procedures for both pre- and post-harvest activities (LeJeune and Rajala-Schultz, 2009).

## 2.8 Milk Marketing Systems in Ethiopia

Ethiopia's milk marketing system consists of both formal and informal channels, with the informal sector being the most dominant. The informal market operates with minimal government intervention, where producers sell fresh milk directly to consumers in both rural and urban areas. Despite regulations requiring pasteurization, a significant portion of milk is sold raw without proper hygienic measures.

Approximately 95% of the country's milk is marketed through informal, unprocessed channels, while only 5% is sold as liquid milk due to limited infrastructure, especially in rural areas. Traditional dairy processing, particularly of soured butter, remains prevalent. Overall, milk production is primarily non-market oriented, with most of it consumed at the household level (Debrah & Berhanu, 1991; Omore *et al.*, 1999; Zegeye, 2003; SNV, 2008; Diriba Tola Teka, 2024).

# 3 MATERIALS AND METHODS

## 3.1Description of the Study Area

The study was conducted in Gurage Zone, in one administrative town, Wolkite, and two districts of the zone, namely Gumer and Ennor. The study areas were chosen purposely based on the dairy cow's potential. Wolkite town is located in the central Ethiopia region, which is the zonal seat town. The town is located at a distance of 158 km from Addis Ababa, the capital city of Ethiopia. The area is a lowland area with an average annual temperature of 25°C and 800 mm of rainfall. It has a livestock population of 12,091 cattle, 1,046 sheep, and 1,190 goats (GZPEDD, 2019). Gumer district is located at a distance of 65 km from Wolkite town in the southeast direction and has a temperature range of 12.6–22.5°C, rainfall of 1,001–1,400 mm, and livestock including 87,514 cattle, 105,878 sheep, and 568 goats. Ennor district is 42 km away from Wolkite and has a temperature range of 12.6–25°C, rainfall of 801–1,400 mm, and the highest livestock population, with 351,290 cattle, 31,842 sheep, and 231,595 goats (GZPEDD, 2019).

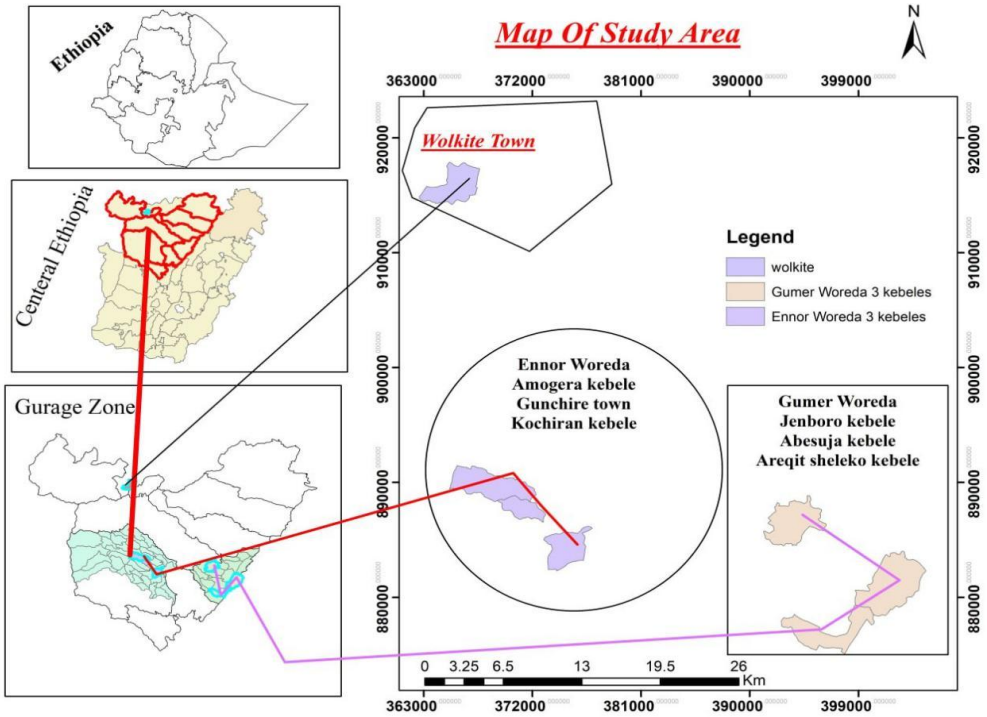



**Figure 2: Map of the Study Area**

## 3.2 Study Design , Sample Size Determination and Methods of Data Collection

### 1.6.1 Study Design and sample size determination

The study was purposively conducted in selected areas of the Gurage Zone, including Wolkite town Administration, Gummer and Ennor districts, focusing on areas with high dairy cattle population potential. In Wolkite Town, three sub-cities; Bekur, Addis and Gubre were selected for the study. Similarly, in Gummer District, which comprises 21 kebeles of these three kebeles Jemboro, Abesuja and Arekit Town were purposively chosen. In Ennor District, which consists of 46 kebeles, three kebeles; Amogera, Kochiran and Gunchre were also purposively identified for inclusion in the study. The study targeted smallholder dairy farmers and milk vendors, with participant selection based on specific criteria; including the presence of lactating cows, availability of milk at the time of sampling, willingness to participate and accessibility of key stakeholders for data collection through questionnaires and milk sampling.

A well-structured cross-sectional study design was employed to assess hygienic milk handling practices and marketing chain dynamics. Additionally, a laboratory-based analysis was conducted to evaluate the physicochemical properties, microbial quality and Isolations of pathogenic bacteria contamination of raw cow milk.

1.6.1.1 Questionnaire survey

A semi-structured questionnaires were used to gather information on potential risk factors for microbial contamination of milk, with a focus on hygienic milking practices, milk handling and marketing assesment. Key risk factors assessed, included the sanitary conditions of barns and milking environments, the hygiene of milking cows' udders and milk handlers, as well as the cleanliness of milking equipment, storage and transportation. Data were collected through face-to-face interviews and direct observations.

The sample size for this study was determined using Yamane's (1967) formula, ensuring a statistically valid representation with a 95% confidence level and a 5% margin of error. From a total population of 2,178 households and vendors, 338 participants were selected purposively. A purposive sampling technique was employed to select smallholder dairy farmers and milk vendors, ensuring the inclusion of key stakeholders directly engaged in milk production and vending. The final sample distribution consisted of 200 small-scale producers and 2 vendors from Gummer District, 116 small-scale producers and 2 vendors from Ennor District and 10 small-scale producers along with 8 vendors from Wolkite town administration.

The Yemane formula was used to determine the sample size (Yemane, 1967).

In order to determine the sample size;

$$n = \frac{N}{1+N(e)\,2}$$

Where; n =designates the sample size the researcher uses;

N= designates total number of households and Vendors

E =designates maximum variability or margin of error 5 %

1=designates the probability of the event occurring.

Therefore ,the sample size is; $n = \frac{N}{1+N\,(e)2} = \frac{2178}{1+2178*(0.05)2} =$ **338**

1.6.1.2 Milk sample collection

To determine the sample size for this investigation, Kothari's (2004) formula for an unknown population ($n = Z^2SD^2/e^2$) was applied. where Z is the estimated standard variation at the 95% CI, which was thought to be the normal distribution point that corresponded to the significance level (Z=1.96). The estimated standard deviation (SD) was 0.15, or 15%, while the estimated error, e, was 0.05, or 5%. Consequently, the sample size "n" was determined as follows:

$n = \frac{(1.96)2*(0.15)2}{(0.05)2} = 34.6$ approximately n = 35 samples in Gurage zone of selected study areas. Based on the calculated sample size, 35 milk samples were initially required from the Gurage zone of three study areas. However, to enhance precision and reliability, the raw cow milk sample size was increased to **60**.

Based on the above calculations; A total of 60 raw cow milk samples were collected from producers and vendors across three selected sites in the Gurage Zone (Wolkite, Gummer and Ennor) in Central Ethiopia Region. The final sample distribution consisted of 26 small-scale producers and 2 vendors from Gummer District, 20 small-scale producers and 2 vendors from Ennor District and 4 small-scale producers along with 6 vendors from Wolkite town administration, all selected purposively. Milk samples were collected in the morning directly from individual household farms and retail vendors to ensure freshness. Each sample consisted of 500 mL of raw cow milk, which was thoroughly homogenized and placed in sterile polyethylene bottles to maintain sample integrity for laboratory analysis.

The samples were then thoroughly mixed, labeled and coded before being securely stored in sterile containers. To maintain quality and prevent microbial alterations, all samples were transported in an icebox to Wolkite University's Food Engineering and Biotechnology Laboratory for further analysis. Upon arrival, the samples were immediately refrigerated at 4°C and analyzed within six hours of collection to ensure accuracy and reliability in the laboratory investigation.

## 3.3 Laboratory Analysis Methods

### 3.7.1 Evaluation of raw cow milk's physicochemical quality

3.7.1.1 PH value

Using a digital pH meter and the method outlined by O'Connor (1995) and Sime Tessema and Seda Bedasa (2019), the pH of the milk samples was measured in the lab. A digital pH meter was used to measure the milk's pH. The pH meter was calibrated using pH 7 and pH 4 buffers. Following calibration, the electrode was submerged in a 100 mL amount of prepared milk sample in a beaker and left there until a consistent reading was obtained (Sime Tessema and Seda Bedasa, 2019).

3.7.1.2 Titratable acidity of milk ( TA )

The procedure developed by the Association of Official Analytical Chemists (AOAC, 1990) was used to analyze the milk samples for titratable acidity. Three to five drops of 1% phenolphthalein indicator were added to nine milliliters of milk sample that had been pipetted into a beaker. After that, a 0.1N NaOH solution was used to titrate the milk samples until a slight pink hue remained. The following formula was ultimately used to determine the titratable acidity, which was expressed as a percentage of lactic acid (AOAC, 1990; Gemechu, 2016).

$$TA\% = \frac{\frac{N}{10}NaOH(ml) * 0.009}{Weight\ of\ milk\ sample} * 100$$

3.7.1.3 Specific gravity ( SG )

An assessment of milk fraud through the measurement of specific gravity and fat content, the adulteration of the raw milk samples that were obtained was assessed. Identifying Specific Gravity: Using a lactometer, density (g/ml) was measured. A lactometer and a controlled milk temperature were used to test for water adulteration using specific gravity (SG). In order to obtain equilibrium and take readings below the meniscus, the lactometer was left to float freely in a cylinder filled with enough milk sample (O'Connor, 1995; Tekliye and Gizaw, 2017). The specific gravity of the milk was thus determined using the following formula.

**Specific gravity = (L/1000) + 1**

where L is the temperature-adjusted lactometer reading. The lactometer reading was adjusted by subtracting 0.2 for each degree below 15.56°C and adding 0.2 for each degree above that temperature.

Crude protein determination

Protein content determination The total protein level was ascertained using the formaldehyde titration method. A beaker was filled with ten milliliters of milk. The milk was then mixed with 0.4 ml of 0.4 percent potassium oxalate and 0.5 ml of 0.5 percent phenolphthalein indicator. The samples were then titrated with 0.1N sodium hydroxide solution using a digital dispenser/burette. The titration was carried on until the pink color's intensity rose. (C.B. O'Connor, 1994). The burette readings were finally recorded. A factor of 1.74 was applied to the readings (Foley et al., 1974; Gemechu, 2016).

$$Percent\ protein = Burette\ reading \times 1.74$$

3.7.1.5 total solids /TS/

To find the total solids content, 5 g of freshly mixed cow milk was placed in a flat-bottom crucible that had been previously weighed and dried (AOAC, 1990). For three hours, the milk samples were dried at 102 degrees Celsius in a hot air oven. The dried samples were then removed from the oven and allowed to cool to room temperature in desiccators. The samples were then weighed once more, and the total solids were calculated using the formula below (Richardson, 1985).

$$\text{Total solids} = \frac{\text{Crucible weight+Ovendry sample weight - Crucible weight}}{\text{Sample weight}} \times 100$$

3.7.1.6 Determination of Ash Content

By igniting the dried milk samples used for the total solids measurement in a muffle furnace and gradually raising the temperature to 550°C for five hours, the total ash was measured gravimetrically. Until the carbon (black color) disappeared or the ash residue turned white, the sample was burned. Finally, the furnace's ash was removed and placed in desiccators to cool. The ash content was calculated as

$$\text{Percent ash} = \frac{Weight\ of\ residue}{Weight\ of\ sample} * 100$$

Finally, the result will be recorded (Richardson, 1985; Gemechu, 2016)).

### 3.7.2 Bacterial Quality Analysis of Raw Cow Milk

3.3.1.1 Methylene blue reduction (MBR) test

Each test tube was filled with 10 ml of milk sample and 1 ml of methylene blue, and it was then placed in a water bath set between 35 and 37°C. Milk samples were checked for decolorization after 30 minutes of incubation, and readings were then taken every hour thereafter. It was noted how long it took for methylene blue to completely change color from the previous inversion to decolorization. The color change's duration was noted. The microbial load in the milk and the entire metabolic processes of the microorganism are referred to as the dye reduction time (Ombui *et al.*, 1995; Hawaz *et al.*, 2015; Yonas Hailu, 2015; Hussien *et al.*, 2021).

**Table 6: The Suggested Classification of Milk Quality Based on Methylene Blue reduction (MBR) Test.**

| Milk Grade | Decolorization time |
|---|---|
| **Excellent** | Not decolorized in 8 hours |
| **Good** | Decolorized in less than 8 hours but not less than 6 hours |
| **Fair** | Decolorized in less than 6 hours but not less than 2 hours |
| **Poor** | Decolorized in less than 2 hours |

Hawaz *et al*. 2015; Yonas hailu, 2015; Hussien *et al.*, 2021)

3.3.1.2 Standard plate count (SPC)

The Standard Plate Count (SPC) is one of the most popular microbiological quality tests for milk and milk products. By introducing 1 milliliter of milk sample to a sterile test tube containing 9 milliliters of peptone water, the total number of bacteria was determined. Following thorough mixing, duplicate samples (1 ml) were pour-plated using 15-20 ml of autoclaved Standard Plate Count Agar (SPCA) solution, and the sample was serially diluted up to $1:10^{-7}$. After allowing the plated sample to settle, it was incubated for 48 hours at 37°C. The colony counter (Marth EH, 1978; Teklegiorgis, 2018) was used to count the colonies. The American Public Health Association's formula was used to determine the

quantity of bacteria in a millimeter of milk after the bacterial colonies in each petri dish had been counted and recorded (APHA, 1992) and (Tekilegiorgis, 2018).)

$$N = \frac{\sum C}{[(n1*2)+(0.1*n2]} *d$$

Where N is the number of colonies per milliliter of milk, ∑C is the total number of colonies on the counted plates, n1 is the number of counted plates on the lower dilution, n2 is the number of counted plates in the next higher dilution, and d is the dilution used to obtain the initial counts.

When computing TBC, only the first two significant digits were recorded and the bacterial count was reported as colony forming unit per milliliter of milk (CFU/ml).

3.3.1.3 Total coliform count (CC)

A sterile test tube with nine milliliters of peptone water was filled with one milliliter of milk sample, and the mixture was thoroughly mixed up to a serial dilution of $10^{-7}$. After surface plating duplicates of the proper decimal dilutions and incubating them on Violet Red Bile Agar (VRBA) for 24 hours at 37°C, dark red colonies that typically measured at least 0.5 mm in diameter on uncrowned plates (Richardson, 1985) were identified as coliforms and counted. This was followed by a confirmatory test by transferring and incubating four to five typical colonies from each plate transferred into tubes containing 2% Brilliant Green Lactose Bile Broth (BGLB). Gas production within 48 hours of incubation at $35^0$C was considered as sufficient evidence for the presence of coliforms (Richardson, 1985, Gemechu, 2016; Tekilegiorgis, 2018).

The formula provided by the American Public Health Association (APHA, 1992) and Tekilegiorgis (2018) was used to determine the number of bacteria in millimeter milk.

$$N = \frac{\sum C}{[(n1*2)+(0.1*n2]} *d$$

Where, n1 = number of plates on lower dilution counted, n2 = number of plates in next higher dilution counted, d = dilution from which the first counts are acquired, and ∑C = total of colonies on plates counted.

Colony forming units per milliliter of milk (CFU/ml) was the bacterial count, and only the first two significant figures were recorded when calculating CC.

3.3.1.4 Yeast and Mold counts ( YMC).

Milk samples were serially diluted using the same methods as for the total bacterial count but using Potato Dextrose Agar (PDA) for surface plating. For three to five days, the dried plates were then incubated at 25°C. Molds and yeasts were identified by counting colonies with a blue-green tint; yeasts were identified as creamy to white/gray colonies, while molds were identified as filamentous (fuzzy) colonies of different colors (yellow, green, and light brown) (A. E. Yousef and C. Carlstrom, 2003; Fereja et al., 2023).

### 3.7.3 Detection and Enumeration of Pathogenic Bacteria

3.7.3.1 Isolation and Identification of E. Coli:

The methods used in isolation and identification of E. coli were according to the techniques recommended by Abunna (2019). The manufacturer's instructions were followed to make the MacConkey agar medium, which is utilized as a differential medium for E. coli identification. Milk and swab samples were then streaked onto agar plates. The plates were then turned upside down and incubated for twenty-four hours at 37°C. Following incubation, the plates were checked for colonies of E. coli. E. Coli colonies cultivated on MacConkey Agar were found to be medium in size, dry, pink and able to grow alone or in groups. The suspected colonies were once more subcultured on Levine Eosin Methylene Blue (EMB) Agar, a selective medium and incubated for twenty-four hours at 37°C. For biochemical validation, morphologically typical E. Coli colonies that were generating metallic sheen were further subcultured onto Nutrient Agar. To differentiate E.coli from others biochemical tests like grams stain, urease, TSI, Catalase, Starch hydrolysis, MR, VP tests were performed to well isolated colony from nutrient agar plates to confirm the presence of E. coli in the test samples(Fereja *et al*., 2023).

3.7.3.1 Isolation and Identifications of Staphylococcus Aureus

Staphylococcus aureus. The raw milk sample was serially diluted up to $10^{-10}$ by adding 1 mL of it to a sterile tube with 9 mL of sterile peptone water. A 0.1 ml aliquot from this dilution was then transferred to mannitol salt agar (MSA) plates that were appropriately labeled. The plates were spread and incubated (inverted) at 37°C for 48hrs, typical Staphylo coccus aureus colonies appeared as golden yellow, smooth, circular of 2-3mm diameter, convex, and moist and surrounded by an opaque halo, clear zones, un-crowded plates were counted. For confrmation, four to five of typical colonies per MSA plate were streaked on Mannitol salt agar which was followed by Gram-staining tests like grams stain, urease, TSI, Catalase, Starch hydrolysis, MR, VP tests were performed to well isolated colony from nutrient agar plates to confirm the presence of S.aureus in the test samples( ISO, 1999; Yousef and Carlstrom, 2003; Tekliye & Gizaw, 2017; Fereja *et al*., 2023).

3.7.3.2 Salmonella spp.

Isolation and identification of Salmonella spp involved three steps based on ISO6579, 2000. First, 25ml of milk sample was pre-enriched with 225ml of buffered peptone water (BPW) and incubated for 24hrs at 37°C. A portion (0.1ml) of the preenriched cultured was transferred to10 ml of Rappaport Vassilidis (RVs) broth and incubated at 42°C for 24hrs. Finally, a loop full of a culture broth was streaked on the surface of a dry Xylose Lysine Deoxycholate (XLD) agar plate. Then the plates were incubated at 37°C for 24hrs and the incubation was prolonged to 48 hrs for those that did not show any growth during the 24hrs of incubation. Characteristic Salmonella spp. colonies, having a slightly transparent zone of reddish color and a blackShorten center, were sub-cultured on nutrient agar (Oxoid, CM0003). To confirm the presence of Salmonella spp. further tests were made including gram staining and biochemical tests (ISO, 2003)(Yirda *et al.*, 2020) .

## 3.8 Data Management Analysis

The gathered information was thoroughly examined in order to produce insightful interpretations, findings, and suggestions. Microsoft Excel was used to create graphical and tabular representations. Prior to statistical analysis, logarithmic values (log10) were used to represent the data from microbial counts. After that, SAS's General Linear Model (GLM) process was used to assess the physicochemical property data and the converted microbial count values (SAS, 2009). When analysis of variance revealed significant differences across means, mean separation was performed using the Least Significant Difference (LSD) technique, with differences deemed significant at $p < 0.05$. To analyze the physicochemical characteristics and microbiological quality analysis and pathogenic bacteria identification of raw cow milk, the following model was employed:

$$Yij = \mu + \partial i + \beta j + eij$$

Where, Yij = The value of the respective variable mentioned above on the I Woreda and Town(i=3, Wolkite Town, Gummer and Ennor districts), $\mu$ = Overall mean of the respective variable, $\alpha i$ = The effect of ith Town & Woreda (Wolkite, Gummer and Ennor) on the respective variable, $\beta j$ = milk source in the three study areas ( Wolkite, Gummer and Ennor), eij = The error term.

# 4 RESULTS AND DISCUSSION

## 4.1Milk Handling practices

In order to keep dairy products safe and high-quality for consumers, milk cleanliness is crucial. Table 7., reveals that the hygienic practices of raw cow milk handling in selected areas of Gurage zone of which Wolkite town Administration, Gummer and Ennor districts. The findings highlight both positive practices and critical shortcomings in milk hygiene, which have significant implications for milk safety and public health.

### 4.1.1 Demographic Characteristics of the Respondents

The study revealed that milking cows in the study areas were predominantly managed by women (95.86%), with only 4.14% of respondents being male. This finding highlights the crucial involvement of women in managing milking cows, feeding, hygiene and overall milk production. Women not only handle milking but also ensure that lactating cows receive proper nutrition and care. One of the notable traditional practices observed was that women isolate the milking cow from the rest of the herd and provide it with special feeding and management. They understand that every drop of milk is directly linked to the quality and quantity of feed given to the cow. This practical knowledge demonstrates their understanding of dairy management and their role in ensuring optimal milk production.

The age of respondents varied across the study areas, highlighting differences in participation among different age groups. The largest proportion of respondents fell within the 41–59 years category, comprising 50.59% (171 individuals), suggesting that middle-aged individuals were the most engaged in the study areas. This could be attributed to their established roles in society, making them more accessible and willing to participate. The second most represented group was the 31–40 years category, accounting for 25.44% (86 respondents), indicating a moderate level of engagement. The younger age group (20–30 years) had a significantly lower representation, making up only 16.27% (55 respondents), which could be due to migration for employment opportunities and low accessibility of their dairy herd. Meanwhile, the oldest age group, those above 60 years, constituted only 7.7% (26 respondents), reflecting potential limitations such as mobility issues or reduced involvement in dairy cattle rearing activities.

The dominance of middle-aged participants suggests that they are the most active and more accessible in participating dairy cattle rearing.

Education significantly influences the adoption of improved dairy management techniques and better milk handling trends. The majority of respondents (78.70%) had basic literacy (read and write), while 15.09% had elementary education, 5.33% attended secondary school and only 0.88% had higher education. The predominance of low educational levels indicates that many farmers have limited access to information on modern dairy practices, hygiene and quality control.

Feeding practices play a critical role in milk production and quality. The study found that the majority (98.77%) of respondents fed their cows with natural grass, crop residue and Enset, while 1.23% supplemented with natural pasture and Enset. No respondent practiced free grazing alone, the dominance of natural grass, crop residues and Enset-based feeding systems suggests a reliance on available resources and shift towards mixed feeding systems. While integrating crop residues with Enset provides some nutritional benefits, the lack of high-quality forage supplementation may limit milk yield and quality. Encouraging farmers to incorporate protein-rich supplements and balanced feeding strategies can help improve dairy productivity and nutritional quality. Most of the dairy farmers operate at a smallholder level, with 66.27% owning only one milking cow, 19.23% having two cows and 10.95% owning three or more cows. The predominance of small herd sizes indicates low-scale milk production, which may limit market supply and household income and consumptions. Expanding access to improved breeds, veterinary services and proper feeding strategies can help smallholder farmers enhance productivity and profitability.

**Table 7: Demographic Characteristics of producers and vendors of participants**

| Variable & Category | Study Areas | | | | | | Total N= 338 | % |
|---|---|---|---|---|---|---|---|---|
| | Wolkite n=18 | | Gummer n= 202 | | Ennor n= 118 | | | |
| | P, 10 | V,8 | P,200 | V,2 | P,116 | V, 2 | | |
| **Sex of the respondents** | | | | | | | | |
| **Female** | 8 | 7 | 192 | 2 | 113 | 2 | 324 | **95.86** |
| **Male** | 2 | 1 | 8 | 0 | 3 | 0 | 14 | **4.14** |
| **Age in years** | | | | | | | | |
| **20-30** | 3 | 1 | 30 | 0 | 20 | 1 | 55 | **16.27** |
| **31-40** | 4 | 4 | 43 | 1 | 33 | 1 | 86 | **25.44** |
| **41-59** | 2 | 3 | 113 | 1 | 52 | 0 | 171 | **50.59** |
| **>60** | 1 | 0 | 14 | 0 | 11 | 0 | 26 | **7.7** |
| **Level of Education** | | | | | | | | |
| **Read and Write** | 8 | 0 | 172 | 0 | 86 | 0 | 266 | **78.70** |
| **Elementary School** | 0 | 6 | 20 | 1 | 24 | 0 | 51 | **15.09** |
| **Secondary School** | 1 | 2 | 8 | 1 | 4 | 2 | 18 | **5.33** |
| **Higher Education** | **1** | **0** | **0** | **0** | **2** | **0** | **3** | **0.88** |

Where, n= number of participants; P= milk producers; V= vendors

Table 8., **Feeding practices and Numbers of milking cows**

| Variables & Category | Study Areas | | | T, P = 326 | % |
|---|---|---|---|---|---|
| | Wolkite **P=10** | Gummer p=200 | Ennor p=116 | | |
| **Feeding Regime** | | | | | |
| Free grazing of natural pasture only | 0 | 0 | 0 | 0 | 0.00 |
| Supplement with natural pasture including Enset | 4 | 0 | 0 | 4 | 1.23 |
| Natural grass, crop residue and Enset and some times supplement | 6 | 200 | 116 | 322 | 98.77 |
| **Number of Milking Cows** | | | | | |
| One | 0 | 138 | 86 | 224 | 66.27 |
| Two | 2 | 42 | 21 | 65 | 19.23 |
| Three and Above | 8 | 20 | 9 | 37 | 10.95 |

Where, T = total; n= number of participants; P= milk producers; V= vendors

### 4.1.2 Status of Milk Handling Practices, Storage or Transportation Conditions and marketing Assesment

#### 4.1.2.1 Milking Practices and Hygiene

The study revealed that 97.55% of respondents milked their cows within the cows' barn, while only 2.45% milked outside the barn. Although barn-based milking is widely practiced, it poses a high risk of milk contamination if hygiene is not properly maintained. A small number of respondents preferred a more controlled environment for milking, especialy Wolkite town administration **stood out slightly**, with a small number of farmers practicing **outside of the barn milking**, which is generally more hygienic. This indicates a modest shift toward better milking practices in Wolkite compared to Gummer and Ennor, which can help reduce exposure to external dirt and dust, highlighting the need for improved hygiene practices during milking. However, without regular barn cleaning, the risk of bacterial contamination remains significant. 76.07% of farmers milked their cows twice a day, while 23.93% milked once a day. Milking twice daily is generally associated with better feeding practices, early lactation stages and also it has benefited increased milk yield. Notably, in **Wolkite town administration**, **100% of the respondents practiced twice-daily milking**, showing a **stronger commitment to improved dairy management**.

Hand hygiene plays a critical role in ensuring milk safety and quality. The study revealed that 83.13% of respondents washed their hands before milking, while 16.87% did not. Among those who practiced handwashing, 92.25% used only cold water, whereas 7.75% used cold water with detergent. Notably, no respondents used hot water and with detergent, which is the most effective method for eliminating bacteria. The low usage of detergent and reliance on cold water highlight a significant gap in hygiene awareness, emphasizing the need for educational interventions to promote proper milking hygiene practices. Proper cleaning of milking equipment is essential for preventing milk contamination. The results showed that 78.83% of respondents washed their milking devices before use, but 21.17% did not wash them at all. Among those who washed the devices, 87.94% used only cold water, while 8.95% used cold water with detergent. Similar to handwashing, no farmers used hot water with detergent, which highlights a gap in proper sanitation practices.

A crucial factor in milk hygiene is cleaning the cow's udder and teats before milking. However, the study found that only 17.79% of respondents practiced udder and teat washing, while a significant 82.21% did not. The primary reasons for neglecting this practice were: 63.43% believed that calf suckling was sufficient for cleaning the udder and teats, 26.87% cited water shortages as a barrier, 9.70% lacked awareness and did not understand the risk of bacterial contamination in milk. This indicates a widespread misconception that calf suckling alone is enough to clean the udder, which may increase the risk of bacterial contamination in milk. Awareness creation and access to clean water sources are crucial for improving udder hygiene. Proper drying of the udder and teats is essential for maintaining milk hygiene. However, the study found that only 0.61% of respondents used towels, and even in these cases, a communal towel was used instead of individual towels for each cow. The vast majority (99.39%) relied on bare hands for massaging and drying the udder, which increases the risk of bacterial contamination from hands to milk. Encouraging the use of clean, individual towels for each cow can significantly improve milk hygiene by minimizing the transfer of dirt and pathogens, ultimately enhancing milk safety and quality.

100% of farmers used plastic containers for milking, while none used stainless steel or aluminum containers. Plastic containers are more difficult to clean and can harbor bacteria, posing risks to milk quality and safety. The lack of stainless steel or aluminum containers suggests limited awareness or access to more hygienic alternatives. Encouraging farmers to transition to stainless steel or aluminum containers, which are easier to sanitize and maintain, could significantly improve milk hygiene. Proper milk storage is essential for preserving quality and preventing spoilage. The study found that 77.51% of respondents stored milk in plastic containers and clay pots, while 22.49% used plastic containers only, for transportation and stored. The complete absence of stainless steel or aluminum storage containers suggests a lack of awareness or accessibility to proper storage materials. Storing milk in non-food-grade plastic or porous clay pots can increase the risk of contamination and spoilage. Farmers should be encouraged to use safe, food-grade storage containers to enhance milk safety and shelf life. The majority of participants (42.60%) rely on river water as their main source. Spring water is used by 10.36% of participants offering a cleaner more natural alternative where available. Tap water is utilized by 16.86% of the sample, indicating the presence of water supply infrastructure, though it's not commonly accessible. Pond or stagnant water is used by 14.20% of participants, a concerning source due to its potential contamination. Finally, 15.98% depend on hand-dug wells, highlighting the reliance on manual water extraction in areas with limited infrastructure. The source of water is crucial as contamination, particularly with bacteria or harmful pathogens, can directly affect public

health. Contaminated water can lead to the spread of diseases, negatively impacting both human health and livestock. For instance, milk that comes into contact with contaminated water during the production process may become a carrier of harmful bacteria, endangering consumers. Ensuring safe and clean water sources is therefore vital to improve overall health outcomes and prevent the spread of contamination.

**Table 9: Milking Practices and Storage Conditions**

| Variable & Category | Study Areas | | | T | Percent % |
|---|---|---|---|---|---|
| | W n= 10 | G n= 200 | E n= 116 | n= 326 | |
| **Milking Place** | | | | | |
| Within the cows' barn | 6 | 197 | 115 | 318 | 97.55 |
| Outside of cows' barn | 4 | 3 | 1 | 8 | 2.45 |
| **Frequency of milking per day** | | | | | |
| Once | 0 | 35 | 43 | 78 | 23.93 |
| Twice | 10 | 165 | 73 | 248 | 76.07 |
| **Washing hands before milking** | | | | | |
| Yes | 8 | 174 | 89 | 271 | 83.13 |
| No | 2 | 26 | 27 | 55 | 16.87 |
| **How hands were washed before milking** | | | | | |
| With cold water only | 7 | 160 | 83 | 250 | 92.25 |
| Cold water and detergent | 1 | 14 | 6 | 21 | 7.75 |
| Hot water with detergent | 0 | 0 | 0 | 0 | 0.00 |
| Hot water only | 0 | 0 | 0 | 0 | 0.00 |
| **Washing the milk device before milking** | | | | | |
| Yes | 10 | 160 | 87 | 257 | 78.83 |
| No | 0 | 40 | 29 | 69 | 21.17 |
| **If Yes, how Washing the milk device before milking** | | | | | |
| With cold water only | 7 | 145 | 74 | 226 | 87.94 |
| Cold water and detergent | 2 | 11 | 10 | 23 | 8.95 |
| Hot water with detergent | 0 | 0 | 0 | 0 | 0.00 |

| | | | | | |
|---|---|---|---|---|---|
| Hot water only | 1 | 4 | 3 | 8 | 3.11 |
| **Practice of washing udder and teats** | | | | | |
| Yes | 10 | 30 | 18 | 58 | 17.79 |
| No | 0 | 170 | 98 | 268 | 82.21 |
| **How the udder and teats were washed** | | | | | |
| With cold water only | 10 | 30 | 18 | 58 | 100 |
| Cold water and detergent | 0 | 0 | 0 | 0 | 0.00 |
| Hot water with detergent | 0 | 0 | 0 | 0 | 0.00 |
| Hot water only | 0 | 0 | 0 | 0 | 0.00 |
| **Reasons for not washing udder and teats** | | | | | |
| Awareness problem | 0 | 10 | 16 | 26 | 9.70 |
| Belief leaving the cal1fs to suckling is sufficient | 0 | 120 | 50 | 170 | 63.43 |
| Shortage of water access | 0 | 40 | 32 | 72 | 26.87 |
| **Use of towel for drying udder and teats** | | | | | |
| Yes | 2 | 0 | 0 | 2 | 0.61 |
| No | 8 | 200 | 116 | 324 | 99.39 |
| **How the towel was used for drying and what other methods used for drying** | | | | | |
| Individual towel for each cow | 0 | 0 | 0 | 0 | 0.00 |
| Communal towel | 2 | 0 | 0 | 2 | 0.61 |
| Used massage with bare hand | 8 | 200 | 116 | 324 | 99.39 |
| **Type of devices used for milking** | | | | | |
| Plastic devices and clay pot | 0 | 0 | 0 | 0 | 0.00 |
| Plastic devices | 10 | 200 | 116 | 326 | 100 |
| Stainless steel/Aluminium | 0 | 0 | 0 | 0 | 0.00 |

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| Variable & Category | Study Areas | | | | | | T, n = 338 | % |
|---|---|---|---|---|---|---|---|---|
| | Wolkite n=18 | | Gummer n= 202 | | Ennor n= 118 | | | |
| | P, 10 | V, 8 | P, 200 | V, 2 | P, 116 | V, 2 | | |
| **Type of devices used for milk storage and transportation** | | | | | | | | |
| Plastic devices and clay pot | 0 | 0 | 173 | 0 | 89 | 0 | 262 | 77.51 |
| Plastic devices | 10 | 8 | 27 | 2 | 27 | 2 | 76 | 22.49 |
| Stainless steel/Aluminium | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| **Sources of water used** | | | | | | | | |
| River water | 0 | 0 | 81 | 0 | 63 | 0 | 144 | 42.60 |
| Spring water | 0 | 0 | 15 | 0 | 20 | 0 | 35 | 10.36 |
| Tap water | 10 | 8 | 20 | 2 | 15 | 2 | 57 | 16.86 |
| Pond or stagnant water | 0 | 0 | 40 | 0 | 8 | 0 | 48 | 14.20 |
| Hand-dug water | 0 | 0 | 44 | 0 | 10 | 0 | 54 | 15.98 |

Where, W= Wolkite; G= Gummer;E= Ennor;P= producers; V= vendors; n= number of participants; T= total participants.

### 4.1.3 Barn Situations

The study found that 79.75% of farmers clean their barns daily, while 20.25% do so every two days. Notably, no respondents reported cleaning only once a week, suggesting moderate barn hygiene practices. Maintaining clean barns is crucial for ensuring milk quality and minimizing bacterial contamination. Regarding barn flooring, the majority of farmers (79.14%) use stone slabs, while 20.86% have concrete floors. No respondents reported using earthen floors, indicating an improvement in overall hygiene and sanitary conditions in dairy farming. While concrete floors are preferred due to their ease of cleaning and disinfection, stone slabs remain a common choice due to their affordability and local availability.

**Table 10: Barn Cleaning Regularity, Barn Type, and Awareness of Raw Milk Risks**

| Variables/ Category | Wolkite (n=10) | Gummer (n=200) | Ennor (n=116) | Total (n=326) | Percent (%) |
|---|---|---|---|---|---|
| **Barn Cleaning Regularity** | | | | | |
| **Daily** | 10 | 150 | 100 | 260 | 79.75 |
| **Once in two days** | 0 | 50 | 16 | 66 | 20.25 |
| **Once in a week** | 0 | 0 | 0 | 0 | 0.00 |
| **Barn Type** | | | | | |
| **Concrete floor** | 10 | 36 | 22 | 68 | 20.86 |
| **Stone slab** | 0 | 164 | 94 | 258 | 79.14 |
| **Earthen** | 0 | 0 | 0 | 0 | 0.00 |

**Where, n = number of participants of producers.**

### 4.1.4 Milk Marketing Practices and Raw Cow Milk Consumption Trends

The study assessed milk marketing practices in Wolkite, Gummer and Ennor, focusing on consumption, processing, storage and challenges. Findings indicate that most milk is marketed in raw or processed form through informal channels rather, especially in Gummer and Ennor. This processed form driven by shelf-life concerns and traditional consumer preferences. with > 30%; Milk processing is widely practiced, particularly in Gummer and Ennor, where over 94.76% of producers process more than 75% of their milk into dairy products like cheese, butter after fermented in to yogurt. The main reasons cited for processing were increasing shelf life and aligning with consumer preferences rather than raw milk (94.71%). However, in Wolkite, 5.03% of producers sell milk in raw form without any processing. Storage remains a challenge, with 98.52% of producers storing milk at room temperature and only 1.48% using refrigeration. Marketing challenges were notable, with 93.49% of respondents stating that traditional beliefs discourage raw milk trade. Religious fasting periods also impact demand, as noted by 6.51% of producers. Additionally, 5.03% of respondents identified a lack of proper storage facilities as a key issue. However, price fluctuations and transportation problems were not reported as major concerns, suggesting that market access is relatively stable. Raw milk consumption along side of "kocho" and others remain high, with 97.6% of respondents reported consuming raw cow milk regularly. This habit is particularly strong in Gummer and Ennor, this widespread practice is largely attributed to a lack of awareness about pasteurization and boiling and traditional beliefs in the nutritional benefits of raw milk. Addressing these misconceptions through education and awareness campaigns is essential to improving public health and dairy safety.

**Table 11., Milk Marketing Practices and Raw Cow Milk Consumption Trends**

| Variables/ Category | Wolkite | | Gummer | | Ennor | | Total Frequency | Percentage (%) |
|---|---|---|---|---|---|---|---|---|
| | P, 10 | V, 8 | P, 200 | V, 2 | P, 116 | V, 2 | 338 | |
| **How much of produced raw milk is used for household consumption?** | | | | | | | | |
| Less than 10% | 8 | - | 83 | - | 42 | - | 133 | 40.80 |
| 10-30% | 2 | - | 117 | - | 74 | - | 193 | 59.20 |
| 31-50% | - | - | - | - | - | - | - | - |
| More than 50% | - | - | - | - | - | - | - | - |
| **How much of your produced raw milk is processed into other dairy products (cheese, butter, yogurt)?** | | | | | | | | |
| None, I sell majority of cow milk in raw form | 10 | - | 4 | - | 3 | - | 17 | 5.21 |
| None, I sell all cow milk in raw and yogurt form | - | - | - | - | - | - | 0 | 0 |
| Less than 20% | - | - | - | - | - | - | 0 | 0 |
| 21%-75% | - | - | 88 | - | 35 | - | 123 | 37.73 |
| More than 75% | - | - | 108 | - | 78 | - | 186 | 57.06 |
| **If you process raw milk into other products, what is your main reason?** | | | | | | | | |
| To increase shelf life and prevent spoilage | - | - | 196 | - | 113 | - | 309 | 94.79 |
| To get a higher price for processed products | - | - | - | - | - | - | 0 | 0 |
| Consumer preference for processed dairy products | - | - | 196 | - | 113 | - | 309 | 94.79 |
| I do not process milk into other products | 10 | - | 4 | - | 3 | - | 17 | 5.21 |

| Variables/ Category | Wolkite | | Gummer | | Ennor | | Total Frequency | Percentage (%) |
|---|---|---|---|---|---|---|---|---|
| | P, 10 | V, 8 | P,200 | V, 2 | P, 116 | V, 2 | 338 | |
| **How do you store raw cow milk before selling it?** | | | | | | | | |
| Refrigeration | 0 | 4 | 0 | 0 | 0 | 1 | 5 | 1.48 |
| Stored at room temperature | 10 | 4 | 200 | 2 | 116 | 1 | 333 | 98.52 |
| **What are the main challenges you face in selling raw cow milk?** | | | | | | | | |
| Low market demand during religious fasting periods | 10 | 8 | 0 | 2 | 0 | 2 | 22 | 6.51 |
| Price fluctuations | - | - | - | - | - | - | 0 | 0 |
| Transportation issues | - | - | - | - | - | - | 0 | 0 |
| Lack of proper storage facilities | 10 | 4 | 0 | 2 | 0 | 1 | 17 | 5.03 |
| Tradition and beliefs negatively affecting raw cow milk trade | 0 | 0 | 200 | 0 | 116 | 0 | 316 | 93.49 |
| **Habit of Raw Milk Consumption along side of kocho and others** | | | | | | | | |
| Yes | 4 | 6 | 200 | 2 | 116 | 2 | 330 | 97.6 |
| No | 6 | 2 | 0 | 0 | 0 | 0 | 8 | 2.4 |

Where, p= producers, v =vendors

## 4.1 Raw Cow Milk's Physical Characteristics and Chemical Compositions

The physicochemical properties of raw cow milk samples collected from selected areas of the Gurage zone, including Wolkite town, Gummer, and Innor districts, revealed the PH, TA, SG, TS, SNF, protein, and ash content of producers and vendors.

### 4.2.1 Physical Quality of Raw Cow Milk

The physical properties of raw cow milk obtained from different sampling locations, including Producers and Vendors of Wolkite Town, Gummer and Ennor District, were analyzed and compared against the standard quality range ( Table 12). The results highlight variations in key milk quality parameters, which can be attributed to factors such as handling practices, feeding systems, environmental conditions and milk adulteration.

4.2.1.1 PH

As shown in Table 12, the average pH values of milk samples from Producers in Wolkite, Gummer and Ennor were 5.50, 6.50, and 6.05, while those from Vendors were 5.40, 6.07 and 5.75, respectively. Statistical analysis showed a significant difference ($p < 0.05$) in milk quality among the three locations for both producers and vendors. According to O'Connor (1995), FAO (1999) and Alganesh (2016), both European Union (EU) and Ethiopian Standards (ES) set an ideal pH range of 6.6–6.8 for raw cow milk. However, the current study's pH values (5.40–6.50) fall below these standards, with Gummer Producers (6.50) nearing the lower limit but still not meeting it. Compared to previous Ethiopian studies, which reported pH values of 6.39 (Terfa et al., 2015), 6.32 (Gemechu et al., 2015) and 6.195 (Legesse et al., 2017), the milk in this study appears less fresh. The lower pH suggests acidification, likely due to poor handling, delayed testing and inadequate cooling. Milk from vendors consistently had lower pH values (5.40–6.07) than producers (5.50–6.50), indicating further quality deterioration along the supply chain, likely due to microbial activity from insufficient refrigeration, below the normal pH range of fresh cow milk, indicating that the milk was most probably not obtained directly from households shortly after milking.

Fresh cow milk has a pH value that ranges from 6.6 to 6.8 (O'Connor, 1995, and FAO, 1999). The pH values higher than 6.8 indicate mastitic milk, and pH values below 6.6 indicate increased acidity of milk due to bacterial multiplication (O'Connor, 1995).

Milk pH below 6.6 is less suitable for consumption and processing, as acidification leads to spoilage and off-flavors. The results emphasize the need for improved handling, including rapid cooling post-milking and maintaining a cold chain during transport. Gummer Producers had the highest pH (6.47 ± 0.04), nearing the recommended range, while Wolkite Vendors had the lowest (5.40 ± 0.08), indicating stay after milking. The findings suggest milk quality in Wolkite, Gummer and Ennor is declining compared to earlier research. Strengthening cold chain management and storage practices could help preserve milk freshness and align quality with national and international standards.

4.2.1.2 Titratable acidity (TA)

The overall acidity of milk is measured by titratable acidity (TA), which reflects the activity of microorganisms that convert lactose to lactic acid. It serves as a key indicator of freshness and spoilage. According to EU and Ethiopian Standards (ES), fresh cow's milk should have a TA of 0.14%–0.16% (O'Connor, 1995). However, this study found TA values ranging from 0.183 to 0.265, showed significant differences of producers and vendors across the three study locations, the overall TA was significantly higher in vendors' milk (0.24 ± 0.007) compared to producers' milk (0.2022 ± 0.003). This suggests that milk from vendors undergoes microbial fermentation ( due to longer holding times during handling, transportation and storage) and potential contamination, leading to increased acidity. Gummer producers recorded the lowest TA (0.183 ± 0.0041), indicating minimal spoilage, while Ennor vendors had the highest TA (0.265 ± 0.0065), exceeding the early spoilage threshold.

Compared to previous Ethiopian studies, the findings align with some but differ from others. Teshome Gemechu et al. (2015) reported TA values of 0.18–0.22%, similar to Gummer producers and the overall producer mean. However, vendor TA values in this study align more with Legesse et al. (2017), who reported TA levels of 0.21–0.25%, reinforcing concerns about acidity in vendor milk.

The significantly higher TA in vendors, especially in Ennor, suggests prolonged exposure to warm temperatures, accelerating bacterial fermentation. Since milk with TA above 0.2% is less suitable for consumption and processing, these results highlight the urgent need for improved handling, refrigeration, and transportation. The clear rise in acidity from producers to vendors signals a decline in milk quality along the supply chain, emphasizing the importance of cold chain management to meet national and international standards.

4.2.1.3 Specific gravity (SG)

Specific gravity (SG), a key indicator of milk density and solids content, was analyzed to assess quality and potential adulteration in raw cow milk from producers and vendors in Wolkite, Gummer, and Ennor. The SG values ranged from 1.01225 ± 0.00085 (Ennor vendors) to 1.03073 ± 0.00022 (Gummer producers), with producers consistently exhibiting higher SG than vendors across all study areas. The overall mean SG for producers (1.0297 ± 0.00019) was significantly higher than for vendors (1.01665 ± 0.00185), highlighting a decline in quality along the supply chain.

The higher SG in producer milk, particularly in Gummer (1.03073), aligns with the fresh, unadulterated milk range of 1.028–1.032, indicating a higher concentration of solids and better quality at the point of milking. In contrast, the lower SG in vendor milk, especially in Ennor (1.01225), falls below this range, suggesting possible adulteration likely water addition that dilutes milk solids and compromises density.

Compared to previous Ethiopian studies, these findings show both consistency and deviation. Gurmessa Terfa et al. (2015) reported an SG of 1.032, and Teshome Gemechu et al. (2015) recorded 1.028–1.034, values similar to Gummer producers and the overall producer mean. However, vendor milk SG in this study (e.g., 1.0165 in Wolkite, 1.01225 in Ennor) is lower than findings by Legesse et al. (2017) (1.026–1.031), indicating increased dilution in the current vendor samples.

The notably low SG in vendor milk likely results from intentional dilution to increase volume, with Ennor's SG (1.01225) falling far below Ethiopian Standards (1.026 minimum) and EU benchmarks (1.028–1.032). This adulteration reduces nutritional value and milk quality, while producer milk SG (1.0297) meets quality standards, reflecting minimal tampering. Gummer producers' higher SG suggests richer milk, potentially due to better feeding and handling, whereas lower vendor values indicate post-production challenges in maintaining quality.

The significant difference ($P < 0.00001$) underscores the impact of vendor practices, such as water addition or poor storage conditions, leading to solids loss and degraded milk quality. This has practical consequences: milk with SG below 1.026 is substandard for processing and consumption, affecting marketability and safety. While producer SG aligns with high-quality milk, vendor SG signals a worrying decline, emphasizing the need for stricter monitoring, supply chain improvements, and anti-adulteration measures to preserve milk density and overall quality.

The reduced SG, particularly among Vendors, likely reflects water adulteration a common practice to increase volume (Tekliye & Gizaw, 2017). This lowers SG, degrades milk quality, and introduces economic and health risks through chemical and microbial contamination. Enhanced monitoring and handling practices are critical to maintain SG within standards.

**Table 12: Physical analysis (Mean$\pm SE$) of raw cow milk produced and Sold in the study areas**

| Sample areas | Participants | Parameters | | |
|---|---|---|---|---|
| | | **PH** | **TA** | **SG** |
| **Wolkite** | Producers n= 4 | 5.5 ± 0.103 | 0.2575 ± 0.005 | 1.02875 ± 0.001 |
| | Vendors n=6 | 5.3983 ± 0.085 | 0.26 ± 0.005 | 1.0165 ± 0.001 |
| **Gummer** | Producers n= 26 | 6.4652 ± 0.042 | 0.1829 ± 0.004 | 1.03073 ± 0.0002 |
| | Vendors n= 2 | 6.0675 ± 0.053 | 0.195 ± 0.007 | 1.023 ± 0.001 |
| **Ennor** | Producers n=20 | 6.0526 ± 0.070 | 0.2097 ± 0.006 | 1.02834 ± 0.0004 |
| | Vendors n= 2 | 5.75 ± 0.071 | 0.265 ± 0.006 | 1.01225 ± 0.001 |
| **Producers** | | 6.1719 ± 0.047 | 0.2022 ± 0.003 | 1.0297 ± 0.0002 |
| **Vendors** | | 5.623 ± 0.076 | 0.24 ± 0.007 | 1.01665 ± 0.002 |
| **F-value** | | 40.55 | 18.27 | 194.68 |
| **P-value** | | < 0.00001 | 0.00004 | < 0.00001 |

Where, TA= titratable acidity; SG= specific gravity; SE= standard error

### 4.2.2 Chemical Analysis of Raw Cow Milk

The chemical properties of raw cow milk collected from producers and vendors in the study areas (Wolkite, Gummer, and Ennor) were analyzed and compared against national (NS) and European (EU) standards. The results are presented in Table 12.

4.2.2.1 Total solids (TS)

The mean TS value of raw milk samples was significantly different ($P < 0.05$) among the three different study areas as well as producers and vendors. The TS content was highest in producer milk from Gummer (13.19±0.17%) and lowest in vendor milk from Wolkite (9.65±0.30%). The overall TS mean for producer milk (12.57±0.14%) was higher than that of vendors (10.01±0.25%). Compared to NS (10.5–14.5%) and EU (>12%) standards, vendor milk had a lower TS content, suggesting dilution and poor quality.

4.2.2.2 Solids-not-fat (SNF)

The mean SNF value of raw milk samples was significantly different ($P < 0.05$) among the three different study areas as well as producers and vendors. The SNF values ranged from 7.81±0.09% (Ennor) to 10.32±0.45% (Wolkite) in producer milk, while vendor milk had lower SNF values (7.40±0.14% to 7.81±0.13%). The overall SNF content in producer milk (8.76±0.07%) was within the NS standard (>8.0%) and EU standard (>8.5%), but for vendor milk (7.5±0.13%), implying possible adulteration.

4.2.2.3 Ash content

The mean ash content of raw milk samples was significantly different ($P < 0.05$) among the three different study areas as well as producers and vendors. The ash content ranged from 0.51±0.012% (Wolkite) to 0.74±0.006% (Gummer) in producer milk and from 0.56±0.015% (Wolkite) to 0.71±0.006% (Gummer) in vendor milk. The overall ash content for producer milk (0.68±0.01%) was within the NS (0.6–0.9%) but slightly below the EU standard (>0.69%), while vendor milk (0.58±0.02%) was below both standards, indicating possible mineral loss or adulteration.

According to O'Connor (1995), the ash level in cow milk is 0.7 to 0.8% and is influenced by the animal's nutrition, breed, and lactation stage. The milk's composition can change based on the animal's breed, the time between milkings, the completeness of milking, the stage of lactation, the animal's diet, and the age and health of the milking cows. The breakdown of milk's proteins and lipids by microbes can also alter the milk's composition (O'Connor, 1995).

4.2.2.4 Protein content

The mean protein content of raw milk samples was significantly different ($P < 0.05$) among the three different study areas as well as producers and vendors.The protein content of producer milk ranged from 2.84±0.001% (Wolkite) to 3.38±0.027% (Gummer), whereas vendor milk exhibited lower protein levels, with the lowest being 2.51±0.19% (Ennor). The overall mean protein content for producer milk (3.27±0.04%) met the EU standard (>2.9%), but vendor milk (2.78±0.09%) was below the required level. The reduction in protein content in vendor milk could be due to poor handling practices or adulteration with water.

Compared to the previous findings of Abd Elrahman et al. (2009), who found a protein content of 3.48% for milk produced in dairy farms, the average protein level of raw milk acquired from the Wolkite, Gummer, and Ennor study sites was lower. Fikrineh et al. (2012) found that milk samples from households with local and crossbred cows had a lower protein content (3.46 ± 0.04%). The total protein level of unprocessed whole milk must not

be less than 2.9% in order to meet European Union quality criteria (Tamime, 2009). As a result, the average protein level of the vendor's milk sample (2.78 ± 0.059%) was slightly below the suggested norm, while the producers' milk sample (3.27 ± 0.04%) was within the approved range across the three regions. For one thing, the price per liter of milk is determined by the amount of protein it contains. As a result, smallholder raw cow milk farmers now have an excellent bulletin.

Overall, the significant variation in the chemical composition ( TS, SNF, Ash and Protein) of raw cow milk between producers and vendors, as well as across study areas, were influenced by factors such as farm management practices, feed quality and animal health. Producers generally have more control over these factors, leading to higher quality milk with better protein and solid content. In contrast, vendors often source milk from multiple producers, which can lead to greater variability. Geographical differences, including pasture quality and climate, also play a role in the milk's composition. Additionally, storage practices further contribute to these variations. The statistical analysis confirms these differences with high significance.

**Table 13: Mean ± Standard Error (SE) Values of Chemical Compositions of Raw Cow Milk**

| Stusy area | Participants | TS | SNF | ASH | PROTEIN |
|---|---|---|---|---|---|
| **Wolkite** | Producers n= 4 | 11.64 ± 0.45 | 10.32 ± 0.390 | 0.51 ± 0.012 | 2.99 ± 0.019 |
| | Vendors n= 6 | 9.65 ± 0.303 | 7.40 ± 0.138 | 0.56 ± 0.015 | 2.84 ± 0.013 |
| **Gummer** | Producers n= 26 | 13.19 ± 0.165 | 8.57 ± 0.044 | 0.74 ± 0.006 | 3.38 ± 0.027 |
| | Vendors n= 2 | 10.88 ± 0.005 | 8.48 ± 0.175 | 0.71 ± 0.006 | 2.96 ± 0.041 |
| **Ennor** | Producers n= 20 | 11.86 ± 0.220 | 8.53 ± 0.054 | 0.65 ± 0.011 | 3.18 ± 0.095 |
| | Vendors n= 2 | 10.38 ± 0.275 | 7.81 ± 0.134 | 0.61 ± 0.051 | 2.51 ± 0.198 |
| **Producers** | | 12.57 ± 0.141 | 8.76 ± 0.068 | 0.68 ± 0.008 | 3.27 ± 0.038 |
| **Vendors** | | 10.01 ± 0.254 | 7.68 ± 0.127 | 0.58 ± 0.018 | 2.78 ± 0.059 |
| F-value | | 88.63 | 56.87 | 22.98 | 17.51 |
| P-value | | < 0.00001 | < 0.00001 | < 0.00001 | 0.00005 |

Where Ts= total solid; SNF; solid not fat; SE= standard error

## 4.3 Microbiological Quality Analysis

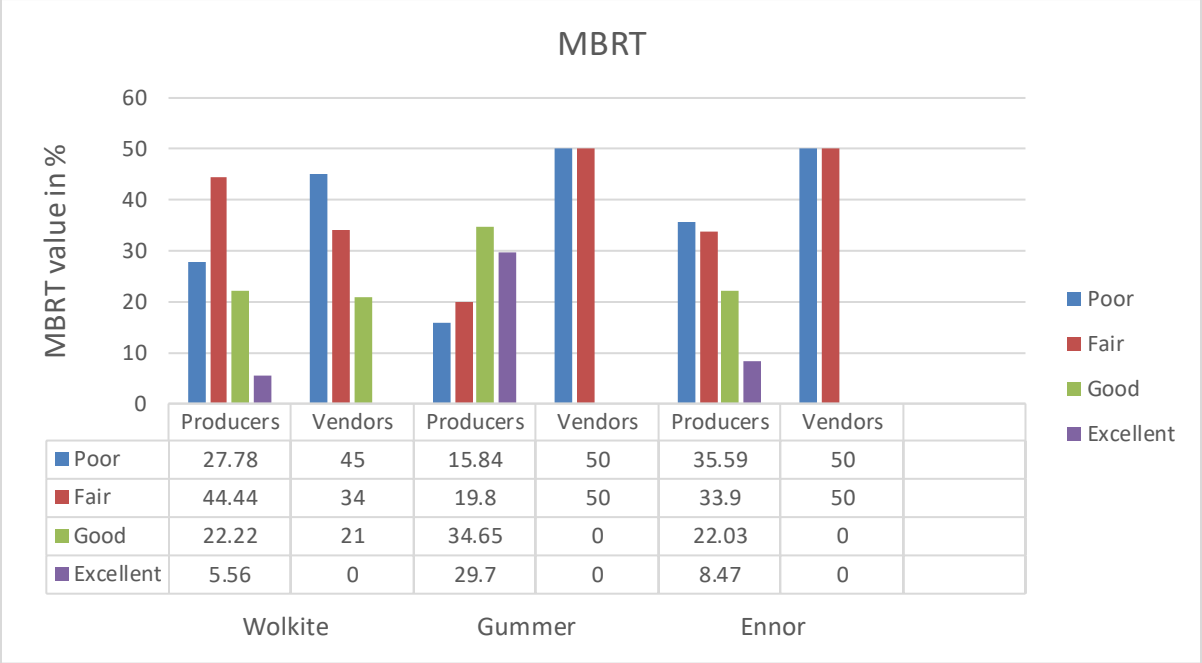
Using accepted techniques, microbiological studies were performed to look into the safety and microbial quality of milk samples from various sources in the study area. The methylene blue reduction (MBR) test, total bacterial count (TBC), coliform count (CC), yeast and mold count (YMC), E. coli, S. aureus, and Salmonella were all subjected to microbial enumeration, isolation, and identification. According to Tamirat (2018), Fufa *et al*. (2019), and Alganesh (2016), in order to ascertain TBC, CC, and YMC. A 1 ml milk sample was diluted in 9 ml of sterile peptone water to assess the microbiological quality analysis. Serial dilutions were then made in sterile peptone water diluents until the desired level of 15–300 counts was reached. A sample of milk was placed on the sterile plate from a chosen dilution. The sample was then carefully mixed with 15–20 ml of plate count agar media, which was then placed onto the plate and left to harden for 15 minutes. After that, the plates were inverted and incubated for 48 ± 2 hours at 35 °C. Colonies were then manually counted (FDA, 2003).

### 4.3.1 Methylene Blue Reduction (MBR) Test

In order to ascertain the microbial load in milk, the dairy industry frequently uses the Methylene Blue Reduction Test. Since the color that is added to milk by adding a dye like methylene blue will eventually fade, this blue-colored reagent is used to estimate the bacterial population of a particular milk sample. The color fades as a result of the bacterial metabolism that removes oxygen from milk and produces reducing chemicals (Anwer *et al.*, 2018). After adding a known dosage of the methylene blue solution to the milk sample, it is observed at certain intervals until the blue hue goes away. Depending on the type and quantity of organisms present, the milk's blue color will diminish over time. Prior to pasteurization or processing, raw milk is typically graded using this test. Raw milk is assessed according to this test (Mahari and Yemane, 2016; Hawaz *et al.*, 2015; Yonas Hailu, 2015; Hussien et al., 2021). Excellent Not decolorized after eight hours, Very good: Decolorized in less than 8 hours but not less than 6 hours. fair: Decolorized in less than 6 hours but not less than 2 hours; and poor: Decolorized in less than 2 hours.

The Methylene Blue Reduction (MBR) Test results for raw cow milk in the Gurage Zone revealed significant differences in microbial load & milk quality among the three different study areas as well as producers and vendors ( Fig 3) . In Wolkite, producers had 27.78% Poor, 44.44% Fair, 22.22% Good, and 5.56% Excellent samples. However, vendors showed a much higher proportion of Poor-quality milk (45%), with no samples in the Excellent category, indicating issues in milk handling and storage, highlighting the need for improved hygiene and storage. In Gummer, a highland area with a cooler climate, producers demonstrated the highest milk quality, with 64.35% of samples rated as Good (34.65%), Excellent (29.70%), indicating better hygiene and storage practices. However, 15.84% of the samples were Poor, likely due to the cold environment helping preserve milk quality, suggesting some gaps in hygiene consistency for further improvementy. However, vendors in Gummer faced similar challenges, with all samples falling into Poor or Fair categories, suggesting that improper handling was a key factor. In contrast, Ennor had the lowest quality, producers with 35.59% of samples classified as Poor, the highest among the three locations. Additionally, 33.90% were Fair and only 8.47% were Excellen and vendors having 50% poor samples. These results pointed to a higher bacterial load, likely due to inadequate sanitation and improper storage.

Overall, producers consistently showed better milk quality than vendors, and the cold climate in Gummer helped maintain higher standards. The findings emphasize the urgent need for improved milk handling practices, better hygiene training for farmers and proper storage facilities. Since shorter MBR times are associated with higher microbial contamination, interventions like cooling systems, enhanced sanitation and regular quality checks are crucial for ensuring safe, high-quality milk production. Strengthening these measures will not only improve milk safety but also enhance market value.




| | Producers | Vendors | Producers | Vendors | Producers | Vendors |
|---|---|---|---|---|---|---|
| Poor | 27.78 | 45 | 15.84 | 50 | 35.59 | 50 |
| Fair | 44.44 | 34 | 19.8 | 50 | 33.9 | 50 |
| Good | 22.22 | 21 | 34.65 | 0 | 22.03 | 0 |
| Excellent | 5.56 | 0 | 29.7 | 0 | 8.47 | 0 |

**Figure 3: Methylene Blue Reduction (MBR) Test**

### 4.3.2 Total Plate Count (TPC) of Raw Cow Milk

The total plate count (TPC) is a crucial indicator of the microbial load and overall hygiene of raw cow milk. In this study, the microbial quality of raw cow milk revealed significant differences in microbial load & milk quality among the three different study areas as well as producers and vendors. The highest mean TPC was recorded in Ennor, where vendor milk exhibited 6.66 ± 0.029 log cfu/ml, while the lowest was observed in Gummer among producers (6.34 ± 0.003 log cfu/ml). The overall mean TPC for producer and vendor milk samples was 6.4 ± 0.017 log cfu/ml and 6.5 ± 0.026 log cfu/ml, respectively. These results suggest a marginal increase in microbial contamination in vendor milk, likely due to contamination during storage, transportation, poor hygienic practices and prolonged exposure to ambient temperature (Table12). The elevated TPC reflects inadequate sanitation, including unclean utensils, uses of plastic containers, contaminated udders or hands and suboptimal milking environments. The increase from producers to vendors indicates additional contamination during storage and transport, highlighting the need for improved hygiene and cold chain management.

Compared to previous Ethiopian studies, the microbial loads in this study are generally lower but still concerning. Ahmed et al. (2008) reported a TPC of 9.089 ± 0.281 log10 cfu/ml in Khartoum State, while Zelalem (2010) found 9.10 log10 cfu/ml in various Ethiopian regions. Haile et al. (2012) and Teklemichael (2012) reported TPC values of 10.28 and 9.137 log10 cfu/ml in Hawassa milk storage point and vendor milk samples, respectively. far exceeding the current findings. However, Debebe (2010) recorded a TPC of 6.98 ± 0.15 log10 cfu/ml for producer milk, closer to the Ennor vendor value (6.66 log cfu/ml) but higher than the Gummer producer value (6.23 log cfu/ml). Studies by Teshome and Tesfaye (2016) and ESAP (2021) reported TPC values of 7.235 ± 0.277, 7.222 ± 0.156, and 6.817 ± 0.381 log10 cfu/ml in milk from Mizan Aman, Debub Bench and Shei Bench, respectively, higher than the current means but aligning with Ennor vendors (6.66 log cfu/ml). Asmarech Dolango et al. (2021) observed an increase from 5.87 log10 cfu/ml at the udder to 7.63 log10 cfu/ml at the vendor level in Jimma town. Similarly, Eshetu et al. (2019) reported a mean TPC of 5.48 ± 0.54 log10 cfu/ml along the milk value chain in Haromaya, while Fereja (2023) recorded a lower range of 4.18–4.99 log cfu/ml in Assosa

District. Tesfaye Debelu et al. (2022) reported an overall mean of 4.513 ± 0.170 log10 cfu/ml in milk from households and collection spots.

Milk produced under hygienic conditions should have a TPC ≤ 4.7 log10 cfu/ml ($5 \times 10^4$ bacteria/ml) (O'Connor, 1993). However, all samples in this study exceeded this threshold, indicating significant microbial contamination. The high TPC may be attributed to poor sanitation practices, such as unclean milking utensils, Uses of plastic containers, initial contamination from the cow's udder or milkers' hands, improper cooling systems and poor hygiene in the milking area.

### 4.3.3 Yeast and Mold Count (YMC)

Yeasts and molds are spoilage microorganisms that can impact milk quality and shelf life (Gamal *et al.,* 2015). This study revealed significant differences among the three different study areas as well as producers and vendors, found that YMC levels were highest in Ennor vendor milk (4.12 ± 0.013 log cfu/mL) and lowest in Gummer producer milk (3.15 ± 0.025 log cfu/mL) (Table 12). The overall mean YMC for producers and vendors was 3.44 ± 0.028 log cfu/mL and 4.03 ± 0.018 log cfu/mL, respectively. These values exceed the acceptable limit of <10 CFU (Mostert and Jooste, 2002; Eshetu *et al.,* 2019).

In comparison, Solomon et al. (2015) reported significantly lower mean log10 CFU/mL values of 0.74 ± 0.03, 0.46 ± 0.035, and 0.62 ± 0.09 (overall mean: 0.622) for samples collected from hotels, farmers and cooperatives in the Dawa Chefa District of the Amhara Region. Teshome and Tesfaye (2016) recorded a higher overall mean of 3.902 ± 0.477 log10 CFU/mL in three districts of the Bench-Maji Zone, Southwestern Ethiopia. However, Haile et al. (2012) reported even higher yeast and mold counts (4.65 log cfu/mL) in milk samples collected from storage containers in Hawassa.

The elevated YMC in vendor milk could be attributed to extended storage times, inadequate refrigeration and unhygienic handling practices, such as improper washing of milking and storage utensils, poor sanitary conditions in milking areas, poor personal hygiene of milkers and vendors and mixing of old and newly drawn milk. Yeasts and molds thrive in milk when cooling is insufficient and when contamination occurs due to

improperly sanitized containers. These findings indicate that milk sourced from informal markets tends to have elevated fungal counts due to poor hygiene and exposure to external contaminants.

Across all study areas, vendor milk exhibited higher microbial loads (both TPC and YMC) than producer milk, suggesting that contamination increases as milk moves through the supply chain. Factors such as the use of plastic containers, frequent opening of milk containers, and exposure to fluctuating temperatures likely contributed to the higher microbial counts. Ennor recorded the highest microbial loads, indicating potential issues with milk handling practices in this area. Warm environmental conditions, longer distances to laboratory testing facilities, and limited access to cooling facilities may have further exacerbated microbial growth. In contrast, Gummer had the lowest microbial counts, possibly due to better milk-handling practices and improved cooling conditions.

**Table 14: Total Plate Count ( TPC) & Yeast And Mold Count (YMC) (Mean±SE) In Milk Samples (Log10 CFU $Ml^{-1}$).**

| Study Areas | Participants | TPC | YMC (log cfu/ml) |
|---|---|---|---|
| **Wolkite** | Producers n= 4 | 6.34± 0.003 | 3.67±0.065 |
| | Vendors n= 6 | 6.48±0.018 | 4.01±0.011 |
| **Gummer** | Producers n= 26 | 6.23±0.011 | 3.15±0.025 |
| | Vendors n= 2 | 6.37±0.002 | 3.92±0.006 |
| **Ennor** | Producers n= 20 | 6.55±0.019 | 3.61±0.031 |
| | Vendorsn= 2 | 6.66±0.029 | 4.12±0.013 |
| **Overall** | Producers | 6.40±0.017 | 3.44± 0.028 |
| | Vendors | 6.50±0.026 | 4.03±0.018 |
| **F-value** | | 9.47 | 169.88 |
| **P-value** | | 0.0026 | <0.0001 |

Where, TPC = total plate count; YMC = yeat and mould count; CFU = coliform unit; SE = standard error.

### 4.3.4 Coliform Count(CC) (Test)

The coliform level analysis showed significant differences in microbial load & milk quality among the three different study areas as well as producers and vendors, ranging from 4.95 ± 0.06 $log_{10}$ CFU/mL in Gummer producers to 5.77 ± 0.03 $log_{10}$ CFU/mL in Wolkite vendors. The overall means were 5.06 ± 0.04 for producers and 5.64 ± 0.06 for vendors. A clear trend emerged: vendors consistently exhibited higher coliform counts than producers, with Wolkite vendors showing the highest contamination level (5.77 $log_{10}$ CFU/mL), followed by Ennor (5.59 $log_{10}$ CFU/mL). Gummer had the lowest coliform count (4.95 $log_{10}$ CFU/mL), likely due to better sanitation and a cooler environment ( Table 13 ).

The coliform counts observed in this study were higher than the acceptable limits set by the American Public Health Service: <2 $log_{10}$ CFU/mL for Grade A milk and 2–2.3 $log_{10}$ CFU/mL for Grade B milk (APHA, 1992). Compared to previous studies, the coliform range in this study (4.95–5.77 $log_{10}$ CFU/mL) was broader and higher than values reported by Teshome and Tesfaye (2016) for Mizan Aman (5.203 ± 0.230 $log_{10}$ CFU/mL), Debub (5.187 ± 0.211 $log_{10}$ CFU/mL) and Shei Bench Woredas (4.911 ± 0.324 $log_{10}$ CFU/mL). The results were also slightly different from those of Asaminew and Eyassu (2011), who reported 4.49 $log_{10}$ CFU/mL in Bahir Dar Zuria and Mecha Districts. Other studies found coliform counts of 4.03 $log_{10}$ CFU/mL (Abebe *et al.*, 2012) and 4.84 $log_{10}$ CFU/mL (Rahel, 2008) in Ezha District of Gurage and Wolayita Zones, respectively, with significant differences across agro-ecologies, where vendors consistently had higher counts.

The findings of this study also exceeded those of Abdalla and Elhagaz (2011), who reported coliform counts of 2.23 ± 0.136 $log_{10}$ CFU/mL in milk from Khartoum State dairy farms. However, they were lower than the 6.57 $log_{10}$ CFU/mL reported by Zelalem and Bernard (2006) for raw cow's milk in central Ethiopia, indicating regional variability.

According to European Union standards, coliform counts in raw milk should be below $10^2$ CFU/mL (or 2 $log_{10}$ CFU/mL) (Fernandes, 2009; ESAP, 2021). The results of this study (4.95–5.77 $log_{10}$ CFU/mL) significantly exceed these standards, raising public health concerns. Factors Contributing to High Coliform Counts indicates contamination from fecal matter, unclean udders and teats, improper sanitation of milking equipment, poor hygiene in the milking environment and contaminated water sources (Jayarao *et al.*, 2004;

Bintsis *et al*., 2008; Biruk *et al*., 2009). While it is impractical to produce completely coliform-free milk, even under high hygiene standards, excessive coliform presence suggests potential health risks (Godefay and Molla, 2000). The detection of coliform bacteria in raw milk underscores the need for strict hygiene measures in dairy farming. Proper sanitation, effective milking practices and ensuring clean water sources are essential for maintaining milk safety and preventing health risks associated with bacterial contamination.

**Table 15: Mean ± Standard Error (SE) Values Of Coliform Count (CC) Log10 CFU/Ml In Raw Cow Milk**

| Study Area | Participants | Mean ± SE (log10 CFU/ml) | F-value | P-value |
|---|---|---|---|---|
| **Wolkite** | Producers n= 4 | 5.16 ± 0.014 | 3.26 | < 0.001 |
| | Vendors n= 6 | 5.77 ± 0.03 | | |
| **Gummer** | Producersn= 26 | 4.95 ± 0.06 | | |
| | Vendors n= 2 | 5.12 ± 0.014 | | |
| **Ennor** | Producers n=20 | 5.19 ± 0.06 | | |
| | Vendors n=2 | 5.59 ± 0.028 | | |
| **Producers mean** | | 5.06 ± 0.04 | | |
| **Vendorsmean** | | 5.64 ± 0.06 | | |

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Where, CC = coliform count; CFU = coliform unit; SE = standard error.

## 4.4 Pathogenic Microbial Identification in Milk Samples

60 raw cow milk samples were collected for Pathogenic microbial identification: 10 from Wolkite (4 producers, 6 vendors), 28 from Gummer (26 producers, 2 vendors), and 22 from Ennor (20 producers, 2 vendors). Fewer vendor samples were collected due to limited accessibility. Fig. 4., The microbiological analysis of raw cow milk from different study areas in the Gurage Zone revealed significant contamination levels of *E. coli*, *Staphylococcus aureus*, and *Salmonella*. The results varied based on location and whether the samples were collected from producers or vendors.

### 4.4.1 Escherichia coli (*E. coli)* Contamination

*Escherichia coli* was detected in all study areas, in Wolkite, both producers and vendors had an equal Escherichia coli contamination level of 50%, Gummer was lower among producers (33.17%) but remained high among vendors (50%), Ennor showed the highest *E. coli* contamination among producers (60.17%), reflecting serious hygiene issues, while vendors also had a 50% contamination rate. Various studies conducted in Ethiopia have also reported the presence of *E. coli* in raw milk. For instance, *E. coli* contamination rates of 13.2% were identified by Deresse (2011) in and around Hawassa town and by Tollessa *et al*. (2012) in Abaya District of the Borana pastoral area. Alebel *et al*. (2013) and Shunda *et al*. (2013) reported contamination rates of 30.9% and 44.4%, respectively. Similarly, Alehegne (2004) found *E. coli* levels ranging from a minimum of 3.9% to a maximum of 83.3% in raw cow's milk from smallholder dairy farms in Debre Zeit.

The current study's high E. coli incidence points to fecal contamination, which is most likely the result of inadequate sanitation during milk handling, dirty water sources, and poor milking practices. Both humans and warm-blooded animals' intestinal tracts normally include the bacteria *E. coli*. Its presence in milk and dairy products, however, is a clear sign of fecal contamination (Soomro *et al*., 2002; Benkerroum *et al*., 2004; Teshome Gemechu, 2016). Because enteropathogenic strains of E. coli can cause severe diarrhea and vomiting, especially in newborns and young children, their discovery in milk indicates a possible health risk. 20., 14.3, 9.1.

### 4.4.2 Staphylococcus aureus Contamination

Staphylococcus aureus, a major cause of foodborne illnesses, was most prevalent . In Wolkite, contamination was slightly higher among vendors (25%) compared to producers (20%), whereas Gummer, *S. aureus* was found in 14.3% of producer samples, but none of the vendor samples tested positive and Ennor 9.1% of producer samples, the lowest among all study areas, while vendor samples had a 50% contamination rate. The high *S. aureus* count in Wolkite may be attributed to direct contamination from milk handlers, improper storage and poor udder hygiene. Several studies across Ethiopia have reported the presence of *S. aureus* in raw milk. Shunda *et al*. (2013) identified *S. aureus* in 44.4% of samples collected from dairy farms, vending shops and homes/cafeterias in Mekelle town. Similarly, Alehegne (2004) and Deresse (2011) found contamination rates ranging from 15.8% to 75% in Debre Zeit and 27.5% to 31.3% around Hawassa town, respectively. Debebe (2010) also reported a 24.4% prevalence in milk samples from producers and street vendors in and around Addis Ababa (Kotebe, Bishoftu and Chancho). Teklemichael *et al*. (2013) found *S. aureus* in 25% of dairy farm milk samples and 50% of vendor samples, while Tollessa *et al*. (2012) reported contamination rates ranging from 6.78% to 7.29% in Abaya District of Borana pastoral area. A higher prevalence (28.7%) was recorded by Zeryehun *et al*. (2013) from 118 quarter milk samples in and around Addis Ababa.

The presence of *S. aureus* in raw milk is not uncommon, as it is frequently isolated from mastitic cows. Studies indicate that mastitis affects up to 50% of cows and 25% of quarters worldwide (Radostitis and Arundel, 2000), with *S. aureus* responsible for nearly 40% of mastitis cases in Ethiopia (Workineh *et al.*, 2002; Dego and Tarke, 2003; Getahune *et al.*, 2008; Abera *et al.*, 2010). The higher prevalence of *S. aureus* in vendor milk samples could be linked to poor handling practices, contamination during transportation and unhygienic conditions at vending sites. In dairy farms, its presence is often associated with inadequate udder preparation and poor milking hygiene. *S. aureus* is a significant foodborne pathogen, capable of causing a range of diseases in both humans and animals. It can lead to mild skin infections or severe conditions such as pneumonia and septicemia (Lowy, 1998). Staphylococcal food poisoning, caused by *S. aureus*, is characterized by nausea, vomiting, abdominal cramps, and diarrhea, with symptoms appearing rapidly, typically within 1 to 6

hours of consuming contaminated food (Cliver, 1990). The high prevalence of *S. aureus* in raw milk highlights the urgent need for improved hygiene and milk handling practices to minimize health risks.

### 4.4.3 Salmonella Contamination

he presence of *Salmonella* in all study areas raises serious food safety concerns, as it is a major cause of foodborne illness. In Wolkite, contamination was relatively lower, with 31.58% of producer samples and 25% of vendor samples testing positive. In Gummer, *Salmonella* was found in 49.75% of producer samples, and vendor samples showed an even higher contamination level at 50%, suggesting that milk contamination increases during handling and storage. The highest contamination levels were recorded in Ennor, where 58.82% of producer samples and 50% of vendor samples were positive for *Salmonella*. The high prevalence of *Salmonella* in vendor samples across all areas highlights the need for improved hygiene and storage conditions to prevent the risk of milkborne infections.

Salmonella, a serious pathogenic bacterium,in Wolkite, contamination was relatively lower with 31.58% of producer samples and 25% of vendor samples testing positive. In Gummer, 49.75% of producer samples and vendor samples showed an even higher contamination level at 50%, The highest contamination levels were recorded in Ennor, where 58.82% of producer samples and 50% of vendor samples were positive. The presence of Salmonella suggests potential contamination from animal waste, inadequate handling, and poor sanitation in barns and milk storage facilities.

**Table 16: pathogenic bacteria distributions**

| Study Area | Participants | E. coli | S. aureus | Salmonella |
|---|---|---|---|---|
| **Wolkite** | Producers n= 4 | 50 | 20 | 31.58 |
| | Vendors n= 6 | 50 | 25 | 25 |
| **Gummer** | Producers n= 26 | 33.17 | 14.3 | 49.75 |
| | Vendors n= 2 | 50 | 0 | 50 |
| **Ennor** | Producers n= 20 | 60.17 | 9.1 | 58.82 |
| | Vendorsn= 2 | 50 | 50 | 50 |

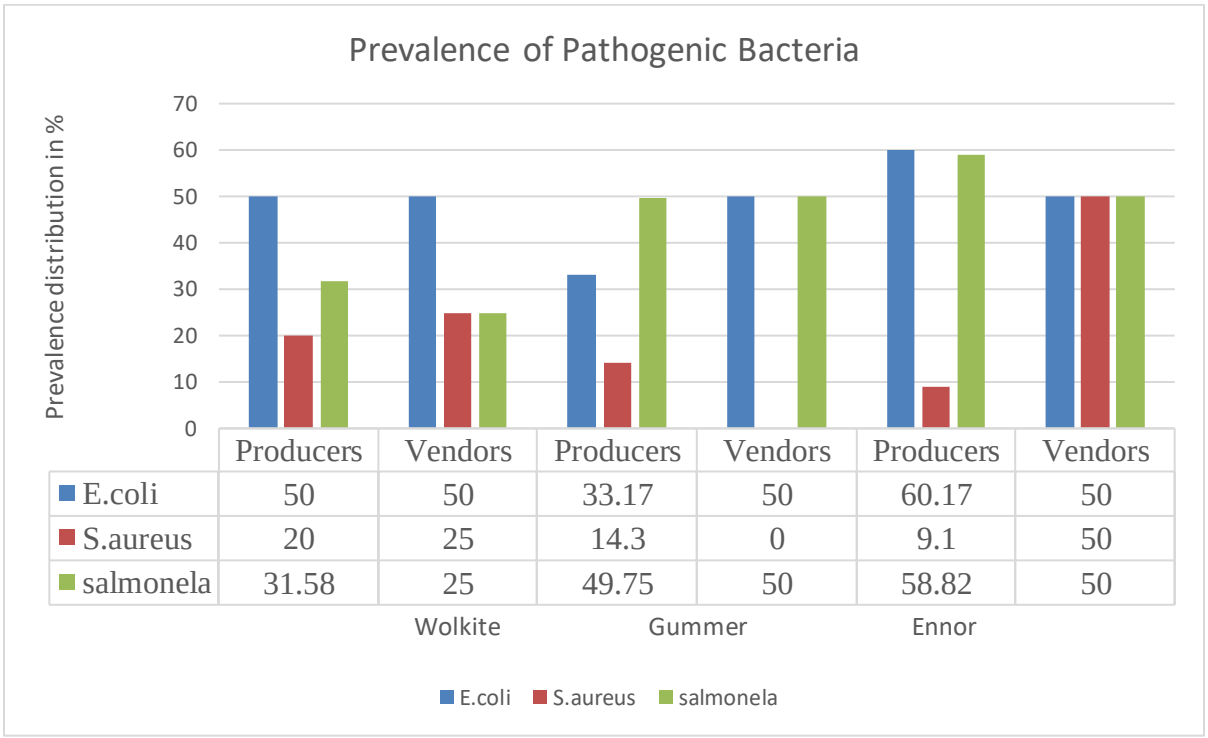


**Figure 4: Pathogenic Bacteria Distributions Chart**

# 5. CONCLUSION AND RECOMMENDATIONS

The findings of this study reveal significant concerns regarding the handling, quality and safety of raw cow milk in the selected areas of Gurage Zone, particularly in Wolkite town, Gummer and Ennor districts. Traditional milk handling practices, coupled with limited hygiene awareness and inadequate storage conditions, contribute to a decline in milk quality. The results in this study indicate that milk from vendors is more prone to deterioration than milk from producers, as evidenced by lower pH, higher acidity, and reduced total solids, protein and ash content. Furthermore, the high microbial load, including the presence of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*, poses serious public health risks. Among the study areas, Gummer demonstrated relatively better milk quality, while Wolkite & Ennor showed the highest contamination levels. The widespread consumption of raw milk, particularly with "kocho". further increases the risk of foodborne illnesses. These findings highlight the need for urgent intervention to improve milk handling, hygienically and infrastructure provision or organize (produsers) to ensure the safety and quality of raw milk.

Based on the the finding of this study, the following recommendations remarks are forwarded

- Providing adequate training on proper milking, handling, storage and transportation techniques should be provided for producer and vendors.
- Use of detergents and clean water for washing hands, milking equipment and storage containers should be encouraged.
- Replacing traditional clay pots and plastic materials with food-grade plastic or stainless-steel containers can help maintain milk quality.
- Establishing milk cooling facilities at both the farm especialy who emerges to dairy farm and vendor levels should be considered to prevent bacterial growth and preserve freshness.
- Promoting boiling of milk before consumption is essential to minimizing health risks associated with microbial contamination.
- Developing collection centers equipped with refrigeration can help maintain milk quality before it reaches consumers.
- Authorities must establish monitoring systems for microbial contamination in raw milk and ensure strict compliance with safety regulations.

Additionally, certification programs for dairy farmers and vendors who meet hygienic standards can further promote best practices in milk handling.

- Conducting public awareness campaigns on the dangers of consuming raw milk and the benefits of boiling should be given a due attention.

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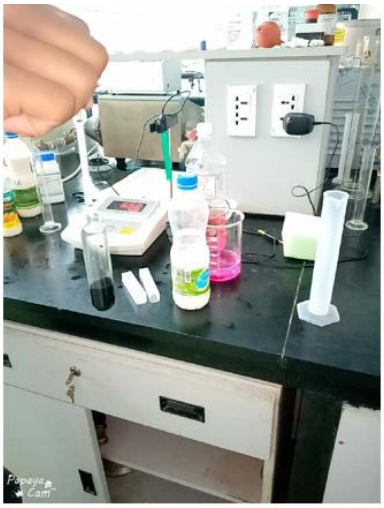
# 7. LIST OF FIGURES IN APPENDEX

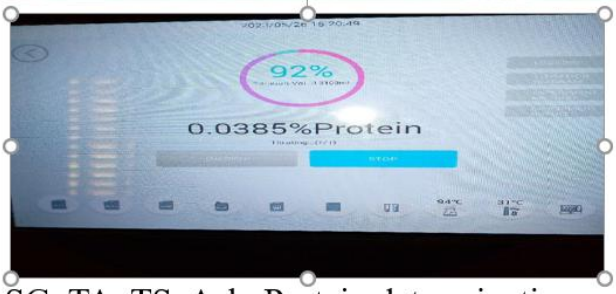
## 7.1 Fotos' Taken from Physicochemical Properties, Microbial Analysis and Identifications of Pathogenic Bacteria

The physicochemical quality parameters were analyzed based on various manufacturers' guidelines, and the results are presented below. The microbial quality analysis and identifications of pathogenic bacteria were conducted to assess the safety and hygiene of the raw cow milk samples in different selected areas of Gurage zone (Wolkite, Gummer and Ennor); this included MBRT on MBR, total plate count on PCA, Yeast and mold count on PDA, Escherichia coli on EMB , Staphylococcus aureus on MSA and salmonella on mackonkay agar, after enumeration and identification, clear images of the microbial colonies were obtained under here.



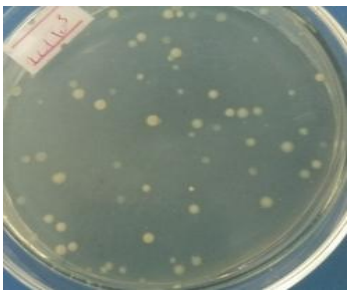

BIOBASE


**Figure 1, Photos taken during lab analysis of Physicochemical and microbial analysis and Pathogenic bacteria Identifications**

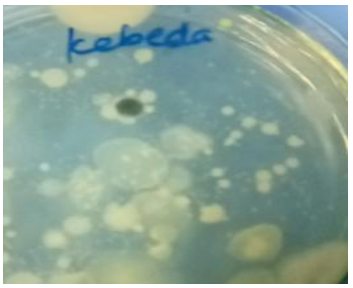



SG, TA, TS, Ash, Protein determination results

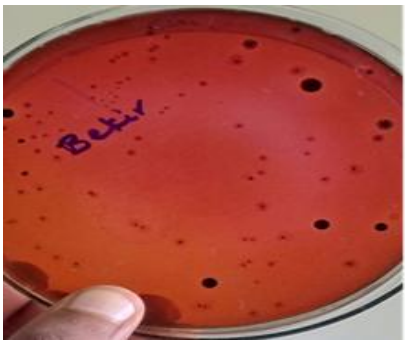
**A. MBRT on MBD**



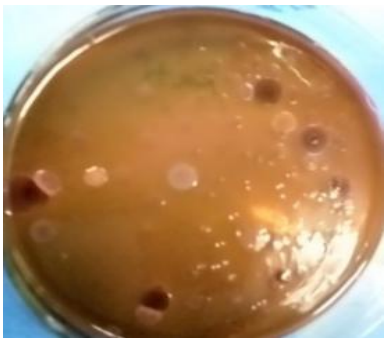
B. TPC on PCA



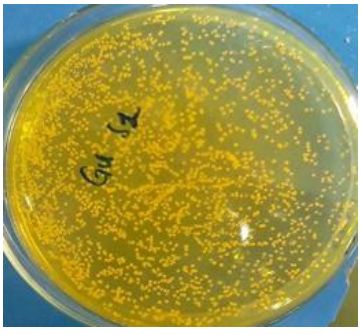

**C**. YMC on PDA



**D.** E.coli on EMB



**E.** Salmonella on mackonkay agar



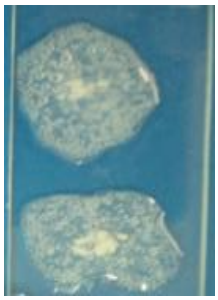
F. S.Aureus on MSA

morphological and biochemical tests

A. S.Aereus subcultured on MSA

B. Pure colcolonies

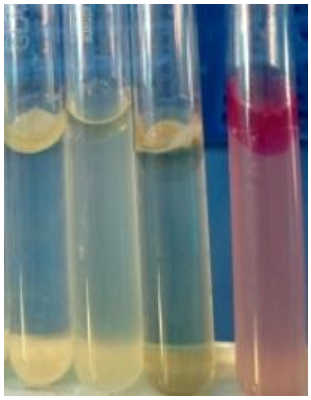
C. MR test



D. Catalase test

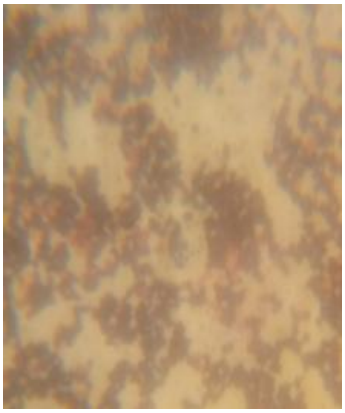
E. Starch hydrolysis test

F. TSI test



G.VP test

H. Coagulase +ve S. Aereus



I. S. aureus gram stain

J.coliform test

k. Konfirmatory for coliform

L. Indole test for coliform

Figure 2: Photos taken during morphological and biochemical conformation tests