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ORIGINAL ARTICLE

Physicochemical and Bacterial Properties of Pasteurized Milk Samples Collected from Tabriz, Northwestern Iran

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KEYWORDS

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ABSTRACT: Milk and dairy products are important components of a balanced diet. Milk does have distinct physicochemical, biological and microbial characteristics. The bacterial contamination of milk not only reduces the nutritional quality but its consumption threatens health of the society. In this study, 100 pasteurized milk samples were collected randomly from Tabriz City, northwestern and were analyzed for total plate count (TPC), coliform, *E. coli* and some physicochemical properties (pH, titratable acidity and density). 33.3% of samples had unacceptable microbial contamination in both warm and cold seasons. *E. coli* contamination was not detected in all milk samples, but 54% of pasteurized milk samples were contaminated with coliforms. The pH value (6.6-6.8) and titratable acidity (0.14-0.16%) were in acceptable range. The means value of samples' density was 1028.79±1.04. Lower microbial contamination level in this area indicates that the dairy factories are concerned about appropriate sanitary practice and pasteurization process.

INTRODUCTION

Milk is one of the most valuable sources of nutrients. It contains a wide variety of nutrients with several functions in the body [1]. Moreover, all of substances that our body need is in an easiest assemble form in milk [2]. Continuous consumption of milk and its product improve growth, physical endurance and learning ability. Therefore, it is strictly recommended for all groups of people [3]. Milk is a suitable medium for growth of a board range of microorganism which may cause it to spoil [4]. The presence of microorganisms in milk will have unpleasant effects on its organoleptic properties and might lead to economic losses [5].

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According to food safety and hygiene measures, the most important effect of microorganisms in milk is their pathogenic effects on consumers. Some of the most important bacterial pathogens of contaminated dairy products are *Salmonella* spp., *Brucella* spp., *Staphylococcus* spp., *Listeria* spp., *Escherichia coli* and coliforms. Coliforms and *E. coli* are as indicator microorganism. Coliforms are known as normal flora of large intestine [6].

Some pathogenic microorganisms can survive during manufacturing processes [7]. Foodborne infection has been increased over last 20 yrs., which shows the importance of food hygiene and quality control. Psychotropic microorganisms are considered as one of the undesired groups of microorganisms in milk industry suspected to spoil the milk such as *Pseudomonas* spp [8]. Presence of such microorganisms in milk indicates an unhygienic condition and insufficient pasteurization process as well as fecal contamination. Moreover, a wide variety of sources like workers' hands and milk containers are contaminating milk products [9].

In current study, microbial contamination of milk specimens were assessed using conventional counting of colonies on plates illuminated by transmitted light and reported as colony forming unit per milliliter ($cfu.ml^{-1}$) [10]. In this study we aimed to evaluate both the physicochemical properties of pasteurized milk samples and presence of total count of microorganisms and coliforms and *E. coli* bacteria in pasteurized milk samples in East Azerbaijan, Iran.

MATERIALS AND METHODS

Milk collection

In this study, 100 pasteurized milk samples, processed in East Azerbaijan region, were purchased from randomly selected supermarkets in Tabriz, East Azerbaijan, Iran.

Determination of some physicochemical properties of pasteurized milk samples

To determine pH, a Tacussel digital pH-meter was used and titratable acidity measured by hydroxide sodium solution (1/10 N) in the presence of phenol phetaleine 1%. Total Dry Extract (TDE) was measured after evaporation of liquid phase and expressed as percentage mass. The density was measured by Thermo lacto dencimeter type Dornic [11].

Microbial evaluation of pasteurized milk samples

All 100 pasteurized milk samples were studied and cultured according to Iran national standards' guidelines (No. 9415). To start test, 3 10-fold dilutions of milk samples (0.1, 0.01, 0.001 v/v) were prepared [12].

According to Iran National Standard (No. 5484), Plate Count Agar (PCA) was used to quantify the bacterial population in test samples. Plates were incubated at 30 °C for 72 h.

Coliforms and *E. coli* bacteria were isolated according to the No. 5486 and 5234 guidelines of Iran National Standard, respectively. To detect *E. coli*, 1 ml of milk sample inoculated to Lauryl Sulfate Tryptose broth (LST) then, incubated at 37ºC for 24 h. In case of observing gas and acid in durham tubes, 1 ml inoculated to Brilliant Green Broth (BGB) and incubated at 37 □C. differential media such The Eosin Methylene Blue (EMB), TSI, urea were used as confirmation tests [13,14]. To detect coliforms, 1 ml of milk was inoculated to selective medium (VRBL) Violet Red Bile Agar. After incubating plates at 30 □C, plates with more than 10 colonies and less than 150 colonies were selected. Then suspected colonies inoculated to Lactose Bile Brilliant Green Broth and incubated at 30 □C for 24 h to observe gas and acid in Durham tubes as a confirmation step [13].

STATISTICAL ANALYSIS

All experiments were conducted in triplicate, and statistical analysis was performed using SPSS 17.0 (Chicago, IL, USA). All results were computed as mean standard deviation and were subjected to one-way analysis of variance to establish whether the differences in experimental results were significant or not. The Statistical significance was determined at $P<0.05$.

RESULTS

Physicochemical Results

The pH value and titratable acidity were in a normal and acceptable range. The pH value should be in a range of 6.6-6.8 and acidity can be varied from 0.14% - 0.16%. A reduction in density of 13% of samples was observed. The minimum and maximum results of physicochemical parameter are shown in Table 1.

Table 1. Mean Value and maximum and minimum for physicochemical quality of pasteurized milk samples

	Max	Mean ± SD	Min
pH	6.72	6.59±0.09	6.41
Acidity	19.5	16.67±0.66	15
Density	1031	1028.79±1.04	1026
Total Dry Extract%	14.39	16.67±0.66	10.4

Microbiological analysis

All 100 pasteurized milk samples were studied. Contaminations with total bacteria, coliforms and *E. coli* were observed in 33/3%, 54% and 0% of samples, respectively.

According to Iran National Standard Guidelines, presence of coliforms in pasteurized milk should not be more than 10 Cfu/ml. All 54 coliform contaminated samples were acceptable according to the above-mentioned cut-off point.

Logarithmic distribution of the total microorganisms from all pasteurized milk samples based on the two halves of the year and the maximum and minimum values of isolated total bacteria of all 100 samples, coliforms and *E. coli* is shown in Tables 2 and 3, respectively. The highest rate of total bacterial count was related to samples which were produced in warm seasons.

Table 2. Distribution of total count bacteria during 2014 year in Tabriz, northwestern Iran

	Samples sizes	Maximum	Minimum	Mean ± SD	Samples more than legal level (10^3)
Hot seasons	50	4.50	0	2.808 ± 0.34^a	33.3%
Cold seasons	50	4.47	0	2.545 ± 0.54^b	33.3%

Means ± SD in the same column with different letters are significantly different ($P < 0.05$).

Table 3. Distribution of the isolated organisms from 100 pasteurized milk samples in Tabriz, northwestern Iran.

	Sample sizes	*E. coli*		Coliform		
		Contaminated samples (N)	Legal Level (cfu/ml)	Contaminated samples (%)	Legal Level (cfu/ml)	Samples more than legal level
Hot seasons	50	0	0	31(62%)	10	0
Cold seasons	50	0	0	23(46%)	10	0

Table 4 shows the highest number of coliforms in samples, which is less than standard level. *E. coli* was not isolated from any of the samples.

Table 4. Maximum and minimum level of isolated microorganisms.

	Max (Cfu/ml)	Min (Cfu/ml)
Total bacteria	11.3×10^3	0
E.coli	0	0
Coliform	2	0

DISCUSSION

The normal pH value shows the freshness of milk, which was in acceptable range in our study. The mean value of titratable acidity of milk was 16.67 ± 0.6, which was slightly higher than normal range and indicated poor transportation and handling practice. The reduction in density was observed in 13% of samples that might be due to added water to increase the volume [11].

In this study, total microbial count, coliforms and *E.coli* was observed 33.3%, 0, and 0 respectively in all 100 pasteurized milk samples. Overall, 54% of samples were contaminated with coliforms, but they were within the acceptable level. In a study on 42 milk samples in India the logarithmic average microbial loads was 3.8 [15]. In another study, the logarithmic average microbial load of raw milk on 75 milk specimens was 2.4 [16]. Similar studies on milk contaminations to indicate organisms like *E. coli* was performed which showed the amount of *E. coli* in raw milk was 24 samples out of 178 [17].

Presence of *E. coli* in milk indicates fecal contamination which may be related to insanitary condition in factory or poor pasteurization process. Milk pasteurization is a significant step to prevent potential health risks to consumers. Without heat processing, important pathogens like *E. coli* and *Listeria* and coliforms may not be killed. The highest level of total bacterial count in raw milk was recorded in 3.79 to 9.05% of samples at <0.0055 to 1.1 CFU/ml [18]. The reason could be that ambient temperature is high and lack of good cooling systems or lower effectiveness of pasteurization [19]. Total bacterial count and *E. coli* count of raw milk, was 67% (73 of 109) samples and 10.1% (11 of 109) samples, respectively (20). Total bacteria and *E. coli* in heat-treated samples was not detected in heat-treated [20]. In a study, risk factors associated with milk and milk products contaminated with *Staphylococcus* were assessed. The results of this study showed that 24% of samples were positive. Milk container sanitation, mastitis, travel time to collection center were considered to be in association with contaminations [21]. It is because of the poor cleaning system, contamination of milk containers and low level of hygiene [22]. In a study, contamination with *E. coli* and coliforms were 42% and 36% for raw milk and 9% and 2% for pasteurized milk, respectively [3].

CONCLUSIONS

Lower distribution of microorganisms in pasteurized milk samples demonstrates the high quality of pasteurized milk in Tabriz. Moreover, it indicates the importance of pasteurization in order to obtain high quality milk and also of the demand for practicing HACCP guidelines in all stage of milk production to control the risk of human infection with zoonotic diseases.

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The authors declare that there is no conflict of interests.

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