Quality and Safety Assessment of Bangladeshi Pasteurized Milk

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HIGHLIGHTS

- Statistically significant differences were recorded among different brands in Bangladesh for all physicochemical parameters except specific gravity.
- The highest and lowest total bacterial counts in different brands were 1150400 and 35500 CFU/ml, respectively.
- This survey revealed that different brands of Bangladeshi milk did not maintain the standard and acceptable quality.

ABSTRACT

Background: Milk is considered as one of the highly nutritious food for human. This study was undertaken to evaluate the physicochemical as well as the microbial quality of pasteurized milk of different brands available in Chittagong, Bangladesh.

Methods: Five types of branded pasteurized liquid milk were collected from retail markets of Chittagong, Bangladesh. Physicochemical analyses were carried out in order to determine the levels of pH, acidity, fat, protein, casein, specific gravity, Solids-Not-Fat (SNF), and total solids of the samples. Also, the samples were analyzed microbiologically to assess the total microbial loads and coliforms. Statistical analysis was done using SPSS software version 23.0.

Results: The ranges of physicochemical parameters of the samples were determined, including specific gravity (1.024-1.031), pH (5.8-6.7), acidity (0.17 ± 0.01-$0.37\pm0.01\%$), total solids content (8.17-12.27%), SNF (7.28-8.49%), fat (0.89-3.78%), protein (3.42 ± 0.09-$3.63\pm0.02\%$), and casein content (2.66 ± 0.07-$2.82\pm0.02\%$). Statistically significant differences ($p<0.05$) were recorded among different milk brands for all physicochemical parameters except specific gravity. The highest and lowest amounts of total bacterial counts were 1150400 and 35500 CFU/ml, respectively. A significant difference ($p<0.05$) was found in bacterial loads among different brands of pasteurized milk.

Conclusion: This survey revealed that different brands of Bangladeshi pasteurized milk did not maintain the standard and acceptable quality.

Introduction

Milk is considered as one of the highly nutritious food for human. It is often regarded as a perfect food as it is rich in almost all forms of nutrients. Water, fat, protein, lactose, vitamins, and minerals can be found in milk at a balance proportion (Guetouache et al., 2014). Whole milk contains 88.13% water, 3.15% protein, 3.27% fat,

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5.05% total sugar as well as 0.4% vitamins and minerals. The percentage may vary with breed, age and health, type of feed, stage of lactation, milking time, and completeness of milking. Milk is also one of the best dietary sources of calcium, vitamin D, and potassium. Cow milk also supplies all essential amino acids vital to the human body health (Barłowska et al., 2011; Haug et al., 2007).

Being a highly nutrient-rich food, milk can be an ideal source of pathogenic bacteria. Microbial presence could also be high due to the addition of mastitis milk with fresh milk (Jeffrey and Wilson, 1987). Several pathogens in contaminated milk, including *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Bacillus cereus*, etc. might be responsible for food-borne outbreaks (Chye et al., 2004). Life-threatening bacteria, like *Escherichia coli* O157:H7 was also found in raw cow milk (Lye et al., 2013). Hence, pasteurization, as a heat treatment at a specific temperature for a certain period, is important to maintain the edible quality of milk as well as to extend the shelf-life. This process can destroy spoilage microorganisms except for bacterial spores. In addition, pasteurization treatment only has a negligible impact on the nutritional quality of milk (Fuquay et al., 2011).

Unfortunately, adulteration of milk is not uncommon in Bangladesh. Milk can be adulterated by adding water, extraction of fat or addition of other components such as alkali materials, flours, etc. Unhygienic handling or filthy ambiance of processing area can also contribute to pathogenic load even after pasteurization. Many studies have been conducted to assess the quality of raw milk produced in different areas of Bangladesh. But, there is meager information on the quality characteristics of branded milk. Today, people are more motivated to buy packaged milk in consideration of food safety and quality. Therefore, this study was undertaken to evaluate the physicochemical as well as the microbial quality of pasteurized milk of different brands available in Chittagong, Bangladesh.

Materials and methods

Sample collection

Five types of branded pasteurized liquid milk (Lm-FR, Lm-MV, Lm-AR, Lm-PR, and Lm-LC) were collected from retail markets of Chittagong, Bangladesh. The milk samples including three samples from each brand were kept in a refrigerator at 3 ℃ until beginning the study. All analysis were carried out in triplicate.

Physicochemical analysis

-Determination of pH

The pH of collected samples was determined by using a digital pH meter.

-Determination of acidity

About 10 ml milk sample from each brand was taken in a beaker. Then, a titration was done against 0.1N NaOH. Phenolphthalein was used as an indicator. The acidity percentage of milk was calculated by using the following formula given below (AOAC, 1990):

$$\% \text{Acidity} = \frac{\text{ml of alkali using} \times (\text{N}) \text{ of NaOH} \times 0.09}{\text{ml milk sample reuired}} \times 100$$

-Determination of fat

Fat percentage of milk was determined by Gerber method (Fuquay et al., 2011). At first 10 ml concentrated H_2SO_4 was taken in a butyrometer. Then, about 11 ml of well mixed milk sample and 1 ml amyl alcohol were added. The butyrometer was shaken properly and placed in a water bath at 65 ℃. The sample was centrifuged in Gerber centrifuge machine (Funke Gerber, Germany) at 1100 rpm for 5 min. The fat percentage was recorded from the butyrometer reading.

-Determination of protein and casein content

About 10 ml well mixed milk sample was taken in a conical flask. Then, 0.4 ml potassium oxalate was added, mixed, and kept for two min. Few drops of phenolphthalein indicator were added and titration was done against 0.1 N NaOH solution till the appearance of faint pink color. After that, 2 ml formaldehyde solution was added to the mixture and kept for 30 min. Again titration was done following the same procedure after adding 2-3 drops of phenolphthalein indicator. Amount of protein required was recorded and the protein and casein percentages were calculated as indicated below (Fuquay et al., 2011).

Casein percentage=ml of alkali required×1.32
Protein percentage=ml of alkali required×1.70

-Determination of specific gravity

Milk sample was mixed well and poured into the lactometer jar up to its brim. The lactometer was placed in the jar in rotating moment and the reading was taken at stationary phase. The temperature of the milk was recorded with the help of dairy thermometer. Corrected Lactometer Reading (CLR) was calculated by adding 0.2 with Lactometer Reading (LR) for each degree Fahren-

heat above 80.6 ℉ or by subtracting 0.2 for each degree Fahrenheit below 80.6 ℉ (Fuquay et al., 2011).

$$CLR=LR\pm(\Delta\ ℉\times0.2)$$

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

-Determination of Solids-Not-Fat (SNF) and total solids

Total solid content of milk sample was determined by following formula:

Percentage of SNF=$\frac{CLR}{4}$+0.25 F+0.6

CLR=Corrected Lactometer Reading at 80.6 ℉

F=Percent of fat content of milk

CLR was calculated by above mentioned way. Total solid content of milk was calculated by adding SNF content and percentage of fat content of milk (Fuquay et al., 2011).

Total solid (%)=SNF (%)+Fat (%)

Microbiological analysis

-Sample dilution

Ten ml of pasteurized milk was added to 90 ml of freshly prepared buffer peptone water to prepare 10 times decimal diluted solution. Then, 1 ml of 10^{-1}, 10^{-2}, and 10^{-3} diluted solutions were prepared by following methods. The whole experiment was done aseptically.

-Standard plate count

Pour plate technique was performed with some modification to determine the bacterial load according to Batt and Tortorello (2014).

-Detection of coliform bacteria

For each sample, a set of 9 test tubes was prepared. About 10 ml of McConkey broth (HiMedia, India) was dispensed in each test tube containing a Durham tube at inverted position. After sterilization of whole set, about 1 ml of sample from the 10^{-1}, 10^{-2}, and 10^{-3} diluted solution was taken in separate test tubes. Triplicate analysis was done for each diluted solution. This whole process was done for the total 10 milk samples from different brands (two samples from each brand). All the tubes were incubated at 37 ℃ for 24 h. Then, each test tube was examined to detect gas formation in the Durham tube (Batt and Tortorello, 2014).

-Isolation of coliform bacteria

In this test, inoculum from each positive McConkey broth tube of the confirmatory test was streaked on Eosin Methylene Blue (EMB) agar and incubated at 37 ℃ for 24 h. Each plate was examined for the presence of

typical colonies. Also, required biochemical tests were carried out based on Batt and Tortorello (2014).

Statistical analysis

SPSS software version 23.0 (SPSS Inc., Chicago) was used to perform Analysis of Variance (ANOVA) test in order to understand the significant difference between different samples. The level of significance was set at ≤0.05.

Results

The specific gravity of all pasteurized milk samples was in the range of 1.024-1.031. The pH value for pasteurized milk varied between 5.8 and 6.7. Acidity percentage was found in the range of 0.17±0.01 to 0.37±0.01%. The average value of total solids content was between 8.17 and 12.27%. The average SNF and fat content was in the range of 7.28-8.49 and 0.89-3.78%, respectively. The present study also revealed a protein content range of 3.42±0.09 to 3.63±0.02% and casein content range of 2.66±0.07 to 2.82±0.02% in various pasteurized milk samples. Statistically significant differences ($p<0.05$) among different milk brands were recorded for all physicochemical parameters except specific gravity (Table 1).

The highest amount of total bacterial count was detected in sample Lm-MV (1150400 CFU/ml) followed by sample Lm-PR and Lm-FR. On the other hand, the lowest amount of bacteria was found in sample Lm-AR (35500 CFU/ml). A significant difference ($p<0.05$) was found in bacterial loads among different brands of pasteurized milk. Regarding coliform detection test, almost all samples exhibited the existence of coliform bacteria except sample Lm-AR (Table 2).

Discussion

The physicochemical properties of some pasteurized milk samples marketed in Chittagong, Bangladesh were not within standard levels. For instance, some milk samples showed low specific gravity which may be due to fraudulent addition of water in the milk. Also, lower pH and high acidity value of sample Lm-LC specified it as low quality milk. Similarly, a previous study in Bangladesh indicated that some milk samples did not have acceptable acidity percentage (Dey and Karim, 2013).

Generally, cow milk has a total solid content of 10.5-14.5% and high total solid contents are also related to high yield of different dairy products (Fuquay et al., 2011). Total solids content does vary with fat and SNF content of milk. Bangladesh Standards suggest that

Table 1: Physicochemical parameters of different brands of pasteurized milk marketed in Chittagong, Bangladesh

Parameters	Lm-FR	Lm-MV	Lm-AR	Lm-PR	Lm-LC
pH	6.28±0.09 [a]	6.56±0.09 [b]	6.58±0.08 [b]	6.7±0.09 [b]	5.8±0.14 [c]
Acidity%	0.22±0.01 [a]	0.17±0.01 [b]	0.17±0.04 [b]	0.18±0.00 [ab]	0.37±0.01 [c]
Fat%	3.78±0.09 [a]	2.9±0.02 [b]	3.03±0.13 [b]	0.89±0.04 [c]	3.08±0.11 [b]
Protein%	3.51±0.06 [ab]	3.53±0.05 [ab]	3.42±0.09 [a]	3.51±0.05 [ab]	3.63±0.02 [b]
Casein%	2.73±0.04 [ab]	2.74±0.04 [ab]	2.66±0.07 [a]	2.73±0.04 [ab]	2.82±0.02 [b]
Specific gravity	1.024±0.05	1.026±0.049	1.025±0.061	1.024±0.041	1.031±0.099
SNF%	8.49±0.1 [a]	8.08±0.1 [b]	7.8±0.1 [c]	7.28±0.06 [d]	7.56±0.04 [e]
TS%	12.27±0.17 [a]	10.98±0.11 [b]	10.79±0.28 [b]	8.17±0.06 [c]	10.64±0.08 [b]

Mean±SD within a row with different superscripts are significantly different ($p<0.05$)

Table 2: Total bacterial count in different brands of pasteurized milk marketed in Chittagong, Bangladesh

Brand	Average total bacterial count (CFU/ml)	Standard total bacterial count (CFU/ml)*
Lm-FR	335000 [a]	
Lm-MV	1150400 [b]	
Lm-AR	35500 [c]	≤20000*
Lm-PR	500000 [d]	
Lm-LC	67750 [c]	

* According to Bangladesh Standards and Testing Institution
Mean values within a column bearing different superscripts are significantly different ($p<0.05$)

pasteurized liquid should contain not lower than 8% SNF content (BDS, 2002). However, only sample Lm-FR (8.49%) and Lm-MV (8.08%) satisfied the standard limit (≥8%). In our study, sample Lm-FR had significantly higher fat content (3.78%) than that of other samples. On the other hand, a much lower fat content (0.89%) was observed in the sample Lm-PR which might be due to high skimming. A lower fat content in pasteurized milk marketed in Bangladesh was also reported by Prodhan et al. (2016). Another study carried out in Ethiopia showed fat content in pasteurized milk varying from 2.9-4.75% (Woldemariam and Asres, 2017). Similarly, fat content was found at much lower level in milk samples of Western Cape, South Africa at an average of 2.87% (El Zubeir et al., 2007). Generally, fat content of milk possesses a high value and the price range of milk varies according to the fat content of milk. The variability of fat and SNF content in our study might be due to the difference in animal breeds, lactation stage or seasonal periods. Cow milk usually contains 3.2-3.4% protein including about 80% casein content (Fuquay et al., 2011). In contrast, sample Lm-FR had the lowest percentage of protein (3.51%) and casein (2.73%). Prodhan et al. (2016) showed protein percentage in different Bangladeshi brands of pasteurized milk ranging from 3 to 3.4%. Average protein percentage in pasteurized milk was found at

2.17% in South Africa (El Zubeir et al., 2007) and 4.14% in Ethiopia (Woldemariam and Asres, 2017). In our study, the protein and casein content was in acceptable range in all samples. The highest percentage of protein and casein was found in sample Lm-LC at 3.63% and 2.82%, respectively. Similar investigation in Pakistan showed the amount of protein and casein content at an average of 3.28% and 2.46%, respectively (Imran et al., 2008).

In terms of microbial quality, the present investigation confirmed that all of the brands were contaminated with high amount of bacteria. Bacterial load of all samples exceeded the standard limits (≤20000 CFU/ml) set by the Bangladesh Standard and Testing Institution (BDS, 2002). Coliform bacteria which their absence generally indicates the cleanliness and sanitary practices during processing, was also found in our milk samples. Unacceptable bacterial contaminations ranging from 18000 to 98000 CFU/ml were previously reported in Bangladeshi pasteurized milk (Hasan et al., 2015). Several studies carried out in Kuwait (Al-Mazeedi et al., 2013), Jamaica (Anderson et al., 2011), and India (Sarkar, 2015) exhibited the presence of coliforms, especially *E. coli*, in pasteurized milk indicating considerable public health risk. High bacterial loads in pasteurized milk samples pointed out that hygienic practices were not maintained properly

in processing areas. Poor processing techniques, dirt in equipment's, worker's less knowledge on hygiene practices can contribute to the microbial loads in processed milk. Moreover, initial concentration of bacteria in raw milk and post processing contamination have a great effect on microbial quality of pasteurized milk. Also, the water used for cleaning purposes must be free from bacterial contamination (Fuquay et al., 2011; Sarkar, 2015).

Conclusion

The present investigation on physicochemical and microbial quality of pasteurized milk revealed that different brands of pasteurized milk did not maintain the standard quality. Physicochemical parameters varied significantly among different brands. Low levels of fat, SNF, and specific gravity in different samples may imply probable water adulteration in pasteurized milk. High microbial loads and the presence of coliforms in Bangladeshi pasteurized milk showed a significant health concern for the local people. In such condition, heat processing before consumption of milk should be carried out by the people. Dairy industries in Bangladesh must focus on the prevention of post processing contamination. Also, monitoring the quality of pasteurized milk must be carried out in regular basis by the national authorities.

Author contributions

S.A. analyzed the data and edited the final manuscript; A.F.M.I.U.Z. and M.S.A wrote the primary draft of manuscript; A.F.M.I.U.Z., S.R., S.G., and A.C. performed the experiments; M.S.A. supervised the project and was in charge of overall direction. All authors revised and approved the final manuscript.

Conflicts of interest

There is no conflict of interests in this research.

Acknowledgements

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