Seasonal variations in some quality parameters of milk produced in Khorasan Razavi Province, Iran

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Abstract

In this trial, 60 samples of raw cow's milk collected during summer and winter in five collective centers from Khorasan Razavi Province of Iran were analyzed for penicillin residues, somatic cell counts (SCC), mesophilic counts (TMC) and adulteration materials. The mean penicillin level (0.57 ± 0.20 µg/L) was much lower than the maximum permitted level (MPL) recommended by the Codex Alimentarius Commission (4 µg/L). However, the mean SCC and TMC (Log 5.71 ± 0.08 cells/mL and Log 6.18 ± 0.11 colony forming units (CFU)/mL, respectively) exceeded their respective MPLs adopted in Iran for grade C milk (Log 5.69 cells/mL and Log 6.0 CFU/mL, respectively), with higher counts ($P < 0.05$) in the summer. High frequencies of samples containing salt (53-60%), detergents (16-20%) and formalin (7-10%) were observed in milk samples, stressing the need for stringent control measures to improve the quality of milk delivered in the evaluated collection centers.

Keywords: antibiotic residue, somatic cell counts, mesophilic counts, milk adulteration.

Practical Application: Occurrence data on penicillin residues, mesophilic bacteria, SCC and adulterants in Iranian milk.

1 Introduction

Cow's milk as the most important product of dairy systems is among the main foods for human consumption all over the world (Pereira, 2014). Because of its high nutritional value, milk offers excellent conditions for microbial growth, thus requiring proper conservation to avoid rapid deterioration by spoilage microorganisms and pathogenic bacteria (Lee et al., 2019). One of the most important contaminants of milk and dairy products are mesophilic bacteria, including several types of pathogens like *Salmonella* spp. and *Mycobacterium* spp. (Lee et al., 2012; Moosavy et al., 2019; Mullan, 2019). Moreover, milk may also contain increased somatic cell counts (SCC), mainly formed by leukocytes from blood origin and epithelial cells from scaling-off as a consequence of inflammation of the mammary gland (Fagundes et al., 2011). In this context, total mesophilic count (TMC) and SCC have been defined as the most reliable indicators for evaluating the quality of raw milk received in dairy processing factories worldwide, especially in the developed countries (Coelho et al., 2017). In the Iranian milk production system, raw milk is categorized into four grades (excellent, A, B and C), depending on maximum permitted levels (MPL) for SCC and TMC (Institute of Standard and Industrial Research of Iran, 2014). The higher MPLs were established for grade C milk, which should contain maximum SCC and TMC levels of 10^5 cells/mL (Log 5.69 cells/mL) and 10^6 colony forming units (CFU)/mL (Log 6.0 CFU/mL), respectively (Institute of Standard and Industrial Research of Iran, 2014).

The main contributing factors for microbial contamination of raw milk are related to milking hygiene and parlous conditions, cow udder soundness, raw milk collection, reserving and handling to the processing factories (Tassew & Seifu, 2011; Kara & Aslan, 2020). Additionally, milk and dairy products are exposed to different chemical contaminants such as antibiotics and other drug residues, neutralizing and preservatives residues (Iqbal, 2017; Müller et al., 2020). Among these contaminants, antibiotics residues represent a worldwide concern, since they are broadly used to control, treat or prevent different diseases, increase growth rate, regulate reproduction, or even improve the production efficiency of the dairy animals (Oliveira et al., 2020). The issue of antibiotic residues in milk is not a new problem. In the early 1960's, over 6% of the raw milk marketed in the United States were contaminated with antibiotic residues, although recent surveys indicated that the contamination percentage in the country decreased to 3.7% (Grunwald & Petz, 2003). Previous studies conducted in Iran indicated that antibiotic residues have been occasionally detected in raw cow's milk (Ghanavi et al., 2013; Moghadam et al., 2016).

Adulteration of milk is known as unethical activities conducted by producers aiming to increase the milk yield or reduce losses due to milk spoilage during storage and transportation (Swathi & Kauser, 2015). Typical examples of milk adulteration include the addition of water to increase milk yield, incorporation of salt or other thickening agents

regions contained penicillin G residues (mean level: 1.2 µg/L). In our study, three samples (two collected in the winter and one collected in the summer) were higher than the MPL (4 µg/L) recommended by the Codex Alimentarius Commission (2020). These data reinforce the need for Iranian regulations as well as the adoption of control strategies to prevent high levels of antibiotic residues in the milk produced in the country.

Table 2 presents the SCC obtained in cow's milk samples collected in Province of Khorasan Razavi. Considering the SCC obtained in both seasons, the overall mean (Log 5.71 ± 0.08 cells/mL) was above the MPL established by European Union (EU) (Log 4.0 cells/ml). In summer, the mean SCC in milk samples (Log 5.75 ± 0.07 cells/mL) was higher ($P < 0.05$) than the value obtained in the winter season (Log 5.69 ± 0.06 cells/mL), with 63% of all values above the MPL of Log 5.69 cells/mL adopted in Iran for grade C milk (Institute of Standard and Industrial Research of Iran, 2014). A previous study conducted in Mashhad city indicated no significant differences in the milk SCC collected in summer or winter seasons (Najafi et al., 2009).

Our results are in agreement with those described by Salman & Elnasri (2011) in the Khartoum State of Sudan, where higher SCC was found in bulk raw milk samples collected in summer (Log 5.78 - 5.90 cells/mL), compared with winter (Log 5.72 - 5.88 cells/mL). The authors hypothesized that the higher SCC found in summer samples of bulk milk was associated with mixtures of individual milks from different farms with high incidence of subclinical mastitis. However, it is well known that higher SCC in summer coincides with increased cases of clinical mastitis in summer months (Olivo et al., 2005; Fagundes et al., 2011), which could explain the season effect on the SCC data obtained in the present study.

Table 3 presents the TMC of cow's milk produced during the winter and summer seasons in Khorasan Razavi Province of Iran. The overall TMC mean was Log 6.18 CFU/mL, which is above the MPL of Log 6.0 CFU/mL adopted by Iranian regulations for grade C milk (Institute of Standard and Industrial Research of Iran, 2014). The samples collected in summer had higher ($P < 0.05$) mean TMC (Log 6.05 to 6.38 CFU/mL) than the mean value obtained in winter samples (Log 5.99 to 6.33 CFU/mL). Importantly, all samples had TMC above the EU standard guidelines (Log 5.69 CFU/mL). These results indicate that most of the traditional milk production in the studied region is accomplished under unsatisfactory sanitary conditions. This is illustrated by the fact that the majority of cow's milk producers in the Province of Korazan Razavi transport and deliver the raw milk to collective central in small containers, which are not submitted to sanitizyn procedures but only rinsed with water. Therefore, the poor hygienic conditions of raw milk transportation may be considered among the most important contributing factors for the high TMC observed in the present study. Highly contaminated raw milks may contain many pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*, which are among the major causes of foodborne illness worldwide (Shabbir et al., 2020). Coherently, the higher TMC and SCC in raw milk samples collected in summer as described here are similar to the data reported in Sudan (Salman & Elnasri, 2011) and Ethiopia (Tassew & Seifu, 2011). In addition, the microbiological quality of raw milk depends on the proper management of dairy herds, hygienic milking of healthy dairy cows and immediate cooling of milk in the farm (Camargo et al., 2014). Since in the present study the sampling procedures were conducted in collection centers, it remains to be determined to what extent these farm-related factors could have contributed for increasing the TMC in the raw milk samples evaluated.

In this study, raw milk samples were evaluated for the detection of unhealthy and inexpensive additives that may be used with the porpoise of extending the raw milk shelf-life or hide fraud practices. The results are present in Figure 1, indicating that raw milk collected in winter and summer was contaminated with several extraneous materials such as detergents (16 and 20%, respectively), hydrogen peroxide (6 and 3%, respectively), salt (53 and 60%, respectively) and formalin (7 and 10%, respectively). However, the analysis of other additives in milk samples including starch and sodium bicarbonate tested negative. Overall, there was no difference ($P > 0.05$) in the percentages of positive samples for adulteration parameters in the summer and winter. These results indicate that adulteration practices of raw milk are frequently adopted over the year in the milk production system of Khorasan Razavi, as previously reported by Mahmoudi et al. (2015) and Panahzadeh et al. (2016) regarding the milk produced in the Iranian provinces of East Azerbaijan and Qazvin, respectively.

Similarly to our results, milk adulteration has been widely reported in developing countries such as Sudan (Ibtisam et al.,

Table 2. Somatic cell counts (SCC) of cow's milk produced during the winter and summer seasons in Khorasan Razavi province of Iran.

Season	SCC mean ± SD (Log cells/mL)	SCC concentration range (Log cells/mL)	n > MPL (%)
Summer ($N = 30$)	5.75 ± 0.07 [a]	5.65 – 5.87	22 (73)
Winter ($N = 30$)	5.69 ± 0.06 [b]	5.54 – 5.79	16 (53)
Total ($N = 60$)	5.71 ± 0.08	5.54 – 5.87	38 (63)

[a-b] Mean values within rows with no common superscript differ significantly ($P < 0.05$). SD = Standard deviation. MPL: Maximum permitted level (Log 5.69 cells/mL) adopted by Iranian regulations for grade C milk (Institute of Standard and Industrial Research of Iran, 2014).

Table 3. Total mesophilic counts (TMC) of cow's milk produced during the winter and summer seasons in Khorasan Razavi province of Iran.

Season	TMC mean ± SD (Log CFU/mL)	TMC concentration range (Log CFU/mL)	n > MPL (%)
Summer ($N = 30$)	6.25 ± 0.07 [a]	6.05 – 6.38	30 (100)
Winter ($N = 30$)	6.10 ± 0.11 [b]	5.99 – 6.33	26 (87)
Total ($N = 60$)	6.18 ± 0.11	5.99 – 6.38	56 (93)

[a-b] Mean values within rows with no common superscript differ significantly ($P < 0.05$). cfu: colony forming units. SD = Standard deviation. MPL: Maximum permitted level (Log 6.0 CFU/mL) adopted by Iranian regulations for grade C milk (Institute of Standard and Industrial Research of Iran, 2014).
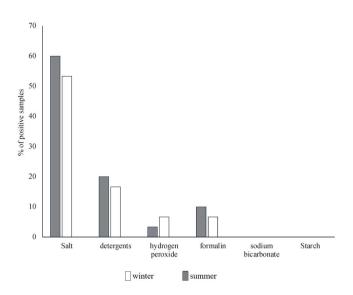
Figure 1. Percentages of samples with detectable adulterants in cow's milk from Khorasan Razavi province of Iran, collected in winter and summer (n = 30 in each season). Values among winter or summer seasons were not different (P > 0.05).

2009), India (Swathi & Kauser, 2015) and Pakistan (Barham et al., 2014; Iqbal, 2017). However, the percentage of positive samples containing formalin (2.4%) and hydrogen peroxide (0.4%) obtained by Ibtisam et al. (2009) in Sudan was lower than those reported here. High percentages (up to 100%) of adulterants including water, starch and detergents were found in milk from local vendors in India (Swathi & Kauser, 2015). In a similar study conducted in Pakistan, Barham et al. (2014) observed higher percentages of samples containing adulterants such as detergents (41%), starch (27%), formalin (20%), hydrogen peroxide (15%), caustic soda (11%), glucose (10%) and hypochlorite (5%). These data indicate that continuous monitoring programs are required in Iran among other developing countries, to inhibit milk adulteration practices. Given the diversity of potential adulterants in milk, the use of automatic instruments should be considered for robust detection of frauds in raw milk (Nascimento et al., 2017).

4 Conclusion

Although penicillin residues were frequently detected in all samples analyzed, the levels found were low and only three samples had concentrations above the MPL adopted in European countries (4.0 µg/L). There were seasonal variations in the TMC and SCC in milk, with higher levels observed in samples collected in summer. However, TMC and SCC obtained in both seasons were higher than the MPLs set up by European Communities and Iran regulations. These results in association with the high occurring percentages of adulterants in raw milk such as hydrogen peroxide, salt, formalin and detergents, stress the need for urgent control measures in the milk production system in Khorasan Razavi province, with special oversee on the collective centers of raw milk.

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