JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 4 (2017) 53-57

Chemical and Microbial Quality of Iranian Commercial Pasteurized Milk Samples at Their Expiration Date

M. Azizkhani [*✉], F. Tooryan

Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

HIGHLIGHTS

- Mean pH value of the milk samples was 6.6.
- Mean sensory score of the milk samples was 6.4 according to nine point hedonic scale.
- Mean coliform count of the milk samples was 0.77 $\log_{10}$ Colony Forming Unit (CFU)/ml.
- Mean *Escherichia coli* count of the milk samples was 0.01 $\log_{10}$ CFU/ml.
- No significant relationship was found between various studied parameters and different milk brands.

Article type
Original article

Keywords
Milk
Food Quality
Food Preservation
Food Safety

Article history
Received: 2 Mar 2017
Revised: 13 Apr 2017
Accepted: 3 May 2017

Acronyms and abbreviations
CFU= Colony Forming Unit

ABSTRACT

Background: Milk can provide a good medium for growth and proliferation of various spoilage and pathogenic microorganisms. The main aim of this study was to evaluate the chemical and microbial quality of Iranian commercial pasteurized milk samples at their expiration date.

Methods: Hundred samples of pasteurized milk packaged in polyethylene pouches were randomly collected from local markets of Amol, North of Iran. Acidity, pH, total viable, coliforms and *Escherichia coli* counts were carried out at expiration date of the samples according to the standard procedures. Data were statistically analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA).

Results: The mean value of pH, acidity, and sensory score of the samples were obtained as 6.6, 0.15 g lactic acid/100 ml, and 6.4, respectively. Also, the mean value of total viable count, coliforms and *E. coli* counts of the samples were 4.11, 0.77, and 0.01 $\log_{10}$ colony forming unit/ml, respectively. No significant relationship (p>0.05) was found between various studied parameters and different milk brands.

Conclusion: Storage of milk pouches out of refrigerator in supermarkets of Iran must be strictly avoided in order to improve chemical and quality of this product. Also, proper pasteurization process and reduction of post-pasteurization contamination are the other keys to produce high-quality pasteurized milk.

Introduction

As milk is a rich source of nutrients, it provides a good medium for growth and proliferation of various spoilage and pathogenic microorganisms which may cause early physicochemical and microbial deterioration. Pasteurization is the heat treatment of milk in order to destroy or reduction of microorganisms and also deactivation of undesirable enzymes. Several factors that influence the shelf-life of pasteurized milk such as chemical and microbial quality of the raw milk are duration of storage of raw milk before heat-treatment, the quality of heat-treatment, the population of heat-resistant bacteria, cross contamination after heat process, filling as well as packaging system, storage conditions of the product during distribution, and also maintenance of desirable temperature during the entire cold chain. Generally, shelf-life of pasteurized milk is 2 up to 20 days (Sarkar, 2015), but in Iran its shelf-life is usually determined

[*] Corresponding author. ✉ m.azizkhani@ausmt.ac.ir

To cite: Azizkhani M., Tooryan F. (2017). Chemical and microbial quality of Iranian commercial pasteurized milk samples at their expiration date. *Journal of Food Quality and Hazards Control*. 4: 53-57.

about 3-5 days. Microbiological analysis of pasteurized milk have been attempted in many researches showed presence of pathogenic microorganisms (Aglawe and Wadatkar, 2012; Anderson et al., 2011; De Oliveira et al., 2011; Singh et al., 2011; Vahedi et al., 2013). Several reports have indicated that inadequate pasteurization and post-pasteurization contamination result in incidence of pathogenic bacteria in pasteurized milk and therefore milk-borne outbreaks have been occurred (Da Silva et al., 1998; Silva et al., 2010; Upton and Coia, 1994).

Numerous studies have evaluated the chemical and microbiological quality of pasteurized milk in different countries (Anderson et al., 2011; Belli et al., 2013; Breurec et al., 2010; Dey and Karim, 2013; Martin et al., 2012; Ranieri and Boor, 2009; Silva et al., 2010) and also some researches have been conducted in Iran (Karajibani et al., 2016; Teymori et al., 2014; Zolfaghari et al., 2012). However, based on our knowledge no microbial and chemical analysis have been carried out at expiration date of pasteurized milk distributed in Mazandaran province, Northern Iran that is the pole of dairy industry of the country; so, the main aim of this study was to evaluate the chemical and microbial quality of Iranian commercial pasteurized milk samples at their expiration date.

Materials and methods

Sample collection

In this cross-sectional study carried out in 2016, totally 100 samples of pasteurized milk from 10 different brands packaged in polyethylene pouches were randomly collected from local markets in different parts of Amol, Mazandaran province, North of Iran. The samples were transported to the laboratory in a cool box and kept in the refrigerator (4-8 °C) until their expiration date indicated on the package.

pH determination

The pH values of the samples were measured using a digital pH meter (Jenway, UK), calibrated routinely with fresh pH 4.01 and 6.86 standard buffers (ISIRI, 2006).

Acidity determination

The amount of lactic acid, as a result of lactose fermentation was measured through titration with NaOH according to ISIRI (2006).

Total viable count

Total viable counts were determined using 1 ml of suitable dilutions (10^{-1} to 10^{-3}) of milk samples on pour-plates of plate count agar (Merck-Darmstadt, Germany) incubated at 32 ºC for 48 h. Dishes that contained 30-300 colonies were selected for colony counting. All results were reported as $\log_{10}$ Colony Forming Unit (CFU)/g as described by ISIRI (2000a; 2008).

Coliform count

Coliform count was determined using 1 ml of prepared dilutions (10^{-1} to 10^{-3}) from milk samples on pour-plates of violet red bile agar (Merck-Darmstadt, Germany) incubated at 30 ºC for 24 h. The results were reported as $\log_{10}$ CFU/g. Dishes that contain no more than 150 colonies were selected for colony counting. For the coliform confirmation test, brilliant green bile lactose broth (Merck-Darmstadt, Germany) with Durham tube was used and incubated at 30 ºC for 24 h (ISIRI, 2000b).

Escherichia coli detection

Tryptophan broth (Merck-Darmstadt, Germany) was used in the detection of *E. coli* from coliform positive tubes. The inoculated tubes were incubated for 48 h. Conversion of tryptophan into the indole by *E. coli* was recognized by adding Kovacs indicator as well as appearance of pink layer at top of the broth medium (ISIRI, 2000c).

Sensory evaluation

The sensory properties were evaluated through consumer taste panels using nine point hedonic scales. Twenty trained participants between the ages of 18-25 (14 females and 6 males) from staff of Amol University of Modern Special Sciences, Amol, Iran were recruited to take part in consumer taste panel. Consumer acceptance of the experimental products was evaluated using a hedonic scale of 1-9 where 1 corresponds with “dislike extremely” and 9 corresponds “like extremely” in two consumer panels. Sensory scores ≥5 were considered as acceptable.

Statistical analysis

All experiments were repeated in triplicate. Data were statistically analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). Difference between data was significant if the *p* value was found to be <0.05.

Results

Chemical quality and sensory score of pasteurized milk samples at the expiration date is shown in Table 1. The mean value of pH, acidity, and sensory score of the samples were obtained as 6.6, 0.15 g lactic acid/100 ml, and 6.4, respectively. The detail results of microbiological quality assessment of the pasteurized milk samples at the expiry date are presented in Table 2. The mean value of

total viable count, coliforms and *E. coli* counts of the samples were 4.11, 0.77, and 0.01 $\log_{10}$ CFU/ml, respectively. No significant relationship (p>0.05) was found between various studied parameters and different milk brands.

According to limitation levels described in the Iran national standards, the level of pH, acidity, total viable count, coliform, and *E. coli* count exceeded in 30, 20, 40, 20, and 10% of the milk samples, respectively. Also, 50% of the samples had acceptable quality according to the national criteria recommended for pasteurized milk.

Discussion

In the present research, we analyzed microbial and chemical parameters of pasteurized milk distributed in Amol, Northern Iran. In this regards, there are many works carried out previously in Iran and other countries having some similarity and also controversy with each other. For instance, most of milk samples in our study were within acceptable pH range, whereas Aggad et al. (2010) announced that 47.8% pasteurized milk samples in Algeria failed to meet the official standards for pH; conversely, Mirzaalizadeh et al. (2017) showed that pH of all studied pasteurized milk samples in Zanjan, Iran was within standard limits during the whole period of storage. However, being within acceptable pH value does not alone guarantee good quality and freshness of the milk product; because some adulterant actions (such as adding alkaline agents to low-quality milk tanks) which are unfortunately not scarce in Iran, can simply change pH into its normal range. Based on the scientific facts, pH of milk samples is decreased due to producing lactic acid towards the end of shelf-life period; but, in this study we found that in some milk samples, there were no considerable pH changes in spite of their great bacterial counts indicating suspicious of probable adulteration mentioned above. However, more analysis should be carried out to express exact and conclusive statement in this regards.

In the current work, the percentage of lactic acid in 80% samples was in the acceptable range (0.14-0.16%) according to national standard. In Algeria, 74% samples of pasteurized milk were not within acceptable acidity range (Aggad et al., 2010). In another investigation, the acidity of all the pasteurized milk samples from India was higher than the standard limit (Saxena and Rai, 2013).

Table 1: pH value, acidity, and sensory score of pasteurized milk samples distributed in Amol at the expiration date

Brand	pH	Acidity (g lactic acid/100 ml)	Sensory score
1	6.75±0.3	0.14±0.01	8.5±0.3
2	6.61±0.51	0.14±0.05	7±0.16
3	6.25±0.14	0.17±0.01	3.5 ±0.27
4	6.81±0.45	0.15±0.04	8±0.39
5	6.68±0.28	0.16±0.01	4.5±0.48
6	6.70±0.35	0.15±0.03	7.5±0.13
7	6.64±0.19	0.15±0.08	8±0.14
8	6.05±0.54	0.19±0.05	4±0.25
9	6.73±0.36	0.14±0.02	9±0.48
10	6.78±0.27	0.16±0.01	4±0.21

Table 2: Total viable count, coliforms and *Escherichia coli* ($\log_{10}$ CFU/ml) of pasteurized milk samples distributed in Amol at the expiration date

Brand	Total viable count	Coliforms	*Escherichia coli*
1	2.53±0.20	0.27±0.16	0
2	3.15±0.16	0.39±0.08	0
3	5.67±0.22	1.73±0.10	0
4	3.35±0.19	0.42±0.13	0
5	4.95±0.15	0.86±0.15	0
6	3.86±0.43	0.60±0.09	0
7	3.71±0.36	0.61±0.12	0
8	6.72±0.24	2.08±0.11	0.1±0.04
9	2.16±0.25	0.25±0.10	0
10	5.05±0.31	0.55±0.17	0

Chatterjee et al. (2006) reported that all of the pasteurized milk samples in India were in good microbial quality due to high quality pasteurization. In another work, Silva et al. (2010) reported presence of coliforms in 57% of pasteurized milk samples in Brazil. In a similar survey conducted in West Azerbaijan province, North-West of Iran, acceptable microbiological quality was reported in about 85% of pasteurized milk samples (Teymori et al., 2014) which is comparable with our findings. In the present study, 10% of the studied milk samples were contaminated with *E. coli* showing variability comparing with findings stated by some researchers who detected *E. coli* in 39.5, 9, 0, and 3.9% of milk products marketed in Shahrekord of Iran (Shojaei and Yadollahi, 2008), Sari of Iran (Vahedi et al., 2013), India (Surve et al., 2011), and South Africa (El Zubeir et al., 2008). The results of microbial count obtained in this study were lower than those of reported by Karajibani et al. (2016) and Zolfaghari et al. (2012) who analyzed microbiologically the pasteurized milk and dairy products marketed in Zahedan (Iran) and Qom (Iran), respectively. Lower bacterial counts observed in our samples could be attributed to the climate condition of the area where the samples were collected; Zahedan and Qom are two main hot cities in Iran and so maintenance of cold chain of commercial milk in this condition is more difficult than that of Amol city with mild and relatively cold climate. On the other hand, in spite of pasteurized milk bottles which are always refrigerated, Iranian commercial pasteurized milk pouches may be stored out of the refrigerator (at room temperature) until they are sold highlighting the probable role of climate condition in milk quality. However, some other factors such as the quality of hygienic practices and handling in dairy facilities can affect the milk quality.

It is worth noting that in spite of all above mentioned studies in which the milk samples had obtained within their shelf-life period, we have chosen the milk samples at their expiration time. Logically, in the near of expiration time, the food products are too perishable and susceptible comparing to the earlier periods. In this regards, Jeppu et al. (2015), showed lower bacterial load in fresh milk than that of those were close to expiration date. Thus, although microbial and chemical quality of milk samples in this study were in close with the findings of the other researches, but it is probably assumed that if the milk are obtained within their shelf-life, microbial and chemical quality of milk samples of Amol, Northern Iran will be improved.

Conclusion

In conclusion, storage of milk pouches out of refrigerator in supermarkets of Iran must be strictly avoided in order to improve chemical and quality of this product. Also, proper pasteurization process and reduction of post-pasteurization contamination are the other keys to produce high-quality pasteurized milk.

Conflicts of interest

The authors have declared that no conflict of interest exists.

Acknowledgements

This work has been supported by a research grant (No. T.8.374) from the Amol University of Special Modern Technologies, Amol, Iran. This research was ethically approved by the local institutional review board. We are also grateful to Dr. Andrew MacCabe of the Instituto de Agroquímica y Tecnología de Alimentos (IATA), Valencia, Spain for critical reading of the manuscript.

References

Aggad H., Bridja Bouhai Aek M., Benaouali M., Djebli A. (2010). Some quality aspects of pasteurized milk in Algeria. *World Journal of Dairy and Food Sciences.* 5: 21-24.

Aglawe P.P., Wadatkar C.M. (2012). Microbial examination of milk sample from Nagpur region with reference to coliform. *Food Science and Technology Letters*. 3: 24-26.

Anderson M., Hinds P., Hurditt S., Miller P., McGrowder D., Alexander-Lindo R. (2011). The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. *Asian Pacific Journal of Tropical Biomedicine*. 1: 205-211.

Belli P., Cantafora A.F.A., Stella S., Barbieri S., Crimella C. (2013). Microbiological survey of milk and dairy products from a small scale dairy processing unit in Maroua (Cameroon). *Food Control*. 32: 366-370.

Breurec S., Poueme R., Fall C., Tall A., Diawara A., Bada-Alambedji R., Broutin C., Leclercq A., Garin B. (2010). Microbiological quality of milk from small processing units in Senegal. *Foodborne Pathogens and Diseases*. 7: 601-604.

Chatterjee S.N., Bhattacharjee I., Chatterjee S.K., Chandra G. (2006). Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. *African Journal of Biotechnology*. 5: 1383-1385.

Da Silva M.C.D., Hofer E., Tibana A. (1998). Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, Brazil. *Journal of Food Protection*. 61: 354-356.

De Oliveira L.P., Soares e Barros L.S., Silva V.C., Cirqueira M.G. (2011). Study of *Staphylococcus aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. *Journal of Food Processing and Technology*. 2: 128-135.

Dey S., Karim M.H. (2013). Study on physicochemical and microbial quality of available raw, pasteurized and UHT milk during preservation. *International Journal of Science Inventions Today*. 2: 150-157.

El Zubeir I.E.M., Gabriechise V., Johnson Q. (2008). Comparison of chemical composition and microbial profile of raw and pasteurized milk of the Western Cape, South Africa. *International Journal of Dairy Science*. 3: 137-143.

Institute of Standards and Industrial Research of Iran (ISIRI). (2000a). Milk and milk products- Enumeration of colony forming units: colony count technique at 30 °C. National

Standard No. 5484. URL: http://www.isiri.gov.ir/portal/files/std/ 5484.htm. Accessed 25 October 2016.

Institute of Standards and Industrial Research of Iran (ISIRI). (2000b). Milk and milk products- Enumeration of coliforms part 2: most probable number technique at 30 ℃. National Standard No. 5486. URL: http://www.isiri.gov.ir/portal/files/std /5486-1.htm. Accessed 25 October 2016.

Institute of Standards and Industrial Research of Iran (ISIRI). (2000c). Milk and milk products- Enumeration of presumptive *Escherichia coli*: most probable number technique. National Standard No. 5234. URL: http://www.isiri.gov.ir/portal/file/?427423/5234.pdf. Accessed 25 October 2016.

Institute of Standards and Industrial Research of Iran (ISIRI). (2006). Milk and milk products- Determination of titrable acidity and pH value- Test method. National Standard No. 2852. URL: http:// http://www.isiri.gov.ir/portal/files/std/2582.htm. Accessed 25 October 2016.

Institute of Standards and Industrial Research of Iran (ISIRI). (2008). Pasteurized milk- Specifications and test methods. National Standard No. 5282. URL: http://www.isiri.gov.ir/portal/files/std/2582.htm. Accessed 25 October 2016.

Jeppu U., Jayaram N., Satya S., Purayil A.K. (2015). Microbiological evaluation of Ultra-High-Temperature (UHT)-treated milk close to expiry date and routine home practices for preservation of milk. *Indian Journal of Community Medicine*. 40: 174-176.

Karajibani M., Merkazee A., Montazerifar F., gomrekee M., Gholamee A. (2016). Microbial contamination of pasteurized milk and dairy products in Zahedan. *Journal of Mazandaran University of Medical Sciences*. 26: 165-169. [Persian full text with English abstract]

Martin N.H., Ranieri M.L., Wiedmann M., Boor K.J. (2012). Reduction of pasteurization temperature leads to lower bacterial outgrowth in pasteurized fluid milk during refrigerated storage: a case study. *Journal of Dairy Science*. 95: 471-475.

Mirzaalizadeh A., Tajkei J., Satei N., Zamani A., Hejazi J. (2017). Evaluation of chemical and microbial characteristics of pasteurized yogurt drink and milk products in Zanjan province during the years of 2012-2014. *Journal of Food and Nutrition Sciences*. 14: 115-123. [Persian full text with English abstract]

Ranieri M.L., Boor K.J. (2009). Short communication: bacterial ecology of high-temperature, short-time pasteurized milk processed in the United States. *Journal of Dairy Science*. 92: 4833-4840.

Sarkar S. (2015). Microbiological considerations: pasteurized milk. *International Journal of Dairy Science*. 10: 206-218.

Saxena M., Rai P. (2013). Microbiological and chemical analysis of raw, pasteurized and UHT milk during preservation in India. *International Journal of ChemTech Research*. 5: 2804-2809.

Shojaei Z., Yadollahi A. (2008). Physicochemical and microbiological quality of raw, pasteurized and UHT milks in shops. *Asian Journal of Science and Research*. 1: 532-538.

Silva R., Cruz A.G., Faria J.A., Moura M.M., Carvalho L.M., Water E.H., Sant'Ana A.S. (2010). Pasteurized milk: efficiency of pasteurization and its microbiological conditions in Brazil. *Foodborne Pathogens and Diseases*. 7: 217-219.

Singh V., Kaushal S., Tyagi A., Sharma P. (2011). Screening of bacteria responsible for the spoilage of milk. *Journal of Chemical and Pharmaceutical Research*. 3: 348-350.

Surve V., Patil M., Gaikwad S. (2011). Microbiological quality of milk marketed in Goa state. *Journal of Dairy Foods and Home Sciences*. 30: 75-76.

Teymori R., Ghazanfarirad N., Dehghan K., Kheyri A., Hajigholizadeh G., Kazemi-Ghoshchi B., Bahmani M. (2014). Monitoring microbial quality of commercial dairy products in West Azerbaijan province, Northwest of Iran. *Asian Pacific Journal of Tropical Disease*. 4:S824-S829.

Upton P., Coia J. (1994). Outbreak of *Escherichia coli* 0157 infection associated with pasteurised milk supply. *The Lancet*. 344: 1015-1022.

Vahedi M., Nasrolahei M., Sharif M., Mirabi A.M. (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. *Journal of Preventive Medicine and Hygiene*. 54: 120-123.

Zolfaghari M.R., Gaeini R., Kalhor N., Khalilian M., Razavian M.H., Sasani M.S. (2012). Determination of microbial contamination of the milk and pasteurized dairy products produced in the Qom province. *Journal of Microbial World*. 5: 47-57. [Persian full text with English abstract]