Research Inventy: International Journal Of Engineering And Science
Vol.3, Issue 11 (November 2013), PP 15-19
Issn(e): 2278-4721, Issn(p):2319-6483, Www.Researchinventy.Com

Microbiological Status of Raw and Pasteurized Milk in the State of Kuwait

[1]Hani M. Al-Mazeedi, [2]Fadheelah A. Gholoum, [3]Batool H. Akbar
[1](Kuwait Institute for Scientific Research, Kuwait)
[2](Kuwait University, Kuwait)
[3](Kuwait Institute for Scientific Research, Kuwait)

ABSTRACT: *The microbiological quality of raw (87) and pasteurized (201) milk samples were collected randomly from 9 dairy farms in Kuwait. The aerobic plate count (APC), psychrotrophs, pseudomonas and B. cereus counts of raw milk ranged from ($1X10^1$ to $9X10^5$), (<3 to $9X10^5$), (3 to $<10^5$) and (3 to $<10^5$) cfu/ml respectively. In pasteurized milk samples, the number of coliforms ranged from <3 to <10 organisms/ml. The raw milk being produced on 5 out of 9 dairy was found to be of poor quality. B. cereus was counted in 23 out of 201 pasteurized milk samples which represent a health hazard.*

I. INTRODUCTION

Milk, secreted by mammals for the nourishment of their young ones, is considered to be a nearly complete food. It, therefore, is a highly perishable commodity and presents a challenge to the modern milk processor for turning it to a variety of products with a reasonable shelf-life [1]. Cows are milked at least twice a day on farms worldwide. Milk is being produced at ambient temperatures ranging from sub-zero, where it is necessary to protect milk in cans from freezing to 30°C or higher (e.g. in Kuwait), where without refrigeration, it is impossible to cool milk much below 25°C. Milk being a nutritious medium, presents a favorable physical environment for the growth of micro-organisms, results in its contamination by a broad spectrum of microbial types. The temperature and duration of milk storage on Kuwaiti dairy farms can vary widely, so that the numbers and types of microorganisms present when the milk leaves the farm differ, often unpredictably, even under apparently similar transportation conditions. No such study has so far been reported on the microbiological status of raw and pasteurized milk being produced in the State of Kuwait. The main objective of this investigation was to study the microbiological quality of raw and pasteurized milk being produced in the state of Kuwait. Also the aim of this study was to indicate the sanitary condition of raw milk in different dairy farms in Kuwait.

II. MATERIALS AND METHODS

2.1 Sample collection

A total 87 raw milk samples from 9 dairy farms and 201 pasteurized milk samples from three dairy companies were analyzed microbiologically. Each raw milk sample was filled in a sterile test tube, while the pre-packaged pasteurized milk samples were purchased from different cooperative societies. The collected samples were taken to the laboratory under aseptic conditions at 4°C and analyzed within 6 h of sampling.

2.2 Sample Preparation

One ml each of thoroughly mixed milk sample was mixed in 9 ml of 0.1% sterile peptone water to obtain a dilution of 1:10. Serial dilutions were prepared from the original dilution by transferring 1 ml to each of a series of sterile test tubes containing 9 ml of 0.1% sterile peptone water. Microbiological investigations of raw and pasteurized milk samples were done as per the procedures given in the [2]. All microbiological media used were from Oxoid. The following tests were conducted: aerobic plate count (APC), psychrotrophs, coliforms, pseudomonas, *E. coli* and *Bacillus cereus* counts.

III RESULTS

3.1 Raw Milk

The aerobic plate (APC), psychrotrophs, pseudomonas and B. cereus counts (log_{10}CFU/ml) of raw milk collected from 9 dairy farms have been presented in Table 1. The APC for the total examined samples (87) from these dairy farms ranged from 1 X 10 to 9 X 10^5 cfu/ml. Out of 87 milk samples tested from all dairy farms, a total of 52 samples (59.8%) had APC per ml ranged from 1 X 10^4 to 9 X 10^5.

Nineteen samples (21.8%) of raw milk samples had psychrotrophs counts ranging from <3 to less than 10, while the counts in 65 samples (74.7%) ranged from 1 X 10^1 to <1 X 10^4 cfu/ml. The remaining 3 samples (3.5%) had psychrotrophs ranged from 1 X 10^4 to 9 X 10^5 cfu/ml. On the other hand, the counts of both pseudomonas and *B. cereus* in 44 raw milk samples ranged from 3 to <1 X 10^5 cfu/ml (Table 1).

Table 2 shows the frequency distribution of coliform and *E. coli*. Coliform counts ranged from <3 to more than 1 X 10^3 cfu/ml. *E. coli* was isolated from 69 samples (79.3%) out of the total raw milk samples tested. Milk samples with the highest prevalence of *E. coli* were detected in 28 (32.2%) raw milk samples.

The total aerobic plate counts are usually used to assess the overall sanitation and storage quality of raw milk. The International Dairy Federation [3], has reported that the APC values may range from <10^3cfu/ml, where contamination during production is minimal, to >1 X 10^6 /ml of milk. Consequently, high initial APC values in milk, e.g > 100 000/ml are evidence of serious faults in production hygiene, whereas the production of milk having APC values <20 000/ml reflects good hygienic practices. Comparing with the International Dairy Federation, the aerobic plate counts of the raw milk samples were considerably higher in 5 out of 9 dairy farms. In many countries a standard for grade A or grade 1 raw milk is an aerobic plate count <1 X 10^5cfu/ml, and this may be obligatory for raw milk intended for heat treatment before liquid consumption. The total aerobic counts for the raw milk samples ranged from 10 to 9 X10^5 cfu/ml, with 73 samples (83.9%) out of the total of 87 samples, having less than 1 X 10^5 cfu/ml (Table 1).

The term psychrotrophs or psychrotrophic bacterium was introduced to indicate bacteria capable of growth at approximately 5^{o}C, whatever their optimum growth temperature. In our study, the count of psychrotrophs and pseudomonas of raw milk ranged from <3 to 9 X 10^5 and 3 to <10^5 cfu/ml, respectively (Table 1). The most commonly occurring psychrotrophs in fresh raw milk are Gram-negative rods. Pseudomonas spp. account for about 50% of the Gram-negative genera [4]. Some of these psychrotrophs, when growing in refrigerated milk, produce extracellular heat-resistant lipase, as well as proteinases which may degrade casein.

The aerobic plate counts (APC) do not indicate the sources of bacterial contamination in milk. To identify of production faults, counts of psychrotrophs, spore-formers and coliforms may assist in the diagnosis of the faults in the quality of milk.

The number of coliforms and *E. coli* in raw milk ranged from <3 to >10^3 ml^- (Table 2). According to the proposed standard [5], it is clear that the highest coliform counts were observed in 45 out of 87 total examined samples. *E. coli* was detected in 69 out of 87 raw milk samples and the count ranged from 3 to >1 X 10^3/ml.

a. Pasteurized Milk

The frequency distribution of the APC, psychrotrophs, pseudomonas, coliform, *E. coli* and *Bacillus cereus* of 201 samples of pasteurized milk representing three dairy companies (I, II and III) are presented in Tables 3, 4 and 5. The mean counts of the aerobic bacteria (cfu/ml) in the pasteurized milk from dairy companies I, II and III were 3 X 10^4, 9 X 10^1 and 5 X 10^3, respectively (Table 3). The frequency distribution of APC in these dairy companies is also shown in Table 3. In case of company II, counts were generally very low in all samples (range <10 to <10^3 cfu/ml), but 75 milk samples (76.5%) out of 98 samples of company I, had counts ranging from 1 X 10^3 to <1 X 10^7 cfu/ml. The APC of 18 milk samples from company III, ranged from <10 to <1 X 10^5 cfu/ml.

Pasteurization is overwhelmingly the most common heat treatment applied to fluid milk products. Although its primary function is to ensure the microbiological safety of milk, it is also an essential element for ensuring that fluid milks have a commercially acceptable shelf-life. For pasteurized milk in England and Wales, the following criteria for defining unacceptable quality of freshly pasteurized milk must be met: APC should be less than 3 X 10^4 cfu/ml and it should be less than 1 X 10^5 cfu/g after incubation at 6^{o}C for 5 days [5]. As per the Kuwaiti Standard, the total aerobic count of pasteurized milk should be <10^5 cfu/ml [6]. According to the standard of England and Wales [5] the APC of 10 out of 98 examined samples of the pasteurized milk from company I are not acceptable (counts equal or more than 3 X 10^4 cfu/ml) and according to Kuwaiti standard, only one sample could be rejected (more than 10^5 cfu/ml). On the other hand, according to the standards of England and Wales or Kuwait, the APC in all examined samples of companies II and III were satisfactory.

Table 4 shows the frequency distribution of psychrotrophs and pseudomonas counts of pasteurized milk samples. Psychrotrophs and pseudomonas counts of company I ranged from <1 to less than 1 X 10^2 cfu/ml. In all the samples of the company II, the count of psychrotrophs and pseudomonas are less than 1 cfu/ml. In company III, the counts of psychrotrophs ranged from <1 to < 1 X 10^2 cfu/ml, while the counts of pseudomonas ranged from <1 to <1 X 10^3 cfu/ml .The majority of pasteurized milk samples (from all three companies), had psychrotrophs and pseudomonas counts of less than one cfu/ml (Table 4). Properly applied pasteurization not only kills all common pathogens in milk but also eliminates the Gram negative psychrotrophs which are the most common cause of spoilage in raw and thermized milk [7]. The results of our study are in agreement with the one reported by [8]. They mentioned that most psychrotrophs are generally killed during heat treatment and their presence in heat-treated milk is due to secondary contamination.

The coliform counts of the pasteurized milk samples of company I ranged between <3 and <1 X 10^5 cfu/ml with 95 (96.9%) of the 98 samples having less than 3 cfu/ml (Table 5). The counts of coliforms in all the pasteurized milk samples (87 samples) of the company II were less than 3 cfu/ml. Among the samples from the company III, the coliform counts were less than 3 cfu/ml in 17 out of 18 total samples; the counts in the remaining sample were 4 cfu/ml. *E. coli* was not detected in all examined samples of the three companies (count less than one cfu/ml). These results agreed well with those required by the Kuwaiti standards.

The incidence of coliforms and *E. coli* in raw milk has received considerable attention, partly on account of their association with contamination of faecal origin and the consequent risk of more pathogenic faecal organisms being present therein; partly because of the spoilage their growth in milk at ambient temperatures could produce. [9]. noted that *E. coli* can be found in soil and water, on plants, in the intestinal tracts of animals, and in various foods, especially animal products and foods handled by humans. Though the *E. coli* count is a suitable indicator of the microbiological quality of foods, but to assure the safety of food product, specific pathogen testing is necessary.

The frequency distribution of *B. cereus* counts (cfu/ml) of three dairy companies (I, II and III) is given in Table 5. The counts of *B. cereus* in 80 samples representing company I ranged from <1 to <1 X 10^5 cfu/ml, while for the total examined samples (73) of company II, the counts of *B. cereus* ranged from <1 to <1 X 10^4 cfu/ml. For 11 samples (68.8%) out of 16 pasteurized milk samples representing company III, the counts of *B. cereus* ranged from 3 to <1 X 10 cfu/ml. The remaining 5 samples (31.2) out of the total samples (16) had *B. cereus* count ranging from 1 X 10^3 to <1 X 10^4 cfu/ml.

Due to the heat resistance of *B. cereus*, its potential pathogenic character, the capability to grow in milk and reported diseases upon consumption of dairy products, the organism should be considered as hazardous in pasteurized milk [10]. The range of *B. cereus* counts in raw milk was 3 to <1 X 10^5 Table 2. This finding disagreed with the suggested microbiological standard that was proposed by [6]. They suggested that the count of *B. cereus* is <1/ ml. On the other hand, the count of *B. cereus* of 169 pasteurized milk samples (representing the three dairy companies) ranged from <1 to <1 X 10^5 cfu/ml. Pasteurization kills most spoilage organisms with the exception of spore forming bacteria. Control of such organisms is difficult and no simple technique has yet emerged [7].

B cereus is important to the dairy and food industries for two reasons. Firstly, under certain circumstances, the organism can cause food poisoning due to enterotoxins production. Secondly, *B. cereus* can give rise to "bitty" cream and sweet curdling in pasteurized milk, due to spores surviving the pasteurization treatment. Upon germination, the vegetative cells of this organism produce extracellular phospholipase (lecithinase) and protease, which leads to spoilage of pasteurized milk. Higher counts of *B. cereus* observed in the raw as well as pasteurized milk samples in Kuwait is a matter of concern from safety point of view and this problem needs to be addressed properly.

IV. CONCLUSION

The bacteriological quality and chemical properties of raw milk are the basis for the quality of dairy products. Considerable variations in the microbial content of raw milk and pasteurized milk were noticed. High incidence of coliform and *E. coli* in raw milk was recorded which indicated poor hygienic standard being observed during milk production and handling. Rapid multiplication of these bacteria is likely to affect the keeping quality of the raw milk and of products derived from it. For these reasons, the quality of milk delivered to the dairy processing plants in the State of Kuwait needs improvements.

The presence of large numbers of coliform bacteria in raw milk with many as 80% of raw milk samples were contaminated with *E. coli*, a finding hardly surprising, since *E. coli* reflects the degree of contamination. Furthermore, the presence of large numbers of coliform bacteria in milk provides an index of hygienic standard used in the production of milk, as unclean udders and teats can contribute towards coliforms. Furthermore, the presence of large numbers of coliforms may give indications that the raw milk may carry serious pathogenic organisms such as

Salmonella spp. and as few as one organism per 100 ml of contaminated milk may result in human infection disease [11].

According to the new EC directive [12] the milk produced on farms should have the mean APC (of four consecutive sampling) of <100,000/ml). Based on these requirements, it can be concluded that milk from most of the dairy farms in the State of Kuwait was consistently poor in quality. Therefore, improving the hygienic conditions on the dairy farms can greatly ameliorate the sanitary status of the raw milk and pasteurized milk being produced in the country

Table 1. Frequency Distribution of the Aerobic Plate, Psychrotrophs, Pseudomonas and *B. Cereus* Counts of Raw Milk (Log_{10}cfu/Ml)

APC	Psychrotrophs		Pseudomonas		*B. cereus*		Range	
	No	%	No	%	No	%	No.	%
<3	-	-	14	16.1	-	-	-	-
3to<10 -	-	5	5.7	9	20.4	32	72.7	
10 to $<10^2$	2	2.3	32	36.8	17	38.6	3	6.8
10^2 to $<10^3$	11	12.6	21	24.1	9	20.5	3	6.8
10^3 to $<10^4$	22	25.3	13.8	4	9.1	5	11.4	
10^4 to $<10^5$	38	43.7	2	2.3	5	11.4	1	2.3
10^5 to $9X10^5$	14	16.1	1	1.2	-	-	-	-
Total	87		87		87		44	

Table 2. Frequency Distribution of Coliform and *Escherichia Coli* Counts (Log_{10}cfu/Ml) for Raw Milk

Range	Coliform		E. coli	
	No	%	No	%
<3	3	3.5	18	20.7
3 to <10	27	31.0	10	11.5
10 to $<10^2$	12	13.8	31	35.6
10^2 to 10^3	35	40.2	20	23.0
$>10^3$	10	11.5	8	9.2
Total	87		87	

Table 3. Frequency Distribution Of The Aerobic Plate Counts (Apc) Of Pasteurized Milk Samples Collected From Local Dairy Plants (I & II) And One Importer (III)

Range	I		II		III	
	No	%	No	%	No	%
<10	2	2.0	5	5.8	1	5.6
10 to $<10^2$	1	1.0	57	65.5	2	11.2
10^2 to $<10^3$	30	30.6	25	28.7	4	22.4
10^3 to $<10^4$	42	42.9	-	-	10	55.6
10^4 to $<10^5$	22	22.5	-	-	1	5.6
10^6 to $<10^7$	1	1.0	-	-	-	-
Total	98		87		18	

Table 4. Frequency Distribution Of Psychrotrophs And Pseudomonas Counts Of Pasteurized Milk Samples Collected From Local Dairy Plants (I & II) And One Importer (III)

Source	I				II				III			
Range	Psychrophiles		Pseudomonas		Psychrophiles		Pseudomonas		Psychrophiles		Pseudomonas	
	No	%	No	%	No.	%	No.	%	No.	%	No.	%
<1	93	95.9	76	95	84	100	70	100	15	83.3	11	68.7
10:<10^2	4	4.1	4	5					3	16.7		
10^2:<10^3											5	31.3
Total	97		80		84		70		18		16	

Table 5. Frequency Distribution of Coliform, *E. Coli* And *B. Cereus* Counts (Log_{10}cfu/Ml) of Pasteurize Milk Samples Collected from Local Dairy Plants (I & II) and One Importer (III)

Range	I						II						III					
		Coliform		*E. coli*		*B. cereus*		Coliform		*E. coli*		*B. cereus*		Coliform		*E. coli*		*B. cereus*
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
<1	-	-	97	99	33	41.3	-	-	87	100	42	57.5	-	-	18	100	-	-
<3	95	96.9	-	-	11	13.8	87	100	-	-	12	16.4	17	94.4	-	-	-	-
3:<10	2	2.1	-	-	29	36.3	-	-	-	-	8	10.9	1	5.6	-	-	11	68.8
10:<10^2	-	-	-	-	3	3.8	-	-	-	-	8	10.9	-	-	-	-	-	-
10^3:<10^4	-	-	-	-	-	-	-	-	-	-	3	4.1	-	-	-	-	5	31.2
10^4:<10^5					4	5	-	-	-	-	-	-	-	-	-	-	-	-

REFERENCES

[1]. W. Banks, and D.G. Dalgleish, Milk and Milk Processing. In *The Microbiology of Milk*. (R.K.Robinson, ed.) (London : Elsevier Applied Science, 1990) 1-35

[2]. Food and Drug Administration. *Bacteriological Analytical Manual*, 7th ed. Food and Drug Administration. 7th ed. (Arlington :Association of Official Analytical Chemists, 1992)

[3]. International Dairy Federation, Bacteriological quality of cooled bulk milk. (Doc. No. 83, IDF, Brussels, 1974)

[4]. A. Gilmour, and M.T. Rowe, Micro-organisms associated with milk. In *The Microbiology of Milk*. (R.K.Robinson, ed.) (London: Elsevier Applied Science, 1990) 37-75

[5]. H. Luck, and H. Gavron, Quality control in the dairy industry. *Dairy microbiology*, 1990, 345-392.

[6]. ANON, Kuwaiti Standards for liquid milk. Ministry of Commerce and Industry, Kuwait, 1979.

[7]. D.D. Muir, The shelf-life of dairy products: 1, Factors influencing raw milk and fresh products. *Journal of the Society of Dairy Technology, 49*, 1996, 24-31.

[8]. T. Mahari, and B. Gashe, Survey of the microflora of raw and pasteurized milk and sources of contamination in a milk processing plant in Addis Ababa, Ethiopia. *Journal of Dairy Research, 57*, 1990, 233-138

[9]. G.J. Banwart, *Basic Food Microbiology. (2nd Edition.)* (New York: Van Nostrand Reinhold, 1989) 371.

[10]. S. Notermans, J. Dufrenne, P Teunis, R. Beumer, M. Tegiffel, and P. Peeters Weem, A risk assessment study of *Bacillus cereus* present in pasteurized milk. *Food Microbiology 14*, 1979, 143-151.

[11]. R.E. Fountaine, M.L. Cohen, W.T. Martin, and T.M. Vernon, Epidemic salmonellosis from cheddar cheese:surveillance and prevention. *American Journal of Epidemiology, 111*, 1980, 247-253.

[12]. ANON. Proposal for a council Regulation (EEC) laying down the health rules for the production and placing on the market of raw milk, of milk for the manufacture of milk-based products and of milk-based products. Official Journal of the European Community Com (89) 776, 1990, 118.