JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2017. Vol. **11**(1), p. 37-42

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.

http://dx.doi.org/10.22207/JPAM.11.1.06

(Received: 23 November 2016; accepted: 16 January 2017)

Pasteurized milk is consumed widely in all socioeconomic classes and consider as the main source of milk for people in Iran. In recent years increased price of pasteurized milk caused by elimination of subsidies resulted decrease in 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).

* To whom all correspondence should be addressed. Tel: + 98 (21) 22376473; Fax: +98 (21) 22376473; E-mail: mr_koushki@yahoo.com

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 ±=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 (9.8×10^6) 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 1. Percentages of compliant and noncompliant pasteurized milk samples regarding microbiological parameters of Iranian National Standard (ISIRI, 2008)

	Compliant	Noncompliant
Coliform counts	78.6 (81)	21.4 (22)
E. coli	92.2 (95)	7.8 (8)
Total microbial counts	40.8 (42)	59.2 (61)

*The total number of milk samples analyzed were 103. Within parentheses are number of samples.

Table 2. Distribution factors of total microbial counts and coliform counts (CFU/mL)

	Mean	Standard deviation	Minimum	First quartile	Median	Third quartile	Maximum
Total microbial counts	6.2×10^7	2.1×10^8	50	9.8×10^3	3.1×10^5	1.7×10^7	1.8×10^9
Coliform counts	9.8×10^6	7.5×10^7	0	0	0	0	7.4×10^8

Table 3. Percentages of pasteurized milk microbial contamination have been marketed in fruit and vegetable markets in Tehran according to type of packaging

Type of	Total microbial counts		Coliform counts		*E. coli*	
packaging	Compliant	Noncompliant	Compliant	Noncompliant	Compliant	Noncompliant
Pouch	48.1 (28)	58.2 (39)	76.1 (51)	23.9 (16)	91 (61)	9 (6)
Bottle	38.9 (14)	61.1 (22)	83.1 (30)	16.7 (6)	94.4 (34)	5.6 (2)
Total	40.8 (42)	59.2 (61)	78.6 (81)	21.4 (22)	92.2 (95)	7.8 (8)

*The total number of milk samples analyzed were 103. Within parentheses are number of samples.

Table 4. Percentages of pasteurized milk samples contaminated with *E.coli* regarding shelf life (days)

E. coli	Shelf life (days)		
contamination	4	5	6
Positive	15.8 (3)	5.6 (3)	6.7 (2)
Negative	84.2 (16)	94.4 (51)	93.3 (28)

*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 *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 *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 *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 *E. coli*).

DISCUSSION

High total microbial counts may be attributed to absence of proper hygienic pasteurized milk production. Coliforms and *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 *et al*. 2012). Our results of total bacterial counts was less compliant (40.8%) to the data obtained by their work in 2010. Valbuena *et al*. (2004) reported that mean of total microbial counts and coliform counts in pasteurized milk brands marketed in Maracaibo city in Venezuela were 3.4×10^4 CFU/mL and 2.8×10^2 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±0.62 log CFU/ml and 1.921.86 log CFU/mL respectively (Belli *et al*. 2013). Also in study carried out in Shahrekord, in Iran mean of total microbial counts and mean of coliform counts reported as 7.1×10^5 CFU/mL and 8×10^2 respectively (Shojaei 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 *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 *et al*. 2010). Da Silva *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

This research was supported by National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences Tehran, Iran (Grant No. 0450/72).

REFERENCES

1. Anderson M, Hinds P, Hurditt S, Miller P, McGrowder D, Alexander-Lindo R., The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. *Asian Pac J Trop Biomed* 2011; **1**:205-211
2. Belli P, Cantafora AFA, Stella S, Barbieri S, Crimella C., Microbiological survey of milk and dairy products from a small scale dairy processing unit in Maroua (Cameroon). *Food Control* 2013; **32**:366-370
3. Breurec S, Poueme R, Fall C, Tall A, Diawara A, Bada-Alambedji R, Broutin C, Leclercq A, Garin B., Microbiological quality of milk from small processing units in Senegal. *Foodborne Pathog Dis* 2010; **7**:601-604
4. Da Silva MCD, Da Silva JVL, Ramos ACS, Melo RD, Oliveira JO., Microbiological and physicochemical characterization of pasteurized milk for the milk program in the State of Alagoa.

Ciencia Tecnol Alime 2008; **28**:226-230
5. Da Silva VAM, Rivas PM, Zanela MB, Pinto AT, Ribeiro MER, Silva FFP, Machado M., Evaluation of physical, chemical and microbiological quality of raw and pasteurized grade A milk and points of contamination at a dairy farm in RS. *Acta Sci Vet* 2010; **38**:51-57
6. Fadaei AAM, Jamshidi E, Kheiri S., Comparison of bacterial contamination of raw and pasteurized milk used in Shahrekord in 2006. *J Shahrekord Univ Med Sci* 2009; **10**:37-44 (In Persian)
7. Filimon MN, Borozan AB, Borodean DM, Popescu R, Gotia SR, Verdes D, Morariu F, Treitli S., Quality assessment of raw and pasteurized milk using microbial parameters. *Anim Sci Biotechnol* 2011; **44**:412-416
8. Ghafari FM., Annual milk production in Tehran province. Jehad and Agricultural Organization, Tehran, Iran (In Persian), 2011.
9. ISIRI., Microbiology of food and animal feeding stuffs-detection and enumeration of Escherichia coli- most probable number technique, Iran's National Standards No. 2946 (2nd revision). Institute of Standards and Industrial Research of Iran, Karaj, Iran, 1999.
10. ISIRI., Milk and milk products-Enumeration of coliforms part 2: most probable number technique at 30°C, Iran's National Standards No. 5486-2 (1nd edition). Institute of Standards and Industrial Research of Iran, Karaj, Iran, 2000.
11. ISIRI (2000) Milk and milk products-Enumeration of colony-forming units of microorganisms-colony-count technique at 30°C, Iran's National Standards No. 5484 (1nd edition). Institute of Standards and Industrial Research of Iran, Karaj, Iran
12. ISIRI., Microbiology of milk and milk products-Specifications-Test method, Iran's National Standards No. 2406 (2nd revision). Institute of Standards and Industrial Research of Iran, Karaj, Iran, 2008.
13. Koushki MR., Technology of milk, meet and egg. Gholami Publisher and Distributer, Tehran, Iran (In Persian), 2010.
14. Koushki MR, Kohi-Kamali-Dehkordi P., Microbiological quality of pasteurized milk in west of Tehran (Winter 2013). *J Food Sci Technol* 2016; **50**:115-120 (In Persian).
15. Martin NH, Carey NR, Murphy SC, Wiedmann M, Boor KJ., A decade of improvement: New York State fluid milk quality. *J Dairy Sci* 2012; **95**:7384-7390
16. Mohammadi M, Jalali H, Naafchi AM., Assessment of physicochemical and Microbial properties in pasteurized milk available in Shahrood city stores. Proceeding of 21st National Congress of Food Science and Technologies. Shiraz, Iran, 2013.
17. Nikoozadeh H, Ghodrati N, Eslami A, Yazdani A, Jalilvand M., Study on microbial quality of milk and dairy products in North Khorasan Province. Proceedings of 2nd International Congress of Food Hygiene. Tehran, Iran, 2011.
18. Papachristou C, Badeka A, Chouliara I, Kondyli E, Kourtis L, Kontominas MG., Evaluation of polyethylene terephthalate as a packaging material for premium quality whole pasteurized milk in Greece. *Eur Food Res Technol* 2006; **224**:237-247
19. Petrus R, Loiola C, Silva C, Oliveira C., Microbiological and sensory stability of pasteurized milk in Brazil. *Chem Eng Trans* 2009; **17**: 939-944
20. Petrus RR, Loiola CG, Oliveira CA., Microbiological shelf-life of pasteurized milk in bottle and pouch. *J Food Sci* 2010; **75**:M36-40
21. Shojaei ZA, Yadollahi A., Physicochemical and microbiological quality of raw and pasteurized and UHT milks in shops. *Asian J Sci res* 2008; **1**: 532-538
22. Silva R, Cruz AG, Faria JA, Moura MM, Carvalho LM, Water EH, Sant'Ana AS., Pasteurized Milk: Efficiency of Pasteurization and Its Microbiological Conditions in Brazil. *Foodborne Pathog Dis* 2010; **7**:217-219
23. Simon M, Hansen AP., Effect of Various Dairy Packaging Materials on the Shelf Life and Flavor of Pasteurized Milk. *J Dairy Sci* 2001; **84**:767-773
24. Surve VV, Patil MU, Gaikwad S., Microbiological quality of milk marketed in Goa state. *J Dairy Foods Home Sci* 2011; **30**:75-76
25. Valbuena E, Castro G, Lima K, Acosta W, Brinez W, Tovar A., Bacteriological quality of main pasteurized milk brands distributed in Maracaibo City, Venezuela. *Rev Cient-Fac Cienc Vet* 2004; **14**:59-67
26. Vassila E, Badeka A, Kondyli E, Savvaidis I, Kontominas MG., Chemical and microbiological changes in fluid milk as affected by packaging conditions. *Int Dairy J* 2002; **12**:715-722
27. Zanella GN, Milkcha JMG, Bando E, Siqueria VLD, Machinski MJR., Occurrence and Antibiotic resistance of Coliform Bacteria and antimicrobial residues in pasteurized Cow's milk from Brazil. *J Food Prot* 2010; **73**:1684-1687
28. Zygoura P, Moyssiadi T, Badeka A, Kondyli E, Savvaidis I, Kontominas MG., Shelf life of whole pasteurized milk in Greece: effect of packaging material. *Food Chem* 2004; **87**:1-9.