Microbiological Quality of Pasteurized Milk Marketed in Tehran Urban Area

Mohammad Reza Koushki

Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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Pasteurized milk is consumed widely in all socioeconomic classes and considered as the main source of milk for people in Iran. In recent years, increased price of pasteurized milk caused by elimination of subsidies resulted in decreased milk consumption and also a possibility of adulterations which may lead to low microbial quality of the products. The present investigation envisaged the microbiological quality of 103 pasteurized milk samples collected randomly from fruit and vegetables markets in Tehran and evaluated them by enumeration of aerobic mesophilic microorganisms, coliforms and detection of Escherichia coli on expiration date. Coliforms, Escherichia coli and aerobic mesophilic microorganisms were in compliance with Iranian National Standard limits in 78.6%, 92.9% and 40.8% of the studied samples, respectively. No significant difference was found between pouches and bottles in terms of Escherichia coli. To conclude, the present study confirmed the partial satisfactory quality of pasteurized milk packages in Tehran urban area. Promotion of public awareness about consumption of pasteurized milk and precise implementation of HACCP and its prerequisite programs to achieve high quality pasteurized milk is recommended.

Keywords: Pasteurized milk, Microbiological quality, Packaging, Expiration date.

Milk is a whole worthy food which is a part of human diet at any age and have great protein content and minerals specially calcium. Milk also is a good medium for survival and growth of different kinds of microorganisms. Microbial quality directly affect milk taste, its physicochemical properties, dairy products and in case of poor quality can be pathogenic for humans. The thermal process method is based on prevention of microbial growth and eventually maintaining desired food quality. With regard to these points microbial quality of pasteurized milk have to be studied constantly (Koushki 2010).

Several studies had been conducted in different countries on microbial quality of raw milk, pasteurized milk and other dairy products. Microbial and physicochemical qualities of pasteurized milk marketed in Shahroud city in Iran were investigated by culturing and polymerase chain reactions applied in order to detect coliforms especially E. coli (Mohammadi et al. 2013). Total number of germs, coliform counts, and E. coli identification tests were carried out in raw and pasteurized milk samples collected from food markets in Timisoara city, Romania (Filimon et al. 2011). Different microbiological tests including standard plate counts, coliform counts, purity plate culture, Gram staining, and biochemical tests were performed to analyze unexpired pasteurized milk samples randomly selected from supermarkets in Kingston, Jamaica (Anderson et al. 2011). Microbiological quality and physicochemical
properties of raw and pasteurized grade A milk were evaluated. Total and fecal coliforms counts of the pasteurized milk samples were above the legal standards in RS, Brazil (Da Silva et al. 2010). Also a study in Senegal showed that pasteurized milk samples, raw milk samples, and sour milk samples were not compliant with official standards (Breurec et al. 2010). Several samples of milk and other dairy products (including 100 milk samples, 47 doogh (fermented milk) samples, and 33 yogurt samples) were tested in terms of coliform counts, *Escherichia coli* (*E. coli*) counts, *Staphylococcus aureus* counts, and yeast and mold counts and the results were compared with Iran’s national standards (Nikoozadeh et al. 2011). The effect of storage temperature on microbiological stability of homogenized whole pasteurized milk (75°C for 15 s) packaged in high density polyethylene (HDPE) and low density polyethylene (LDPE) pouch were evaluated through mesophilic and psychrotrophic counts (Petrus et al. 2010). Also microbiological quality of pasteurized milk marketed in West of Tehran city were evaluated (Koushki et al. 2016).

Tehran province, with an annual production of 840 thousand tons of milk ranks first in milk production in Iran (Ghafari 2011). Of about 8.5 million tons annual milk production in Iran, a portion of 25% to 30% is allocated to pasteurization and sterilization (Koushki 2010). On the other hand, increased price of pasteurized milk and dairy products caused by elimination of governmental subsidies resulted decrease in milk consumption and also a possibility of adulterations which may lead to low quality and microbial instability of the products.

The aim of the present study was to evaluate microbial quality of pasteurized milk (pouches and bottles) randomly selected from different fruit and vegetables markets in Tehran urban area on their expiration date. The tests’ results then compared with Iranian National Standard levels and their compliance were analyzed statistically.

**MATERIALS AND METHODS**

A number of 103 pasteurized milk samples collected randomly from several fruit and vegetables markets in five different regions north, south, east, west and downtown of Tehran the capital of Iran in winter 2013 and spring 2014. The collected samples which had two kinds of packaging (pouches and bottles) brought to the lab at 4°C and held at this temperature in proper condition until the expiration date which microbial tests accomplished. Samples were in 13 different brands in total. The samples then analyzed in terms of microbial quality according to Iranian National Standard No. 2406 (ISIRI, 2008). All the samples were evaluated for total microbial count (TMC) using plate count skim milk agar (PCA), total coliforms using violet red bile lactose agar (VRBL) and *E. coli* using Lauryl sulfate tryptose broth (LS), EC broth and Tryptone water with regard to Iranian National Standard No. 5484 (ISIRI, 2000), No. 5486 (ISIRI, 2000), and No. 2946 (ISIRI, 1999) respectively.

Each sample were completely shaken and then distributed accurately into test tubes providing serial dilutions for total microbial count and total coliforms tests. The amount of 1 mL of each dilution transferred into separate petri dishes and mixed with proper amount of VRBL or PCA in accordance with pour plate method. Then PCA and VRBL plates incubated at 30°C for 72 h and 24 h, respectively. Presumptive colonies detected on VRBL plates were confirmed using brilliant green bile broth (Iranian National Standard No. 5484, ISIRI, 2000 and No. 5486, ISIRI, 2000).

In order to detect *E. coli* in samples, 1 mL of each prepared sample were added into test tubes containing sterile LS medium and incubated at 37°C for 48 h. In case of presence of turbidity and gas bubbles LS medium inoculated into EC broth and turbidity and gas bubbles were checked after incubation at 44°C for 24h. Positive results then followed by inoculation in Tryptone water cultures and indole positive ones confirmed the presence of *E. coli* (ISIRI, 1999).

Quantitative variables were reported as means, standard deviations, minimum, and maximum, while qualitative variables reported in counts and percent. Statistical tests applied in this study were Kruskal-Wallis for distribution of three groups and Mann-Whitney U test for comparing two groups. To investigate the relationship in qualitative variables and also in quantitative ones Pearson’s chi-squared test and determination of correlation coefficient were used.
respectively. The significance level was $\pm 0.05$ in this study and all the statistical analyses were performed in SPSS (version 17.0).

**RESULTS**

Microbial quality of 103 milk samples in 13 different brands collected from fruit and vegetables markets in Tehran urban area were studied. Of 103 samples collected, 65% (67 samples) were packed in pouches and the rest 35% (36 samples) in bottles. Regarding fat content 47.6% (49 samples), 18.4% (19 samples), and 34% (35 samples) were low fat (1.5% or lower), reduced fat (2%) and whole fat (3% or greater) respectively. In case of shelf life 18.4% (19 samples), 52.4% (54 samples), and 29.1% (30 samples) had shelf life of 4, 5 and 6 days respectively.

In this study 103 milk samples were evaluated which indicate the *E. coli*, coliform counts and total microbial counts 7.8, 21.4 and 59.2 of the samples respectively were noncompliant according to Iranian National Standards (Table 1).

Table 2 indicate that although mean of coliform counts ($9.8 \times 10^6$) was higher than standard levels, most of the analyzed samples met Iranian National Standard levels (at least 75%) of the samples contained no coliform, in other way only few sample had higher coliform counts. And although mean of TMC was higher than Standard limit, third quartile showed higher TMC.

As shown in Table 3 most of samples were not contaminated with *E. coli* and no statistically significant relationship ($p=0.710$) observed between type of packaging and contamination with *E. coli*. Distribution of total microbial counts between pouches and bottles was not significant statistically ($p=0.981$). Similarly there was no significant relationship between coliform counts and type of packaging ($p=0.540$).

<table>
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<tr>
<th>Table 1. Percentages of compliant and noncompliant pasteurized milk samples regarding microbiological parameters of Iranian National Standard (ISIRI, 2008)</th>
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<td>Compliant</td>
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<td>Coliform counts</td>
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<td><em>E. coli</em></td>
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<td>Total microbial counts</td>
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*The total number of milk samples analyzed were 103. Within parentheses are number of samples.

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<th>Table 2. Distribution factors of total microbial counts and coliform counts (CFU/mL)</th>
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<td>Mean</td>
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<td>Total microbial counts</td>
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<td>Coliform counts</td>
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<th>Table 3. Percentages of pasteurized milk microbial contamination have been marketed in fruit and vegetable markets in Tehran according to type of packaging</th>
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<td>Type of packaging</td>
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<td>Pouch</td>
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<td>Bottle</td>
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*The total number of milk samples analyzed were 103. Within parentheses are number of samples.
Results indicated that there was no statistically significant difference between samples with various fat contents (low fat, reduced fat, and whole fat) and distribution of coliform counts \( (p=0.699) \). Similar results obtained for distribution of total microbial counts in accordance with samples with different fat contents \( (p=0.813) \).

The relationship between shelf life and microbial qualities was investigated in this study. More than 84% of the samples with different shelf life (4, 5, and 6 days) were not contaminated with \textit{E. coli} and most of the contaminated samples had less shelf life (Table 4). There was no significant relationship between shelf life and contamination with \textit{E. coli} \( (p=0.348) \). Results indicated that there was no statistically significant difference between distribution of coliform counts and samples with different shelf life \( (p=0.084) \). Also there was no significant difference between distribution of total coliform counts and shelf life duration of samples \( (p=0.201) \).

In this study sample collection was included 13 different brands which 7 brands had the most number of samples. Brands are recognized by their codes in this paper. Brands with codes 5, 8, and 10 had the most noncompliant samples regarding to total microbial counts and codes 9 and 11 had the most noncompliant samples in case of coliform counts. Contamination with \textit{E. coli} was observed in samples belonged to codes 2, 4, 8, and 11. Brand numbers 2, 4, 8, 9, 10, and 11 had samples which were noncompliant with Iranian National Standard in all three microbial properties tested (total microbial counts, coliform counts, and \textit{E. coli}).

### DISCUSSION

High total microbial counts may be attributed to absence of proper hygienic pasteurized milk production. Coliforms and \textit{E. coli} represent fecal contamination which indicates post contamination, probable incomplete pasteurization or defective packaging. Microbial quality of pasteurized milk from markets is rarely investigated in Iran. In this study, of the 103 pasteurized milk samples collected, 59.2% (61) of the samples were noncompliant in terms of total microbial counts according to Iranian National Standard (ISIRI, 2008).

A study on microbial quality of milk in New York State showed total bacterial counts compliant with standard limits (21%) in 2002 raised to (48.6%) in 2010 (Martin \textit{et al.} 2012). Our results of total bacterial counts was less compliant (40.8%) to the data obtained by their work in 2010. Valbuena \textit{et al.} (2004) reported that mean of total microbial counts and coliform counts in pasteurized milk brands marketed in Maracaibo city in Venezuela were \( 3.4 \times 10^4 \) CFU/mL and \( 2.8 \times 10^2 \) CFU/mL respectively. In Cameroon mean of total microbial counts and mean of coliform counts in pasteurized milk collected from a dairy processing unit were \( 3.72 \pm 0.62 \) log CFU/ml and \( 1.921.86 \) log CFU/mL respectively (Belli \textit{et al.} 2013). Also in study carried out in Shahrekord, in Iran mean of total microbial counts and mean of coliform counts reported as \( 7.1 \times 10^4 \) CFU/mL and \( 8 \times 10^2 \) respectively (Shojaii and Yadollahi 2008). The mean of total microbial counts and mean of coliform counts obtained in this study were higher than above mentioned studies and Iranian National Standard limits. Although the mean of coliform counts are higher than standard limits in this study, 78.6% of the samples were compliant with the standard and only 21.4% was unacceptable. Our results in case of compliant samples was in accordance with the data obtained by Da Silva \textit{et al.} (2008) in Brazil.

Efficiency of pasteurization and microbial quality of 120 milk samples were investigated in Brazil 70.8%, 57.5%, and 40% of the samples were not compliant in terms of coliform counts in 35°C, coliform counts in 55°C, and mesophilic bacteria counts respectively (Silva \textit{et al.} 2010). Da Silva \textit{et al.} (2010) reported that 50% and 33.3% of pasteurized grade A milk samples were higher than Brazil’s standard levels in terms of total and fecal coliform counts respectively while only 2 samples reported as noncompliant in terms of mesophilic bacteria counts. Out of 260 milk samples, 105 samples (40.4 %) were not suitable to consume.
according to Brazil’s legal standards in case of coliforms and antimicrobial residues (Zanella et al. 2010). Present investigation showed lower noncompliance in case of coliforms compared to aforementioned studies.

Results showed that 7.8% of the samples were contaminated with E. coli. In a similar study, 39.5% of pasteurized milk samples collected in Shahrekord city in Iran were contaminated with E. coli which was 5 fold higher than our findings (Shojaei and Yadollahi 2008) whereas another study in Shahrekord reported no presence of E. coli in evaluated milk samples (Fadaei et al. 2009). A considerable number of milk samples evaluated in Romania also demonstrate presence of E. coli (Filimon et al. 2011). Of 20 pasteurized milk samples analyzed in Jamaica only one sample showed presence of E. coli (Anderson et al. 2011). Similar study in India indicated no presence of coliforms and E. coli in packaged milk samples, though 26.4% of the samples collected from pasteurized milk tankers were contaminated with aforementioned bacteria (Surve et al. 2011).

Our study demonstrate that distribution of total microbial counts, coliform counts and presence of E. coli was not statistically significant in relationship with type of packaging (pouches and bottles) which is in agreement with Papachristou et al. (2006) who found no statistically significant differences in mesophilic counts obtained for three type of polyethylene terephthalate and one type of paperboard cartoon packaging material in pasteurized milk samples during first 4 days of storage at 4°C. Similar results reported by Vassila et al. (2002) in case of using different pouch materials during 7 days of storage. Likewise, Zygoura et al. (2004) reported no significant differences in mesophilic counts recorded for milk samples in all five formulated packaging materials. Another study evidenced that different packaging boards did not affect (P> 0.05) bacterial growth rate in pasteurized milk stored at 6.7°C for 4 weeks (Simon and Hansen 2001). A study reported that HDPE bottle was better for storing pasteurized milk in comparison with LDPE pouches in case of shelf life extension (Petrus et al. 2010). Also HDPE bottle help maintain for abuse storage temperature as compared to LDPE pouch (Petrus et al. 2009).

No significant difference was found between different fat contents in terms of distribution of total microbial counts and coliform counts. No literature found on this case.

**CONCLUSION**

Coliform counts and E. coli were in conformity with Iranian National Standards, whereas more than half of samples were not compliant in terms of total microbial counts. It should be noted that although the mean of coliform counts were higher than the standard limits, only 21.4% were noncompliant in accordance with Iranian national standard. To conclude, this study confirm the relevantly acceptable overall microbial quality of pasteurized milk samples marketed in Tehran urban area evaluated on expiration date. It’s recommended to increase public awareness about consumption of pasteurized milk and precise implementation of GHP, GMP and HACCP to achieve high quality pasteurized milk in the dairy industry.

**ACKNOWLEDGEMENTS**

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