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# CHEMICAL AND MICROBIOLOGICAL QUALITY ASSESSMENT OF RAW AND PROCESSED LIQUID MARKET MILKS OF BANGLADESH

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# ABSTRACT

Twelve different liquid market milks of Bangladesh were examined to evaluate their chemical and sanitary quality. Six of these were open raw milk bought from local daily markets and the other six were processed packet milk (both pasteurized and UHT [Ultra High Temperature] - processed) available in shops. The twelve samples were examined for the determination of percentage of water, total soluble solids (TSS), fat, solids-non-fat (SNF), lactose, protein, and ash; measurement of titratable acidity; detection of adulterants; enumeration of total bacterial count, staphylococcal, coliform, fecal coliform, Salmonella and Shigella, Aeromonas hydrophila, and psychrophilic count. Results revealed that most of the raw and pasteurized milks were substandard in both chemical and sanitary quality whereas the quality of UHT-treated milks was excellent. Majority of the raw and pasteurized milks contained fair amounts of lactose, protein and ash, but a number of these had lesser amount of fat. All the raw and pasteurized milks were found to be contaminated with bacterial loads exceeding the acceptable limit. The indicator organisms i.e. coliforms and fecal coliforms were present in most of these samples in large numbers. Pathogenic bacterial genera (Aeromonas, Salmonella, and Staphylococcus) were also identified in some of these. High counts of psychrophilic bacteria were also found in the raw and pasteurized milk. But none of the UHT-processed milks contained any bacteria. Water had been added to five raw and one pasteurized milk whereas sucrose was found in five of the six heat-treated samples.

KEYWORDS: Adulteration, bacterial distribution, chemical composition, pasteurized milk, sanitary quality, titratable acidity, UHT-milk.

# INTRODUCTION

Milk is considered as nature's single most complete food (O'Mahony 1988) and is definitely one of the most valuable and regularly consumed foods. But at the same time, it is highly vulnerable to bacterial contamination and hence is easily perishable (Kim et al 1983; OECD 2005). Moreover, in Bangladesh, milk adulteration is pretty common. Quality evaluation of milk is thus vital.

Consumers prefer wholesome and nutritious food produced and processed in a sound and sanitary manner such that it is free from pathogens. For fulfilling consumer's demand, production of quality milk is essential. Quality milk is the milk of normal chemical composition, completely free from harmful bacteria and harmful toxic substances, free from sediment and extraneous substances, has lower degree of titratable acidity, of good flavor, adequate in preserving quality, and low in bacterial counts. In Bangladesh, milk is produced mostly in nonstandardized way and is usually supplied to the consumers from the urban and rural areas by milkmen. Although there is little milk pockets specially milk vita, and some established dairy farms where surplus milk is readily available, this perishable product has never received particular attention in hygienic distribution to the consumers (Khan et al 2008). But milk is an excellent growth medium for bacteria and can easily be contaminated by many different sources including the udder and body of cows, dust from the air, litter, floor, flies, insects and rodents, water supply, hands and clothes of the milker, utensils, bottles, atmosphere etc. (Ensminger et al 1994; Heineman 1919; Cousin 1982). Thus milk and the dairy products can be important sources of food borne pathogens (Oliver et al 2005). Moreover, adulteration of milk with water, which is very common in Bangladesh, not only causes dilution of milk reducing the milk solids, but also involves the risk of introducing germs into the milk, further decreasing its quality. So, it is naturally of great importance that such a valuable and easily-damaged food be delivered to the consumer in a wholesome and unadulterated form. Not only because the abstraction of cream and the adulteration with water diminish the food value of the milk, but

because there is great danger in the latter case of the germs of infectious and contagious diseases being introduced into the milk, and so disseminated (Ghosh and Maharjan 2002; Barthel 1910).

The Bangladesh Standards and Testing Institution (BSTI) obliges various chemical and sanitary requirements for the pasteurized milk (BSTI 2002). However, no standard is known to be established for the raw and UHT-treated milk.

So far, no work had been reported on the quality evaluation of liquid market milks of Bangladesh. The objectives of this study were to determine the bacterial load, nutritive value and degree of cleanliness surrounding the production and handling of the milk samples as well as to find out the differences among the raw, pasteurized and UHT-processed milks in terms of chemical composition and bacterial distribution.

# MATERIALS AND METHODS

# Collection of samples

In Bangladesh, milk is generally sold in two ways. In most cases, the farmers bring milk in open pots and sell it directly in the market without any processing and packaging. In other cases, milk companies collect milk from the farmers or dairy farms, process it via pasteurization or UHT treatment and package the processed milk which is then sold in shops under specific brand name. In this study, raw milks were purchased from a local daily market while brand milks were bought from different shops. The samples were chosen at random. A total of twelve samples were examined. Six (designated as R-1, R-2, R-3, R-4, R-5, and R-6) were raw milk bought from different vendors. Of the remaining six, three (P-7, P-8, P-9) were pasteurized milks each from different brand and the other three (U-10, U-11, U-12) were UHT-processed also from different brands. All the samples were collected in the sellers' usual form (plastic packets), instantly transported to the laboratory maintaining cold state and examined immediately.

Chemical analysis

Percentage of water was determined by subtracting the value of total soluble solids (TSS) from 100.

TSS was determined by using refractometer (portable refractometer, model: FG 103, Brix 0-32%; Comecta S. A.) (Daniel 2010).

Milk fat was measured by Rose-Gottlieb's method (Barthel 1910).

Amount of solids-non-fat (SNF) was determined by subtracting the amount of fat from TSS.

Lactose was determined by volumetric method (Heineman 1919). 20 ml of milk was diluted with water to a volume of 400 ml and 8-16 drops of a 10% solution of acetic acid added. The precipitate was filtered off and washed with cold water (4°C). The filtrate was boiled in a flask and the albumin precipitated. This was filtered off also and the precipitate was washed with cold water (4°C). The filtrates and wash water (water obtained upon washing the precipitate with cold water) were mixed and measured accurately. A portion of this mixture was placed in a burette, and this was run into a boiling mixture of 20 ml Fehling's solution and 80 ml water. After the copper had been completely precipitated, the number of ml used was read. 20 ml Fehling's solution corresponds to 0.135 gram milk-sugar.

Protein was measured by Kjeldahl method (Crampton and Harris 1969; Jacobs 1973; Mitchell 1972; Pearson 1977).

Ash content was determined by the method described by Maynard (1970).

Acidity was measured by titration with 0.1 N sodium hydroxide solution and using 1% ethanol solution of phenolphthalein as indicator (Lampert 1947).

Water content of milk is usually 87.25% (Eckles et al 1951) and it ranges from 84.0 to 89.0% (Lampert, 1947). In this paper, samples with water content of more than 90% were regarded as adulterated with water.

Presence of the other adulterants was tested by specific qualitative tests (www.dairyforall.com):

Neutralizers: 20 ml of milk was taken in a silica crucible, the water was evaporated and the contents were burnt in a muffle furnace. The ash was dispersed in 10 ml distilled water and it was titrated against decinormal (N/10) hydrochloric acid using phenolphthalein as an indicator. If the titre value exceeded 1.2 ml, then it was construed that the milk was adulterated with neutralizers.

Formalin: 10 ml of milk was taken in test tube and 5 ml of conc. sulphuric acid was added on the sides of the test tube without shaking. Appearance of a violet or blue ring at the intersection of the two layers indicated the presence of formalin.

Sucrose: 10 ml of milk was taken in a test tube and 5 ml of hydrochloric acid was added along with 0.1 g of resorcinol. Then the test tube was shaken well and placed in a boiling water bath for 5 min. Appearance of red colour indicated the presence of added sugar in milk.

Starch: 3 ml milk was taken in a test tube and boiled thoroughly. Then milk was cooled to room temperature and added with 2 to 3 drops of 1% iodine solution. Change of colour to blue indicated that the milk was adulterated with starch.

Glucose: 3 ml of milk was taken in a test tube and 3 ml Barford's reagent was added and mixed thoroughly. Then it was kept in a boiling water bath for 3 min and then cooled for 2 min by immersing in tap water without disturbance. Then 1 ml of phosphomolybdic acid was added and shaken. If blue colour was visible, then glucose was present in the milk sample.

Salt: 5 ml of silver nitrate (0.8%) was taken in a test tube and added with 2 to 3 drops of 1% potassium dichromate and 1 ml of milk and thoroughly mixed. If the contents of the test tube turned yellow in colour, then milk contained salt in it. If it was chocolate coloured, then the milk was free from salt.

#### Bacteriological analysis

Standard Plate Count (SPC) method recommended for dairy products (APHA 1960) was followed for quantitative analysis of bacteria:

Enumeration of total viable bacteria: Nutrient agar medium (Difco) was used for enumeration of total viable bacteria. pH of the medium was adjusted at 6.8 prior to sterilization. Inoculated plates were incubated at 37°C for 24 to 72 hours to facilitate viable bacterial growth. After incubation, the inoculated plates having 30 to 300 colonies were considered for counting using colony counter (Gallenkamp, England) and following back calculation total count was expressed as colony forming units per milliliter (cfu/ml).

Enumeration of total coliform bacteria: Total coliform was determined by the same method used in the enumeration of total viable bacteria. The medium used for coliform was MacConkey agar. Inoculated plates were incubated at 37°C for 24 hours. After incubation, typical pinkish and centrally red colonies were counted by using colony counter and total coliform was calculated.

Enumeration of total fecal coliform bacteria: Fecal coliform (mFc) agar medium was used for the enumeration of fecal coliform. The media were inoculated and after incubation at 44°C for 24 hours, typical bluish colonies were counted using colony counter and using back calculation, total fecal coliform count determined.

Enumeration of total *Staphylococcus* bacteria: *Staphylococcus* medium was used for the enumeration of *Staphylococcus* bacteria. Media were inoculated and after incubation at 37°C for 24 hours, colonies were counted using colony counter and following back calculation, total *Staphylococcus* count was obtained in cfu/ml.

Enumeration of total Salmonella and Shigella: *Salmonella* and *Shigella* agar (SSA) medium was used for enumeration of *Salmonella* and *Shigella*. Media were inoculated and after incubation at 37°C for 24 hours colonies were counted using colony counter and following back calculation, the total *Salmonella* and *Shigella* count was obtained.

Enumeration of Total *Aeromonas hydrophila*: Starch ampicillin agar medium was used for the enumeration of total viable Aeromonas bacteria. Agar plate media were inoculated and after incubation at 37°C for 24 hours,

typical pinkish honey colonies were counted using colony counter and following back calculation, total aeromonas count was determined.

Enumeration of Total Psychrophilic Bacteria: Nutrient agar medium was also used to enumerate total psychrophilic bacteria. Inoculated plates were incubated at 4°C for 15 days to facilitate the growth of psychrophilic bacteria. After incubation, colonies were counted using colony counter and following back calculation, total psychrophilic bacterial count was determined.

For obtaining single colony isolate, the method described by Sharp and Lyles (1969) was used. Morphologically dissimilar well-spaced colonies were picked up with the help of a sterile loop from the plates, which had from 30 to 300 colonies. Each colony was streaked on to freshly prepared plates of the same media and incubated at 37°C for 24 hours or more. After incubation, typical pure colonies were taken as isolates.

The selected isolates were then purified through repeated streak plating. When plating produced only one type of colony in a particular plate, it was considered to be pure. The purified isolates were then transferred to nutrient agar slant in one drum screw capped culture vial and preserved as stock culture.

Identification was done up to genus by following the 'Bergey's manual of determinative bacteriology,' (Buchanan and Gibbons 1974). For identification, different morphological characteristics including shape, size, form, texture, opacity, edge, elevation of the isolated colonies were studied carefully and after Gram staining, microscopic examination was carried out. The biochemical tests performed were catalase test, oxidase test, methyl-red test (MR Test), Voges-proskauer test (VP Test), production of hydrogen sulphide (KIA Test), hydrolysis of starch and fermentation tests.

# **RESULTS AND DISCUSSION**

Chemical composition

The percentage of water, total soluble solids (TSS), fat, solids-non-fat (SNF), lactose, protein, and ash has been presented in Table 1.

Five (R-2 to R-6) of the six raw milks contained more than 90% water which is above the usual range i.e. 84.0 to 89.0% (Eckles et al 1951), suggesting that they were adulterated with water. Among the heat-treated milk, only P-7 contained high percentage of water i.e. 90.83% (Table 1).

Addition of water dilutes milk reducing its TSS content. Reduced TSS was observed in five raw (R-2 to R-6) and one pasteurized (P-7) milk; none of these samples had TSS over 9.5% though milk TSS usually ranges from 10.5 to 14.5% (O'Mahony 1988). The UHT-milks were comparatively rich in TSS content each having at least 11.0% TSS (Table 1).

Commercially, the fat of milk is unquestionably the most valuable constituent of milk. Milk having a fair amount of fat is more valuable as a food than milk which is poor in fat. The Food and Drug Administration (FDA) requires not less than 3.25% milk fat for fluid whole milk. The U.S. public health service (USPHS) Milk Ordinance and Code also recommended a minimum of 3.25% butterfat in farm milk (Graf 1976). However, in this study, three of the raw milks (R-2, R -3, and R-6) contained less than 3.25% fat. The other three (R-1, R -4, and R-5), however, satisfied the criterion each having at least 3.3% fat. The BSTI (2002) requirement for fat content of pasteurized milk is a minimum of 3.5% which is fulfilled by only one (P-9) of the three pasteurized milks. The other two (P-7 & P -8) had fat contents of 3.34% and 3.4% respectively. The fat content of U-12, one of the UHT-processed milks, was even less (3.09%). Data have been presented in Table 1.

FDA standard for SNF content of whole milk is a minimum of 8.25% (Graf 1976). None of the raw milks maintained this standard. Five of these even contained SNF of less than 6.5% indicating that these might have been adulterated with water. The pasteurized milks also failed to maintain the minimum SNF requirement set by BSTI (2002) which is 8.0%. Two (P-8 and P-9) of these had SNF values of more than 7%, whereas SNF of the other (P-7) was exceptionally low, 5.83%. In case of the UHT processed milk, two (U-11& U-12) had SNF contents of more than 8.0% whereas SNF of the other (U-10) was near to 8.0% (Table 1).

The percentage of lactose of most of the raw milks was around 4.25%, similar to that reported by Lingathurai et al (2009). The lactose content of milk though can range from 3.6 to 5.5% (O'Mahony 1988). The specifications

for pasteurized milk, established by BSTI (2002), require at least 4.4% lactose in milk. All the three pasteurized milks fulfilled the requirement. The lactose content of the UHT-milks was even higher, around 4.9%, the highest being 4.97% obtained in U-10 (Table 1).

Sample	% of constituents							
	Water	TSS	Fat	SNF	Lactose	Protein	Ash	
R-1	89.0	11.0	3.75	7.25	4.83	3.57	0.80	
R-2	91.0	9.0	3.15	5.85	4.30	3.16	0. 70	
R-3	91.0	9.0	3.18	5.82	4.14	3.07	0. 74	
R-4	90.50	9.50	3.41	6.09	4.21	3.20	0. 72	
R-5	90.73	9.27	3.30	5.97	4.53	3.26	0.74	
R-6	90.77	9.23	3.12	6.11	4.10	3.30	0.69	
BSTI Std. for Pasteurized milk			3.50	8.0	4.4	3.3	0.70	
P-7	90.83	9.17	3.34	5.83	4.65	3.35	0.64	
P-8	89.0	11.0	3.40	7.60	4.82	3.49	0.67	
P-9	89.17	10.83	3.72	7.11	4.78	3.51	0.71	
U-10	88.0	12.0	3.62	8.38	4.97	3.68	0.75	
U-11	88.0	12.0	3.44	8.56	4.80	3.52	0.75	
U-12	89.0	11.0	3.09	7.91	4.88	3.43	0.69	

The protein content of the raw milks varied from 3.07% to 3.57%. Lingathurai et al (2009) reported slightly higher (3.77%) protein content. The three pasteurized milks were of acceptable quality with respect to protein content according to BSTI (2002) norms (not lower than 3.3%). All the pasteurized milks satisfied this requirement each containing a minimum of 3.35% protein. The UHT-milks were also of good quality regarding protein content each having at least 3.4% of protein (Table 1).

It was interesting to find that the raw milks, though inferior in fat, sugar and protein contents in most cases, had minerals greater than the pasteurized milks. The ash content of the raw milks varied from 0.69% to 0.8% which falls within the usual range of 0.6 to 0.9% (O'Mahony 1988). But It is higher than that (0.33- 0.69%) found by Elmagli and El Zubeir (2006). Ash content of the pasteurized milks ranged from 0.64% to 0.71%, whereas BSTI (2002) demands at least 0.7% of ash for the pasteurized milk. On the other hand, two of the UHT-milks (U-11 & U-12) were quite rich in the mineral content, each containing 0.75% of ash (Table 1).

# Acidity

Table 2 depicts the titratable acidity of the samples.

	Titratable Acidity				
Sample	(% lactic acid)				
R-1	0.216				
R-2	0.180				
R-3	0.200				
R-4	0.171				
R-5	0.135				
R-6	0.162				
BSTI Std. for pasteurized milk	Max 0.150				
P-7	0.144				
P-8	0.162				
P-9	0.162				
U-10	0.189				
U-11	0.144				
U-12	0.175				

Table 2: Titratable acidity of the samples.

Titratable acidity is a measure of freshness and bacterial activity in milk. Popescu and Angel (2009) reported that high quality milk has to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one sample to another. The highest value was 0.216 % (R-1) indicating high bacterial activity and the lowest was 0.135% (R-5) indicating it's relatively better quality with regards to freshness. The acidity of the pasteurized milks ranged from 0.144% to 0.162%, where BSTI (2002) allows a max. acidity of 0.15% for the pasteurized milks. Elmagli and El Zubeir (2006) observed a greater range of acidity (0.14 to 0.86%) in pasteurized milks. No bacteria were found in the UHT-milks U-10 and U-12, but both these showed high degree of titratable acidity (0.189% and 0.175% respectively) suggesting that the high acidity might have developed prior to the heat treatment (Table 2).

Titratable acidity of milk has long been recognized and employed as an indicator of quality (Jaynes et al 1980). It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity (O'Mahony 1988; Lampart 1947). Within a short time after milking, the acidity increases perceptibly due to bacterial activity. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing

acid formation. Therefore, the amount of acid depends on the cleanliness of production and the temperature at which milk is kept. For this reason, determination of acid in milk is an important factor in judging milk quality. Acidity affects taste as well. When it reaches about 0.3%, the sour taste of milk becomes sensible. At 0.4% acidity, milk is clearly sour, and at 0.6% it precipitates at normal temperature. At acidity over 0.9%, it moulds ((Heineman 1919; Tzouwara-Karayanni 2000).

## Adulteration

Results for the presence of adulterants are given in Table 3.

Table 3: Presence of adulterants.								
Sample	Added water	Neutralizer	Formalin	Sucrose	Starch	Glucose	Salt	
R-1	-	-	-	-	-	-	-	
R-2	+	-	-	-	-	-	-	
R-3	+	-	-	-	-	-	-	
R-4	+	-	-	-	-	-	-	
R-5	+	-	-	-	-	-	-	
R-6	+	-	-	-	-	-	-	
P-7	+	-	-	+	-	-	-	
P-8	-	-	-	+	-	-	-	
P-9	-	-	-	-	-	-	-	
U-10	-	-	-	+	-	-	-	
U-11	-	-	-	+	-	-	-	
U-12	-	-	-	+	-	-	-	
'+' = Present, '-' = Absent								

No neutralizer, preservative, added sugar, glucose, starch, or salt was found in raw milks. Five (R-2, R-3, R-4, R-5, and R-6) of the raw milks, however, had been adulterated with water which is very common in Bangladesh particularly in case of raw milk. Addition of water dilutes the amount of total solids in milk and it also involves the danger of introducing germs into milk including the pathogens. Adulteration of milk with water therefore may introduce chemical or microbial hazards to health. It reduces nutritional and processing quality, palatability as well as marketing value of milk (Swai and Schoonman 2011).Water had also been added in one (P-7) of the pasteurized milks. The other adulterant detected was added sugar (sucrose) which was found in five (P-7, P-8, U-10, U-11, and U-12) of the six processed milks (Table 3).

#### Bacterial distribution

The results of bacterial distribution in the samples are presented in Table 4.

All the raw milks had high bacterial load which ranged from  $1.75 \times 10^6$  to  $1.22 \times 10^8$  cfu/ml. The most frequent cause of high bacterial load is poor cleaning of the milking system. Bacterial count was high due to milking dirty udders, maintaining an unclean milking and housing environment, and failing to rapidly cool milk to less than 40°F. The TVBC (total viable bacterial count) of the pasteurized milk samples ranged from  $7.5 \times 10^7$  to  $1.24 \times 10^8$  cfu/ml, much higher than that recommended by BSTI and USPHS (not exceeding 20,000 cfu/ml) (BSTI 2002; Jay 2003). The reason for high bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers. However, TVBC of each of the UHT-processed milks was nil, indicating their excellent sanitary quality (Table 4).

Coliforms are considered as 'indicator organisms' because their presence in food indicates some form of contamination. Coliform count in the raw milks ranged from  $4.5 \times 10^3$  to  $2.03 \times 10^6$  cfu/ml. These results are higher than that obtained by Saitanu et al (1996), who found TCC (total coliform count) of <1000 cfu/ml. However, TCC obtained in the study of Sraïri et al (2006) varied from less than 30 to  $2.08 \times 10^7$  cfu/ml in raw milk. Poor herd hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment can all lead to higher coliform counts in raw milk (CDFA 2008). Pasteurized milk P-8 didn't contain any coliform, whereas TCC of the other two pasteurized milks were  $3.7 \times 10^4$  and  $1.2 \times 10^6$  cfu/ml though the standard has been set by BSTI (2002) at less than 10 colonies/ml. USPHS allows not over 10 colonies for 'Grade A' pasteurized milk indicates recontamination after pasteurization. The UHT-milks were completely free from any coliform (Table 4).

Table 4: Distributio	on of bacteria (c	fu/ml)					
Sample	TVBC (cfu/ml)	TCC (cfu/ml)	TFCC (cfu/ml)	TSC (cfu/ml)	TSSC (cfu/ml)	TAHC (cfu/ml)	TPBC (cfu/ml)
R-1	1.22×10 <sup>8</sup>	1.36×10 <sup>6</sup>	3.3×10 <sup>5</sup>	2.84×10 <sup>5</sup>	1.4×10 <sup>5</sup>	1.63×10 <sup>7</sup>	2×10 <sup>5</sup>
R-2	3.3×10 <sup>7</sup>	5×10 <sup>5</sup>	9.5×10 <sup>4</sup>	1.45×10 <sup>5</sup>	2.9×10 <sup>5</sup>	6×10 <sup>4</sup>	9.2×10 <sup>4</sup>
R-3	1.75×10 <sup>6</sup>	4.5×10 <sup>3</sup>	3.8×10 <sup>3</sup>	1.2×10 <sup>5</sup>	Nil	Nil	1.6×10 <sup>4</sup>
R-4	2.9×10 <sup>7</sup>	5.4×10 <sup>5</sup>	1×10 <sup>5</sup>	1.48×10 <sup>6</sup>	Nil	Nil	$1.98 \times 10^{4}$
R-5	6.1×10 <sup>7</sup>	3.6×10 <sup>5</sup>	Nil	1.54×10 <sup>5</sup>	Nil	Nil	1.02×10 <sup>5</sup>
R-6	2.4×10 <sup>7</sup>	2.03×10 <sup>6</sup>	4.8×10 <sup>5</sup>	5.7×10 <sup>4</sup>	5.9×10 <sup>5</sup>	4.4×10 <sup>5</sup>	2.08×10 <sup>4</sup>
BSTI Std. for pasteurized milk	$\begin{array}{c} Maximum \\ 2 \times 10^4 \end{array}$	Less than 10					
P-7	7.5×10 <sup>7</sup>	3.7×10 <sup>4</sup>	5.5×10 <sup>3</sup>	1.4×10 <sup>3</sup>	6.1×10 <sup>4</sup>	Nil	4.9×10 <sup>3</sup>
P-8	8.3×10 <sup>7</sup>	Nil	Nil	1.6×10 <sup>3</sup>	Nil	Nil	4×10 <sup>2</sup>
P-9	1.24×10 <sup>8</sup>	1.2×10 <sup>6</sup>	1×10 <sup>4</sup>	8.1×10 <sup>4</sup>	1.2×10 <sup>6</sup>	Nil	$1.87 \times 10^{4}$
U-10	Nil	Nil	Nil	Nil	Nil	Nil	Nil
U-11	Nil	Nil	Nil	Nil	Nil	Nil	Nil
U-12	Nil	Nil	Nil	Nil	Nil	Nil	Nil

cfu/ml = colony forming units per milliliter, TVBC = Total viable bacterial count, TCC = Total coliform count, TFCC = Total fecal coliform count, TSC =Total staphylococcal count, TAHC = Total *Aeromonas hydrophila* count, TSSC = Total *Salmonella* and *Shigella* count, TPBC = Total psychrophilic bacterial count

Among the raw and pasteurized milks, two samples, R-5 and P-8, didn't have any fecal coliform but others showed quite high count, higher than that found by Sraïri et al (2006) in the raw milk of some dairy farms in Morocco. The fecal coliforms are more closely related to fecal contamination than are the total coliforms. The organisms can originate from improperly sanitized working surfaces in a processing plant. In these cases, their presence would reflect the quality of sanitation and not the direct pollution of the product (Banwart 2004). The UHT-milks didn't contain any fecal coliform (Table 4).

A large percentage of all cases reported as food poisoning or food infection is actually *Staphylococcus* poisoning and many people encounter this illness during their lifetime. The staphylococcal food intoxication accounted for over 17% of all the outbreaks and almost 34% of the cases of reported foodborne illnesses in the United States in 1981 (Frazier and Westhoff 2005; Banwart 2004). In this study, *Staphylococcus* was found in all of the raw and pasteurized milks but not found in the UHT-milk. TSC (total staphylococcal count) in the raw milks ranged from  $5.7 \times 10^4$  to  $1.48 \times 10^6$  cfu/ml. These counts are less than the findings of Khan and Abdul (2002) where the mean staphylococcal counts were  $4.7 \times 10^6$  cfu/ml in raw milk, but higher than that of Sraïri et al (2006) where TSC ranged from less than 30 cfu/ml to 10820 cfu/ml. In the pasteurized milks TSC ranged from  $1.4 \times 10^3$  to  $8.1 \times 10^4$  cfu/ml (Table 4).

The UHT-milks, three raw milks (R-3, R-4, and R-5) and one pasteurized milk (P-8) didn't show TSSC (total *salmonella* and *shigella* count). However, in the remaining three raw milks TSSC varied from  $1.4 \times 10^5$  to  $5.9 \times 10^5$  cfu/ml. P-7 and P-9 also showed high TSSC (Table 4). The salmonellae are said to be ubiquitous, being worldwide and found in or on soil, water, sewage, animals, humans, processing equipment, feed, and various food products (Banwart 2004). Khan and Abdul (2002) though did not obtain any *Salmonella* or *Shigella* in raw milks in their study on 'Microbiological quality of milk, vegetables and fruit juices'. Members of *Salmonella* are potentially pathogenic for humans. The transmission of the disease is usually from animals to humans by ingestion of food of animal origin. Raw milk has been the vehicle for salmonellae causing salmonellosis throughout the world, whereas a widespread outbreak in 1985, affecting over 16,000 people in several states, involved pasteurized milk. This incident should make us aware that pasteurization systems and proper procedures for handling pasteurized milk are needed to prevent salmonellosis and perhaps other illnesses (Banwart, 2004).

Three of the six raw milks (R-1, R-2, and R-6) contained *A. hydrophila* with the total count ranging from  $6 \times 10^4$  to  $1.63 \times 10^7$  cfu/ml (Table 4). The *Aeromonas* spp. are often introduced from water which is thought to be the main source of contamination (Bizani and Brandelli 2001). The isolation of *Aeromonas* from raw milk has been reported by Khalil (1997). Nahla (2006) also reported the presence of *Aeromonas* in raw and pasteurized milks. In this study, however, the pasteurized and UHT-processed milks didn't contain any *A. hydrophila*. Fourteen species of *Aeromonas* have been described, five of which, including *A. hydrophila*, are currently recognized as human pathogens (Janda and Abbott 1998).

TPBC (total psychrophilic bacterial count) of raw milks varied from  $1.6 \times 10^4$  to  $2 \times 10^5$  cfu/ml and that of the pasteurized milks ranged from  $4 \times 10^2$  to  $1.87 \times 10^4$  cfu/ml. The UHT-milks didn't possess any psychrophiles as usual (Table 4). Presence of psychrotrophs in the pasteurized milks indicates post-pasteurization contamination. Psychrotrophs are becoming increasingly dangerous to the dairy industry because they produce extracellular heat- resistant lipases and proteases. Milk altered by the activity of these enzymatic systems is depreciated and must be eliminated from processing. TPBC is used as a supplementary indicator of milk quality. Data on TPBC are required by some dairies because of specific technological requirements and quality-dependent payment for raw milk supplies. The current EU standards for top quality milk require that TPBC shall not exceed 5,000 CFU/ml. (Cempírková 2002).

A major reason of the poor bacterial quality of the raw milks is adulteration with addition of water. Water is added to milk to increase its volume. Addition of water reduces the percentages of the soluble solids including fat and the other vital components in milk and at the same time it involves the danger of introducing germs that may even be pathogenic. Diluting milk with pure water, however, may lead only to malnutrition; but adding impure water may cause intestinal problems (KDB Training guide 2004, Kurwijila 2006).

### CONCLUSIONS

The UHT-treated milks were much better than the raw and pasteurized milks particularly from sanitary point of view and two of these, U-10 and U-11, were the bests of all samples considering most parameters. The hygienic standard of the raw and pasteurized milks was very poor. All the raw and pasteurized milks had high bacterial loads and some contained pathogenic bacteria. The UHT milks didn't contain any. Few of the raw and pasteurized milks were also inferior in fat content. Two adulterants, added water and sucrose, were identified in a number of raw and pasteurized milks. The presence of the pathogenic organisms, the high counts of coliforms and the high levels of adulteration are indicative of a potentially hazardous product which is likely to be posing a serious health risk to the consumers. The government therefore should conduct frequent inspection of the marketed milks to check whether they meet the minimum legal standards and should monitor the overall

hygienic condition surrounding the production and handling of milk. Realistic standards for the raw milks need to be devised and appropriate training should be given to the raw milk producers in hygienic handling of milk.

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