RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 44 Special Issue: 168-174 (November 2025)

Adulteration and Microbiological Quality of Raw Milk from Local Markets in Hebron, Palestine

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10.18805/ajdfr.DRF-516

ABSTRACT

Background: Milk is an essential item in daily life. It is not only a source of good quality protein, but also of calcium and riboflavin besides other nutrients. This study aims to evaluate the quality of raw milk sold in Hebron district, Palestine. Milk may be infected with pathogenic microorganisms, or adulterated with various adulterants which forms a risk to consumers.

Methods: Milk components, freezing point, density and added water were determined using an ultrasonic milk analyzer. The microbial quality of milk was evaluated by assessing it for total bacterial count (TBC) and total coliform count (TCC). Adulterants (carbonate, formalin, starch and salt) were also tested.

Result: The mean TBC and TCC were log 6.65 and 4.04 cfu/ml respectively, indicating the deterioration of milk quality along the dairy value chain. The overall mean for fat, solid-not-fat (SNF), protein and lactose per cent were 3.57±0.8, 8.51±0.9, 3.14±0.5 and 4.56±0.5, respectively. Moreover, none of the samples were contaminated with any adulterants. Hence, when compared to the standard level, the chemical composition results were deemed adequate, while the microbial results are higher than anticipated and disappointing. Therefore, enhancing the attitude and educational attainment of milk handlers is a crucial milestone to enhance milk quality and prevent dairy-related illnesses.

Key words: Adulterants, Coliform, Milk analyzer, Raw milk, Total bacterial count.

INTRODUCTION

The dairy industry is one of the main food businesses in Palestine, with yearly milk production exceeding 212,000 tons (Palestinian Ministry of Agriculture, 2021). Adulteration is an act changing the quality of food either by adding lower-quality materials or by the removal of some valuable components (Amin, 2016). Milk adulteration may be intentional to increase the profit or accidental due to unhygienic and faulty production and handling practices (Eman *et al.,* 2015). Some of the major adulterants in milk having critical health impairment are urea, carbonate, formalin, detergents, ammonium sulphate, boric acid, salicylic acid, benzoic acid, sugars, melamine and hydrogen peroxide (Debnath *et al.,* 2015). Urea added to increase SNF and whiten milk results in abnormal physiological activity in young children (Debnath *et al.,* 2015). However, hydrogen peroxide, carbonates, formalin, caustic soda or antibiotics may be added as preservative (Mohanty *et al.,* 2020). Some adulterants, like detergents, are added to milk to improve its aesthetic qualities. Artificial detergents are used to give milk a foamy appearance because water reduces the milk's foamy appearance (Francis *et al.,* 2020). This immoral practice is frequently changed to prevent monetary losses brought on by milk spoiling while being transported and sold.

Milk contains fats, proteins, lactose and minerals that support microbial growth under poor hygiene or storage. Minerals like calcium, phosphorus, iron and zinc also play key roles in metabolism and biological functions (Singh *et al.,* 2023). Several factors contribute to the poor microbiological quality of raw milk, including hygiene practices during milking, storage conditions, transportation and the inability to cool milk quickly to below 4.5°C all result in increased total bacterial and coliform count in raw milk, causing spoilage during storage and transportation (Mohamedsalih and Abubaker, 2021). Finding pathogens and coliform bacteria in milk suggests that the udder, milk utensils, or water source may have been contaminated with bacteria (Bonfoh *et al.,* 2003). However, microbial loads in fresh milk from a healthy cow are typically low (less than 1×10^3 cfu/ml), but after being stored for a while at room temperature, they can rise by 100 times or more (Chye *et al.,* 2004).

In Hebron, a major city located in south of West Bank in Palestine, raw milk is frequently available in local markets and is a staple for many residents. However, there is inadequate data on the extent of adulteration and the microbial condition of raw milk in this area. Investigating

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How to cite this article: Tubesha, Z.A. (2025). Adulteration and Microbiological Quality of Raw Milk from Local Markets in Hebron, Palestine. *Asian Journal of Dairy and Food Research*. **44 (Special Issue):** 168-174. doi: 10.18805/ajdfr.DRF-516.

Submitted: 04-03-2025 **Accepted:** 19-05-2025 **Online:** 26-06-2025

the prevalence of these concerns is critical for developing strategies to guarantee food safety and protect community health. This research aims to assess the adulteration practices, physiochemical and the microbiological properties of raw milk vended in Hebron's markets, offering important insights into the safety and reliability of this commonly consumed product.

MATERIALS AND METHODS

Study period and site

The research was carried out between March and May 2023 in the Food Chemistry and Analysis Laboratory, Faculty of Agricultural Sciences and Technology, Palestine Technical University, Palestine.

Collection of samples

According to Palestinian Ministry of National Economy records, there are 21 registered retail raw milk sellers in Hebron district, all of them were invited to participate in the study, thirteen of them responded positively to our invitation. An amount of 1000 ml of raw milk packed in plastic bags (the usual packaging) was purchased from each sales point. All samples were stored at temperatures between 4-8°C in ice box and transported to be analyzed immediately after arrival to the laboratory.

Microbial analysis

The microbiological tests considered for evaluation of the bacterial load in raw milk samples were total bacterial count and total coliform count. For these two methods, HiMedia plate count agar (PCA) and violet red bile agar (VRBA) were utilized, respectively. Peptone water was used as diluent to dilute the samples.

Total bacterial count

In short, a sterile test tube containing 9 ml of peptone water was filled with 1 ml of milk sample. Appropriate decimal dilution (10^{-4}-10^{-7}) of milk samples were pour-plated with 15 mL of PCA in duplicate, allowed to solidify and incubated in inverted positions at 30°C for 48 h (Negash *et al.,* 2012). Colony counts were made using colony counter (Stuart, China).

Coliform count

In brief, a sterile test tube holding 9 ml of peptone water was filled with 1 ml of raw milk sample. Appropriate decimal dilutions (10^{-1}-10^{-4}) of milk samples were pour-plated on 15 ml VRBA. The medium was allowed to solidify before incubating at 30°C for 24 h. Lastly, a colony counter was used to count the colonies. Typical dark red colonies were considered as coliform colonies (Hyera, 2015).

For both tests, bacterial counts between 25 and 250 were used for calculating the number of colony forming units (cfu/ml) according to the formula:

$$cfu = \frac{n}{v \times d}$$

Where,

n = Number of colonies counted per plate.

v = Volume of inoculant in each plate (ml).

d = Dilution factor.

The mean value of the countable colonies after the incubation period of the duplicate plates was applied in the calculations (Ledo *et al.,* 2020).

Physicochemical analysis

Ultrasonic Milk Analyzer (Lactoscan SP, china) was used to analyse milk for compositional parameters including the protein, lactose, fat, SNF, density, freezing point and presence of added water.

Milk adulterants

In this study, various milk adulterants were identified, such as carbonates, formalin, starch and salt.

Detection of carbonates

To about 5 ml of milk sample in a test tube, 5 ml of methanol and 5 drops of methanolic solution of rosalic acid (1% w/v) were added and then mixed well. Appearance of a pinkish red color suggested the existence of sodium carbonate/ sodium bicarbonate and hence unsuitable for individual intake (Debnath *et al.,* 2015).

Detection of formalin

10 ml of milk was taken in test tube and 5 ml of concentrated sulphuric acid was added on the walls of the test tube without shaking. Formation of a blue or violet ring at the junction indicated the presence of formalin (Mohanty *et al.,* 2020).

Detection of starch

3 ml of milk was taken in a test tube and boiled carefully. Then milk was cooled to ambient temperature. Appearance of blue color after adding 2-3 drops of 1% iodine solution indicated presence of starch (Mamun *et al.,* 2016).

Detection of salt (sodium chloride)

5 ml of 0.8% silver nitrate was taken in a test tube and added with 3 drops of 1% potassium dichromate and 1 ml of milk and thoroughly blended. Appearance of yellowish hue suggests presence of dissolved chloride (Parekh *et al.,* 2020).

RESULTS AND DISCUSSION

Total bacterial and coliform count

The total bacterial count (TBC) in milk samples was measured and compared to palestinian standard (PS 600:2024) and California "grade A" milk standards (California Department of Food and Agriculture, 2025) for raw cow's milk as shown in (Fig 1). All samples exceeded the Palestinian Standard limits (log 6.00 cfu/ml). The TBC values ranged from log 6.14±0.20 cfu/ml (S8) to log 6.98±0.09 cfu/ml (S6) with average value log 6.65 cfu/ml, indicating high bacterial contamination across all samples.

In addition, all samples also exceeded the California milk standards (CS) of log 4.70 cfu/ml. The results in this current study are inline with those done in raw milk by Aaku *et al.* (2004) in Botswana, Al-Tarazi *et al.* (2003) in Jordan and Karimuribo *et al.* (2005) in Tanzania which reported higher bacterial count above recommended level by standards in most of the samples that were tested. The high TBC values in all samples suggest significant bacterial contamination, likely due to poor hygiene practices during milking, storage, or transportation. The lack of compliance with both PS and CS standards highlights the need for improved handling and cooling practices to reduce bacterial growth (Sarkar, 2016).

Coliforms are gram-negative, aerobic or facultative anaerobic, non-spore-forming rods that ferment lactose and produce gas and acid at 35°C. They typically live in the gastrointestinal tracts of both humans and animals (Mounier *et al.,* 2019). For more than a century, coliforms have been used to detect fecal contamination in dairy, water and other food items (Martin *et al.,* 2023). These organisms are a sign of unhygienic production and/or inappropriate handling of milk or milk utensils when they are found in milk and milk products (Al-ledani and Ghazi, 2016). Since Coliform limits are not specified in the Palestinian raw milk standard (PS 600:2024), our results were evaluated against the stringent California standards. According to these international standards, the acceptable limit for coliforms in raw milk is typically ≤ log 2.88 cfu/ml. In this study, TCC values (Fig 2) ranged from log 1.50 cfu/ml (S6) to log 5.59 cfu/ml (S11) with average value log 4.04 cfu/ml. The results showed that, 31% raw milk samples coincided with the CS, while the remaining 69% had TCC above the standard. The results obtained are lower to those of Al-Tarazi *et al.* (2003), who obtained mean values of TCC log 5.5 cfu/ml and higher to the findings of Ismail *et al.* (2024), who obtained mean values of TCC log 3.64 cfu/ml. The high TCC values in most samples further emphasize the need for better hygiene practices, such as proper cleaning of milking equipment, udder sanitation and immediate cooling of milk after collection.

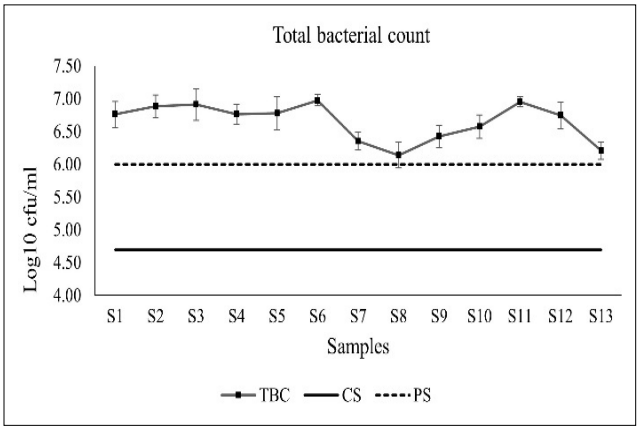


Fig 1: Total bacterial count (TBC) in raw milk samples, measured in log10 cfu/ml compared to standards (CS = California standard and PS = Palestinian standard).

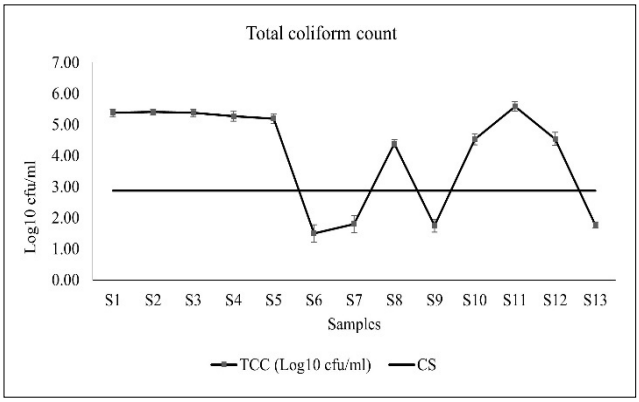


Fig 2: Total coliform count (TCC) in raw milk samples, measured in log10 cfu/ml compared to California standard (CS).

Physicochemical analysis

Chemical properties

The physicochemical properties of raw milk samples in the study were shown in Table 1. The average fat content (3.57±0.8 per cent) meets the PS (≥3 per cent), even though the range (2.32 to 4.60 per cent) shows that some samples are below the requirement. According to this study, 77% of raw milk samples coincided with the standard, while the remaining 23% had fat content below the standard. The average fat content in this study is similar with earlier findings of Yakubchak *et al.* (2021) and Malacarne *et al.* (2013) who reported a fat content of 3.5±0.1% and 3.71± 0.3 for milk obtained in private households and raw bulk milk samples respectively. Though, higher values of fat content (4.3%) was reported from milk of cows from dairy farms were reported by Lindmark-Månsson *et al.* (2003) as compared to the present study. However, the average fat content in the study were greater than the earlier findings of Kazeminia *et al.* (2023) who reported a fat content of 3.13±0.36% from milk collection centers in Qazvin, Iran. Because the cost per liter of milk is determined in part by its fat content. The current outcome is therefore great news for smallholder producers of raw cow milk. Mean SNF content was 8.51±0.9%, with a range of 6.63 to 10.20% observed for minimum and maximum values in the samples. The PS (≥8.5 per cent) is nearly met by the mean SNF content. Approximately similar results were reported by Duguma (2022), Gemechu (2016) and Mohammed and Abdel-Aall (2024).

The average protein content of milk samples ranged from 2.43% to 4.37% with an average of 3.14±0.5. This result is in line with 3.12 and 3.14% reported by Dehinenet *et al.* (2013) and Azeze and Tera (2015) respectively. However, this results lower than 3.40 and 3.98% reported by Haftu and Degnet (2018) and Gemechu and Amene (2021), in cow milk of Western Shewa and Bench Maji

Table 1: Physicochemical properties of raw milk samples.

Parameter	Min	Max	Mean ± SD	PS
Fat (%)	2.32	4.60	3.57±0.8	≥3
SNF (%)	6.63	10.20	8.51±0.9	≥8.5
Protein (%)	2.43	4.37	3.14±0.5	N/A
Lactose (%)	3.65	5.56	4.56±0.5	N/A
Density (g/cm^3)	1.020	1.035	1.027±0.01	1.028 to 1.035
Freezing point (°C)	-0.576	-0.419	-0.522±0.04	(-0.520) to (-0.560)

Table 2: Chemical adulterants in raw milk samples.

S. no.	Carbonate test	Formalin test	Starch test	Salt test
S1	-ve	-ve	-ve	-ve
S2	-ve	-ve	-ve	-ve
S3	-ve	-ve	-ve	-ve
S4	-ve	-ve	-ve	-ve
S5	-ve	-ve	-ve	-ve
S6	-ve	-ve	-ve	-ve
S7	-ve	-ve	-ve	-ve
S8	-ve	-ve	-ve	-ve
S9	-ve	-ve	-ve	-ve
S10	-ve	-ve	-ve	-ve
S11	-ve	-ve	-ve	-ve
S12	-ve	-ve	-ve	-ve
S13	-ve	-ve	-ve	-ve

region respectively. The genetic variability of the milking cow or environmental factors such as feed, lactation stage, milking interval, season and location could be the cause of this variation.

Another important component was lactose, a sugar found in milk. In this study the overall average of lactose content was 4.56±0.5 per cent. According to the European Union Quality standards for unprocessed whole milk the lactose content should not be less than 4.2 per cent (Assen and Abegaz, 2024; Tamime, 2009). Conversely, (O'Connor, 1995) recommended the normal range of cow milk lactose content as 4.7 to 4.9%. The present study found that the lactose content of milk was higher than that of the study conducted in the Peja region (Loshi *et al.,* 2023), which found 4.34±0.2 per cent and comparable to that of the study conducted in Basrah, Iraq (Al-Iedani and Ghazi, 2016), which found 4.61±0.6. Milk samples appears to contain a moderate amount of lactose, which is within the typical range for dairy products. The composition of milk can fluctuate due to interval between milking, stage of lactation, age and health of the cow. Milk lactose is influenced considerably by the health of the udder as well as the cow's metabolic and energy balance (Antanaitis *et al.,* 2024).

Physical properties

Based on the physical characteristics (Table 1) of milk, the most widely used techniques include the density and freezing point (Ionescu *et al.,* 2023). As a legal standard, the freezing point is measured to ascertain whether milk has been diluted with water. When water is adulterated with the milk, the salts dissolved in the serum are diluted and the freezing point is raised (Kamthania *et al.,* 2014). In this study, although some samples de*via*te with values as low as -0.576°C and as high as -0.419°C, the average freezing point (-0.522±0.04) falls within the PS typical range (-0.520°C to -0.560°C). However this results agreed with 0.520±0.001 reported by Abd Elrahman *et al.* (2009) for freezing points of raw milk in Blue Nile Dairy Company (Sudan).

The density of milk samples ranged from 1.020 to 1.035 with an average of 1.027±0.01, close to the standard of 1.028 to 1.035 g/cm^3. About 46% of the samples were lower than the normal density value which is partially due to adulteration by addition of water. However, low density of the milk does not automatically mean it has been adulterated with water and may for an example be the result of a cow suffering from mastitis (Sharma *et al.,* 2011). This study is similar to one conducted by (Orregård, 2013), whose findings indicated that approximately 33% of farmer samples did not fall within the acceptable density limit. Two of the thirteen tested milk samples were discovered to be tampered with by adding water. The milk's density could be diminished due to this adulteration.

Milk adulterants

For a variety of reasons, although mostly to mislead consumers and boost profits, milk is adulterated with ingredients like carbonates, formalin, salt and starch (Momtaz *et al.,* 2023). Carbonates, are used to cover the acidity and sour taste of the milk to avoid rejection in dairy collection hubs and production units (Irshad *et al.,* 2021). Even though formalin, a formaldehyde solution, is extremely toxic, act as preservatives and extend the milk's freshness (Ionescu *et al.,* 2023). Sodium chloride, or salt, is added mainly to get a correct lactometer reading by increasing the milk density. However, starch is added to raw milk to increase its viscosity and texture, especially when the milk has been diluted (Kailasapathy, 2015). Ingestion of adulterated milk may have a harmful consequence on human health because of the entrance of toxic substances or loss of nutritional value (Abbas *et al.,* 2024).

The presence of chemical adulterants in raw milk samples was tested and the results are presented in Table 2.

All samples tested negative for carbonate, formalin, starch and salt adulteration. This indicates that the milk samples were free from these common chemical adulterants, which is a positive finding. However, continuous monitoring is necessary to ensure that milk remains free from such adulterants, as their presence can have serious health implications. These results suggest that the owners of raw milk stores in the Hebron district have moral and religious convictions, particularly in light of the infrequent and limited inspection visits from the relevant ministries to these establishments. These finding agree with those reported by El-Bessary (2006) and Mansour *et al.* (2012), while Salama *et al.* (2023) found that 26.67% and 13.33% of market raw milk samples were formalin and carbonate positive respectively.

CONCLUSION

The results indicate that the raw milk samples had high bacterial and coliform counts, exceeding standard limits, which suggests poor hygiene practices during production and handling. The current study's poor bacteriological quality necessitates more research on the health of the animals, particularly mastitis and the importance of the impact of containers in determining their role in microbial quality. The physiochemical properties and presence of adulterants in the collected raw cow milk samples were closely to the recommended levels of PS. To improve the quality and safety of raw milk, it is essential to implement stricter hygiene practices, regular monitoring and adherence to quality standards.

ACKNOWLEDGEMENT

The author wishes to thank Palestine Technical University for funding this research. I am also profoundly thankful to Jamila Ismail and Tasneem Hijazi for their exceptional assistance and collaboration, particularly in sample collection and laboratory analysis.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The author declares no conflict of interest.

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