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Microbiological and Physicochemical Properties of Raw Milk Produced from Milking to Delivery to Milk Plant

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The aim of this study was to determine the microbiological and physicochemical properties of raw milk in Mazandaran province, Iran, during 6 months from winter 2013 to spring 2014. A total of 253 raw milk samples were collected, from dairy farms, milk cans, milk collection centers, and delivery milk tankers. The samples were analyzed for microbial quality, total plate count (TPC), and physicochemical properties including titrable acidity, pH, content of fat and protein, solids nonfat (SNF) level, specific gravity, percentage of water adulteration, and alcohol testing. The mean of TPC was 48×10^7 CFU/mL. The higher TPC (23×10^8) was found in Chalous city during winter season. TPC less than 10^6 was used as a basic standard limit by Institute of Standards and Industrial Research of Iran (ISIRI). Significant effect of region was observed on all physicochemical properties except pH ($p<0.05$). The mean counts of titrable acidity, pH, fat, protein, SNF, specific gravity, and percentage of water adulteration were 15.38, 6.66, 3.42%, 3.04%, 8.33%, 1.029 and 2.11%, respectively, and alcohol stability result of 22.1% of the samples was positive. It can be assumed that raw milk in the study area had poor bacteriological quality according to the Iranian National Standard (ISIRI ; therefore, it may be hazardous for human consumption. This finding shows the necessity to implement good hygiene practices.

Keywords: Raw milk, Physicochemical properties of milk, Microbiological quality of milk, Iran.

The most important matter for the dairy industry is factors affecting the quality of milk products, especially fermented dairy products. High quality raw milk is the initial prerequisite to produce high quality fermented milk products. The quality of raw milk defines by microbial quality and physicochemical properties that vary in the milk samples of different dairy farms; these differences are technologically important to make fermented milk products (Stulova *et al*., 2010). There is a relationship between the properties of used raw milk and produced pasteurized milk (Abd Elrahman *et al*., 2009). Raw milk attributes and seasonal variation affect the composition and properties of raw milk during processing (Chen *et al*., 2014). Many microorganisms can grow in milk. Special composition of milk, its high water content, and the neutral pH value allows the growth of many microbes (Quigley *et al*., 2011). These microorganisms enter milk from a variety of sources, and can play different roles such as causing spoilage (Quigley *et al*., 2013). Milk is highly perishable and poor management causes health

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threat and economic losses; therefore, hygienic practices is needed throughout the production to consumer chain (Swai and Schoonman, 2011). Seasonal variation affects raw milk quality. In warm weather, microbial load of raw milk increases (Yarahmadi *et al*., 2008). Recently, some studies have been done to assess the quality of raw milk, but no scientific and comprehensive research has been carried out so far on raw milk quality in Mazandaran province, Iran. The aim of this study was to determine the microbiological and physicochemical quality of raw cow milk in this province during winter 2013 and spring 2014

MATERIALS AND METHODS

Sampling: Totally, 253 raw cow milk samples were collected according to random sampling design, monthly, from winter 2013 to the end of spring 2014. Sampling was carried out from the dairy farms, milk cans, milk collection centers, and milk delivery tankers in Mazandaran province, Iran. Each milk sample consisted of 10 mL of raw milk poured into a sterile syringe for microbial evaluation and 700 mL in to a sterile container for chemical analysis . The samples were delivered to the laboratory of Haraz Milk Plant, Mazandaran province in a cool box, and tested immediately on arrival.

Physicochemical analysis

The pH of milk was recorded using pH meter (Metrohm, Switzerland). pH and titrable acidity were measured according to Iranian National Standard (NSI) (ISIRI, 2006). Fat content was determined by Gerber method (ISIRI, 1991). Protein content was determined using Kjeldahl method, INS No. 639 (ISIRI, 2013), and SNF was measured according to the INS No. 637 (ISIRI, 2013). Specific gravity of the milk samples was measured using Lactodensimeter (SlW, Germany) according to the INS (ISIRI, 1993); also alcohol test was conducted according to the NSI (ISIRI, 2005). Water adulteration was measured by Milkoscan (Ekomilk Total EON- Bulgaria).

Microbiological analysis

For microbiological analysis, TPC of the samples was evaluated by plate count agar (Merck, Germany); then they were incubated at 37°C for 72 hours. Colony counting was carried out using colony counter (ISIRI, 2008). The total plate count according to ISIRI, 2008 is maximum 10^6 CFU/ml acceptable.

Statistical analysis

Statistical analysis was performed using Minitab software and analysis of variance(ANOVA). Tukey Test was used for comparison of averages, as well as the effects and interactions of factors. Excel and Minitab software were used for drawing charts.

RESULTS

Microbiological quality

The mean of total plate count of raw cow milk in Mazandaran province during two seasons was 48×10^7 CFU/ml (Table 1). However, the microbial count of three regions (Chalous in winter, and spring and Tonekabon in winter) were higher than the other regions, but there was no significant difference in the total microbial count of different regions and in two seasons ($p>0.05$).

Physicochemical properties

Fat content: Fat content of the samples varied between 2.95 and 3.89% with an average of 3.42±0.03% (Table 1). There was a significant difference in fat content of the raw milk samples in different regions and two seasons ($p<0.05$). The samples collected in winter had higher fat content than those collected in spring. Fat content of the samples collected from Salmanshahr was significantly lower than those collected from other cities, and the samples collected in Nour, Tonekabon and Chalous had higher fat content than other regions.

Protein content: The results indicated that protein content of the samples varied between 2.47 and 3.41% with an average of 3.04±0.02% (Table 1). The results of ANOVA showed a significant difference between the mean of protein content in different regions ($p<0.05$). Protein content of the samples collected from Nowshahr was significantly lower than other cities, the samples collected from Nour and Sari had higher protein content than other regions. No significant difference was observed between protein content of the samples collected in two seasons ($p>0.05$).

Acidity and pH: Mean of acidity in different regions was 15.38° D (Table 1). Data analysis showed a significant difference in different regions, and between the mean of acidity of two

Table 1. Means of microbiological and physicochemical parameters of the raw milk samples according to season and sampling region

Sampling region	Season	TPC (CFU/ml)	Fat (%)	Protein (%)	Acidity	pH (%)	SNF gravity	Specific	Water adulteration (%)
1. Sari	Winter	23×10^{6} a*	3.64 a	3.15 a	14.08 e	6.70 a	8.60 a	1.030a	0.00 b
	Spring	9×10^{6} a	3.27 a	3.07 a	13.66 f	6.75 a	8.64 b	1.030a	0.25 b
2. Babolsar	Winter	18×10^{7} a	3.40 a	3.08 a	15.53 f	6.69 a	8.42 a	1.030a	0.00 b
	Spring	28×10^{7} a	3.17 a	3.01 a	13.33 g	6.69 a	8.22 a	1.029a	1.16 a
3. Amol	Winter	28×10^{7} a	3.36 a	3.00 a	14.27 d	6.70 a	8.20 a	1.029a	3.55 a
	Spring	13×10^{7} a	3.30 a	3.04 a	14.35 d	6.71 b	8.32 a	1.029a	1.75 a
4. Noor	Winter	78×10^{6} a	3.89 a	3.10 a	14.84 c	6.68 a	8.48 a	1.030a	25.2 a
	Spring	19×10^{7} a	3.50 a	3.12 a	15.25 b	6.71 a	8.52 a	1.030a	0.00 b
5. Nowshahr	Winter	29×10^{7} a	3.43 a	2.91 b	15.26 b	6.68 a	7.96 a	1.028b	5.23 a
	Spring	26×10^{7} a	3.18 a	3.00 a	16.56 a	6.60 a	8.19 b	1.029a	4.25 a
6. Chalous	Winter	11×10^{8} a	3.72 a	3.07 a	16.03 a	6.64 b	8.39 a	1.029a	1.38 a
	Spring	23×10^{8} a	3.39 a	3.05 a	16.42 a	6.61 c	8.35 a	1.030a	0.53 b
7. Salmanshahr	Winter	44×10^{7} a	2.95 b	3.05 a	15.11 b	6.68 a	8.32 a	1.030a	6.66 a
	Spring	35×10^{7} a	3.36 a	3.13 a	16.25 a	6.66 a	8.51 a	1.030a	3.25 a
8. Nashtarood	Winter	38×10^{7} a	3.54 a	3.05 a	16.28 a	6.65 b	8.34 a	1.029a	1.74 a
	Spring	52×10^{7} a	3.27 a	3.04 a	17.45 a	6.62 b	8.31 a	1.029a	3.40 a
9.Tonekabon	Winter	13×10^{8} a	3.73 a	2.99 a	15.63 a	6.65 b	8.18 a	1.029a	1.63 a
	Spring	52×10^{7} a	3.47 a	3.03 a	16.87 a	6.60 e	8.27 a	1.029a	1.00 a
Total mean		48×10^{7}	3.42	3.04	15.38	6.66	8.33	1.029	2.11

*Different letters within columns are significantly different at $p<0.05$

seasons ($p<0.05$). Mean of pH value for the raw milk samples obtained from various regions was 6.66. There was no significant difference ($p>0.05$) in the mean of pH in various regions (Table 1).

SNF content: Mean of SNF content in different regions was 8.33% (Table 1). However, various SNF amounts were observed in different regions ($p<0.05$) but not for the samples prepared in two mentioned seasons ($p>0.05$).

Specific gravity: Mean of the samples specific gravity for was 1.029 (Table 1). A significant difference was observed in the specific gravity of milk samples in different regions ($p<0.05$) but season did not have significant effect on it ($p>0.05$).

Water adulteration percentage: Mean of water adulteration was 2.11% (Table 1). According to the results, there was a significant difference in the water adulteration mean of various regions ($p<0.05$), indicating the addition of water by producers; however, there was no significant difference between two seasons in this regard ($p>0.05$).

Alcohol test

Alcohol stability of the raw milk sample was evaluated, and the result of 22.1% of 102 samples was positive.

DISCUSSION

Microbiological quality

Total plate count in most of the present samples exceeded the standard limits $\geq10^{6}$ CFU/mL (ISIRI, 2008). The milking process (especially the milking-machine, preservation, collection, transportation, cooling, and equipment associated with it) introduces the greatest proportion of microorganisms in raw milk. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned (using detergent), and disinfected immediately after use. However, keeping the milk in refrigerated temperatures immediately after milking process may delay the increase of the first microbial load (Swai and Schoonman, 2011). In the study of raw milk from collection centers of the three regions in Morocco, 75% of the samples had unsatisfactory quality with respect to TPC and the mean counts of TPC were 1.4×10^{6} CFU/mL (Belbachir *et al* 2015).

The average TPC of raw milk 1.03×10^{6} CFU/mL and 5.5 (±0.2) log CFU/mL were reported from Shahrekord, Iran(Fadaei 2014) and Kamana *et al* (2014) respectively. In another study the mean counts of TPC were between 9.2×10^{4} and 3.6×10^{7} CFU/mL (Pyz-Lukasik *et al* 2015). Chye *et al*. (2004) determined the microbiological safety of raw milk from four regions of Malaysia. The mean of total plate count of the samples was 12×10^{6} CFU/mL that exceeded the limits; however, the present study showed higher bacterial contamination of raw milk. The assessing results of 297 samples from New Zealand's raw milk samples collected from five major dairying regions over a one year period, showed that raw milk supply inevitably contains pathogens; so, control by thermal treatment of raw milk seems essential (Hill *et al*., 2012) even so the contamination of raw milk in New Zealand was lower than other countries and the regions of this study. Pyz-Lukasik *et al*. (2015) examined the microbiological quality of raw cow milk samples in Poland. The mean count of total aerobic bacteria was between 9.2×10^{4} and 3.6×10^{7} CFU/ml that is lower than that of the present study. Kalmus *et al*. (2015) determined the microbiological quality of raw milk in Estonia. The total bacterial count exceeded 100,000 CFU/mL in 21.4% of the bulk milk samples, and in 71.4% of the collected samples, it was at the retail level. O'Connell *et al*. (2015) measured the total bacterial count(TBC) of raw milk samples in Ireland. The average of TBC was 17000 CFU/mL, and showed seasonal trends. The TBC in the above study was lower than in the results of present study. It could be concluded that more hygienic practices are carried out in the herds of Ireland. However, season variation did not affect the microbiological quality of the samples in the present study, which can be due to low temperature difference between the winter and spring seasons. In another study, 50 samples of raw milk were collected from Dehradun city in India. Only 8% of the samples were found in the category of good quality, and 25% of the samples contained 41×10^{7} CFU/mL bacterial count, and were in poor category (Pant *et al*., 2013). A total of 100 raw milk samples in Turkey were analyzed for microbiological and chemical quality. The average of total plate count was 3.95×10^{6} CFU/mL (Tasci, 2011); which is much lower than our results and very closed to the NSI limit. The microbial quality of raw milk produced in Estonia during 4 years was determined; more than 91% of the samples involved less than 5×10^{4} CFU/mL (Stoluva *et al*., 2010); this result is better than that of the present study in this regard. Different microbial loads of raw milk samples of different regions are related to milk utensils, water supply, condition and temperature of raw milk after milking process, especially during keeping and transport (Chye *et al*., 2004). These reasons could also, cause varied microbial loads of the samples of different regions in this study.

Physicochemical properties

Physicochemical properties of raw milk samples of the present study including the titrable acidity, pH, content of fat and protein, solids nonfat level, specific gravity, percentage of water adulteration, alcohol testing were determined. The findings showed that all of the mentioned factors have amounts within normal range. In the present study, the mean of fat content of the collected samples was within the range, and the minimum limit of 3.2% for fat content is acceptable (ISIRI, 2005); therefore, 85% of the regions were within the normal range. Shojaei and Yadollahi (2008) found an overall average of 2.6% fat content of the raw milk samples of three regions in Shahrekord, Iran, which is lower in comparison with the findings of the present study and the ISIRI (2005). There was a significant difference in the fat content of samples of different regions in the present study ($\rho<0.05$). Milk fat content varies because of species of animal, breed, stage of lactation, age, seasonal variations, feeding, management, preservation and transportation of milk. It can also be affected by water adulteration (Javaid *et al*., 2009). Fat content of the samples in the present work was affected by seasonal variations ($\rho<0.05$); similar results were found by other researchers (Yang *et al*., 2013). The protein content of 3-3.3% was set as acceptable range by the ISN (ISIRI, 2005). Shojaei and Yadollahi (2008) reported the same parameter measurement in Shahrekord, Iran. The results of our study showed a significant difference between the mean of protein content in different regions ($\rho<0.05$) and insignificant difference in different seasons ($\rho>0.05$). In the present study, seasonal variation did not affect on protein, probabely due to low temperature difference between winter and spring seasons. Acidity value of 14 –16ºD according to ISIRI (2005) is within

the normal range. Average of pH value for the present samples was 6.6, which is within the normal range, 6.6–6.8 (ISIRI, 2005). Similar results were obtained by evaluation of pH value for the milk samples from Tandojam, Pakistan (Javaid *et al*., 2009). Evaluation of the physical properties of raw milk samples in Turkey showed a mean pH of 6.74 (Tasci, 2011) that is higher than the average of pH in the present investigation. pH variation of milk could be due to addition of water, ice or chemical preservatives to improve its shelf life (Javaid *et al*., 2009). Mean of SNF content of the samples was acceptable (minimum 8%) according to the ISIRI (2005). The results showed significant difference in different regions ($\rho<0.05$). Water adulteration, as reported in the present study, could affect on the SNF content of different region samples. There was no difference in the SNF content of samples collected in two different seasons ($\rho>0.05$), which is not in agreement with the reported results by Matutinovic *et al*. (2011) and Yang *et al*. (2013). It could be because of low temperature difference between the winter and spring seasons in the area (Mazandaran province) this study was carried out. Mean of specific gravity of the present samples was 1.029, which is in the normal range (1.029-1.032) according to ISIRI (2005). Significant difference was observed in the specific gravity of milk samples in different regions ($\rho<0.05$). The result showed water adulteration in milk, since water is lighter than milk, then its addition reduces the specific gravity of milk (Javaid *et al*., 2009). Mean of specific gravity for 100 raw milk samples in Turkey was 1.027 (Tasci, 2011). The present study showed higher specific gravity, and it can be concluded that adulterated water by sellers was lower. Addition of water is carried out commonly, which affects the physicochemical composition of milk by changing the proportion of its different constituents (Javaid *et al*., 2009). Finally, alcohol test of the raw milk samples was determined, and showed alcohol stability of 22.1 % of the raw milk sample was nonresistant, and coagulated therefore, can not tolerate heat treatment during the processing.

CONCLUSION

The results of the present study showed that the raw milk samples had a poor microbiological quality. High contamination affects the keeping quality and safety of raw milk and milk-based products. According to our results, the physicochemical composition of the raw milk samples collected from different regions of Mazandaran province was acceptable according to the ISIRI. Change of milk composition in different seasons could provide scientific pattern for dairy producers and manufacturers to use appropriate raw milk based on the type of production. It is recommended that hygienic practices are implemented during the milking, preservation and transport. Moreover, avoiding the consumption of untreated raw milk could be instructed.

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