Journal of Food Quality and Hazards Control 11 (2024) 214-222

# Physio-chemical and Microbiological Quality Assessment and Detection of Adulterants in Different Sources of Milk in Bangladesh

S. Sultana, A. Hosen, A.S. Afsana, S. Sultana, E.A. Pehan, A. Islam, N. Sultana, M.R. Hassan*

*Bangladesh Livestock Research Institute, Savar, Dhaka - 1341, Bangladesh*

## HIGHLIGHTS

- The higher and lower titratable acidity were in milk samples obtained from Milkman and Branded pocket, respectively.
- The highest content of fat, protein, lactose, Solids-Not-Fat, Total Solids were found in samples obtained from Farmgate.
- Water adulteration was higher in milk samples obtained from Milkman.
- Neutralizers and nitrates were found positive in Milkman, Bazar, and Farmgate milk samples.
- Branded packet samples showed lower mean average of total viable bacteria.

***Article type***
Original article

***Keywords***
Food Quality
Food Contamination
Milk
Bangladesh.

***Article history***
Received: 14 Mar 2024
Revised: 20 Jun 2024
Accept: 18 Sep 2024

***Abbreviation***
BSTI=Bangladesh Standard and Testing Institute
CFU=Colony Forming Unit
ECC=Escherichia coli Count
SNF=Solids Not Fat
TCC=Total Coliform Count
TS=Total Solid
TVC=Total Viable Count

## ABSTRACT

**Background:** The sources of milk play a significant role on overall milk quality. Therefore, the current study was intended to evaluate the quality (physiochemical, compositional, and microbiological) and detect the adulterants in milk.

**Methods:** A total of 200 milk samples were collected during mid of October to mid of December 2022 from four sources viz. Farmgate (50), Milkman (50), Bazar (50), and Branded packet milk (50). Milk samples were examined for various physiochemical, compositional, microbiological properties, and existence of adulterants. Data were analyzed by one way ANOVA and differences were determined by least significant difference test using RStudio.

**Results:** Physiochemical analysis found significant variation in titratable acidity, protein, fat, salt, Solids-Not-Fat, and Total-Solids content among the sources ($p<0.05$), while lactose content was found non-significant. The highest (0.17%) and the lowest (0.13%) values for acidity were found in samples obtained from Milkman and Branded packet milk, respectively. Milk sample collected from Farmgate was found significantly higher in protein, fat, lactose, Solids-Not-Fat, and Total-Solids content than the other sources. In microbiological analysis, significant lower Total Viable Count, Total Coliform Count, and *Escherichia coli* Count were observed in Branded packet milk compared to the milk from Farmgate, Milkman, and Bazar source ($p<0.001$). In adulteration examinations, only neutralizers (sodium carbonate and bicarbonate) and nitrates were found positive in milk sample collected from Milkman, Farmgate, and Bazar, and their frequency was varied significantly ($p<0.001$). Overall, the milk obtained from Farmgate was better in terms of nutrient composition and physiochemical properties than the other sources but in respect to microbiological quality, Branded packet milk was found more hygienic.

**Conclusion:** The study found that the variance in milk sources led to changes in the microbial load, physical appearance, and nutritional content of milk. The present study investigated that the addition of different adulterants and variation in time interval (milking to selling) are the main reasons for the deterioration of milk.



* Corresponding author (M.R. Hassan)
E-mail: mdrakibulhassan@gmail.com
ORCID ID: https://orcid.org/0000-0002-8577-1642

**To cite:** Sultana S., Hosen A., Afsana A.S., Sultana S., Pehan E.A., Islam A., Sultana N., Hassan M.R. (2024). Physio-chemical and microbiological quality assessment and detection of adulterants in different sources of milk in Bangladesh. *Journal of Food Quality and Hazards Control*. 11: 214-222.

**DOI:** 10.18502/jfqhc.11.3.16593 Journal website: http://jfqhc.ssu.ac.ir

## Introduction

Milk is one of the most nutrient-dense and the healthiest food that is widely consumed worldwide. Milk contains a balanced amount of water, fat, protein, lactose, vitamins, and minerals (Guetouache et al., 2014). However, milk is also readily perishable due to its high temperature and inadequate infrastructure, making it extremely susceptible to bacterial contamination (Kim, 1983). Even though Bangladesh dairy industry is expanding quickly, it is still too early to say that the milk produced is safe to drink. Food safety is a public health issue that includes ensuring the safety of food at every stage of the supply chain. It addresses all of the risks that could endanger a consumer's health when eating certain food. The safety and quality of cow milk are complicated matters that include a range of factors such diet and health of animals, milking procedures, transportation, processing, etc. Due to a lack of supervision and guidelines, food adulteration, particularly with regard to raw milk, is becoming a major problem in developing nations (Azad and Ahmed, 2016; Bari et al., 2015). According to Reddy et al. (2017), there may be three factors contributing to milk adulteration including rising demand, heightened competitiveness in the dairy industry, and financial gain. In addition to milk, adulteration is also found in a wide range of other goods, such as oil, spices, cereals, candies, beverages, and more. As a result, the quality of all food products is eventually reduced (Nayak, 2017). According to Moonajilin et al. (2018), urea, formalin, detergents, ammonium sulphate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide ($H_2O_2$), sugars, and melamine levels in milk are some of the main adulterants that have substantial negative health effects. Additional adulterants include salt, sugar, water, starch, chlorine, hydrated lime, sodium carbonate, and different antibiotics (Reddy et al., 2017). Additionally, during different phases of milk procurement, processing, transportation, etc., microorganisms may contaminate the milk. The surfaces of milk handling and storage equipment as well as the udder's exterior are common places for microbial contamination to arise. Raw milk can become contaminated with bacteria from a variety of sources, including the air, feed, feces, dirt, and milking equipment (Marjan et al., 2014). The use of non-potable water increases the risk of germs getting into milk. It is well known that tropical climates, with their hot, muggy weather for large portions of the year, are perfect for the growth and proliferation of numerous bacteria, which causes milk to quickly deteriorate and spoil (Hisham et al., 2022). According to reports, the cleanliness of udders prior to milking and the cooling of milk following milking are related to the number of germs present in milk. The microbiological quality of liquid milk frequently deteriorates in developing nations like Bangladesh, because there is insufficient cooling in these areas, where ambient temperatures are high and cooling systems are frequently unavailable (Corry et al., 2012; Pantoja et al., 2009). However, in Bangladesh, liquid milk can be obtained from four sources such as Farmer, Guala, local market and pasteurized packet milk. According to Reddy et al. (2017), over 68% of the milk supplied to customers in the Indian subcontinent is subpar. According to a survey conducted in Dhaka, Bangladesh, roughly 23% of people drank powdered milk and 64.7% drank loose milk (Moonajilin et al., 2018). It is important for consumers to be aware of the nutritional value and microbiological state of the liquid milk they get from various sources when they gather it. This study was conducted to assess the physiochemical, nutritional, and microbiological condition of milk obtained from Farmgate, Milkman, Bazar, and Branded packet milk in various parts of Bangladesh, as well as to look for adulterants in the milk.

## Materials and methods

### *Collection of samples*

A total of 200 milk samples (volume 250 ml/sample) were collected during 15 October to 15December 2022 from four different sources such as Farmgate (50), Bazar (50), Milkman (50), and Branded packet (50) milk from different areas of Bangladesh. All the samples were collected early in the morning in sterilized bottles and categorized based on the location, time of collection and source of milk. Then, the samples were transported in ice-box to Dairy laboratory, Dairy Research, and Training Center, BLRI, Savar, Dhaka for further analysis. The tests were repeated at least thrice for all the studied parameters.

### *Sources of milk samples*

-*Farm gate:* the milk which is collected directly from dairy farms immediately after milking.

-*Bazar:* bazar refers to the local market where some dairy farmers sale milk to the consumer as retail basis.

-*Milkman:* milkman refers to the man who collects milk from different dairy farms and sale to the consumer as door to door.

-*Branded packet milk:* branded packet milk refers to the pasteurized packet milk that is collected by different companies through their collection centers. The collected milk is cooled at 4 $^{0}$C then transported from collection center to pasteurization plant where milk is pasteurized and pocketed. Finally, pocketed milk is sent to different shops across the country.







[ DOI: 10.18502/jfqhc.11.3.16593 ]

*Organoleptic and physical parameters of milk*

All the collected milk samples were tested for normal appearance, color, odor, and consistency according to the methods as described by Eckles et al. (1951).

*Physiochemical parameters of milk*

*-Determination of specific gravity*

Specific gravity of milk was determined according to the method as described by Fuquay et al. (2011) and by the help of following formula.

CLR=LR±(Δ $^0$F×0.2)

Specific gravity=$\frac{\text{CLR}}{1000}$+1

Where, CLR is the corrected lactometer reading, LR is the lactometer reading, Δ is the difference between sample temperature and standard temperature (20 $^0$C), F is the temperature factor.

*-Determination of acidity*

A 10 ml of milk sample was poured in a clean porcelain cup. About 1 ml of phenolphthalein (HiMedia Laboratories Pvt. Limited, India) was added to it as an indicator. Then, a titration was done against N/10 sodium hydroxide (NaOH) (Merck KGaA, 64271 Darmstadt, Germany). The acidity percentage of milk sample was calculated as described by AOAC (2005).

% Acidity=volume of NaOH used×0.09.

*-Determination of fat*

Fat content of milk sample was measured by Gerber method (Kleyn et al., 2001). At first, 10.75 ml concentrated sulfuric acid ($H_2SO_4$; Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA) was poured in a butyrometer (BENNY, India). Then, 10.75 ml of properly mixed milk sample followed by 1 ml amyl alcohol (Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA) were added. After proper shaking, the butyrometer was placed in a water bath at 65 $^0$C. Later, the butyrometer was centrifuged in a Gerber machine (Funke Gerber, Germany) at a speed of 1,100 rpm for 5 min. The fat percentage was recorded from the butyrometer reading.

*-Determination of protein, Solids-Not-Fat (SNF), lactose, and mineral contents*

The SNF, protein, lactose, and salt contents of milk samples were analyzed by ultrasound technique with the lactoscan ultrasonic milk analyzer (Milkotronic, Bulgaria). Before testing milk samples, lactscan machine was washed with cleaning solutions (everyday by lactodaily solution and weekly by lactoweekly solution) according to manufacturer's instructions. Ten ml of milk sample was mixed well in a plastic container (25 ml) and rotated in a machine. Results were recorded from the screen of the lactoscan machine.

*-Detection of adulterants in milk*

Collected milk samples were assessed for the presence of common adulterants; added water in milk sample was determined using the lactoscan ultrasonic milk analyzer. The presence of starch, glucose, sodium chloride, urea, and nitrates (pond water) were detected followed by the methods as described by FSSAI (2022). Cane sugar, neutralizers, sulphates, formalin, $H_2O_2$, and detergent were detected following the methods as stated by Sharma et al. (2012). Vegetable fats were detected by melting 5 ml of milk fat was and pouring it into a test tube. Later, 5 ml of concentrated hydrochloric acid (HCl; Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA) and 0.4 ml (5%) of furfural solution were added. After vigorous shaking for 2 min, the mixture was allowed to be separated. Development of pink or red color in acidic layer indicated the incidence of vegetable fats, which was further confirmed by the addition of 5 ml of water. The tenacity of color in acidic layer, confirmed the presence of vegetable oil (Bintsis et al., 2008).

*Microbiological properties of milk*

Standard methods agar was used for the enumeration of Total Viable Count (TVC). pH of the medium was accustomed at 6.8 prior to sterilization. Diluted test samples (1ml) of $10^{-3}$ to $10^{-7}$ were transferred into sterile petri dish through dispensing pipette. Warm (45±1 $^0$C) sterile plate count agar medium (12 to 15 ml) was mixed into inoculums and the mixture was allowed to be solidified. Plates were incubated at 37 °C for 24 to 72 h to facilitate viable bacterial growth. The plates (having 30 to 300 colonies) were enumerated with the help of colony counter and the results were expressed as Colony Forming Units (CFU)/ml (Bintsis et al., 2008). Total Coliform Count (TCC): a set of nine test tubes were taken for each sample. An amount of 10 ml of McConkey broth (HiMedia, India) was allotted in each test tube containing a Durham tube at inverted position. After sterilization of whole set, 1 ml of sample from the $10^{-1}$, $10^{-2}$, and $10^{-3}$ diluted solution was taken in separate test tubes. Each of the solution was analyzed in triplicate. The tubes were incubated at 37 ºC for 24 h. After incubation, the test tube was examined to detect gas formation in the Durham tube (Batt and Tortorello, 2014). *Escherichia coli* Count (ECC): 10 ml of raw milk sample was taken a sterile sample container with saline solution. Decimal dilutions were prepared ($10^{-1}$ to $10^{-6}$). Each of the dilutions was used to inoculate MacConkey agar using a spread plate method. Plates were incubated at 37 $^o$C for 24 h. After incubation, the plates were examined for suspicious colonies of *E. coli*.



[ DOI: 10.18502/jfqhc.11.3.16593 ]

Pink to red colonies were suspicious of *E. coli*. For further confirmation, the indole test was conducted. Then, 0.3 ml of Kovac's reagent (HiMedia Laboratories Pvt. Limited, India) was added and checked for dark red color at the top of the test tubes; which are peculiar characteristics of a generic *E. coli* (ISO, 2017). Lastly, the pure colonies were enumerated carefully.

*Statistical analysis*

Data were arranged in Microsoft excel and Shapiro Wilk test was performed to examine the normality of variables. The data of physical parameters of milk samples were analyzed for variation using independence chi-square test. One-way ANOVA was performed for the data of other parameters and differences between treatments were determined by LSD test using RStudio. Differences among the treatments were considered significant at $p$<0.05.

## Results

*Physical examination of milk*

The results of physical examination of milk samples collected from Farmgate, Milkman, Bazar, and Branded Packet milk for physical properties such as general appearance, odor, color, and consistency are shown in Table 1. The highest number of samples was found clear, milky, whitish, and normal in milk samples obtained from Farmgate for appearance, flavor, color, and consistency examinations, respectively. Maximum dirty appearance and watery consistency were found in milk samples obtained from Milkman while the highest sourish flavor was observed in milk samples obtained from Bazar. Overall, physical properties of Branded packet milk were better than the other sources.

**Table 1:** Physical examination of raw milk samples collected from different sources

| Physical parameters | | Farmgate | Milkman | Bazar | Branded Packet | SEM | *p*-value |
|---|---|---|---|---|---|---|---|
| General appearance (%) | Clear | 82.40 $^{b}$ | 76.67 $^{b}$ | 77.27 $^{b}$ | 96.00 $^{a}$ | 4.50 | 0.016 |
| | Dirty | 17.60 $^{a}$ | 23.33 $^{a}$ | 22.73 $^{a}$ | 04.00 $^{b}$ | | |
| Flavor (%) | Milky | 85.16 $^{a}$ | 83.33 $^{a}$ | 81.81 $^{a}$ | 88.89 $^{a}$ | 1.50 | 0.950 |
| | Mild sourish | 14.84 $^{a}$ | 16.67 $^{a}$ | 18.19 $^{a}$ | 11.11 $^{a}$ | | |
| Color (%) | Whitish | 83.33 $^{a}$ | 86.67 $^{a}$ | 86.36 $^{a}$ | 77.77 $^{a}$ | 2.10 | 0.812 |
| | Yellowish | 16.67 $^{a}$ | 13.33 $^{a}$ | 13.64 $^{a}$ | 22.23 $^{a}$ | | |
| Consistency (%) | Normal | 25.93 $^{a}$ | 23.33 $^{a}$ | 25.00 $^{a}$ | 33.33 $^{a}$ | 2.20 | 0.887 |
| | Watery | 74.07 $^{a}$ | 76.67 $^{a}$ | 75.00 $^{a}$ | 66.67 $^{a}$ | | |

*p*-value: Chi-square test at 5 percent level of significance, values with different superscripts are significantly different at $p$<0.05, comparisons are within columns.
SEM=Standard Error of Mean

*Physiochemical properties and proximate composition of milk*

The present results of physiochemical parameters such as specific gravity, titratable acidity, ash, protein, fat, lactose, SNF, and TS contents in milk samples obtained from different sources (Farmgate, Milkman, Bazar and Branded packet) are presented in Table 2. The results found that the titratable acidity, protein, fat, salt, SNF, Total Solid (TS) were significantly different among the treatments ($p$<0.05), while specific gravity and lactose content were found non-significant. The highest titratable acidity was found in Milkman milk followed by Bazar, Farmgate, and Branded packet milk sample, respectively ($p$<0.001). The highest fat contents were found highest in Farmgate milk sample followed by Milkman, Bazar, and Branded packet milk sample, respectively ($p$<0.00016).

**Table 2:** Physiochemical qualities of milk

| Parameter | Farmgate | Milkman | Bazar | Branded packet | SEM | *p*-value |
|---|---|---|---|---|---|---|
| Specific gravity | 1.029 $^{a}$ | 1.026 $^{a}$ | 1.020 $^{a}$ | 1.028 $^{a}$ | 0.03 | 0.0940 |
| Titratable acidity (%) | 0.16 $^{b}$ | 0.17 $^{a}$ | 0.16 $^{ab}$ | 0.13 $^{c}$ | 0.01 | 0.00024 |
| Fat (%) | 3.57 $^{a}$ | 3.14 $^{b}$ | 2.94 $^{b}$ | 2.77 $^{b}$ | 0.13 | 0.00013 |
| Protein (%) | 2.85 $^{a}$ | 2.59 $^{c}$ | 2.71 $^{bc}$ | 2.82 $^{ab}$ | 0.06 | 0.0036 |
| Lactose (%) | 4.21 $^{a}$ | 3.94 $^{ab}$ | 4.09 $^{ab}$ | 4.23 $^{a}$ | 0.09 | 0.1088 |
| Salt (%) | 0.65 $^{a}$ | 0.59 $^{b}$ | 0.61 $^{b}$ | 0.63 $^{ab}$ | 0.01 | 0.0001 |
| SNF (%) | 7.72 $^{a}$ | 7.12 $^{b}$ | 7.42 $^{ab}$ | 7.68 $^{a}$ | 0.15 | 0.0216 |
| TS (%) | 11.29 $^{a}$ | 10.26 $^{b}$ | 10.36 $^{b}$ | 10.45 $^{b}$ | 0.22 | 0.00036 |

Values with different superscripts are significantly different at $p$<0.050, comparisons are within columns.
SNF=Solids Not Fat; TS=Total Solids; SEM=Standard Error of Mean



[ DOI: 10.18502/jfqhc.11.3.16593 ]

*Adulterants in milk*

Frequency of milk samples adulterated with water is depicted in Figure 1. The highest frequency of water adulteration was found in Milkman milk samples followed by in Bazar, Braded packet, and Farmgate milk sample, respectively. The average percent of added water was found 27.07, 25.58, 24.5, 10.62% in milk samples obtained Farmgate, Milkman, Bazar, and Branded packet milk, respectively.

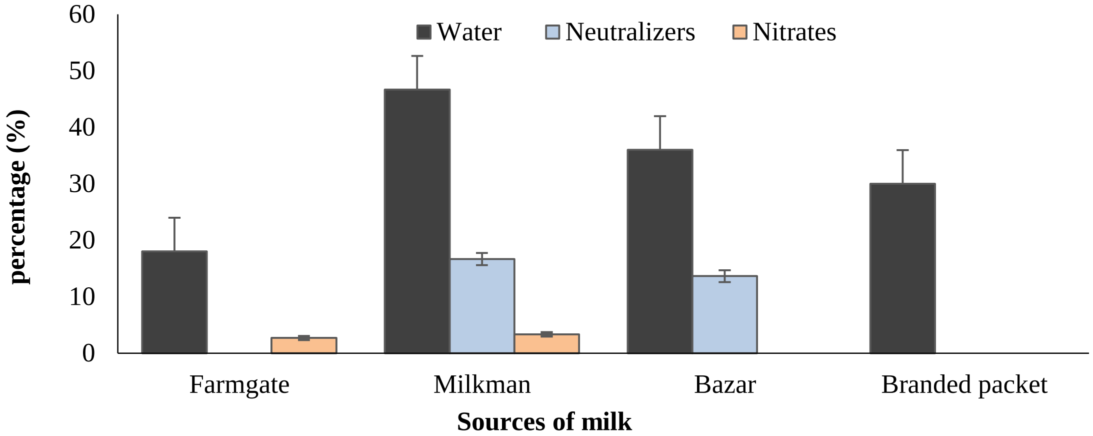


**Figure 1:** Adulterants found in collected milk samples

In chemical adulterants, only neutralizers (sodium carbonate and bicarbonate) and nitrates were found positive. On the other hand, starch, cane sugar, glucose, salt, vegetable fat, urea, neutralizers, sulphates, nitrates, $H_2O_2$, formalin, detergents were not found in the collected milk samples. Neutralizer test was positive for milk samples obtained from Farmgate, Milkman, and Bazar milk but nitrates test was positive for milk obtained from Farmgate and Milkman. The presence of neutralizers was numerically higher ($p$>0.05) in milk collected from Milkman followed by local market and Farm milk, respectively (Figure 1).

*Microbial qualities of milk*

The results of microbial analysis of milk sample are depicted in Table 3. It was found that TVC, TCC, and ECC were statistically different among the sources of milk samples ($p$<0.001). The higher number of TVC, TCC, and ECC were detected in milk samples obtained from Milkman followed by Bazar, Farmgate, and Branded packet milk, respectively ($p$<0.001).

**Table 3:** Microbial quality analysis of different milk samples

| Parameter | Farmgate (log CFU/ml) | Milkman (log CFU/ml) | Bazar (log CFU/ml) | Branded packet (log CFU/ml) | SEM | *p*-value |
|---|---|---|---|---|---|---|
| TVC | 6.16 [c] | 6.93 [a] | 6.4 [b] | 4.08 [d] | 0.08 | 0.00003 |
| TCC | 2.31 [b] | 3.87 [a] | 3.57 [a] | 1.23 [c] | 0.14 | 0.00002 |
| *E. coli* | 1.24 [b] | 2.93 [a] | 2.88 [a] | 0.80 [c] | 0.08 | 0.00001 |

Values with different superscripts are significantly different at $p$<0.05, comparisons are within columns.
CFU=Colony Forming Unit; SEM=Standard Error of Mean; TCC=Total Coliform Count; TVC=Total Viable Count

## Discussion

Quality of liquid milk depends largely on cow (health, breed, age etc.), inputs (feed, water, housing facilities, medications etc.) and handling of milk (milking to final use). Milk is subject to different types of handling based on their sources. Generally, in Bangladesh, source of liquid milks is Farmgate, Milkman, Bazar, and Branded packet milk. Milk quality is greatly influenced by how it is handled and exposed to various sources. The quality of liquid milk has a direct impact on the production of premium dairy products with additional value. Greater yields of various dairy products are likewise associated with greater TS contents (Fuquay et al., 2011). High initial bacterial concentrations shorten the shelf-life of liquid milk by causing it to deteriorate quickly. The production of off flavor and/or color in milk can occur when spoilage organisms reproduce within it. Excessive acid production denatures proteins, making milk less resistant to various


[ DOI: 10.18502/jfqhc.11.3.16593 ]

processes including fermentation and heating (Sperber et al., 2009). In Bangladesh and other parts of the world, milk quality is evaluated using a core set of characteristics and a single source for milk sample collection. In contrast, this study evaluated the quality of liquid milk obtained from many sources.

In physical parameters, general appearance of milk varied significantly (*p*=0.016) among the sources while flavor, color, and consistency was found non-significant. The highest number of samples obtained from branded packet milk was clear in appearance followed by milk obtained from Farmgate, Bazar, and Milkman, respectively. This result might be due the fact that manufacturing companies filter their milk during reception and follow proper storage and transportation and maintain cool chain, while Milkman and Farmer might not filter milk during collection and keep the milk cane open for long time during transportation. Consequently, bacteria remained in milk, deteriorate the milk, resulting off flavor and poor consistency.

The color of milk varies from yellowish (16.67-13.33%) to whitish (83.33-86.67%). These outcomes agree with those of Muntaha et al. (2020), who found that milk color varies from yellowish (19-11%) to whitish (81-89%). In another study, Hasan et al. (2017) reported that milk color ranges from whitish (50%) to yellowish (50%). Milk appears white due to light reflection; however, its yellow color is caused by the presence of the carotene pigment. According to Judkins and Mack (1955), the yellowish white color of normal milk is caused by the presence of fat, casein, and a minor amount of natural coloring agents. The breed, amount of fat, TS, and feed that the animal consumes can all affect changes in milk color (Eckles et al., 1951).

Milk samples collected from Branded packet milk showed the highest milk flavor (88.89%) among all the sources. The higher milk smell was observed in Farmgate milk due to the fact that the farmer practice hygienic measures during milking and not allows the cows to eat some sorts of flavored feed before or during milking. Moreover, Farmgate milk is sold or distributed to buyers within very short time, mostly within 30 min of milking in same cases up to 1 h. Thus, microorganisms enter into milk during milking, cannot grow properly.

Muntaha et al. (2020) showed that milk flavor ranges from normal (70-79%) to very mild (21-30%). Foley et al. (1972) reported that milk from ketosis-stricken cows has a "cowy" flavor, whereas inadequate ventilation in cow sheds causes the milk to taste "barny". Olson (1956) said that if the cow eats onion, bitter weeds, French weeds, green rye, etc. right before milking, feed, and weedy flavors may develop in the milk. The current study found that Farmgate milk had a stronger milky odor due to hygienic practices used by the farmer, who prevented the cows from eating flavored feed either before or during milking.

Physiochemical analysis resulted that titratable acidity, protein, fat, salt, SNF, TSs contents of milk varied significantly (*p*<0.05) among milk sources, while specific gravity and lactose contents of milk were found non-significant. The acidity of normal cow milk ranges from 0.10 to 0.18% with 0.16% on average (Rahman et al., 2016). In the present experiment, values of acidity percentage were within the range though; however, acidity of milk sample obtained from Milkman was significantly higher than Bazar, Farmgate, and Branded packet milk samples. Islam et al. (2016) discovered a similar outcome, showing that milk's acidity ranged from 0.15 to 0.16%. Rahman et al. (2017) also found that milk acidity ranged from 0.11 to 0.13% in another experiment. The amount of time needed from milking to heat treatment may sometimes impact how acidic milk is (Islam et al., 2019). The milk samples from Milkman had the highest acidity levels in the current trial. This is might be due to keeping the milk unheated for longer period of time (milking to selling) which facilitates high microbial activities or enzymatic reactions that might be the reason for increasing the acidity of milk. Islam et al. (2016) reported that the greater content of SNF in milk may proportionately increase the percent acidity of milk and vice versa.

Fat contents of milk were found significantly higher in Farmgate milk than in Milkman, Bazar, and Branded packet milk (*p*<0.001). Similarly, protein, SNF, and TS contents were also found higher in Farmgate milk followed by Branded packet, Bazar, and Milkman, respectively. The Bangladesh Standard and Testing Institute states that cow milk must have a minimum fat level of 3.5% (BSTI, 2002). The fat content of the Farmgate milk samples in this experiment met the BSTI requirement; however, the fat content of the samples from the other three sources did not. The study findings, however, are in line with those of Hasan et al. (2017), who found that milk samples taken from three nearby marketplaces had a fat level of 3.03-3.30%. Islam et al. (2019) reported that milk samples had a mean fat percentage ranging from 3.33 to 3.7%. The milk samples from Farmgate and Branded package milk had a significantly (*p*<0.05) higher protein content than milk samples from Milkman and Bazar milk. Similar outcomes were noted by Rahman et al. (2017) who found that the protein content of milk samples was 1.97-3.27%.

In contrast, Islam et al. (2019) discovered that milk samples had an average protein level ranging from 2.8 to 3.3%. Reduced protein concentration in milk may be caused by the nutritional status of the cows, genetic diversity, and the addition of water (Islam et al., 2016). All of milk samples SNF concentrations (7.16 to 7.8%) were







[ DOI: 10.18502/jfqhc.11.3.16593 ]

discovered to be below the BSTI minimum requirement (8.4%). This result, however, is consistent with the findings of Hasan et al. (2017), who found that milk SNF content varied from 7.54 to 7.93%.

A little higher result was found by Ara et al. (2021) who stated that the mean values of SNF of milk from Milkmen, dairy farms, and pasteurize milk in Dhaka city were 8.33, 8.73, and 8.66%, respectively. The present study found the lowest fat, SNF, and TS contents in Branded packet milk samples. These results, however, are in line with a study by Tesfay et al. (2015), which found that milk samples taken from dairy farms had a considerably ($p$<0.05) greater TS content than milk samples taken from Milkmen and pasteurized milk.

The present investigation suggests that the lower levels of fat, SNF, and TS found in milk samples sourced from Milkman, Bazar, and Branded packet milk might be attributed to a higher milk adulteration with water. Similarly, the higher levels of fat, SNF, and TS found in Farmgate milk could be attributed to decreased milk adulteration with water. The reason for the lowest TS concentration in Branded packet milk could be due to either a reduced fat content or water adulteration of milk. Another reason might be skimming or withdrawing of fat from milk (Islam et al., 2019). In Bangladesh, most of the manufacturer companies skimmed fats from whole milk before selling, resulting in lower fat, SNF, and TS contents in liquid packet milk. Milkman and seller in Bazar have a tendency to increase milk volume by adding water which decreases milk solids accordingly.

Milk sample from all source (Farmgate, Milkman, Bazar, and Branded packet) were found adulterated with water, though their level of admixture varied (10.62-27.07%). Milks purchased from the Milkman had a higher frequency of water adulteration than milks purchased from Bazar, Farmgate, Branded packet, and so on. A related study conducted by Ebner et al. (2016) found that approximately 25% of milk samples had water inconsistencies, with those taken from Bazar having a higher likelihood of irregularities (32.7%) than samples from shops (15.6%). The percentages of soluble solids in milk, including fat and other essential ingredients, decrease as water is added. It also carries the risk of introducing potentially harmful germs at the same time. Kurwijila (2006) explained that adding polluted water to milk can lead to health issues, yet diluting milk with pure water may just lower its nutritional content.

According to Hossain et al. (2011), one of the primary causes of raw milk low microbiological quality is adulteration of the milk with water. According to the current study, Milkman's intention to raise milk volume in order to gain profit may be the reason for the increased water adulteration in milk.

Screening of milk samples for various chemical adulterants such as, starch, cane sugar, glucose, salt, vegetable fat, urea, neutralizers, sulphates, nitrates, $H_2O_2$, formalin, detergents showed that only neutralizers (sodium carbonate and bicarbonate) and nitrates test were found positive. Similar experiment conducted by Chanda et al. (2012) found that about 20% samples were detected as positive for neutralizers in raw milk samples. In this study, the highest percentage of milk samples obtained from Milkman was found positive for neutralizers which might be the usage of neutralizers (Sodium bicarbonate) to normalize the pH and acidity of improperly preserved milk (Ara et al., 2021).

The TVC in milk samples collected from Milkman was significantly higher than other sources while significant lower value was found in Branded packet milk ($p$<0.001). The study results are in line with Kader et al. (2015) findings, which indicated that the average TVC value ranged from 5.914 to 6.256 log CFU/ml. According to Hossain et al. (2011), raw milk samples exhibited a high bacterial load, with values ranging from 6.243 to 8.086 log CFU/ml. Coliforms are known as "indicator organisms" because they are a sign of contamination in food. Hossain et al. (2011) reported similar results for the coliform count, ranging from 3.65 to 6.31 log CFU/ml for raw milk. These elevated bacterial counts are associated with improper handling of milk, contamination from animal bedding, blending of regular milk with milk from sick cows, etc. (Muhammad et al., 2009).

Higher coliform counts in raw milk can be caused by unhygienic milking procedures, contaminated water, dirty herd hygiene, and badly cleaned and maintained equipment (CDFA, 2008). The higher value of TVC, TCC, and ECC in milk sample obtained from Milkman might be unhygienic handling of milk, use of unclean milk containers, and longtime exposure of milk at room temperature. Generally, Milkman keeps milk in an uncovered milking cane at room temperature for a long period of time (milking to selling). Because of uncovered milk cane, there might be a chance of microorganisms from environment to enter into the milk. All these factors may result rapid deterioration of microbiological quality of milk.

## Conclusion

The present study revealed that source of milk significantly affects physiochemical, nutritional, and microbiological quality of liquid milk. The study found that physiochemical and nutritional quality of milk obtained from Farmgate was superior to Milkman, Bazar, and Branded packet milk. However, the microbiological quality of Branded packet milk was superior to the other sources. On the other hand, milk samples from the entire source were found adulterated with water and the level of admixture varied from 10.62-27.07%. Among different chemical adulterants, only neutralizers (sodium carbonate and bicarbonate) and nitrates were detected positive in Farmgate, Milkman, and Bazar milk. Therefore, this study could be concluded that the quality of liquid milk depends on their sources. The study also recommends that milk collected from Farmgate and Branded packet milk was better for nutritional and microbial point of view, respectively. For further research, experiment could be designed including somatic cell count, antibiotic residues, and heavy metals load in liquid milks.

### Author's contributions

S.S. designed and supervised the experiment; A.H. collected the samples, carried out the experiments, analyzed the data, and prepared the manuscript; A.S.A., S.S., E.A.P. and A.I. also collected the samples and conducted the experiments; N.S. coordinated the experiment; M.R.H. monitored and critically reviewed the final manuscript version. All authors read and approved the final manuscript.

### Conflict of interest

The writers affirm that they have no conflicts of interest.

### Acknowledgement

The authors would like to express sincere gratitude to Dairy Research and Training Center, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh for their generous support and providing required facilities of this research. In addition, it could be disclosed that the current study was funded by Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh. The authors would like to sincerely thank Dairy Research and Training Center, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh for their kind cooperation and provision of the facilities needed for this study.

### Funding

The present research was carried out at Dairy Research and Training Center, Bangladesh Livestock Research Institute, Savar, Dhaka under the project entitled "Quality and Safety Assessment of Milk and Milk Products". It should be mentioned that the research was funded by the Bangladesh Livestock Research Institute in Savar, Dhaka, Bangladesh.

### Ethical consideration

Not applicable. In this study, no humans or animals were involved.

### References

Ara T., Uddin M.N., Bithi U.H., Mitra K. (2021). A study on nutritional assessment and adulteration of raw and pasteurized milk collected from local markets in Dhaka city. *North American Academic Research (NAAR) Journal*. 4: 100-111. [DOI: 10.5281/zenodo.5387808]

Association of Official Analytical Chemists (AOAC). (2005). Official methods of analysis. 18th edition. AOAC International, Gaithersburg, MD, USA. URL: Official-methods-of-analysis-of-AOAC-International/oclc/62751475.

Azad T., Ahmed S. (2016). Common milk adulteration and their detection techniques. *International Journal of Food Contamination*. 3: 22. [DOI: 10.1186/s40550-016-0045-3]

Bangladesh Standards and Testing Institution (BSTI). (2002). Bangladesh standard: specification for pasteurized milk. BDS 1702. Dhaka, Bangladesh. URL: http://bsti.portal.gov.bd/sites/default/files/files/bsti.portal.gov.bd/notices/02f028d7_6fa9_49f1_a109_a20bd0ca678d/Draft%20Pasteurized%20milk.pdf

Bari L., Hoque M.R., Reza M.S.A., Hossain M.A., Islam A. (2015). Adulteration of raw milk in selected regions of Tangail district of Bangladesh. *Journal of Environmental Sciences and Natural Resources*. 8:41-44. [DOI: 10.3329/jesnr.v8i1.24662]

Batt C.A., Tortorello M.L. (2014). Encyclopedia of food microbiology. 2nd edition. Elsevier Academic Press, Oxford, UK.

Bintsis T., Angelidis A.S., Psoni L. (2008). Modern laboratory practices–Analysis of dairy products. In: Britz T.J., Robinson R.K. (Editor). Advanced dairy science and technology. Blackwell Publishing, Garsington Road, Oxford, UK. [DOI: 10.1002/9780470697634.ch6]

California Department of Food and Agriculture (CDFA). (2008). New coliform standard for milk sold raw to consumers. California Department of Food and Agriculture, Sacramento. URL: https://www.cdfa.ca.gov/exec/Public_Affairs/Press_Releases/Archive/pr.html?id=07-090.

Chanda T., Debnath G.K., Hossain M.E., Islam M.A., Begum M.K. (2012). Adulteration of raw milk in the rural areas of Barisal district of Bangladesh. 41: 112-115. [DOI: 10.5555/20133112029]

Corry J.E.L., Curtis G.D.W., Baird R.M. (2012). Handbook of culture media for food and water microbiology. 3rd edition. Royal Society of Chemistry, London, United Kingdom.

Ebner P.D., Ghoryar M.A., McNamara K., Amini R., Faqiri E., Azizi A.E., Isaqzai N.A., Rahimi Z., Sakhee N., Sarwary S., Sofizada M. (2016). Quality and safety assessment of raw bovine milk in Herat province, Afghanistan. *Journal of Food Safety and Hygiene*. 2: 15-20.

Eckles C.H., Combs W.B., Macy H. (1951). Milk and milk products, 3rd edition. McGraw-Hill Book Company, Inc, New York. pp: 49-67.

Foley R.C., Bath D.L., Dickinson F.N., Tucker H.A. (1972). Dairy cattle: principles, practices, problems, profits. Lea and Febiger, Philadelphia, USA.

Food Safety and Standards Authority of India (FSSAI). (2022). Manual of methods of analysis of foods - dairy and dairy products. FDA Bhawan, Kotla Road, New Delhi-110002, India. URL: https://fssai.gov.in/upload/uploadfiles/files/Manual_Dairy_07_10_2022.pdf.

Fuquay J.W., Fox P.F., McSweeney P.L.H. (2011). Encyclopedia of

dairy sciences. 2nd edition. Elsevier Science, Netherlands.
Guetouache M., Guessas B., Medjekal S. (2014). Composition and nutritional value of raw milk. *Journal Issues in Biological Sciences and Pharmaceutical Research*. 2: 115-122. [DOI: 10.15739/ibspr.005]
Hasan M., Sarker T., Prabakusuma A.S., Islam S. (2017). Quality of raw milk available at different markets of Bangladesh. *Biological Sciences*. 1: 16-30. [DOI: 10.31058/j.bs.2017.11002]
Hisham M.B., Hashim A.M., Hanafi N.M., Abdul Rahman N., Abdul Mutalib N.E., Tan C.K., Nazli M.H., Yusoff N.F.M. (2022). Bacterial communities associated with silage of different forage crops in Malaysian climate analysed using 16S amplicon metagenomics. *Scientific Reports*. 12: 7107. [DOI: 10.1038/s41598-022-08819-4]
Hossain T.J., Alam M.K., Sikdar D. (2011). Chemical and microbiological quality assessment of raw and processed liquid market milks of Bangladesh. *Continental Journal of Food Science and Technology*. 5: 6-17. [DOI: 10.5281/zenodo.5568945]
International Organization for Standardization (ISO). (2017). Microbiology of the food chain—preparation of test samples, initial suspension and decimal dilutions for microbiological examination—part 1: general rules for the preparation of the initial suspension and decimal dilutions. 2nd edition. ISO 6887-1: URL: https://www.iso.org/standard/63335.html.
Islam A., Harun-Ur-Rashid , Ahmed S., Masum A.K.M. (2016). Quality evaluation of raw milk collected from local markets at Madhupur upazila of Tangail district. *Fundamental and Applied Agriculture*. 1: 70-74.
Islam R., Sarker T., Islam S., Prodhan A.S. (2019). Assessment of the quality and detection of adulteration of raw milk of local markets in Bangladesh. *Discovery Agriculture*. 5: 168-176.
Judkins H.F., Mack M.J. (1955). The Principle of Dairying. John Wiley and Sons, Inc., New York, USA.
Kader A., Deb M., Aziz A., Sohag M.H., Rahman S.R. (2015). Evaluation of physico-chemical properties and microbiological quality of milk collected from different dairy farms in Sylhet, Bangladesh. *Food Science and Technology*. 3: 37-41. [DOI: 10.13189/fst.2015.030301]
Kim H. (1983). Off-tastes in raw and reconstituted milk. Food and Agriculture Organization, Rome, Italy. pp: 1-39.
Kleyn D.H., Lynch J.M., Barbano D.M., Bloom M.J., Mitchell M.W. (2001). Determination of fat in raw and processed milks by the gerber method: collaborative study. *Journal of AOAC International*. 84: 1499-1508. [DOI: 10.1093/jaoac/84.5.1499]
Kurwijila L.R. (2006). Hygienic milk handling, processing and marketing: reference guide for training and certification of small-scale milk traders in Eastern Africa. International Livestock Research Institute (ILRI), Nairobi, Kenya.
Marjan S., Kanta Das K., Kishore Munshi S., Noor R. (2014). Drug-resistant bacterial pathogens in milk and some milk products. *Nutrition and Food Science*. 44: 241-248. [DOI: 10.1108/NFS-05-2013-0061]
Moonajilin M.S., Islam M.S., Paul R. (2018). A study on milk adulteration of Savar Upazila in Bangladesh. *International Journal of Community Medicine and Public Health*. 5: 4670-4675. [DOI:10.18203/2394-6040.ijcmph20184554]
Muhammad K., Altaf I., Hanif A., Anjum A.A., Tipu M.Y. (2009). Monitoring of hygienic status of raw milk marketed in Lahore city, Pakistan. *The Journal of Animal and Plant Sciences*. 19: 74-77.
Muntaha S.T., Iqbal R., Yasmin I., Tehseen S., Khaliq A., Chughtai M.F.J., Ahsan S., Khan W.A., Nadeem M., Hleba L., Rebezov M., Khayrullin M., et al. (2020). Safety assessment of milk and indigenous milk products from different areas of Faisalabad. *Journal of Microbiology, Biotechnology and Food Sciences*. 9: 1197-1203. [DOI: 10.15414/jmbfs.2020.9.6.1197-1203]
Nayak N. (2017). Detection of different adulterant in different milk samples. *Research and Reviews: Journal of Dairy Science and Technology*. 6: 17-29.
Olson T.M. (1956). Elements of dairying. 6th edition. The Macmillan Company, New York, USA. pp: 230-233.
Pantoja J.C.F., Reinemann D.J., Ruegg P.L. (2009). Associations among milk quality indicators in raw bulk milk. *Journal of Dairy Science*. 92: 4978-4987. [DOI: 10.3168/jds.2009-2329]
Rahman A., Habib R., Ali Y., Islam M.A., Rashid H. (2017). Physico-chemical analysis and detection of adulteration in raw milk collected from Goalas of different places of sadar upazila in Mymensingh district. *Research in Agriculture, Livestock and Fisheries*. 4: 99-106. [DOI: 10.3329/ralf.v4i2.33721]
Rahman M.O., Hassan M.N., Siddiki M.S.R., Habib M.R., Rana M.S. (2016). Evaluation of morning and evening milk of Holstein Friesian crossbred cows collected from Bangladesh agricultural university dairy farm. *Asian-Australasian Journal of Bioscience and Biotechnology*. 1: 255-260. [DOI: 10.3329/aajbb.v1i2.61572]
Reddy D.M., Venkatesh K., Reddy C.V.S. (2017). Adulteration of milk and its detection: a review. *International Journal of Chemical Studies*. 5: 613-617.
Sharma R., Rajput Y.S., Barui A.K. (2012). Detection of adulterants in milk: laboratory manual. *Agricultural and Food Sciences*.
Tesfay T., Kebede A., Seifu E. (2015). Physico chemical properties of cow milk produced and marketed in Dire Dawa town, Eastern Ethiopia. *Food Science and Quality Management*. 42: 56-61.