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Original Research Article

Biochemical and microbial quality attributes of cow's milk in respect to regional discrimination in Bangladesh

ABSTRACT

Aims: The aim of this study was to assess inter-regional quality and safety of cow milk of Bangladesh.
Study design: Collection of raw cow's milk from different dairy farms, analysis of samples, and statistical analysis to find out the discrimination among the study regions as quality and quantitative risk assessment.

Place and Duration of Study: Three different regions (South-Central, North-Western and Western regions) of Bangladesh (August to December, 2016).

Methodology: Biochemical composition (protein, total fat, solids-non-fat, total casein, and lactose) and different microbial status of collected cow's milk have been investigated.

Results: Significant differences were observed in the content of milk protein, fat, solids-non-fat, casein, and lactose among those regions. Milk from West region of Bangladesh contains significantly higher content of total protein (4.06%), casein protein (2.91%), solids-non-fat, and lactose compared to other regions. However, South-Central milk possesses significantly higher level of fat (3.84%). The Pearson's correlation indicated that the content of milk protein, casein, lactose and SNF are positively correlated (P=.05 and <0.01) with each other whereas milk fat was found to be negatively correlated with other constituents of milk. The Box plot and canonical discriminate analysis based on biochemical parameters revealed good distinction among individual geographic origin. A significant difference was shown in microbiological quality of raw milk from the three studied regions and South-Central region showed highest microbial contamination compared to North -Western and Western regions.

Conclusion: The present study has strongly indicated regional variation in cow milk quality. Statistical analysis (Canonical Discriminate) of the milk constituents (protein, lactose, casein, fat, and SNF) provides a prospect for geographic discrimination of raw milk and thus showed good regional separation among three studied regions of Bangladesh. The study would be useful to analyze more diverse dairy products in the future leading to more effective determination of geographic origin.

Keywords: Cow milk, Biochemical quality, Box plot, Regional discrimination

1. INTRODUCTION

It is well known that nutritious, safe and adequate diet is essential for the growth and development of child and accordingly for prevention of various diseases during adulthood. Although in developing countries malnutrition resulting wasting and infection remains a critical public health problem. According to reports from UNICEF, WHO and World Bank, about 33.9 million children under 5 years are suffering from wasting and about 66% of the world's stunned children live in lower-middle-income countries mostly in South Asia [1]. Thus, there is a demand for improving nutritional status through providing adequate improved diets and consumer awareness. Consumption of dairy products rich in macro- and micronutrients could provide nutritional benefits to the population of developing countries like Bangladesh where the poor people has limited access to animal-source foods. FAOSTAT, 2012 [2] reported that, milk is a major source of dietary energy, protein and fat, contributing on average 134 kcal of energy/capita per day, 8 g of protein/capita per day and 7.3 g of fat/capita per day. Thus, milk and dairy products are crucial for the

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variation to plant based diets promoting child growth as well as being essential component in formulated foods used for therapeutic feeding of malnourished children. Moreover, epidemiological studies reported that dairy products decrease the prevalence of individual metabolic risk factors such as hypertension, dyslipidemia, and mild hyperglycaemia in adults. Guo-Chong et. al. [3] showed a significant inverse association between dairy products consumption and risk of metabolic syndrome. Higher dairy consumption was significantly associated with 17% and 14% reduced risk of metabolic syndrome in cross- sectional/case-control studies and prospective cohort studies, respectively.

In Bangladesh, the per capita milk availability in 2008 was only 19 kg [4], which was far below the requirements (92 kg/person/year) as indicated by the World Health Organization [5]. Moreover, the consumption of milk was lower (30 g/caput/day) in rural areas compared to urban areas (42 g/caput/day). Therefore, being a substantial part of food intake, especially in children, the regional variations in milk constituents and safety measures may be of value when considering environmental factors in public health. Several factors influence milk quality such as stage of lactation, breeding, seasonal variations, age, feed, and health of the animal [6]. Moreover milk becomes adulterated for extra profit or due to poor hygiene conditions of processing, storage, transportation, and marketing. One of the oldest and simplest forms of milk fraud is through the addition of variable volumes of water to artificially increase its volume for greater profit which can substantially decrease the nutritional value of milk, for infants and children this may be a serious concern as they are at a critical stage of growth and development and are dependent on milk products for supplies of vital nutrients and so may be at risk of malnutrition. If the water added is contaminated by pathogens, there is a risk to human health because of potential waterborne diseases [7].

Food authenticity is another important worldwide issue, which includes geographic and production origins, can influence consumers' purchasing decisions. In recent years, after the advent of food borne diseases there has been an increasing demand in obtaining information on the authenticity, geographical origin and quality of food all over the world [8]. Milk is most susceptible to be mislabeled and their country of origin can sometimes be in question. Consequently determination of the geographical origin of milk is undeniably linked to its authenticity [9]. The geographical origin and authenticity can be ascertained by determining the elemental concentration, isotopic ratio and nutritional values of milk. In case of milk and dairy products, most of the studies have been performed on cheese for origin authentication purposes [10]. Sacco et al. [11] used multi-elemental analysis in combination with stable isotope analysis for differentiation between Southern Italy and foreign milk. However, information on geographical origin of different food stuffs based on nutritional values is limited [12, 13]. Thus with growing consumer concerns, there is also increased awareness of safety and quality issues in milk and dairy products because of possible connections between the consumption of adulterated cow's milk and public health. In line with this issue, the present study was aimed to investigate biochemical and microbiological quality of cow milk with a particular interest in substances suggested to influence public health of South-central, North-Western and Western part of Bangladesh. Biochemical compositions were also used in ascertaining the factors that allow for the determination of geographical origin of milk obtained from three GI in Bangladesh.

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2. MATERIALS AND METHODS

2.1 Milk sampling site and collection

The cow's milk was collected from 9 different dairy farms from three different regions such as Western (n=9), North-West (n=19), and South-Central part (n=15) of Bangladesh (Figure1) during April to July, 2016. The geographic and environmental backgrounds of these regions are given in Table 1. The south-central part is the capital areas, the west and north-west region has been chosen as the dairy cooperative and milk pocket areas of Bangladesh (Figure 1). The farm size of milk sources range from 0.2 to 0.35 hector and the average herd size was 50. The cows of the farm were fed roughages-dry straw and silage and drank ground water. Milking was done manually twice a day at 7.00 am and 5.00 pm. All samples of raw cow's milk (1 liter) were collected from the bulk milk tanks of the farms into cleaned and sterile washed glass bottles within 20 min of milking during morning milking of a test day and immediately placed in a cooler box. Sampling procedure was repeated three times in same condition at three different test days. The collected milk samples were carried to the laboratory for biochemical and microbiological assay.

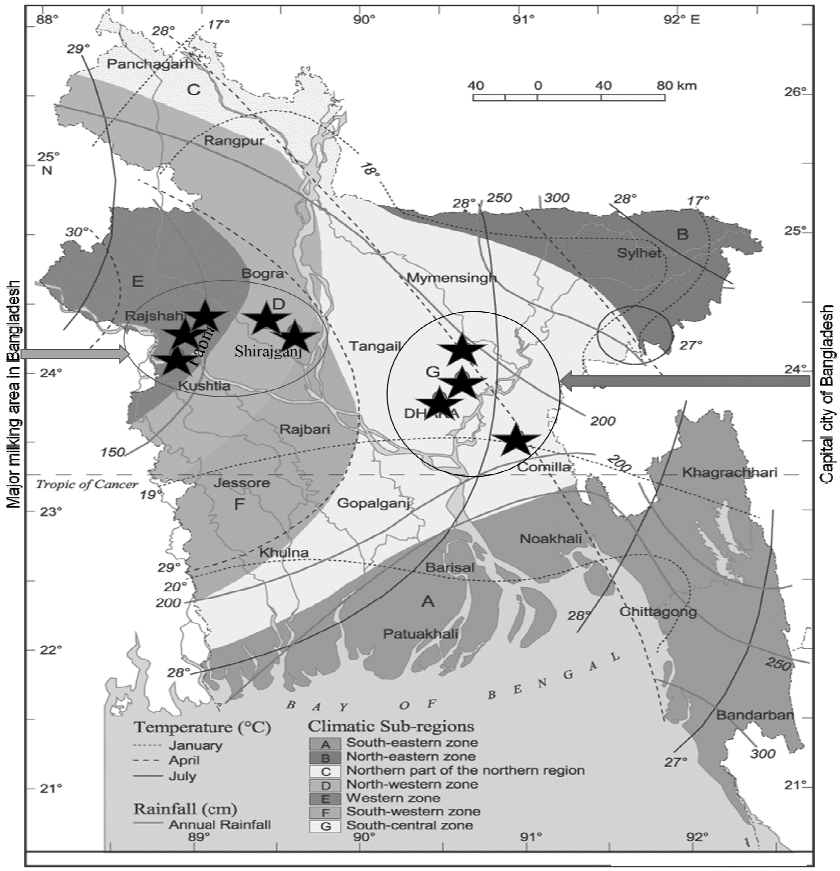

Fig. 1. Sampling areas in three study regions of Bangladesh

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Table 1 Geographic and environmental backgrounds of milk sampling areas

Region	Sampling sites	Geographic Location			Temperature during sampling period**	Rain fall (cm) during sampling period**
		Latitude (DD)*	Longitude (DD)*	Elevation (m)		
West	Ruppur, Pabna	24.063	89.051	22	22.7 - 34.2℃	77 - 308
	Borompur, Pabna	24.047	89.064	15.24		
	Faridpur , Pabna	24.167	89.445	17.84		
North-West	Baghabari, Shirajganj	24.135	89.584	11.99	22 - 34.6℃	74 - 315
	Ullapara, Shirajganj	24.319	89.562	12.32		
South-central	Daudkandi, Comilla	23.53	90.73	8.12	22.5 - 33.9℃	142 - 407
	Mohammodpur, Dhaka	23.75	90.35	4	23.4 - 34.1℃	141 - 388
	Ashulia, Dhaka	23.95	90.28	12		
	Gazipur, Dhaka	23.87	90.40	16		

*DD= Decimal Degree; ** Bangladesh bureau of statistics, 2015.

2.2 Biochemical analysis of whole milk

The collected cooled milk samples were kept in water bath first at 40℃ for ten minutes to liquefy the milk fat and unify the structure. The samples were then immediately cooled to the temperature of measurement (20℃). The percentage of protein, fat, solids-not-fat (SNF) and lactose of milk were determined by using the pre-calibrated Lactostar auto milk analyzer (Funke-Gerber, Berlin, Germany) which is a quick and reliable method to determine the constituent of milk. The measure resolution was 0.01% and the repeatability less than 0.04%. The measurement is based on combined thermo-optical procedure i.e. the milk sample (12 ml to 20 ml, adjustable) is pumped in measuring cells. The casein protein has been determined by precipitating method using ammonium sulphate [14]. Three replicate determinations were carried out on each sample for each method.

2.3 Microbial analysis of milk

The collected milk samples were analyzed for microbial status immediately after arrival at the laboratory. Total Viable Bacterial Count (TVBC) was enumerated by the Standard Plate Count (SPC) method following the method described by Sharp and Lyles [15]. Nutrient agar (Difco^{TM}, USA, pH 7.0-7.4) was used to determine TVBC as well as for isolation purposes. Appropriate dilution of raw milk sample was prepared using 0.9% Sodium chloride solution and pasteurized milk sample was directly inoculated on these media without any dilution. Thereafter, 100 µl of each sample was inoculated in respective culture media using sterile pipette and spreaded on medium using a sterile glass spreader for each sample. Plates were then kept in an incubator at 37ºC for 24-48 hours. Following incubation, plates exhibiting colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVBC. Microorganisms associated with milk sample were expressed as the number of organism of colony forming units per millilitre (cfu/ml). Similarly, Total coliform count (TCC), total *Staphylococcal* (TSC) and total *Listeria* count (TLC) was enumerated at 37ºC using MacConkey agar

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(Acumedia, USA) medium, Mannitol salt agar (Sigma-Aldrich) and Listeria selective agar media (Oxoid, UK) respectively. Viable cell counts (cfu/ml) were the average of at least three independent experiments.

2.4 Statistical analysis

All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. Data were analyzed by the SPSS.18 (Statistical Package for Social Sciences) software. Differences were considered significant at the level of P<0.05. The experimental data were subjected to multivariate statistical analysis to evaluate the canonical discrimination, box plot and correlation analysis of milk samples according to their origin.

3. RESULTS AND DISCUSSION

3.1 Biochemical composition of raw milk

Milk provides essential nutrients and is an important source of dietary energy, high-quality proteins, fats, and a significant contribution to the required intakes for calcium, magnesium, selenium, riboflavin, vitamin B12 and pantothenic acid. According to FAO [16], fat constitutes approximately 3 to 4 percent of the solid content of cow milk, protein about 3.5 percent and lactose 5 percent. In the present study, some important quality parameters of milk such as total protein, total casein, fat, lactose, and Solids-non-fat (SNF) contents of cow milk from different regions of Bangladesh were measured and are summarized in Table 2.

Table 2 Biochemical analysis (%) of cow milk collected from different regions of Bangladesh.

Region	Total Protein	Casein	Lactose	Fat	SNF
South-Central	3.67 ± 0.26[a]	2.53 ± 0.25[a]	5.32 ± 0.38[a]	3.84 ± 0.28[a]	9.78 ± 0.69[a]
West	4.06 ± 0.15[b]	2.91 ± 0.1[b]	5.9 ± 0.23[b]	3.29 ± 1.19[ab]	10.83 ± 0.4[b]
North-West	3.82 ± 0.13[a]	2.25 ± 0.12[a]	5.57± 0.2[a]	2.96 ± 0.52[b]	10.19 ± 0.38[b]

Results are given as the average values± standard deviation of three independent samples.
Different letters in the columns indicate statistically significant differences (*P*<0.05).

Dairy protein from cow's milk has been found predominantly consumed by humans, which consists of around 80% (w/w) casein, 20% (w/w) whey proteins. Robin et al. [17] reviewed that milk protein improve metabolic health by promoting changes in body composition in favor of increased lean body mass and decreased adiposity, particularly during energy-restricted weight loss. Also, branched chain amino acids, present in high levels in milk protein, enhance muscle protein synthesis, lean body mass and skeletal muscle metabolic function. The results revealed that milk from the studied regions has the total protein level (3.67 to 4.06%) as recommended by FAO indicating adequate milk quality for good nutritional status. However, Milk from West region of Bangladesh contains significantly higher content of total protein (4.06%) and total casein protein (2.91%) compared to other region. This might be due to the differences of availability of quality and quantity of roughage feeds throughout the year in studied regions. The

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sampling area, Pabna district within the Western region, is called as milk-shed area of Bangladesh because of having huge grazing lands providing green grasses and legumes to dairy animals especially indigenous Pabna breed that are popular for huge milk production [18]. A comparative study [19] showed that a good feed base region and genotype significantly influence milk protein content. Another study found average protein content as 3.70% in milk from areas of western and central region of Bangladesh respectively [20].

The predominant milk carbohydrate is lactose, which is involved in the intestinal absorption of calcium, magnesium and phosphorus, and the utilization of vitamin D [21, 22]. It appears that the enhancement of calcium absorption by the presence of lactose in the diet is due to a direct action of lactose either on the gut wall or within the intestinal lumen [23]. Lactose also provides a ready source of energy for the neonate. It has been found that milk from South-Central, Western and North-Western regions of Bangladesh has adequate lactose content and milk from the Western region has significantly higher (5.9%) lactose compared to other regions. This may be partly because of environmental factors, but some genetic variation within a breed must exist. Rahman et al. [20] found same amount of lactose (5.29%) in milk from Western and Central part.

Milk fat contributes about half of the energy in whole milk. For this reason, animal milk can play an important role in the diets of infants and young children in populations with a very low fat intake, where the availability of other animal source foods is limited. Moreover, Milk fat carries the fat soluble vitamins A, D, E, and K. The results showed that milk of north-west region has lowest fat content (2.96%) whereas the South-central region milk possess significantly higher level of milk fat (3.84%). The South-central region is the capital city and it is surrounding urban area where the green grazing field is limited and the feed diet was based on hay due to unavailable herbage. Thus increased feeding frequency of low fiber, high grain diets increase milk fat levels [24]. Swathi et al. [25] also reported higher milk fat content in urban areas than in rural areas of India suggesting that milk fat composition is influenced by feed types.

A standard level of Solids-not-fat (SNF) in milk has been defined as 8.25% by the Food and Drug Administration [26]. The obtained SNF contents in the raw milk samples collected from south-central, west and north-west zone of Bangladesh were 9.87, 10.83, and 10.19% respectively which were higher than FDA standard. The statistical analysis showed that SNF content of south-central zone is significantly lower than other areas.

The box plots analysis of milk components (Protein, fat, lactose, casein and SNF) represent the scales of variability within the dataset to assess data distribution according to different sampling areas (Figure 2). Milk fat content greatly varies within the study areas of west region with a median value of 2.60% meaning that 50% of the data is greater than this value. However, fat content of South Central and North West indicated lower deviation within the dataset. The content of total protein and casein in milk of west region has been found significant variation with a median value of 4.06% and 2.86% compared to other regions. This result suggests protein-rich milk of West region could provide daily recommended dietary allowances for protein to the child for their growth and adults to maintain good health of that region. It is

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notable that the content of lactose and SNF in milk also have been found to be higher in Western region of Bangladesh indicating good quality milk as well as health impact for the people of this area and wherever supplied.

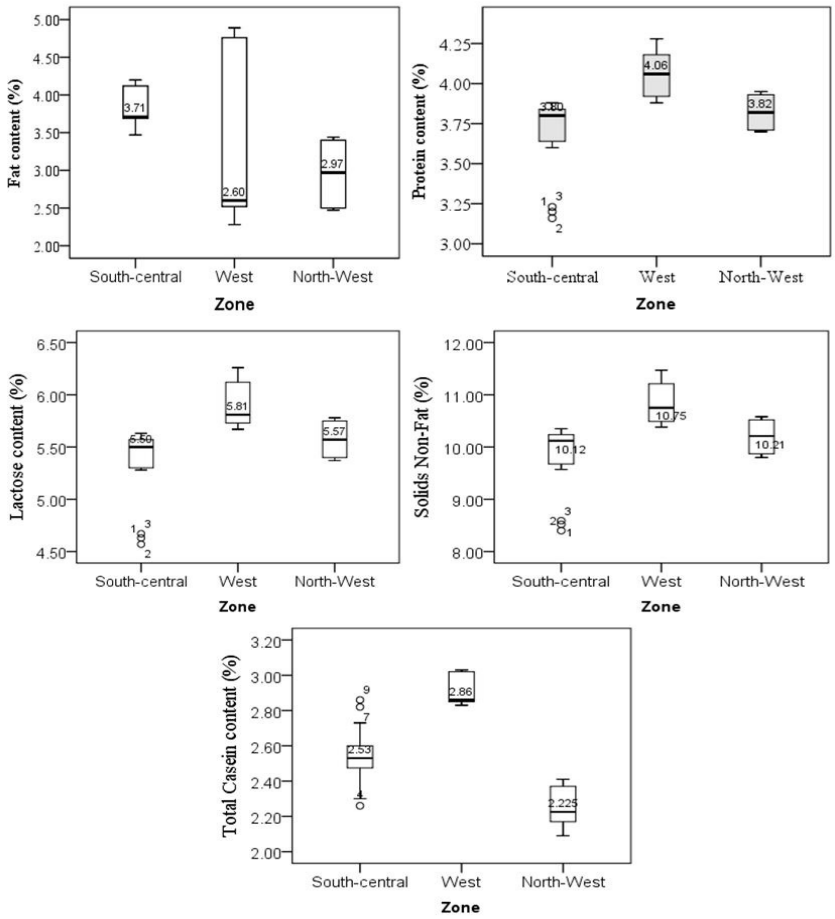

Fig. 2 Box plot statistics of raw milk constituents. The boxes represent the range and the horizontal line inside the box represents the median of the individual value of milk component.

3.2 Correlation analysis of milk constituents

The data of milk components (fat, protein, lactose, SNF and casein) was analyzed in terms of Pearson's logarithmic correlation (<0.05 and <0.01) to indicate the strength level of the correlation among various milk components. The results of the correlation coefficient as presented in Table 3 showed the inter-relations of one component with another at different levels of significance. Strong correlations between some variables out of composition of the milk suggest possible influence on milk quality of the studied regions of Bangladesh. It has been found that milk protein was significantly correlated with the lactose (<0.01), SNF (<0.01) and casein (<0.05) content. Similarly milk lactose is significantly correlated with SNF and casein. The correlation coefficient between milk constituents are 0.996 for protein-lactose, 0.999 for

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protein-SNF, 0.438 for protein-casein, 0.998 for lactose-SNF, 0.445 for casein-SNF. Milk fat was found to be negatively correlated with other constituents of milk. Since, SNF by definition are the total solids other than fat portion, it is logical to have positive correlation between milk SNF with protein, lactose and casein content. Simultaneously, milk fat has been found to be negatively correlated with the SNF content of milk samples. On the other hand Iuliana et al.[27] found that the fat content showed highly positive correlated with protein content, total solids, and casein content. The variation may be due to milk samples being collected from different breed groups and farms and also management practices were different.

Table 3 Correlation coefficients between milk constituents of different regions in Bangladesh

Milk parameter		Fat	Protein	Lactose	SNF	Casein
Fat	Pearson Correlation	1	-.170	-.242	-.212	-.097
	Sig. (2-tailed)		.368	.197	.260	.610
Protein	Pearson Correlation	-.170	1	.996**	.999**	.438*
	Sig. (2-tailed)	.368		.000	.000	.016
Lactose	Pearson Correlation	-.242	.996**	1	.998**	.438*
	Sig. (2-tailed)	.197	.000		.000	.016
SNF	Pearson Correlation	-.212	.999**	.998**	1	.445*
	Sig. (2-tailed)	.260	.000	.000		.014
Casein	Pearson Correlation	-.097	.438*	.438*	.445*	1
	Sig. (2-tailed)	.610	.016	.016	.014	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

3.3 Regional discrimination of cow milk by biochemical quality

Statistical comparison of the constituents of milk sampled from a variety of sources has contributed to the geographical characterization of milk. Canonical Discriminate analysis was applied to the milk constituents (protein, lactose, casein, fat, and SNF) in order to classify milk samples into groups according to their geographical origin. Scores plot (Figure 3) showed good regional separation, suggesting that these five components contained sufficient information to assess the geographical origin of milk that can play role in public health effects of those studied areas. The values for milk collected in South-central region were dispersed broadly, whereas those of West and North-west region samples converged within a very small region. Several studies [28,29] have effectively used combined chemical and statistical analyses to determine the geographic origin of milk and milk products. Matteo et al. [30] identified milk origin based on the discriminant analysis using the isotope ratio of $\delta^{13}C$ and $\delta^{15}N$ for the milk samples and their fractions (fat, casein, and whey).

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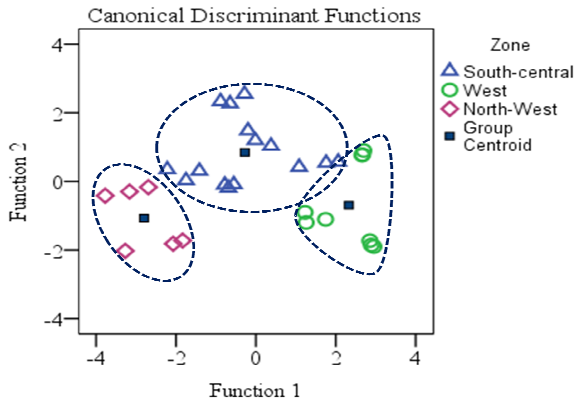

Figure 3. Scatter plot for discrimination among geographical regions using milk constituents (Protein, lactose, casein, fat and SNF). Function 1 represents 81.5 % of variability, while function 2 represents 18.5 % of variability.

3.4 Microbiological evaluation of cow's raw milk

Milk is highly perishable due to microbial contamination. Raw milk can be contaminated with pathogens, either directly through organisms or indirectly. Indirect contamination may be due to some reasons which are (i) the udder and teats may be contaminated by a cow's own fecal matter or fecal matter of other cows (ii) milking clusters contacting surfaces with fecal contamination and (iii) environmental contamination. Therefore, in the present study microbial analysis was carried out to evaluate the level of microbiological quality of raw milk samples collected from different areas of South-Central, West and North-West region of Bangladesh. The total viable bacterial counts (TVBC), total coliform counts (TCC), total *Staphylococcal* counts (TSC), and total *Listeria* counts (TLC) were shown in Table 4.

Table 4 Microbiological quality analysis of raw milk samples collected from three different zones in Bangladesh

Types of Microorganisms	South –central Zone		West Zone		North-west Zone	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
TVBC	3.4×10^3	6.91×10^5	5.76×10^2	2.23×10^4	5.77×10^2	7.133×10^3
TCC	4.7×10^1	2.3×10^4	<1	1.49×10^2	1.12×10^2	1.18×10^2
TSC	1.0×10^1	5.12×10^5	2.07×10^2	1.112×10^3	1.79×10^2	2.39×10^2
TLC	6.3×10^1	4.84×10^5	9.2×10^1	6.250×10^3	7.39×10^2	7.41×10^2

Results are given as the average values of three independent samples.

Total bacterial count was ranged from 5.76×10^2 to 6.91×10^5cfu/ml in raw milk samples from three studied regions. South-central region showed highest bacterial load (3.4×10^3 to 6.91×10^5cfu/ml) which was higher than the recommended value (not exceeding 2×10^4 cfu/ml) of TVBC by Bangladesh Standard and Testing Institution and United States Public Health Service [31,32]. Dairy farms of South-Central region located mainly in industrial zone accommodates the highest number of garments, textile

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mills, tannery and leather industries, chemicals and fertilizers industries still discharge wastes into the nearby water bodies and soil. The discharge of industrial effluent into surface water increases its nutrient stock leads to enhancement of the microbial growth [33]. Moreover, the teat surface is the major path of entry for microorganisms into raw milk. It is also reported that teats might be contaminated by feces and soil in the form of dust or mud [34,35]. However, cattle of South-Central region might be exposed to the contaminated water and soil resulted in highest microbial load in raw milk. Banik et al.[36] found high bacterial load (ranged from 1.3×10^{7} to 5.2×10^{8} cfu/ml) in raw milk samples collected from daily markets of different regions of Bangladesh.

The incidence of coliform in raw milk has gained considerable attention due to their association with contamination of fecal origin, hence it is defined as an hygienic indicator to reflect the general microbiological quality in routine test [37]. In the present study the average coliform count ranged from <1 to 2.3×10^{4} cfu/ml in milk from three different studied regions. South-Central region showed the highest coliform count (4.7×10^{1} to 2.3×10^{4} cfu/ml) compared to North-West and West region which indicated the poor sanitary practices during milking and further handling processes [38]. The standard set maximum amount of coliform bacteria in raw milk is not more than 10 bacteria per milliliter of raw milk. This level is consistent with both national and international public health and food safety requirements[39]. The total coliform counts were too high for all the studied samples. Banik et al. [36] found average coliform count in the raw milk from daily markets of different regions of Bangladesh ranged from 1.0×10^{4} to 2.7×10^{5}cfu/ml which were comparatively higher than the present study.

In the present study TSC count was ranged from1.0×10^{1} to 5.12×10^{5} cfu/ml in South-Central region which was maximum compared to West and North-West region (2.07×10^{2} to 1.112×10^{3} and 1.79×10^{2} to 2.39×10^{2} cfu/ml respectively). Lowest range of TSC count was found in North-West region, it might results from the relatively better hygienic status due to organized dairy farm production system, use of relatively better milking utensil and better quality of drinking water. The presences of *Listeria* spp. cause listeriosis which is a major cause septicemia, meningitis and encephalitis in the infected person [40]. According to the Table 4, highest range of TLC was found in raw milk collected from South-Central region. Poor quality of silage, inadequate frequency of cleaning the exercise area, poor cow cleanliness, insufficient lighting of milking areas might be associated with milk contamination by Listeria.

The entire samples tested were exposed to microbial contamination and they were handled under unhygienic conditions which pose health risk to consumers. Based on these findings, it is strongly recommended that people should desist from taking raw milk. Also, the milk producer should be educated on proper personal and environmental hygiene. However, pasteurization is required and proper refrigeration temperature should be maintained prior consumption of milk for preventing human health hazards.

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4. CONCLUSION

It was revealed that milk from West region showed significantly higher content of protein, casein; lactose and SNF compare to other two regions (South-Central and North-West) indicated good nutritional status. But all milk samples from three studied regions were found to be exposed to different microbial contamination and they were handled under unhygienic conditions which pose health risk to consumers. Safety measurement to minimize the microbial contamination should be practiced according to food safety regulation. The present study has strongly indicated that combining biochemical analysis results with statistical analysis provides an excellent prospect for discriminating the geographic origin of raw milk. Canonical Discriminate analysis of the milk constituents (protein, lactose, casein, fat, and SNF) showed good regional separation among three studied regions of Bangladesh. The study would be useful to analyze more diverse dairy products in the future could lead to more effective determination of geographic origin.

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