Comparative Quality Analysis of Milk Collected from Open Markets and Dairy Industries in Bangladesh

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Abstract
The present study was conducted to analysis the quality indicators of cow’s milk both in open market and processing industries in Bangladesh. Total 84 samples of fluid milk (15 samples from the open market of each district area Sirajganj, Pabna, Tangail and Dhaka and 6 samples of pasteurized milk of 4 different brands) were collected. The determination of physicochemical properties, microbial quality and presence of adulterant of all milk samples were carried out. Highest average milk fat was found in the raw milk collected from the open market at Dhaka district and in Brand 4 as 4.2% and 3.55% respectively. The lowest corrected lactometer reading (CLR) was found in Sirajganj as 25.5. A few of the pasteurized milk and open market milk were being adulterated by skimmed milk, sugar, added water and soda and alcohol respectively. Results pertaining to the enumeration of E.coli, Salmonella and V. Cholera exhibited 56.66%, 45% and 10% of samples of open market and 12.5%, 8.33% and 4.17% of samples of brands respectively. Due to unhygienic milking practice and very poor sanitary facilities, the highest amount of total plate count was 7.9 x 10^6 cfu/ml at Sirajganj district.

Keywords: Milk quality, Pasteurization, Open market, Adulteration, Food safety
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1. Introduction
Milk is the valuable single most complete food but assuring the quality of milk is a significant issue now a days. It is easily perishable and highly vulnerable to adulteration and microbial contamination. The consumer usually suffers for milk adulteration so they want to get fresh, clean and pure milk and pathogen free (Coitinho, Cassoli, Cerqueira, Silva, Coitinho, & Machado, 2017; Ghasemi-Varnamkhasti, Ghatreh-Samani, Naderi-Boldaji, Forina, & Bonyadian, 2017). There are 7,990 number of registered dairy farm in Bangladesh (2009-10). Consumption of milk and milk products makes these foodstuffs targets for potential adulteration with financial gains for unscrupulous producers (Nicolaou & Goodacre, 2011).

Nirwal et al. (2013) was conducted a study to analyze the milk quality, adulteration and mastitis infection in milk sold at 30 different regions of Dehradun. Out of 100 milk samples analyzed for adulteration, adulterants found were glucose (80%), skim milk powder (58%), salt (51%) and urea (35%) while found negative for formalin, salicylic acid, boric acid, starch, soap and ammonium sulphites. It can cause impairments, heart problems, cancer or even death. While the immediate effect of drinking milk adulterated with urea, caustic soda and formalin is gastroenteritis or severe forms of diarrhea (food poisoning). In the long run, these chemicals in milk adversely affect vital organs such as the liver and kidney resulting in organ failure and/or cancer and thus, untimely loss of life. Due to high water activity and nutritional value of milk, it serves as an excellent medium for the growth of many microorganisms, especially Lactobacillus, Streptococcus, Staphylococcus and Micrococcus sp. Bacteria that have been frequently involved in food borne outbreaks associated with the consumption of milk include Listeria monocytogenes, Salmonella, Campylobacter, Staphylococcus aureus, Bacillus cereus, Escherichia coli.
and *Clostridium botulinum*. These pathogens can cause illness to humans while others cause spoilage in milk rendering it unsuitable (unsafe) for human consumption (Kivaria, Noordhuizen, & Kapaga, 2006; Parekh & Subhash, 2008; Bukuku, 2013). In some cases, infections can cause contamination and even make the milk not proper for use. It can conceal life-threatening hazards when it comes to gastroenteritis, diarrhea, typhoid, or bovinetuberculosis (Al-Khatib & Al-Mitwalli, 2009). The total count of bacteria in milk has a decisive effect on the quality and safety of dairy products (Khaton, Hasnat, Rahman & Rahman, 2014). Contamination of milk with high levels of spoilage bacteria is usually unsuitable for further processing since it does not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes) (Nanu, Latha, Sunil, Prejit, Thomas, & Venon, 2007). As a result, total viable bacterial counting has become one of the accepted criteria for grading milk intended for consumption and processing for dairy products.

Milk produced under hygienic conditions from healthy animals should not contain more than 5 x 10^3 CFU/ml (Popescu, Borda, Diugan & Oros, 2014). Whereas total plate counts, total coliform counts and the presence of *Escherichia coli* and *E. coli* O157:H7 were determined in 250 samples of kraals and indigenous milk products in the coastal savannah zone of Ghana. Total plate counts exceeded 10^9 CFU/ml in 45.2% of the samples while coliforms exceeded 10^8 CFU/ml in 66.0% and *E. coli* was detected in 11.2%. Antibiotic residues were detected in 3.1% of raw cow milk samples (Addo, Mensah, Aning, Narrey, Nipah, Bonsu, Akyeh & Smits, 2011). Pasteurized milk contained acceptable numbers of bacteria in >90% of samples indicating pasteurization to be effective in lowering bacterial contamination of milk in processing industry. Spore-forming bacteria are found in a wide range of dairy-associated environments including soil, water, feed, and manure (Scheldeman, Herman, Foster & Heyndrickx, 2006; Huck, Hammond, Murphy, Woodcock & Boor, 2007; Ivy, Ranieri, Martin, Bakker, Xavier, Wiedmann & Boor, 2012; Masiello, Martin, Watters, Galton, Schukken, Wiedmann, Boor, 2014). Management practices significantly associated with increased bulk tank somatic cell count were a lack of use of the California mastitis test at freshening and >25% of cows with dirty udders observed in the milking parlor (Giffel, Wagendorp, Herrewegh & Drieuhs, 2002).

High microbial load and adulteration of milk pose a serious threat to public health of Bangladesh where the health literacy is poor and level of awareness is very low. But there is only a few research has done and no standard data to compare the quality of milk. The study was conducted to analysis the physicochemical, nutritional and microbial quality of open market milk and processed milk, to identify the possible source of infectivity from farm to open market and process industry to consumer.

2. Materials & Methods

2.1 Sample Collection

A total 84 samples of which 60 were collected from the 4 open markets of each Sirajganj, Palba, Tangail and Dhaka district and 24 samples were collected from 4 different dairy processing plants. 250 ml of each sample were collected. Samples are being collected from dairy industries are divided into two sections: raw milk and pasteurized milk. Then, sample were transported to the laboratory in icebox and analyzed immediately on arrival. Each sample of milk (25ml) was taken and mixed well with 225ml of sterile peptone water and was serially diluted according to the manufacturer's directions. MYP agar base, with the addition of Polymyxin-B selective agar and the supplements were purchased from Oxoid, United Kingdom and prepared according to the manufacturer’s directions. MYP agar base, with the addition of Polymyxin-B selective supplements, and Mac Conkey agar were prepared for the isolation of *B. cereus* and *E. coli*, respectively (AOAC, 1999). For the examination of Salmonella and Campylobacter, Salmonella, Shigella agar and Campylobacter blood free selective agar base, with the addition of Campylobacter selective supplement, were used (AOAC, 2001).

2.2 Structured Questionnaire

Structured questionnaire was distributed to 20 milkmen and 12 worker of processing plants. The questionnaire was aimed to obtain first-hand information on the general awareness of the milkmen, adulteration, sources of cattle feed and hygiene practices. The questionnaire for industrial worker also included the practice of washing the equipment, sanitary practices, training on food hygiene and safety.

2.3 Physicochemical Analysis

The physical characteristics and different chemical properties such as lactose, protein, water, and fat of various milk samples were determined shortly after they determinations were carried out according to AOAC 2000. Various physicochemical properties of milk were examined and compared to Bangladesh Standard (BDS-1985) and WHO Standards. Conductivity, freezing point are examined for physical analysis; formalin, sugar, acidity, ph, salt for chemical examination and fat, corrected lactometer reading (CLR), solid non fat (SNF), total solid (TS) for nutritional analysis.

2.4 Microbiological Examination

The specific medium agar and the supplements were purchased from Oxid, United Kingdom and prepared according to the manufacturer’s directions. MYP agar base, with the addition of Polymyxin-B selective supplements, and Mac Conkey agar were prepared for the isolation of *B. cereus* and *E. coli*, respectively (AOAC, 1999). For the examination of Salmonella and Campylobacter, Salmonella, Shigella agar and Campylobacter blood free selective agar base, with the addition of Campylobacter selective supplement, were used (AOAC, 2001).
The inoculated plates were then incubated at 35°C for 24-48 hr. Coliform count was carried using Most Probable Number (MPN) technique. For total coliform count (TCC), 1 ml of each of the three consecutive dilution tubes was inoculated into tubes containing lactose broth (LB) with Durham’s tubes and incubated at 35°C for 48 hr (Uma, Chandrakanth, Indu, Nagalakshmi & Usha, 2009). From positive cultures, a loop-full of suspension was transferred to tubes containing 2% Brilliant Green LB and incubated at 35°C for 48 hr. Positive tube were considered positive to coliform and then CC calculated using MPN table (Blodgett, 2010). Each presumptive positive tube of LB was transferred to tubes of E. coli broth tubes and incubated for 48hr at 45.5°C. Production of gas in an E. coli broth culture was determined as positive for fecal coliform.

2.5 Effect of Sampling Time on Bacterial Load
To analyze the effect of sampling time on the total viable bacterial count (TVC), sampling time was separated into two sections: samples were collected during the morning and afternoon period.

2.6 Test of Adulteration
Raw milk quality and determination of adulterants at the collection point also a major concern of this study. To determine whether the samples were adulterated by mixing various agents, we analyzed the presence of sugar, starch, salt, soda, alcohol, formaldehyde and added water as per AOAC 2000.

2.7 Effect of Industrial Processing on Bacterial Count
Raw milk is collected from the chilling center through tanker and cool at 5°C. Then the milk is taken for standardization, mixing, homogenization, pasteurization, cooling and packaging. 250ml of sample was taken each steps of processing to calculate the bacterial count of milk in different processing steps. TPC and TCC were examined to analysis the effect of thermal process.

2.8 Data Analysis
Mean separated and significance difference determined at the 5% level were analyzed by analysis of variance using SPSS software version 16.0.

3. Results and Discussion
3.1 Questionnaire Survey
A total 20 cattle farm and 4 dairy industries were interviewed to obtain primary data on milking practices, sources of cattle feed, hygiene practices, quality analysis, chain of marketing etc. 95% of the farm on the bank of Baral River at Shahjadpur, Sirajganj have no shade for cattle. They keep them in a closed bamboo fenced farm called “Bathan”. In there all the cattle drink water from river source which is polluted with industrial effluents. Luciana et al visited a group of 22 dairy farms were twice (winter and summer) in order to collect bulk tank milk and post-rinse water samples and swabs from liners and milk receiver (Bava, Zucali, Sandruci, Brasca, Vanoni, Zanini, & Tamburini, 2011). A study showed that, total plate count in farmer’s hand was 178 CFU/ml and in milking pot it was 94 cfu/ml. Total coliform counts in farmer’s hand was 44 CFU/ml and in milking pot 27 CFU/ml (Prodhan, Alam, Sadia, Sultana, Hye, Ahamed, 2016). In this study, >91% of milkmen were used fresh water for washing milk-pot and <7% use warm water or detergent. The presence of insect especially flies are in acute condition in the local farm. When the cattle become sick, the milkmen purchase antibiotic or other medicine according to the recommendation of medicine store.

A survey questionnaire targeting 52 Municipal health services managers or designated persons (directly responsible for milk control) at the various metropolitan municipalities (metros) and district municipalities (DMs) in South Africa was conducted (Agenbag & Lues, 2009). Miller et al. (2015) examined the association between farm management practices and mesophilic and thermophilic spore-formers in raw milk. Farm management factors associated with fewer mesophilic spores, the most abundant spore type found in raw milk, included larger herd size, the use of sawdust bedding, and not fore-stripping during pre-milking routine. 64% of worker of the industries abide by hygiene which is monitored by the safety and sanitation monitoring committee. Male worker are 37% less conscious in using hand and mouth gloves than female worker comparatively. In farm level, <5% of measures are maintained and the hygiene condition was extremely poor.

3.2 Organoleptic, Physical and Major Proximate Analysis
In organoleptic test of collected milk, all the appearance (color, flavour, taste) were examined. All the color of pasteurized milk is creamy white and the color of raw milk is yellowish white. Rashidul et al and Monem et al found the sample collected from Mymensingh and Bogura district respectively were yellowish white also (Rashidul, 2012; Monem, 2012). The shelf life of pasteurized milk was 7 days.
Table 1: Major proximate analysis of raw milk and processed milk of selected open markets and brands

| Sample type | No. of sample | pH | Mean Fat (g/kg) | Lower boundary | Upper boundary | p-value | CLR | Mean SNF | Lower boundary | Upper boundary | p-value | SNF | Lower boundary | Upper boundary | p-value |
|-------------|---------------|----|----------------|----------------|---------------|---------|-----|----------|----------------|---------------|---------|-----|---------------|---------------|---------|
| OMS         | 15            | 4.05 | 4.9 | 5.2 | 0.21 | 5 | 24.5 | 27 | 0.19 | 7.46 | 7.20 | 8.0 | 0.318 |
| OMf         | 15            | 4.0 | 4.0 | 4.15 | 26 | 25.5 | 28.5 | 7.63 | 7.41 | 7.88 |
| OMt         | 15            | 3.85 | 3.78 | 4.1 | 5 | 26 | 26.5 | 27 | 7.90 | 7.17 | 8.1 |
| OMD         | 15            | 4.2 | 4.1 | 4.7 | 27 | 26.5 | 29 | 7.16 | 6.90 | 7.92 |
| Brand 1     | 6             | 3.5 | 3.5 | 3.6 | 20 | 27.5 | 28.5 | 8.15 | 7.92 | 8.34 |
| Brand 2     | 6             | 3.55 | 3.45 | 3.55 | 29 | 23 | 29.5 | 8.02 | 8.01 | 8.15 |
| Brand 3     | 6             | 3.5 | 3.5 | 3.5 | 28 | 26.5 | 28 | 7.95 | 7.83 | 8.10 |
| Brand 4     | 6             | 3.55 | 3.5 | 3.55 | 28 | 28 | 29 | 8.15 | 8.00 | 8.20 |

OMs = Open Market of Sirajganj; OMp = Open Market of Pabna; OMt = Open Market of Tangail; OMD = Open Market of Dhaka district; CLR = Corrected Lactometer Reading; SNF= Solid Non Fat in milk.

The physical and major proximate analysis of selected local farm at Sirajganj, Pabna, Tangail and Dhaka district and 4 commercial brand of fluid milk available in Bangladesh in table 1. Islam et al (2016) studied the milk samples collected from Kakrak Bazar, Tangail district were superior to other markets in terms of fat (36.83±0.29 g/kg), protein (34.17±1.26 g/kg), lactose (45.72±1.02 g/kg) and total solid (124.72±1.95 g/kg) content. In the present study, milk samples collected from the open market contains high quantity of milk fat (3.8-4.2%) than the commercial brands. Statistical analysis showed that there was no significant difference (p<0.01) within the protein content of different milk samples (Motta, Hoff, Barreto, Andrade, Lorenzini, Meneghini, & Pizzolato, 2014). But the CLR and SNF of open market milk is lower than the commercial brands.

Table 2: Physicochemical analysis of raw milk and processed milk of selected open markets and brands

| Sample type | No. of sample | pH | Mean Fat (g/kg) | Lower boundary | Upper boundary | p-value | Conductivity | Mean Freezing point |
|-------------|---------------|----|----------------|----------------|---------------|---------|--------------|-------------------|
| OMS         | 15            | 4.05 | 4.0 | 4.2 | 0.21 | 5 | 24.5 | 27 | 0.19 | 7.46 | 7.20 | 8.0 |
| OMf         | 15            | 4.0 | 4.0 | 4.15 | 26 | 25.5 | 28.5 | 7.63 | 7.41 | 7.88 |
| OMt         | 15            | 3.85 | 3.78 | 4.1 | 5 | 26 | 26.5 | 27 | 7.90 | 7.17 | 8.1 |
| OMD         | 15            | 4.2 | 4.1 | 4.7 | 27 | 26.5 | 29 | 7.16 | 6.90 | 7.92 |
| Brand 1     | 6             | 3.5 | 3.5 | 3.6 | 20 | 27.5 | 28.5 | 8.15 | 7.92 | 8.34 |
| Brand 2     | 6             | 3.55 | 3.45 | 3.55 | 29 | 23 | 29.5 | 8.02 | 8.01 | 8.15 |
| Brand 3     | 6             | 3.5 | 3.5 | 3.5 | 28 | 26.5 | 28 | 7.95 | 7.83 | 8.10 |
| Brand 4     | 6             | 3.55 | 3.5 | 3.55 | 28 | 28 | 29 | 8.15 | 8.00 | 8.20 |

OMs = Open Market of Sirajganj; OMp = Open Market of Pabna; OMt = Open Market of Tangail; OMD = Open Market of Dhaka district; CLR = Corrected Lactometer Reading; SNF= Solid Non Fat in milk.

Following table 2 showed the physicochemical test of selected open market and commercial milk brands. The pH of OMP and OMD is 5.45 and 5.67 respectively which is slightly lower. Due to lack of chilling storage facilities and warm weather the milk of open market make lower pH. Brand 2 showed the highest level of mean conductivity which is 4.6. The mean freezing point of the milk found in Sirajganj district, Dhaka district and Brand 4 is lower as -0.509, -0.505 and -0.515 respectively.

3.3 Quality/safety status of milk samples according to Bangladesh Standard (1702:2002)

Table 3: Quality analysis of milk according to the Bangladesh Standards

| Characteristics | Bangladesh Standards | OMS | OMp | OMt | OMb | Brand 1 | Brand 2 | Brand 3 | Brand 4 |
|-----------------|----------------------|-----|-----|-----|-----|--------|--------|--------|--------|
| Fat             | 3.50% (min.)         | 4.05 | 4.0 | 3.8 | 4.2 | 3.5 | 3.5 | 3.5 | 3.55 |
| pH              | 6.6-6.8              | 6.63 | 6.71 | 6.74 | 6.82 | 6.73 | 6.72 | 6.62 | 6.56 |
| Total Plate Count | <20000/ml         | 7.9 x10^6 | 6.2 x10^6 | 5.8 x10^6 | 4.6 x10^6 | 125 | 485 | 675 | 14500 |
| Total Coliform  | <10/ml               | 4.1 x10^6 | 3.2 x10^6 | 4.6 x10^6 | 3.8 x10^6 | 0 | 2 | 5 | 8 |

Table 3 showed the comparison of pasteurized and raw milk with the Bangladesh Standard 1702:2002. Among the pasteurized milk of 4 brands and raw milk of the open market, the highest amount of fat found in Dhaka and the highest amount of CLR found in Brand 2 is 29.5. According to the Bangladesh Standard (BDS) (BSTI, 2002), both the fat and CLR are above the standard limit. The SNF, acidity and pH are approximate to the standard also. High quality milk essentially needs to have less than 0.14 percent acidity (Popescu & Angel, 2009). The average acidity of the pasteurized milk samples ranged from 0.169±0.010to 0.200±0.013 during the six days examination period, where BSTI (2002) allows a maximum acidity of 0.15% for the pasteurized milks (Dey & Karim, 2013). Milk sample found in Pabna and Dhaka are contaminated with adulterants as soda and skimmed milk powder respectively. Pasteurized milk sample of Brand 2 and Brand 3 were found adulterated with added water and skimmed milk powder also. The distribution of the total viable micro-organisms ranged
from $>10^4$ CFU/ml$^{-1}$ to $10^7$ CFU/ml$^{-1}$ with the highest recorded count at $6.08 \times 10^7$ CFU/ml$^{-1}$ which is much higher than the legislative standard of $5 \times 10^4$ CFU/ml for raw milk intended for consumption (Lues, Beer, Jacoby, Jansen & Shale, 2010). The Coliform limits in the raw milk accepted internationally are less than 100 cell/ml (Salman, 2011; Shojaei & Yadollahi, 2008). Surprisingly found that total plate count and total coliform in local raw milk is $7.9 \times 10^6$ to $4.6 \times 10^6$ cfu/ml & $4.6 \times 10^6$ to $3.2 \times 10^6$ cfu/ml respectively where the BDS is less than 20000/ml & 10/ml respectively. Beside that in pasteurized milk total plate count and total coliform were 125-14500 cfu/ml and nil to 8 cfu/ml respectively.

3.4 Adulteration Test of Milk of Different Sources
Islam et al. (2018) collected raw milk sample in three different market of Jamalpur district, Bangladesh and observed that there was no adulteration in any of the collected raw milk samples. Raw milk had been adulterated with water and sugar found in Bangladesh by T. J. Hossain et al. (2010). Figure 1 showed the adulteration test result of fluid milk in different sources of open market and commercial brands. Positive result found sugar test in 33% and 66% sample of Brand 2 and Brand 3 sample respectively.

![Figure 1: Adulteration test of milk of different sources](image)

Soda found in 13% sample of open market of Pabna and 6.5% sample of Dhaka district. 40% sample of open market of Pabna and 13% sample of Dhaka contain below the standard limit of alcohol. To enhance the specific gravity of fluid milk skimmed milk powder were commonly found in 40%, 33%, 40%, 66% and 13% sample of Open market in Sirajganj, Pabna, Brand 2, Brand 3 and Brand 4. is showed negative results. Naturally the cow’s milk are milking in afternoon contain less fat and CLR than morning. SMP and sugar are added to milk to increase CLR. Popescu et al. (2009) reported that every tank truck of milk is carefully checked concerning the presence of antibiotics prior to the tank being unloaded in USA but there is no routine check of antibiotics in processing of milk in Bangladesh.

3.5 Microbial Safety Status of Milk
Table 4: Detection of E. coli, Salmonella and V. Cholera in liquid milk collected from the local markets of Bangladesh

| No. | E. coli | Salmonella | V. cholera |
|-----|---------|------------|-----------|
|     | Open market | Process milk | Open market | Process milk | Open market | Process milk |
| 1   | 11/15 | 1/6 | 6/15 | 0/6 | 3/15 | 0/6 |
| 2   | 8/15 | 0/6 | 7/15 | 0/6 | 1/15 | 0/6 |
| 3   | 9/15 | 0/6 | 9/15 | 1/6 | 1/15 | 0/6 |
| 4   | 6/15 | 2/6 | 5/15 | 1/6 | 1/15 | 1/6 |
| Total | 34/60 (56.66%) | 3/24 (12.5%) | 27/60 (45%) | 2/24 (8.33%) | 6/60 (10%) | 1/24 (4.17%) |

Table 4 shows, the presence of E. coli, salmonella and V. cholera are detected in selected samples. A total 84 sample are examined which are divided in 2 categories as open market and brands. Marjan et al. (2014) state that out of 80 samples, 74 were found pathogens within a range of 102-104 cfu/ml, including Escherichia coli, Salmonella spp., Staphylococcus aureus and Vibrio spp. In the present study, E. coli, Salmonella and V. cholera are found in 56.66%, 45% and 10% samples in open market respectively. 12.5%, 8.33% and 4.17% sample of brands are found positive result in detection of E. coli, salmonella and V. Cholera respectively.
Table 5: Detection of TPC, TCC and FCC in milk sample

| Sample type | No. of sample | Mean TPC (cfu/ml) | Lower boundary (cfu/ml) | Upper boundary (cfu/ml) | p-Value | Mean TCC (cfu/ml) | Lower boundary (cfu/ml) | Upper boundary (cfu/ml) | p-Value | Mean FCC (cfu/ml) | Lower boundary (cfu/ml) | Upper boundary (cfu/ml) | p-Value |
|-------------|---------------|------------------|------------------------|------------------------|---------|------------------|------------------------|------------------------|---------|------------------|------------------------|------------------------|---------|
| OMS 15      | 15            | 7.9 x 10^6       | 6.8 x 10^6             | 9.3 x 10^6             | 0.5     | 4.1 x 10^6       | 3.1 x 10^6             | 5.5 x 10^6             | 0.6     | 3.0 x 10^6       | 2.9 x 10^6             | 3.6 x 10^6             | 0.01    |
| OMP 15      | 15            | 6.2 x 10^6       | 3.9 x 10^6             | 8.7 x 10^6             | 0.8     | 2.7 x 10^6       | 2.0 x 10^6             | 4.8 x 10^6             | 0.9     | 2.5 x 10^6       | 2.0 x 10^6             | 4.1 x 10^6             |         |
| OMT 15      | 15            | 5.8 x 10^6       | 2.8 x 10^6             | 7.1 x 10^6             | 0.7     | 2.4 x 10^6       | 1.6 x 10^6             | 4.8 x 10^6             | 0.8     | 3.3 x 10^6       | 1.6 x 10^6             | 4.2 x 10^6             |         |
| OMD 15      | 15            | 4.6 x 10^6       | 3.2 x 10^6             | 6.9 x 10^6             | 0.5     | 3.5 x 10^6       | 2.1 x 10^6             | 6.1 x 10^6             | 0.6     | 3.1 x 10^6       | 2.7 x 10^6             | 3.6 x 10^6             |         |
| Brand 1     | 6             | 125              | 80                     | 250                    | ND**    | -                | -                      | -                      | -       | -                | -                      | -                      | -       |
| Brand 2     | 6             | 455              | 110                    | 780                    | 2       | 2                | 3                      | 3                      | ND      | -                | -                      | -                      |         |
| Brand 3     | 6             | 675              | 380                    | 1200                   | 5       | 4                | 6                      | 6                      | ND      | 1                | 1                      | 2                      |         |
| Brand 4     | 6             | 14500            | 130                    | 22000                  | 8       | 7                | 12                     | 12                     | 2       | 1                | 1                      | 4                      |         |

*cfu= colony forming unit; **ND=not detected

Table 5 showed the microbial enumeration of milk for TPC, TCC and FCC. The presence of these organisms in milk and milk products is an indication of unsanitary production and/or improper handling of either milk or milk utensils (EL-zubeir & Ahmed, 2007). Dehinenet et al. (2013) reported that the mean total bacterial count (TBC/ml), total coliform count (TCC/ml) and somatic cell count (SCC) were 1.1x10^8, 3.0x10^4 and 5.5x10^5 respectively in Ethiopia. The open market contain higher amount of TPC, TCC and FCC than all the brands. Maximum TPC found in the samples collected from Sirajganj was 7.9 x 10^6 cfu/ml. The unhygienic condition in milking and the poor sanitary practices of milkmen are the cause of higher TPC, TCC and FCC. The sample collected from the open markets of Tangail contain highest amount of TCC and FCC were 4.6 x 10^6 and 3.3 x 10^6 cfu/ml respectively. It was surprisingly found that the brands contain only 125-14500 cfu/ml due to pasteurization at 85-90°C for 15 seconds. TCC and FCC found in Brand 1 were nil. Brand 4 contains the higher amount of TPC, TCC and FCC among the all 4 brands but all brands complies with Bangladesh Standard (BDS).

3.6 Effect of thermal process on the bacterial growth of milk in different processing steps

3.6.1 Prevalence and enumeration of total plate count in processing steps

![Figure 2: Distribution of total plate count in different steps ofProcessing of milk](image)

The effect of thermal processing i.e. pasteurization on the microbial quality milk is showed [Figure 2]. Most fluid milk consumed in the United States is HTST pasteurized (i.e., 72°C for 15s) with an anticipated shelf-life of 14 to 21 days (Murphy, Martin, Barbano, & Wiedmann, 2016). The consumption of milk that is not pasteurized increases the risk of contracting diseases (Angulo, Lejeune, Rajala-Schultz, 2009). The sample was taken in all the possible way of increasing or decreasing of microbial count. The TPC of taken sample was 5.08 x 10^6 cfu/ml which collected from milk tanker at 5°C. In the receiving section of the processing industry, the milk is passing through the heat exchanger to lose heat to 2°C and goes to receive tank. The total plate count in cooling section was 3.75 x 10^6 cfu/ml. Milk is then pre-heated at 40-45°C and the total counts decrease to 3.52 x 10^6 cfu/ml. In Bangladesh, most of the processes of milk are semi-manual. In the mixing section, fat separated, water added and due to manual mixing the TPC increase to 4.3 x 10^6 cfu/ml. Then it passes through the homogenizer and pasteurized at 85-90°C for 15-20 seconds. At this stage, total plate counts drastically decreases to 16 cfu/ml only. Then the milk cooled to 4°C. Milk is then passes through filling machine and pack finally. The final product contains 21cfu/ml of total plate counts and it stored.
3.6.2 Prevalence and enumeration of total coliform count in processing steps

Figure 3: Distribution of total coliform count in different steps of Processing of milk

Figure 3 showed the average Total Coliform Count (TCC) found in each steps of processing line. The shelf-life at these pasteurization temperatures was more dependent on other factors such as bacterial load, post-pasteurization contamination, and storage conditions (Gandy, Schilling, Coggins, White, Yoon & Kamadia, 2008). Similarly, samples taken from different processing steps to detect TCC. The raw milk sample contain 3363 cfu/ml of TCC which are decrease to 2715 cfu/ml in cooling, 2580 cfu/ml in pre-heat steps and increase to 2640 cfu/ml in mixing steps. Significantly, 3 cfu/ml of TCC found after pasteurization and the final pack contain 4 cfu/ml of TCC.

4. Conclusion
The present study revealed nutritional, physicochemical and microbial quality of milk being sold at open market and the pasteurized milk of different brands of Bangladesh. The average milk fat percentage in open market is slightly higher in raw milk sold in open market but CLR is lower than the pasteurized milk. Usually adulterant were not found in commercial milk except SMP, Sugar and added water is found to improve the CLR and taste of milk. But soda and alcohol was found in open market milk. Most of the farmers and milkmen are illiterate and they have not enough knowledge about personal hygiene and sanitary practices and unconscious about food safety. The sources of pathogenic bacteria come from natural source and unhygienic handling of milk especially improper hand washing of milkman and the unhygienic condition of cows and milking pot. It is remarkably enumerated that the heat treatment and immediate cooling are the most effective ways for microbial lessening of raw milk during industrial processing. It was surprisingly found that the average TPC and TCC come to least and very acceptable quantity in the pasteurized milk. It can be recommend that, regular monitoring should carried out by processing industries to ascertain the physiochemical characteristics including adulteration parameters and nutritional quality of raw milk. In farm level, different initiative should take to establish sanitary facilities for milkmen, arrange targeted training for milkmen and worker on safe farming & hygiene practices and ensure safe drinking water facilities for cattle. To ensure the milk quality requires the necessity and greater emphasis on regulatory aspects with advanced methods of analysis and monitoring milk production and processing have set new goals for quality assurance and food safety.

Conflict of Interest
The authors declare no conflict of interest for this study.

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