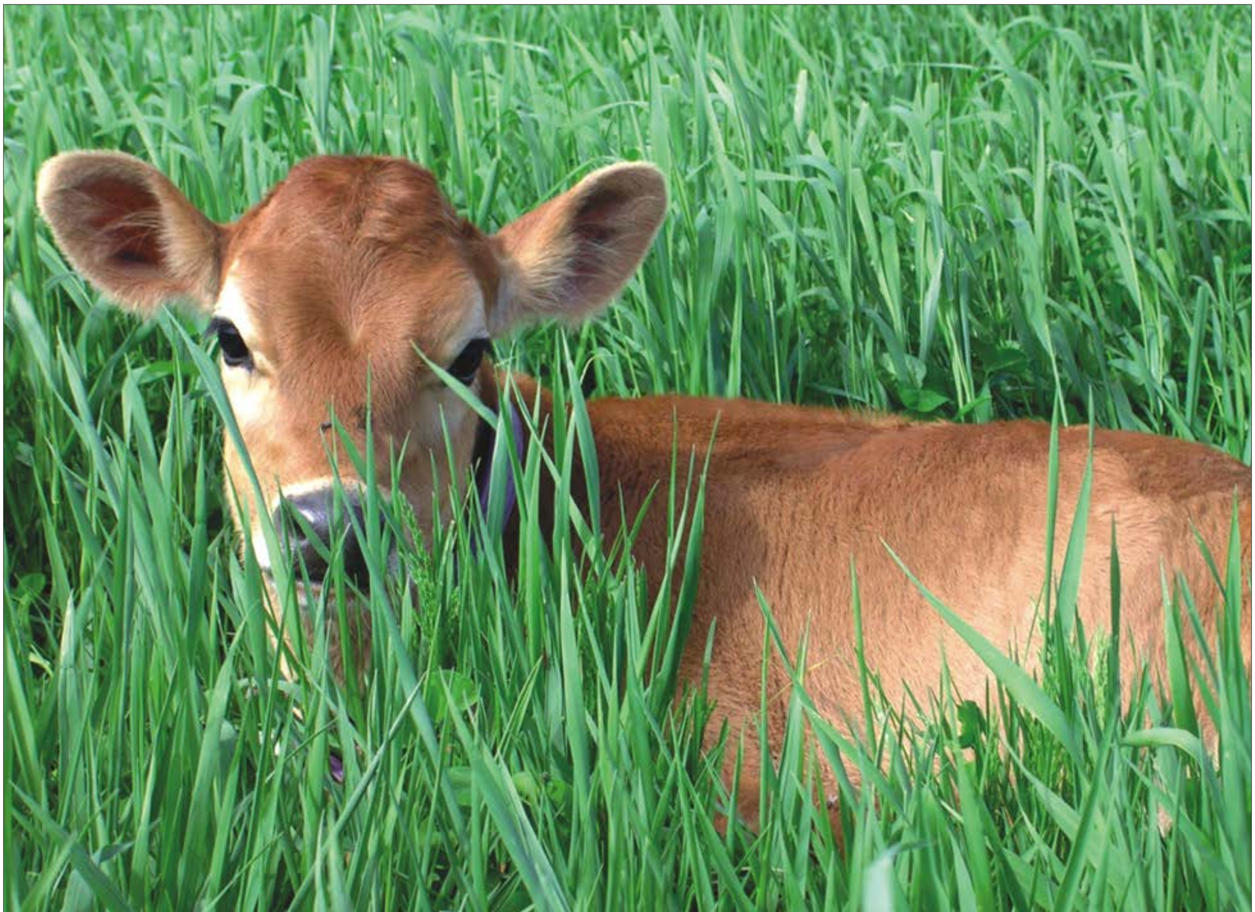

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Rapid Estimation of Quality of Raw Milk for its Suitability for Further Processing in the Dairy Industries of Bangladesh

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ABSTRACT

Milk is prone to rapid microbial spoilage because of having high nutrients. Improper or delayed chilling or long term storage may lead to increased growth of bacteria in raw milk, causing spoilage of milk products. Due to high perishable nature, rapid reception and processing of raw milk is mandatory in the processing factory. Instead of expertise dependent, lengthy, laborious, expensive direct microbiological methods (e.g. standard plate counts, coliform bacterial counts and direct microscopic counts); rapid, simple and inexpensive quality determination methods including physical, chemical tests and Methylene Blue Reduction Time (MBRT) test could be opted. Little interlinked data on physical, chemical and MBRT in relation to raw milk quality are available. This study accordingly estimated the quality of raw milk for its suitability for further industrial processing. Physical examinations including color, flavor, taste and specific gravity (1.030±0.001), chemical and indirect microbiological tests including fat, solids-not-fat, total solids, water, acidity, pH contents and MBRT, respectively revealed their acceptable standard limit. While the yearly average fat, solids-not-fat, total solids and water content were 3.98, 7.4, 11.38 and 88.62%, respectively along with the highest fat percentage 4.06 in the autumn and the lowest 3.83 in the summer season which were not varied significantly ($p<0.05$). On the other hand, the yearly average acidity, pH and methylene blue reduction time were 0.137%, 6.84 and 56.75 min, respectively along with similar dye reduction times in different seasons. The results obtained in this study satisfied close to the minimum standard of raw milk for further industrial processing.

Key words: Raw milk, physical and chemical quality, MBRT, industrial processing

INTRODUCTION

Milk is the nature's single most nearly complete food in the world. Bangladesh is a small milk producing country in the world whose domestic milk production is not sufficient to meet up the national demand. The major volume of milk comes from the milk pocket zones of Bangladesh which is developed by Bangladesh Milk Producers' Co-operative Union Limited (BMPCUL)-popularly known as Milk Vita. In addition to Milk Vita, raw milk collection, processing and marketing throughout the country have been carried by a number of companies likely PRAN (Programme for Rural Advancement Nationally) Dairy Ltd., BRAC (Bangladesh Rural Advancement Committee) Dairy and Food Project, Aftab Milk and Milk Products Ltd., Bikrampur Dairy Ltd., Akij Food and Beverage Limited. Therefore, hundreds of raw milk collection and chilling centers have been established and operated in the milk pocket zones of Bangladesh by these Dairy companies.

Milk is produced in rural or peri-urban areas of the country and transported from point of production to the neighboring chilling centers of different dairy companies of Bangladesh. Dairy cows suffering from subclinical mastitis used to excrete pathogens in raw milk but milk of healthy animal's udder is free from pathogenic bacteria (FAO, 1989). Consequently, pooled milk gets contaminated after mixing with such kind of milk containing pathogens.

Over and above, microbial contaminants in raw fresh milk mainly come from dirty or poorly disinfected milking utensils, environment, animal skin, dirty teat surface, milker's hand, flies, untreated water supplies or adulteration with untreated water etc. (FAO, 1989). Even though sterile milk is let down from the udder of healthy animals, but in practice contamination of the raw milk with ranged from 10^2-10^3 microorganisms/mL is unavoidable. This might be due to the microbial residents in the interior of the udder or in the teat canal (Heeschen, 1996).

The magnitude of microbial loads in raw milk is mainly influenced by contamination during milking, storage temperature and on the time elapsed between milk production and collection. Unhygienic milking, inefficient storage practice will allow higher microbial growth in raw milk as it is highly rich growth medium of microbes (Ensminger, 1993; Nooruddin *et al.*, 2006). Transportation of raw milk from point of production to the collection centers at ambient temperature might leads to high microbial growth, causing quality deterioration of raw milk (Schmidt and Van Vleck, 1974). In principle, higher microbial loads in raw milk are mainly responsible for quality defects in Pasteurized milk (Blankenagel, 1982), UHT milk (Bjorck, 1973; Law *et al.*, 1977; Richardson and Newstead, 1979), dried skimmed milk (Muir *et al.*, 1986), butter (Antila, 1982) and cheese (White and Marshall, 1973; Mohamed and Bassette, 1979). Moreover, both living bacterial population and enzymes including heat resistant proteinases (Fairbairn and Law, 1986) and lipases (Downey, 1980) may also be responsible for the spoilage of dairy products. For these reasons, the standard limit of microbial counts in raw milk has been set by the European Community (EC) at 1×10^5 CFU mL^{-1} or 4×10^5 CFU mL^{-1}, according to the dairy product to be manufactured from it.

Estimation of microbial load in raw milk is highly recommended in relation to its keeping quality. Several laboratory methods to determine bacterial load in raw milk including Direct Microscopic Count (DMC), Most Probable Number (MPN) and Standard Plate Count (SPC) can be opted which are expensive, lengthy and expertise dependent. Consequently, the attention in rapid methods for microbial examination of milk has been growing gradually in the last few decades (Fung, 1995, 1991). Currently a number of instrument aided methods are frequently employed for rapid estimation of bacterial load following metabolic activity, light scattering, electrical impedance, chemiluminescence and fluorescence (Pettipher, 1993; Spencer *et al.*, 1994; Vanderzant and Splittstoesser, 1992) e.g. direct epifluorescent filter technique (Champange *et al.*, 1997) for determination of viable bacterial population in raw milk and detection of metabolic changes in microorganisms in raw milk during incubation (Shelef and Eden, 1996).

However, the need for rapid, simple and economical methods for assessing the quality of raw milk still subsists. The simplest and economic dye reduction tests e.g. Methylene Blue Reduction Time test (MBRT) are still convenient means of detecting the metabolic activity of microorganisms in a relatively short time for grading of raw milk in the commercial dairies of the developing countries (Edmondson *et al.*, 1985; Harding, 1995). Nevertheless, the dye reduction methods are favored over more other precise methods because they are easy to perform and economical indeed and also suitable in rapid grouping of raw milk samples into classes or grades (Dabbah *et al.*, 1967). For example, raw milk can be graded into good and excellent grades based on methylene blue reduction time of 2 to 5 and more than 5 h, respectively and reduction time of around 1 h is considered to be suitable for further processing in the developing tropical and sub-tropical countries (Kotaratititam, 1995). In contrary to other countries milk produced in Bangladesh could be categorized under close to poor grade. Climatic condition, unhygienic milking, flies, disease, improper handling, improper sanitation and cleaning of utensils, together milk itself enriched media, facilitate growth and yield of higher number of bacteria, even the highest number of bacterial load/ml of raw milk in the world (Chanda *et al.*, 2008). Little information is available on the rapid determination of raw milk in the rural dairies for further industrial processing particularly about the convenient methods. Therefore, the study was aimed to rapid determination of physical, chemical and microbiological quality of raw milk for further processing based on some simple, faster and economic methods.

MATERIALS AND METHODS

Study area: Baghabari, Bera, Selonda, Demra of Pabna district and Vatara area of Jamalpur district were selected for this study which are recognized as milk pocket zones of Bangladesh. The Dairy cattle population of these areas is principally composed of local/native cows called Pabna cattle and cross breed of Holstein Friesian and native cows. Farmers were selected and brought under an agreement that they will supply milk to keep smooth flow of milk to the factory which is refereed as contract farmers. These areas are covered with Jamuna River, its branches and submerged under water up to 4 months in a year. During winter, vast areas of these regions are used as pasture land in this period where the dairy cattle are reared under free range system. The farmers those who have more than 100 liters of raw milk production per day and can ensure constant flow of raw milk to the company were selected and contracted (Fig. 1).

Collection of raw milk: Milk Vita and other private dairies including PRAN, BRAC Dairy, Bikrampur Dairy and Aftab Milk and Milk Products Ltd., Shilaidah Dairy and Akij Food and Beverage Ltd. have collected raw milk from the selected areas. The farmers of these regions are more apt in dairy cattle management than the farmers of other areas of the country. This is due to the provision of better animal health care and technical support by the Milk Vita and other private dairy industries of these areas.

However, they failed to maintain standard milking system due to illiteracy of the farmers, lack of awareness about the prevention of microbial contamination of milk and lack of training about the health and hygiene. Milk Vita used to collect milk from its members as the organization run by cooperative system. But the private dairies used to have milk from the contract farmers who are or are not the members of the Milk Vita. Farmers used to perform milking following the traditional method. Then milk is brought to the neighboring chilling centers by loading the milk can through travelling around 10 miles away by rickshaw or van and sometimes by motor vehicles with or

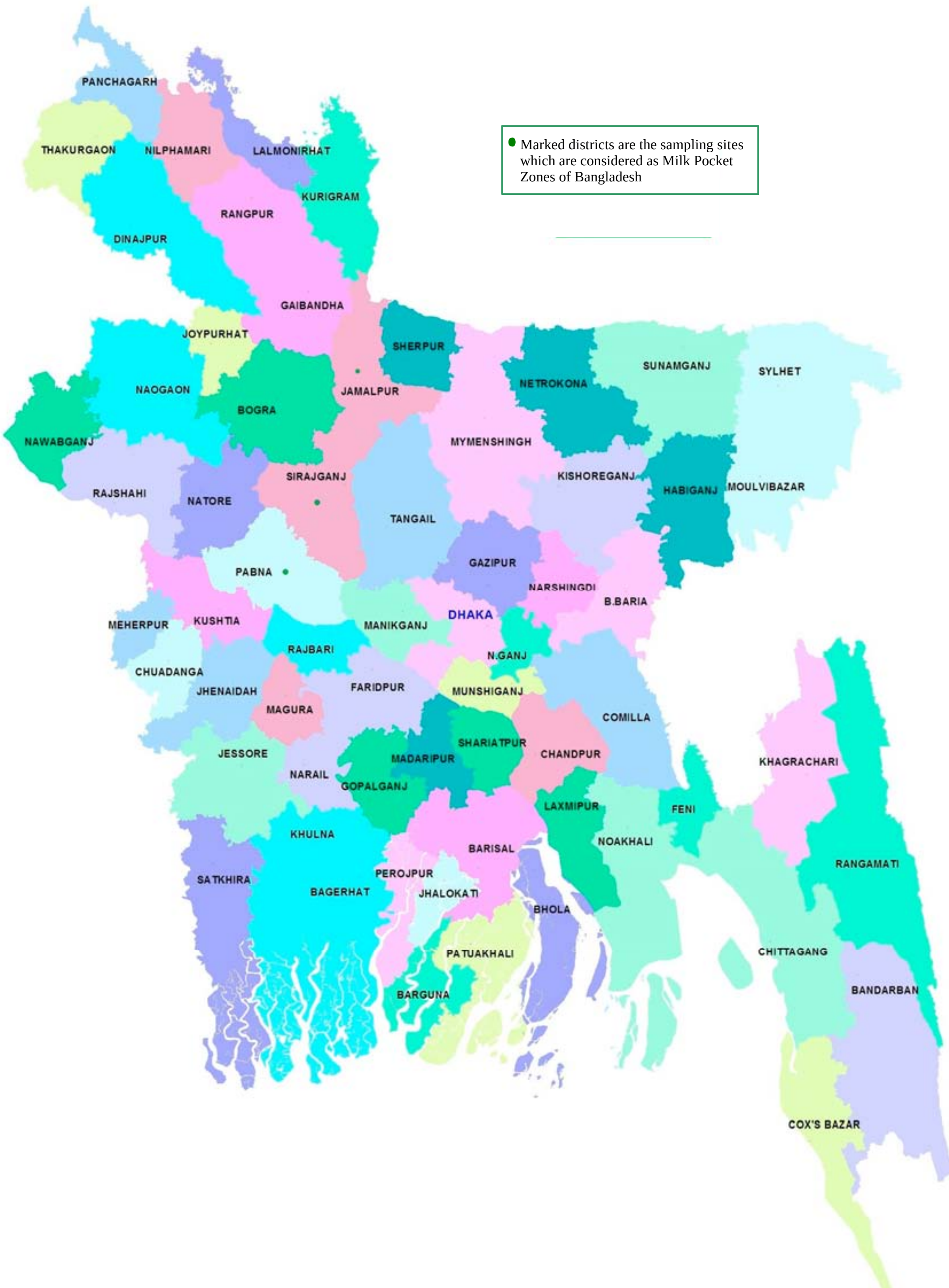

Fig. 1: Study areas (Milk Pocket Zones) indicating in the Map of Bangladesh

without any preservatives. Thus, they accumulate milk in a large vat from different cows of different farmers' house. Raw milk is received by the quality controller based on organoleptic tests and sometimes on the basis of physico-chemical parameters. The price is determined based on fat content of raw milk. Then all the collected milk is being allowed to chill at 4°C in the chilling center. From the chilling center, all of the collected milk brought to the factory by insulated road milk tanker to the factory. The test samples were collected from the road milk tanker by sterilized beaker and dilution bottle both for physico-chemical and indirect microbiological tests and brought under different physico-chemical and indirect microbiological tests immediately.

Samples: A total of 365 mixed samples were collected from the road milk tanker by sterilized beaker and dilution bottle and brought under different tests for reception and commence for processing immediately.

Physico-chemical study

Physical study: Organoleptic tests were carried out with different sensory organs of man which are eyes, tongue and nose. Of which Color, flavor and taste of raw milk were evaluated with the help of eyes, nose and tongue.

Chemical tests: The chemical tests like Alcohol Precipitation Test (APT), Clot on Boiling (COB) test, fat test, acidity test, pH test, Corrected Lactometer Reading (CLR) test and Solids-not-fat (SNF) calculation by using fat% and CLR were done following the instruction of standard methods for the determination of dairy products (Wehr and Frank, 2004).

Microbiological study: Milk is the enriched media for the growth and multiplication of micro-organism. Therefore, for the sake of public health it is important to observe the magnitude microbial load in the raw milk. As Standard Plate Count (SPC) and Coliform Count (CC) are time consuming to interpret about the quality of milk, the rapid method Methylene Blue Reduction Test (MBRT) were carried out following the instruction of the standard methods for the determination of dairy products (Wehr and Frank, 2004).

Adulterants determination: Hydrogen per oxide, sodium hydroxide, formalin, sugar, starch, urea and gelatin were determined in the raw milk following the method described by Wehr and Frank (2004).

Data analysis: Data were analyzed using the statistical package Statistical Program for Social Studies (SPSS). confidence interval at 95% was considered as level of significance (Zar, 1984).

RESULTS AND DISCUSSION

In this study, physical properties of raw milk were found to be satisfactory. On the other hand, the commercial parameters including fat%, SNF%, TS% and water% satisfied the recommended standard of raw milk. Indirect microbiological evaluation based on acidity%, pH values and methylene blue reduction time revealed that raw milk of rural dairies of Bangladesh can be classified as close to the poor grade. Moreover, all of the raw milk samples tested in this study were free from all kinds of adulterants including Hydrogen per oxide, sodium hydroxide, formalin, sugar, starch and urea.

Physical parameters: The color of all milk samples collected from the different regions of Bangladesh was normal (Whitish) which is mainly depends on the breed, the level of fat and solid present and in most cases the nature of feed consumed by Eckles *et al.* (1951). The occurrence of normal flavor of all raw milk samples might be influenced by rapid chilling, prompt preservation and quick transportation and rapid examination of raw milk samples without lingering. In addition, the cows might have not been allowed eating some sorts of odd flavored feed prior to milking them.

The results obtained from this study corroborate findings in other similar studies (Islam *et al.*, 1984; Chanda *et al.*, 2007). The taste of all collected milk samples was found slightly sweet which is similar to the finding of Islam *et al.* (1984) and Chanda *et al.* (2007). The average mean and standard deviation of specific gravity of milk samples were 1.030±0.001 (Table 1). Statistical analysis showed that the difference among specific gravity of milk of the test samples was not significant ($p<0.05$). Islam *et al.* (1984) reported that the mean of specific gravity of milk of Bangladesh Agricultural University (BAU) dairy farm was 1.031 and milk from local market was 1.026. Similarly, Yadav and Sarawat (1982) reported a lower specific gravity in market milk of Varansi town, India. Chanda *et al.* (2007) also reported lower specific gravity in market milk of Chittagong city of Bangladesh. Filliptovic (1953) obtained the specific gravity of 1.032 for cow's milk in Yugoslavia. Commercial industries used to accept raw milk with minimum specific gravity of 1.025.

Chemical and microbiological parameters

Month wise variation of Fat, SNF, Total solids and water contents: The average fat% was found 3.98% (3.98±0.20) which is non-significant and much lower than Jebu and Taurine cattle like Red Sindhi (4.9%), Gir (4.73%), Tharparker (4.55%), Sahiwal (4.55%) and Jersy (5.37%). It is also lower than Crossbred cows (4.5%) but higher than the Friesian (3.4%) cows (ICAR, 2002) (Table 2). The highest fat% was found 4.19% (4.19±0.18) in January and the lowest was found 3.78% (3.78±0.26) in April both of which displayed similarity with the aforesaid results (Table 2).

The average SNF% was found 7.4% (7.4±0.09) which is non-significant and much lower than the milk of Jebu and Taurine cattle like Red Sindhi (8.76%), Gir (8.67%), Tharparker (8.7%),

Table 1: Observed physical qualities of raw milk samples (average)

Physical parameters	Color	Flavor	Taste	Specific gravity
Observations	Whitish 100 %	Normal flavor milky 100%	Slightly sweet 100%	1.030±0.001
Level of significance	ND[a]	ND[a]	ND[a]	NS[b]

[a]ND = No difference, [b]NS = No significance

Table 2: Monthly status of fat%, SNF%, Total Solids (TS) % and Water% of raw milk

Month	Fat %	SNF %	TS %	Water %
January	4.19±0.18	7.38±0.12	11.57±0.51	88.43±0.70
February	4.03±0.19	7.38±0.09	11.41±0.52	88.59±0.65
March	3.93±0.21	7.33±0.09	11.26±0.49	88.74±0.55
April	3.78±0.26	7.32±0.13	11.11±0.48	88.90±0.68
May	3.84±0.25	7.42±0.06	11.26±0.41	88.74±0.38
June	3.88±0.19	7.41±0.03	11.29±0.53	88.71±0.35
July	3.94±0.25	7.45±0.09	11.39±0.36	88.61±0.41
August	3.93±0.19	7.43±0.06	11.36±0.42	88.64±0.82
September	3.98±0.19	7.43±0.06	11.41±0.53	88.59±0.52
October	3.91±0.18	7.31±0.19	11.22±0.32	88.78±0.39
November	4.11±0.16	7.48±0.09	11.59±0.61	88.41±0.35
December	4.17±0.15	7.45±0.09	11.62±0.65	88.38±0.51
Average	3.98±0.20	7.40±0.09	11.38±0.48	88.62±0.52

Values are Mean±SD

Sahiwal (8.82%), Jersy (9.54%), Friesian (8.86%) and Crossbred (8.63%) cows (ICAR, 2002) (Table 2). The highest SNF% was found 7.48% (7.48±0.09) in November and the lowest was found 7.31% (7.32±0.19) in October (Table 2).

The average total solids and water contents were found 11.38% (11.38±0.48) and 88.62% (88.62±0.52) which is lower and higher than the milk of Jebu and Taurine cattle like Red Sindhi (13.66%), Gir (13.30%), Tharparker (13.25%), Sahiwal (13.37%), Jersy (14.91%), Friesian (12.26%) and Crossbred (13.13%) in respect of Total Solids and Red Sindhi (86.34%), Gir (86.7%), Tharparker (86.75%), Sahiwal (86.63%), Jersy (85.09%), Friesian (87.74%) and Crossbred (86.87%) in respect of water% (ICAR, 2002) (Table 2).

Season wise variation of Fat, SNF, Total solids and Water contents: Seasons have had influence on the milk composition where the highest fat% was found 4.06% (4.06±0.16) in the autumn and the lowest has been found 3.83% (3.83±0.23) in the summer. The fat percentage starts to fall gradually from the autumn till the early period of summer. Then it starts again increasing steadily, showing a remarkable rise in the September (Tables 2, 3).

The highest SNF% has been found 7.43% (7.43±0.07) in the rainy Season and the lowest has been found 7.36% (7.36±0.10) in the winter season. Regular seasonal variations have also been reported in the raw milk's content of lactose, ash and citric acid (SNF), but these variations seemed to be comparatively little compared to those of fat and protein. The average citric acid and ash content in milk generally varied over the year at the rate of a couple of hundredths of a percent.

The highest total solid was 11.48% (11.48±0.52) in the autumn and the lowest was found 11.23 (11.23±0.47) in the summer (Table 3). The highest Water% was found 88.79% (88.79±0.47) in the summer and the lowest was found 88.53% (88.53±0.41) in the autumn (Table 3). By and large, these variations generally repeat themselves in the following years, but the variations of fat and SNF contents might differ from one year to another depending on weather and feed.

Month wise variation of Acidity, pH and MBRT of raw milk: The average acidity and pH was found 0.137% (0.137±0.008) and 6.84 (6.84±0.02) which were within the acceptable range while the average MBR time 56.75 minutes (56.75±18.25) (Table 4). However, the highest acidity and lowest pH were found 0.143% (0.143±0.005) and 6.83 (6.83±0.019) in May and the lowest acidity and highest pH were found 0.135% (0.135±0.012) and 6.85 (6.85±0.005) in the month of December.

The development of acidity in milk depends upon the number of available lactic acid and other acid producing bacteria like *Lactobacillus* spp., *Pseudomonas* spp., *E. coli, Streptococcus* spp., *Staphylococcus* spp., Yeasts and Moulds. Improper chilling of raw milk may lead to development of higher acidity and lower pH in raw milk due to increased growth and multiplication of microbes there. While (May) the highest acidity (0.143%) and the lowest pH (6.83)

Table 3: Fat %, SNF %, Total Solids (TS) % and Water% on the basis of season

Season	Fat%	SNF%	TS%	Water%
Winter (Jan.-March)	4.05±0.19	7.36±0.10	11.41±0.50	88.59±0.63
Summer (April-June)	3.83±0.23	7.38±0.07	11.23±0.47	88.79±0.47
Rainy (Jul.-Sept.)	3.95±0.21	7.43±0.07	11.38±0.43	88.61±0.58
Autumn (Oct.-Dec.)	4.06±0.16	7.41±0.12	11.48±0.52	88.53±0.41
Average	3.98±0.20	7.40±0.09	11.38±0.48	88.62±0.52

Values are Mean±SD

Table 4: Reduction time of methylene blue reduction time (MBRT) test, acidity and pH of raw milk on the basis of months

Months	MBRT	Acidity (%)	pH
January	59.11±18.20	0.136±0.002	6.85±0.122
February	56.53±19.00	0.137±0.003	6.83±0.009
March	57.30±18.00	0.136±0.006	6.84±0.009
April	56.42±17.20	0.137±0.008	6.84±0.009
May	55.47±18.20	0.143±0.005	6.83±0.019
June	55.42±19.00	0.136±0.012	6.83±0.018
July	67.25±22.20	0.137±0.002	6.84±0.007
August	54.88±18.00	0.136±0.015	6.84±0.005
September	49.91±16.20	0.137±0.008	6.84±0.008
October	49.00±16.00	0.138±0.008	6.85±0.027
November	57.36±18.00	0.136±0.015	6.84±0.004
December	62.37±19.00	0.135±0.012	6.85±0.005
Average	56.75±18.25	0.137±0.008	6.84±0.020

Values are Mean±SD

Table 5: Reduction time of methylene blue reduction time (MBRT) test, acidity and pH of raw milk on the basis of season

Seasons	MBRT	Acidity (%)	pH
Winter (Jan.-March)	57.65±18.40	0.136±0.0037	6.83±0.047
Summer (April-June)	55.77±18.13	0.139±0.0083	6.83±0.015
Rainy (Jul.-Sept.)	57.35±18.80	0.136±0.0083	6.84±0.007
Autumn (Oct.-Dec.)	56.24±17.66	0.136±0.0117	6.84±0.012
Average	56.75±18.25	0.137±0.0080	6.84±0.020

Values are Mean±SD

then MBR time was found 55.47 minutes (55.47±18.20) which was the lower reduction time among the other months. And while (December) the lowest acidity (0.135%) and the highest pH (6.85) then the MBR time 62.37 minutes (62.37±19.00) which was the highest reduction time in the year (Table 4).

Season wise variation of acidity, pH and MBR time of raw milk: The microbial load, acidity, pH of raw milk has been influenced by the season where the highest acidity and the lowest pH have been found 0.139% and 6.83 in the summer. Because, in the summer the MBR time was found 55.77 (55.77±18.13) min (Table 5). Though the similar acidity and pH have been observed in rainy, autumn and winter season, but the MBR time slightly differs among different seasons.

Adulterants determination: Determination of adulterants revealed in the absence of adulterants such as Hydrogen per oxide, sodium hydroxide, formalin, sugar, starch, urea and gelatin in the raw milk because of rejection of adulterated milk in the collection centers. Adulterants referred that those agents are added in raw milk with an aim to enhance self-life, increase specific gravity or density and volume. This has been practiced by the farmers from the commercial point of view not taking into account of public health issues. Obviously, the adulterants like Hydrogen per oxide, sodium hydroxide, formalin, urea and gelatin are harmful for human body which should be strictly monitored.

As milk is the most perishable product, essential food for infants and olds, it requires rapid reception, processing, packaging and to ensure constant availability in the market. Therefore, convenient and rapid method for the determination of milk quality is essential. Our study explored the chemical composition and the acceptable values of methylene blue reduction test, acidity, pH and other chemical tests and their relation which is acceptable to determine the raw milk quality for reception and further processing satisfying the minimum standard particularly in the rural dairies of the developing countries. Furthermore, quick adulterants determination will ensure supply of safe milk to the consumers. This will be a guideline for determining the quality of raw milk in the rural dairy industries of the developing countries.

CONCLUSION

In conclusion, it can be clearly outlined that commercial and hygienic quality of raw milk determination is crucial prior to reception of raw milk for further processing. Due to high perishable nature, rapid estimation of physical, chemical and microbiological quality of raw milk using fast, cheap and less expertise dependent methods e.g. MBRT has gained remarkable acceptance in contrast to other costly, time-consuming and high skill dependent methods. Therefore, Methylene Blue Reduction Time (MBRT) test can be opted for rapid determination of raw milk quality. Microbiological, Physical and Chemical qualities determined by using MBRT along with other relevant physical and chemical tests have ensured faster and efficient raw milk quality determination exercise in this study. The results of this study explored that physico-chemical and bacteriological qualities of industrial raw milk of Bangladesh close to satisfy the minimum standards which were determined by MBRT and other relevant physical and chemical tests. The qualitative value of raw milk obtained in this study is favorable for further industrial processing.

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