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# Study on Nutrient and Microbial Analysis of Selected Milk Samples from Kottayam District, Kerala, South India

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**KEYWORDS**

Milk quality, Microbial contamination, SNF content, Hygienic Practices

**ABSTRACT:**

This study aimed to evaluate the quality of various milk samples through comprehensive chemical and microbiological analyses. Four distinct sets of data were interpreted, focusing on fat content, solids-not-fat (SNF), water content, adulterants, preservatives, and the presence of pathogenic bacteria. The chemical analyses involved comparing the measured values of fat, SNF, and water in three samples against established permitted levels, while the microbiological analyses assessed the presence of *E. coli, Staphylococcus aureus, Brucella spp.,* and *Shigella spp.* in three different milk sources: Milma milk, Malanadu milk, Elanadu milk and fresh udder milk.

The initial chemical examination showed that all samples had fat content between 6.33% and 6.43%, well above the 3.2% standard. This high fat was accompanied by low SNF readings (6.48% to 6.57%), below the 8.5% limit, and slightly raised water content (86% to 89%), above the 85% limit. A milk composition mismatch could indicate dilution or an imbalanced milk content. An additional data set showed that three samples had fat concentrations slightly above or below the 3.2% limit. This disparity indicated multiple milk samples were analysed. This second dataset also had low SNF and high water content, suggesting dilution. However, further testing yielded near-perfect results. The third chemical analysis was substantially closer to the limits. Fat content was 3.21–3.31%, close to or above the 3.2% standard. SNF levels were from 8.46% to 8.49%, close to 8.5%. Near-85% water content was also observed. All chemical analyses found no adulterants or preservatives. Fat content was highest in Elanadu milk (3.41 % )and lowest in Malanadu milk(3.05%). SNF level was highest in fresh udder milk( 8.47%) and lowest in Milma milk( 6.52%). The water content was high in milma milk and lowest in Malanadu milk.

The microbiological investigation showed a clear distinction between processed and unprocessed milk. Milma Malanadu, and Elanadu milk samples demonstrated excellent bacterial quality, with no detection of *E. coli, Staphylococcus aureus, Brucella spp.,* or *Shigella spp.* Conversely, the fresh udder milk sample tested positive for *E. coli*, suggesting fecal contamination.

This highlights the importance of hygienic milk collection practices for unprocessed milk and validates the efficacy of processing techniques employed in commercial milk production. The absence of *Staphylococcus aureus, Brucella spp.,* and *Shigella spp.* across all samples indicates a relatively low risk of infection from these specific pathogens.

## 1. Introduction

. Milk is a fundamental component of the human diet, providing essential nutrients such as proteins, fats, carbohydrates, vitamins, and minerals. Its consumption is widespread, particularly among vulnerable populations like children and the elderly, making its quality and safety paramount. The composition of milk,

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including fat content, solids-not-fat (SNF), and water content, directly influences its nutritional value and sensory properties. Deviations from permitted levels can indicate dilution, adulteration, or improper processing, compromising its quality. Furthermore, the presence of adulterants and preservatives, often added to extend shelf life or mask poor quality, poses significant health hazards. Consequently, detailed chemical analysis is essential to assess the compositional integrity of milk.

In addition to chemical composition, microbiological safety is a critical aspect of milk quality. Milk can serve as a vehicle for various pathogenic microorganisms, including *Escherichia coli (E. coli)*, *Staphylococcus aureus*, *Brucella spp.*, and *Shigella spp.*, which can cause severe illnesses. The presence of these bacteria indicates unsanitary handling, fecal contamination, or inadequate pasteurization, highlighting the need for stringent microbiological testing.

This research work aimed to comprehensively evaluate the quality of various milk samples through a combined approach of chemical and microbiological analyses. By assessing the fat content, SNF, water content, adulterants, preservatives, and the presence of pathogenic bacteria, this study sought to provide valuable insights into the compositional and microbiological integrity of different milk sources. The findings of this research contribute to the broader understanding of milk quality control and emphasize the importance of implementing effective measures to ensure the safety and nutritional value of this essential food product. This study's results may be utilized to inform consumers, guide regulatory bodies, and improve the overall quality of milk available in the market.

Milk and milk products are excellent high quality foods providing both nutritional and culinary values. Bacterial contamination of raw milk can originate from different sources from animals such as air, milking equipment, feed, soil, feces and grass. Milk microflora includes spoilage and pathogenic microorganisms. Much milk borne diseases such as tuberculosis, brucellosis and typhoid fever are known. Milk is spoiled by a wide range of microorganisms some of which are pathogenic and are responsible for milk borne diseases. The milk is very easily contaminated if collected unhygeinically and handled carelessly leading to quick spoilage.(1)

At the milk outlets, regularly opening containers to sell milk and predisposing the milk to hand contamination increased the risk of contamination by environmental contaminants. Survey was done with 60 respondents, 37 (61.67%) said they were aware of the health risks associated with milk. Twenty-three people (38.33%) said they knew about diseases linked to drinking contaminated milk. It also discovered that there was no formal food hygiene training for any of the food handlers. However, 11.7% of the workers had advanced training in a variety of fields. The microorganisms associated with milk products' spoilage in this study are of economical and public health significance. Some strains of *A. flavus* have been reported to produce potent mycotoxins called ochratoxin that can be harmful to human beings and animals. Cares should be taken on the handling of milk and milk products. And the improved preservation methods should be suggested to enhance the quality of milk products. The findings of the study provide a foundation for developing better milk policies.(2)

The microbiology of raw milk and is confined to the study of the milk of the most common domestic lactating animal species, i.e., cows, goats and sheep. The presence of food-borne pathogens in milk and milk products is due to direct contact with contaminated sources in the dairy farm environment, to excretion from the udder of an infected animal and during processing and handling. Fermented milk products, such as yogurt and cheese, appeared in human diet about 8000–10000 years ago. Up to the 20th century, milk fermentation remained an unregulated process, and, the discovery and characterization of lactic acid bacteria (LAB) have changed the views on milk fermentation(3).

Pathogenic bacteria, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium* have been identified as a cause of food poisoning outbreaks linked to raw milk in several countries. These bacteria can be present in the milk from the start or be introduced at any point during production and processing [9]. Public health strategies focus on reducing the risk of harmful bacteria in raw milk throughout the food supply chain, before it reaches consumers .Though, several studies have shown that consumer food handling can counterbalance food safety practices during processing and culminate in foodborne

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disease. In particular, poor consumer food handling practices, such as leaving refrigerated foods like milk out at room temperature for too long, can create conditions that favor bacterial growth(4).

The milk from cows immunized with human intestinal bacteria (immune milk) contains an anti-inflammatory component that may suppress the inflammatory reaction and modulate cytokine production. Therefore, it was decided to test whether immune milk may have some beneficial effects on controlling the symptoms and signs in OSF patients. Oral administration of immune milk leads to significant improvements of symptoms and signs in OSF patients.(5).

The health of raw milk is in the interest of public, and failure provide high quality milk will reduce the level of public health as well as economic health; therefore, due to the importance of this issue, it is vital to quickly and accurately identify raw milk contamination. Therefore, it is necessary to study the profile of pathogenic microbes phenotypically. Considering the importance of milk as an essential nutrient, it is advisable for the research community to identify pathogenic bacteria such as *Salmonella spp*, *S. aureus* and *Listeria monocytogenes* and to study the spread of antibiotic resistance in these pathogens . Therefore, the current study aimed to evaluate the contamination of raw and pasteurised milk as well as cheese with *Salmonella typhi*, *L.monocytogenes* and *S.aureus* to determine the antibiotic resistance pattern of the isolated bacteria.(6).

*Staphylococcus* aureus is one of the important causes of food-borne diseases in humans, S. aureus is commonly associated with intoxications due to its ability to produce a variety of potent enterotoxin (Balaban and Rasooly, 2000 and Le Loir et al., 2003). Identical S. aureus strains have occasionally been isolated from dairy cows and hands of milking persons (Jorgensen et al., 2005 a), but strains originating from bovine mastitis in general represent a genetically different cluster than the human strains, suggesting host specificity (7).

An increasing number of people are consuming raw unpasteurized milk. Enhanced nutritional qualities, taste, and health benefits have all been advocated as reasons for increased interest in raw milk consumption. However, science-based data to substantiate these claims are limited. People continue to consume raw milk even though numerous epidemiological studies have shown clearly that raw milk can be contaminated by a variety of pathogens, some of which are associated with human illness and disease. Several documented milkborne disease outbreaks occurred from 2000–2008 and were traced back to consumption of raw unpasteurized milk. Numerous people were found to have infections, some were hospitalized, and a few died. In the majority of these outbreaks, the organism associated with the milkborne outbreak was isolated from the implicated product(s) or from subsequent products made at the suspected dairy or source. Development of pre- and postharvest control measures to effectively reduce contamination is critical to the control of pathogens in raw milk. One sure way to prevent raw milk–associated foodborne illness is for consumers to refrain from drinking raw milk and from consuming dairy products manufactured using raw milk(8).

Milk products are rich in calcium, protein, potassium and phosphorus and it is very important for children and adolescents because it contains numerous essential nutrients so it provides around 52–65 % of the dietary reference intake of calcium and 20–28 % of the protein requirement. Some of the bacteria contained in milk (such as *Lactobacillus spp* or *Bifidobacterium spp*.) are also present in the healthy human gastrointestinal tract, aiding in digestion and protection from other infections, while other bacteria can be extremely harmful to human health. Milk can be polluted by *Mycobacterium bovis*, *Brucella* species, *Streptococci* and *Coxiellaburnettifrom* infected cattle. Agents from human sources such as *Salmonella* species, *Shigella* species, *Corynebacterium diphtheria* and *Streptococcus species* can also be presented in milk(9).

Milk is widely consume as nutrient food and it is excellent medium for the growth of microorganisms such balanced diet. Milk becomes contaminated with several types of microorganisms which originate from the soil, water, or skin and hair of the animals or utensils or from the milk maid. Bacterial contamination is brought about by bacteria, virus and parasites. All food carry contaminating microorganisms from natural sources in most instances contamination begins from the start of handling by humans and this continues till the product is consumed .Milk is a complex biological fluid and by its nature,a good growth medium for many microorganisms. Because of the specific production it is impossible to avoid contamination of milk with microorganisms

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therefore the microbial content of milk is a major feature in determining its quality(10).

A milk shake can also be made by adding powder into fresh milk and stirring the powder into the milk. Milkshakes made in this way can come in a variety of flavors, including chocolate, caramel, strawberry, and banana. The manufacture of this product (milk shake) is based on traditional method without any regard to the quality of raw material used and/ or the hygienic quality of the products. Under such conditions many microorganisms can find access to the milk products. Among all microorganisms *E. coli* is frequently contaminating organism, and is reliable indicator of fecal pollution generally in insanitary conditions of water, food, milk and other dairy products. *E.coli* was isolated from milk products like Mawa/ Khoa, Cream Dahi, Cheese, Butter and Gulabjaman. *E. coli* is a normal inhabitant of the intestines of animals and humans but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to sever gastrointestinal disturbance. Its presence in processed foods results from recontamination, because this bacterium usually does not survive food preservation processes. The main reasons for the presence of E. coli in food products are nonobservance of relevant technological regimes, incompliance with recommended process standards, and the lack of personal hygiene (11).

Milk, as every bacteriologist knows, is not only a universal and excellent food-stuff for human beings, but a medium admirably adapted for the growth and multiplication of microbes. This fact is confirmed by the bacteriological examination of the milk sold in London shops, milk which, normal though it may be in appearance, chemical analysis, and taste, is usually found to contain hundreds of thousands of bacteria per cubic centimeter; bacteria which belong to various species and some of which when grown separately in sterile milk cause rapid changes and alter profoundly the character of the milk, *e. g. Bacillus lactis, Proteus vulgaris, Bacillus coli, Bacillus mesentericus,* spores of *Bacillus enteritidis,* etc. If allowed to stand, the milk containing the above mixture of bacteria exhibits even at ordinary temperatures, but in a more marked degree at temperatures of 70° F. and above, those profound changes which are popularly expressed as ‘going bad,’ changes caused by the rapid multiplication of one or other of the above microbes(12).

Even today most cheese is made by traditional methods ,frequently from raw milk,and is much handled by the cheesemaker and other factory personnel.Reliance on the effects of starter competition and of low pH to eliminate pathogens often leads to careless practices.Low pH is not completely effective in destroying pathogens,and in addition there is no certainty that all the cheese reaching the market has developed the pH level characteristic of the variety. Some may never reach the low pH required because the cheese starter has failed and the fermentative processes responsible for the drop in pH were not completed. Further more,in the mould-ripened or mould-infected cheese,and in cheese with surface bacterial smear, there are pockets or layers in or on the cheese where the pH tends to be neutral or alkaline. Similar pockets might also result from the incidental presence of bacteria (13).

Raw milk, meat and plant materials are subjected to high risks of contamination by various pathogenic bacteria and thus their growth prevention is a great challenge in the food industry. Food fermentation by lactic acid bacteria (LAB) besides changing its organoleptic characteristics also helps to eliminate unfavorable microflora and represses growth of pathogens. To the date only few LABs has been reported to exhibit activity against bacteria embedded in the biofilms characterized by extreme resistance to antimicrobials, high exchange rate with resistance genes and represent high risk factor for foodborne disease development(14).

Identification of pathogenic and spoilage microorganisms by rapid and reliable methods is a fundamental aspect of dairy microbiology. Psychrotrophic bacteria are considered as the major concern in ultrahigh temperature (UHT) processing, as they can grow under refrigeration conditions, regardless of their optimal growth temperature Psychrotrophs comprise a large heterogeneous group of Gram negative and Gram positive genera .Among those problematic microbiota, *Pseudomonas* (particularly *Pseudomonas fluorescens*) and *Bacillus* are the leading causes of spoilage of milk and dairy products, and the most frequent isolates from milk and dairy products at the time of spoilage despite comprising less than 10.0% of the original raw milk microbiota .They are ubiquitous in

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nature and can be disseminated into raw milk via soil, water and vegetation at dairy farms(15).

Human milk is the main source of nutrition for infants and the transmission of various microorganisms. The lactic acid bacteria (LAB) in breast milk allow for the establishment of the gut microflora of infants. In this study, we aimed to assess the probiotic potential of LAB strains isolated from breast milk of healthy Chinese women(16).

Food poisoning is a common, yet distressing and sometimes life-threatening problem for millions of people all over the world. More than 250 different diseases can cause food poisoning. Gram's staining test on these colonies to detect the bacterial type. This step showed that all bacterial samples were gram positive bacteria and confirmed that high degree of similarity between the aerobic and faculatitative aerobic samples in the shape .Six different antibiotics were used to study the resistance of the isolated bacteria against these antibiotics. Different concentrations were used in the culture media starting with 0.005 mg/ml. The MIC was according to the antibiotic activity against the examinated bacteria. The lowest minimum inhibition concentration (MIC) of tetracycline (0.005 mg/ml).then , ampicillin and kanamycin (0.025 mg/ml), then neomycin and spectinomycin (0.08 mg/ml) the highest MIC was in case of chloromphenicol (0.125 mg/ml). This result was confirmed that different degree of antibiotic resistance affecting on the bacterial growth, bacteria have the highest resistance to chloromphenicol and moderate resistance to ampicillin , kanamycin, neomycin and spectinomycin while have the lowest resistance to tetracycline(17).

Probiotics are generally used in fermented food production and are considered safe with application in medical and veterinary activities. In the food industry, probiotics are commonly used as starter cultures and have been indexed as part of human microbiota. Yogurt, cheese and fermented milk products are the main sources of probiotics. Lactic acid produced on fermentation of lactose contributes to the sour taste of yogurt by decreasing its pH and enables the formation of the characteristic texture by acting on milk proteins. Traditional dairy products have been used for ages by natives and are the main source of potentially probiotic bacteria(18).

The outbreak isolates belonged to sequence type 1038, clonal complex 101, genetic lineage II. There were no pre-mature stop codons in *inlA*. Isolates contained *Listeria* Pathogenicity Island 1 and multiple internalins. PFGE and multiple whole genome sequencing (WGS) analyses all clustered together food, environmental and clinical isolates when compared to outgroup from the same clonal complex, which supported the finding that *L. monocytogenes* likely persisted in the soft serve ice cream/milkshake maker from November 2014 to November 2015 and caused 3 illnesses, and that the outbreak strain was transmitted between two ice cream production facilities. The whole genome SNP analysis, one of the two species-specific cgMLST, the lineage II-specific cgMLST and the wgsMLST/outbreak-specific cgMLST showed that *L. monocytogenes* cells persistent in the milkshake maker for a year formed a unique clade inside the outbreak cluster. This clustering was consistent with the cleaning practice after the outbreak was initially recognized in late 2014 and early 2015. Putative prophages were conserved among prophage-containing isolates. The loss of a putative prophage in two isolates resulted in the loss of the *Asc*I restriction site in in the prophage, which contributed to their *Asc*I-PFGE banding pattern differences from other (19).

*Bacillus* spp. and related sporeformers are important food spoilage organisms. While use of molecular subtyping methods has provided important information on the ecology and transmission of foodborne pathogens, the lack of rapid, reliable, and affordable subtyping methods for *Bacillus* spp. has limited our ability to understand and control their transmission throughout the food chain. Analysis of subtypes isolated over time in dairy products revealed the presence of both persistent and transient bacterial subtypes, indicating that application of these methods can improve our understanding of the ecology of these spoilage organisms and can help in identification of bacterial niches that may contribute.

## 2. Objectives

To make a study on the selected nutrients in milk samples like Milma milk, Malanadu milk, Elanadu milk and fresh udder milk.

To study the presence of contaminants in the selected milk samples

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To study the presence of various bacteria in the selected milk samples

## 3. Methods

### 3.1.Collection Methods

Milk samples were collected from the market – Three brands viz. Milma milk Malanad milk , and Elanad milk were collected for study. Samples of fresh udder milk were also used for study.

### 3.2. Nutrient Analysis


**Fig. 1. Automatic milk analyser**

Automatic milk analyzers have revolutionized the dairy industry by providing rapid and accurate nutrient analysis of milk. These devices use various technologies, primarily ultrasonic and infrared spectroscopy, to determine the composition of milk quickly and efficiently. Here's a breakdown of how they work and what they analyze:

Automatic milk analyzers typically measure the following key parameters:

**Fat Content:**

This is a crucial parameter for determining the quality and value of milk.The analyzer accurately measures the percentage of fat in the milk sample.

**Solids-Not-Fat (SNF):**

SNF represents the total solids in milk, excluding fat.It includes proteins, lactose, and minerals.SNF is an important indicator of the nutritional value of milk.

**Water Content:**

Some analyzers can detect added water in milk, which is a common form of adulteration.

### 3.3 Isolation And Identification Bacteria

**Sample Collection and Preparation:**

Collect milk samples aseptically using sterile containers.If the milk sample is refrigerated, allow it to reach room temperature before processing.Thoroughly mix the milk sample to ensure even distribution of bacteria.Disinfect the work area with 70% ethanol.

**Serial Dilution (Optional but Recommended):**

Prepare a series of sterile test tubes containing sterile saline or peptone water.Using a sterile pipette, transfer a known volume (e.g., 1 mL) of the milk sample to the first tube.Mix thoroughly.Transfer the same volume from the first tube to the next, repeating the process to create a series of dilutions (e.g., 1:10, 1:100, 1:1000). This is done to achieve a countable number of colonies on the agar plates.

**Plating:**

**Spread Plate Method:**

Using a sterile pipette, transfer a known volume (e.g., 0.1 mL) of the milk sample or diluted sample onto the surface of a prepared agar plate.Using a sterile glass spreader (flame-sterilized and cooled), spread the sample evenly across the agar surface.Allow the plate to dry.

**Incubation:**

Invert the Petri dishes and incubate them at an appropriate temperature (e.g., 37°C for general bacteria, 25°C for environmental bacteria) for 24-48 hours.Observe the plates for bacterial growth.

**Colony Selection and Subculturing:**

Select well-isolated colonies with distinct morphologies.Using a sterile loop, transfer a single colony to a fresh agar plate for subculturing.Incubate the subculture under the same conditions as the original plate.Repeat subculturing to obtain pure cultures.

**Identification**

Perform Gram staining to determine the Gram reaction and morphology of the bacteria.Perform biochemical tests (e.g., catalase test, oxidase test) or use commercial identification kits to identify the bacteria.Microscopic examination of the bacteria.

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## 4. Observation and results

**Table. 1 Milk brand – Milma**

| Item | Sample 1 | Sample 2 | Sample 3 | Permissible limit |
|---|---|---|---|---|
| Fat content Percent m/m | 6.43 | 6.42 | 6.33 | 3.2 |
| S.N.F percent m/m | 6.57 | 6.53 | 6.48 | 8.5 |
| Water Percent | 88 | 89 | 86 | 85 |
| Adulterants Percent | 0 | 0 | 0 | 0 |
| Preservatives Percent | 0 | 0 | 0 | 0 |

**Fat Content (Percent m/m):**

Samples 1, 2, and 3 show fat content of 6.43%, 6.42%, and 6.33%, respectively.The permitted level is 3.2%. All three samples significantly exceed the permitted fat content. This indicates the milk is very high in fat, potentially whole milk, or even cream enriched.

**Solids Not Fat (S.N.F) (Percent m/m):**

Samples 1, 2, and 3 show S.N.F values of 6.57%, 6.53%, and 6.48%, respectively.The permitted level is 8.5%. All three samples are below the permitted level for S.N.F. This indicates a potential dilution of the milk with water, or a naturally low level of solids not fat.

**Water (Percent):**

Samples 1, 2, and 3 show water content of 88%, 89%, and 86%, respectively.The permitted level is 85%. All three samples are slightly above the permitted water level. This reinforces the possibility of dilution, especially when correlated with the low S.N.F values.

**Adulterants (Percent):**

All three samples show 0% adulterants.The permitted level is 0%. No adulterants were detected in any of the samples, which is a positive result.

**Preservatives (Percent):**

All three samples show 0% preservatives.The permitted level is 0%.

No preservatives were detected in any of the samples, which is also a positive result.

**Table. 2 Milk brand – Malanadu milk**

| Item | Sample 1 | Sample 2 | Sample 3 | Permissible limit |
|---|---|---|---|---|
| Fat content Percent m/m | 2.97 | 3.32 | 2.88 | 3.2 |
| S.N.F percent m/m | 7.98 | 7.96 | 7.94 | 8.5 |
| Water Percent | 88 | 88 | 87 | 85 |
| Adulterants Percent | 0 | 0 | 0 | 0 |
| Preservatives Percent | 0 | 0 | 0 | 0 |

**Fat Content (Percent m/m):**

Sample 1: 2.97%Sample 2: 3.32%.Sample 3: 2.88%.Permitted Level: 3.2%.Sample 2 is slightly above the permitted level.Samples 1 and 3 are slightly below the permitted level. This indicates that sample 2 has a higher fat content than the minimum permitted level. samples 1 and 3 are close to the permitted level, but slightly lower.

**Solids Not Fat (S.N.F) (Percent m/m):**

Sample 1: 7.98%.Sample 2: 7.96%. Sample 3: 7.94%. Permitted Level: 8.5%. All three samples are below the

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permitted S.N.F level. This suggests a potential deficiency in the non-fat solid components of the milk.

**Water (Percent):**

Sample 1: 88%. Sample 2: 88%. Sample 3: 87%. Permitted Level: 85%. All three samples exceed the permitted water content. This reinforces the concern raised by the low S.N.F values, potentially indicating dilution.

**Adulterants (Percent):**

.All samples: 0%. Permitted Level: 0%**.** No adulterants were detected in any of the samples, which is a positive result.

**Preservatives (Percent):**

All samples: 0%. Permitted Level: 0%. No preservatives were detected in any of the samples, which is also a positive result.

**Table 3 Milk brand – Elanadu milk**

| **Item** | **Sample 1** | **Sample 2** | **Sample 3** | **Permissible limit** |
|---|---|---|---|---|
| Fat content Percent m/m | 3.32 | 3.35 | 3.56 | 3.2 |
| S.N.F percent m/m | 8.23 | 8.42 | 8.34 | 8.5 |
| Water Percent | 82 | 83 | 83 | 85 |
| Adulterants Percent | 0 | 0 | 0 | 0 |
| Preservatives Percent | 0 | 0 | 0 | 0 |

The analysis of the *Malanad* milk brand based on the given parameters shows the following results:

**Fat Content (% m/m)**

The fat content in all three samples (3.32%, 3.35%, and 3.56%) is slightly higher than the permitted level of 3.2%.This indicates good fat quality in the milk, making it richer and potentially more nutritious.

**Solids-Not-Fat (SNF) (% m/m)**

The SNF values (8.23%, 8.42%, and 8.34%) are slightly below the permitted level of 8.5%.This suggests a minor deviation, possibly due to variations in milk composition. However, the difference is not significantly alarming.

**Water Content (%)**

The water content in the samples (82%, 83%, and 83%) is well below the permitted level of 85%. This indicates that the milk is not excessively diluted and maintains a good balance of natural constituents.

**Adulterants (%)**

All samples tested negative for adulterants (0%), confirming that the milk is free from harmful substances such as starch, urea, or detergent.This is a positive indication of milk purity.

**Preservatives (%)**

No preservatives were detected (0%), ensuring that the milk is fresh and does not contain chemical preservatives.This suggests the milk is natural and safe for consumption.

**Table 4 Milk brand – Fresh Udder milk**

| **Item** | **Sample 1** | **Sample 2** | **Sample 3** | **Permissible limit** |
|---|---|---|---|---|
| Fat content Percent m/m | 3.31 | 3.28 | 3.21 | 3.2 |
| S.N.F percent m/m | 8.48 | 8.46 | 8.49 | 8.5 |
| Water Percent | 86 | 86 | 85 | 85 |
| Adulterants | 0 | 0 | 0 | 0 |

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| | | | | |
|---|---|---|---|---|
| Percent | | | | |
| Preservatives Percent | 0 | 0 | 0 | 0 |

**Fat Content (Percent m/m):**

Sample 1: 3.31%.. Sample 2: 3.28%. Sample 3: 3.21%. Permitted Level: 3.2%. All three samples are very close to, or slightly above, the permitted fat content. This indicates that the fat content of the milk is within an acceptable range, with samples 1 and 2 being slightly higher than the minimum standard.

**Solids Not Fat (S.N.F) (Percent m/m):**

Sample 1: 8.48%..Sample 2: 8.46%.Sample 3: 8.49%.Permitted Level: 8.5%.**Interpretation:** All three samples are very close to, but slightly below, the permitted S.N.F level. This indicates a minor deficiency in the non-fat solid components. However, the results are extremely close to the permitted level.

**Water (Percent):**

Sample 1: 86%.Sample 2: 86%.Sample 3: 85%.Permitted Level: 85%. Samples 1 and 2 are slightly above the permitted water content, while sample 3 is at the permitted level. This slight increase in water content, combined with the slightly lower S.N.F, could indicate a very minor dilution, or just natural variation.

**Adulterants (Percent):**

All samples: 0%. Permitted Level: 0%**.** No adulterants were detected, which is a positive result.

**Preservatives (Percent):**

All samples: 0%.Permitted Level: 0%**.** No preservatives were detected, which is also a positive result.

**Table.4 Bacterial isolates from milk samples**

| Sl. No | Name of Bacteria | Milma Milk | Malanadu Milk | Elanad milk | Fresh udder milk |
|---|---|---|---|---|---|
| 1 | *E. Coli* | - | - | - | + |
| 2 | *Staphylococcus aureus* | - | - | - | - |
| 3 | *Brucella spp* | - | - | - | - |
| 4 | *Shigella spp* | - | - | - | - |

**E. Coli:**

Milma Milk: - (Negative)Malanadu Milk: - (Negative). Elanadu milk -(Negative). Fresh Udder Milk: + (Positive). *E. coli*, a common indicator of fecal contamination, was found only in the fresh udder milk. This indicates potential unsanitary handling or contamination during the collection of this particular sample. The processed milks are shown to be free of *E.coli.*

***Staphylococcus aureus:***

Milma Milk: - (Negative). Malanadu Milk: - (Negative). Elanadumilk -(Negative).Fresh Udder Milk: - (Negative). *Staphylococcus aureus*, which can cause food poisoning, was not detected in any of the samples. This is a positive result, indicating that these milk samples are likely free from this particular pathogen.

***Brucella spp:***

Milma Milk: - (Negative)

Malanadu Milk: - (Negative)

Elanadu milk -(Negative)

Fresh Udder Milk: - (Negative)

*Brucella spp*., the bacteria responsible for brucellosis, was not detected in any of the samples. This is a crucial finding, as brucellosis is a zoonotic disease that can be transmitted through contaminated milk.

***Shigella spp:***

Milma Milk: - (Negative)

Malanadu Milk: - (Negative)

Elanadu milk - (Negative)

Fresh Udder Milk: - (Negative)

*Shigella spp*., which causes shigellosis (dysentery), was not detected in any of the samples. This is a positive result, indicating the absence of this pathogenic bacteria.

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The commercially processed Milma, Malanadu, and Elanadu milk samples show good bacterial quality, with no detection of the tested pathogens. This suggests effective processing and hygiene practices.

The fresh udder milk sample tested positive for E. coli, indicating fecal contamination. This highlights the importance of proper hygiene during milk collection and handling.

The absence of Staphylococcus aureus, Brucella spp., and Shigella spp. in all samples is a positive indication of milk safety.

The fact that the fresh milk had *E.coli*, shows that processing the milk, as is done with the Milma , Malanadu and Elanadu milk, is effective at removing harmful bacteria.

**Discussion**

The results of this study offer a nuanced perspective on the chemical and microbiological quality of diverse milk samples, illustrating both commendable practices and areas requiring attention. The chemical analyses revealed variability in milk composition, particularly concerning fat, SNF, and water content, aligning with observations from other studies highlighting the influence of various factors on milk composition (20).

The initial finding of consistently high fat content, surpassing regulatory minimums, coupled with low SNF and elevated water levels, suggests potential inconsistencies in milk processing or possible dilution. This aligns with concerns raised by early research on milk adulteration (21). While high fat content might appeal to certain consumers, it's vital that milk composition adheres to regulatory standards to ensure nutritional balance and prevent misrepresentation, as emphasized by Codex Alimentarius guidelines (22). The varying fat content found in subsequent tests, from slightly below to slightly above the standard, shows the importance of consistent testing, and the potential for a variety of milk qualities to be tested. The consistently low SNF and high water content in some samples raise concerns about potential dilution, which could significantly diminish the nutritional value of the milk, a practice documented in several studies on milk adulteration (23). However, the last set of chemical testing showed results that were very close to the permitted levels, showing that high quality milk was also tested. The absence of detectable adulterants and preservatives across all samples is a positive finding, indicating adherence to safety standards and minimizing the risk of harmful chemical exposure, aligning with recommendations for food safety (24).

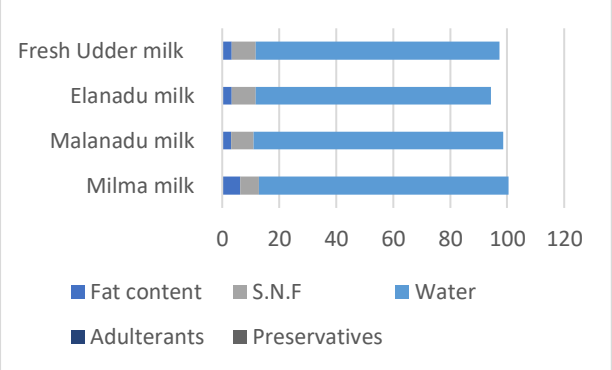


**Fig. 2. Percentage bar diagram showing the selected components of milk**

The above percentage bar diagram indicates the following facts,

**Fresh Udder Milk:**

Shows the highest water content among the four, though the difference is marginal. Has a slightly lower fat content compared to Elanadu and Malanadu. Adulterants and preservatives are present in very small amounts.

**Elanadu Milk:**

Exhibits a slightly higher fat content than Fresh Udder and Milma. Water content is still dominant but slightly lower than Fresh Udder. Low levels of adulterants and preservatives.

**Malanadu Milk:**

Similar composition to Elanadu milk with a slightly higher fat content than Elanadu. Water content is slightly lower than Elanadu..Low levels of adulterants and preservatives.

**Milma Milk:**

Shows the lowest fat content among the four types. Water content is slightly lower than Fresh Udder but higher than Elanadu and Malanadu. Low levels of adulterants and preservatives.

The microbiological analysis provided a stark contrast between processed and unprocessed milk. The absence

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of pathogenic bacteria (*E. coli, Staphylococcus aureus*, *Brucella spp.*, and *Shigella spp.*) in Milma Malanadu and Elanadu milk samples underscores the efficacy of pasteurization and hygienic processing practices. These findings validate the importance of commercial processing in eliminating microbial contaminants and ensuring milk safety, as supported by research on pasteurization effectiveness (25).

Conversely, the detection of *E. coli* in fresh udder milk highlights the vulnerability of unprocessed milk to fecal contamination. This finding emphasizes the critical role of proper hygiene during milk collection and handling, particularly for small-scale producers and consumers who rely on raw milk. *E. coli* is a well-established indicator of fecal contamination, and its presence suggests potential exposure to other enteric pathogens, a risk documented in studies on raw milk consumption (26). While the absence of other tested pathogens is reassuring, the presence of *E. coli* necessitates stringent hygiene measures to prevent further contamination and ensure consumer safety.

The discrepancies in chemical composition observed in some samples could stem from various factors, including variations in animal feed, breed, lactation stage, and processing techniques, as detailed in dairy science literature (27). Further investigation into these factors is warranted to optimize milk production and ensure consistent quality. The positive microbiological results for commercially processed milk highlight the importance of maintaining rigorous quality control measures throughout the production chain, from farm to consumer ( 28)

The findings of this study have implications for both consumers and regulatory bodies. For consumers, the study underscores the importance of choosing reputable milk brands and practicing safe handling of raw milk. Regulatory bodies can utilize these findings to strengthen quality control measures, implement stricter hygiene standards, and enhance consumer awareness about milk safety, as advocated by food safety guidelines (29).

In conclusion, this study provides valuable insights into the chemical and microbiological quality of milk. While commercially processed milk demonstrated satisfactory quality, the presence of *E. coli* in raw milk and variations in chemical composition highlight the need for continuous monitoring and improvement of milk production and processing practices. Further research should focus on identifying the specific factors contributing to variations in milk composition and developing strategies to mitigate microbial contamination in raw milk.

**Conclusion**

This study, through a combined approach of chemical and microbiological analyses, provided a comprehensive evaluation of the quality of various milk samples. The chemical analyses revealed variability in milk composition, particularly concerning fat, SNF, and water content, highlighting the need for consistent monitoring and adherence to regulatory standards. While some samples exhibited deviations from permitted levels, potentially indicating dilution or processing inconsistencies, others demonstrated compliance, showcasing the range of milk qualities available. The absence of adulterants and preservatives across all samples is a positive indicator of adherence to safety protocols.

The microbiological investigation clearly distinguished between processed and unprocessed milk. Commercially processed Milma Malanadu and Elanadu milk samples demonstrated excellent bacterial quality, with no detection of pathogenic bacteria, validating the effectiveness of pasteurization and hygienic processing. Conversely, the presence of *E. coli* in fresh udder milk underscored the vulnerability of raw milk to faecal contamination, emphasizing the critical role of proper hygiene in milk collection and handling.

The findings of this research emphasize the importance of rigorous quality control measures throughout the milk production and distribution chain. For consumers, the study reinforces the significance of choosing reputable milk brands and practicing safe handling of raw milk. For regulatory bodies, the results highlight the need for continuous monitoring, stricter hygiene standards, and enhanced consumer awareness to ensure milk safety.

Ultimately, this study contributes to a better understanding of milk quality and safety. By identifying potential areas of concern and reinforcing effective practices, this research supports efforts to provide consumers with safe, nutritious, and high-quality milk. Future research should focus on further exploring the factors influencing milk composition and developing

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strategies to mitigate microbial contamination, ultimately contributing to improved public health and consumer confidence.

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**Statement of conflict of interest**

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**Outcome to the Society**

Reduced Risk of Foodborne Illnesses: By identifying potential sources of bacterial contamination, particularly *E. coli* in raw milk, the study highlights the importance of hygienic practices and proper pasteurization. This can lead to a reduction in foodborne illnesses associated with contaminated milk consumption.

Improved Nutritional Intake: Ensuring milk quality and safety contributes to the delivery of essential nutrients to the population, particularly vulnerable groups like children and the elderly, supporting their overall health and well-being.

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