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ORIGINAL ARTICLE

The Effect of Seasonal Variation on the Chemical and Microbial Quality of Raw Milk Samples Used in Qazvin, Iran

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ABSTRACT: Although milk and dairy products are considered as nutritious food for human, it serves as a beneficent medium for the growth of many microorganisms such as psychrotrophic microorganisms (PMs). PMs can be affected by the milk quality and dairy products by production thermostable enzymes. The present study aimed to evaluated the psychrotrophic microorganisms count (PMsC) of raw cow milk samples in milk collection centers. A total of 60 raw cow milk samples were collected from 15 main milk collection centers located in Qazvin, Iran for a period of one year (four seasons). The mean±SD of PMs, Ph, and acidity levels for a period of one year was 4.83±0.43 Log_{10} CFU/ml, 6.30±0.41, and 20.13±3.21°D, respectively. PMsC, Ph, and acidity levels in cold seasons (autumn and winter) were greater than warm seasons (spring and summer). It may be expected that PMs values can affect the pH and acidity levels while no significant relationship (P>0.05) was found among PMs, Ph, and acidity levels. Seasonal variations had a significant effect (P<0.05) on PMs values while seasonal variations had no significant effect (P>0.05) on the pH and acidity levels. 55% (33 out of 60) of milk samples were in the accepted limit. Milk quality properties in Qazvin in terms of PMs were relatively within the hygienic standards limit.

INTRODUCTION

Milk and dairy products represent a major source of protein, calcium, phosphorus and fat-soluble vitamins (vehicle for vitamins A and D) and may make a significant contribution to the dietary intakes of other minority such as vitamin C and minerals (magnesium and iodine) [1]. A growing number of customers consume raw milk. The reasons for this decision include enhanced nutritional qualities, taste, and health benefits in raw milk compared to thermal treatment milk (pasteurized or UHT) while many epidemiological studies have clearly stated that raw milk can be contaminated by a variety of pathogens being associated with human diseases [2]. Raw cow milk has favorable physical and chemical media for a range of microorganisms such as a of psychrotrophic microorganisms species which are mainly the members of the genus *Pseudomonas* and other germs infecting milk during milk collection or storage [3].

Psychrotrophic microorganisms (PMs) are defined as the microorganisms with the ability to grow at low temperatures. The optimal temperature for PMs growth is at 15°C or lower, a maximal temperature for growth is at about 20°C, and it has an ability to grow at 0°C or below but for a period short time [4]. For organizational and economic reasons, it is impossible to reach milk to the dairy factories after each milking, thus milk should be stored at low temperatures in the farm bulk or milk

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collection centers (MCCs) for several hours or days after milking. In addition, the milk in the dairy factories is not always processed immediately. Quick cooling and cold storing protect milk against acidifying and pathogen bacteria while prepare suitable conditions for the growth of PMs [5]. In addition, several studies have indicated that predominant microorganisms were PMs as contamination in raw milk stored at low temperatures (below 7°C) [6]. If health conditions such as cleaner cows and cleaning in place, rapid cooling of raw milk, enclosed pipeline milk systems, sanitary design of equipment, using inline plate coolers prior to storage in bulk tanks in MCCs and transportation system (from farm, lorry, and silo) dare not accomplished well, the replication of populations of PMs and others germs may lead to the spoilage of milk [3, 7]. Cultivable PMs in raw milk are represented predominantly by gram-negative such as *Pseudomonas*, *Aeromonas*, *Alcaligenes*, *Serratia*, *Achromobacter*, *Flavobacterium* spp, and *Chromobacterium* and at much lower numbers by gram-positive such as *Lactobacillus*, *Bacillus*, *Streptococcus*, *Clostridium*, *Corynebacterium*, and *Microbacterium* spp [3]. However, putrefactive bacteria including both gram-positive and gram-negative bacteria in most cases have significant effects on the quality of milk and dairy products [8].

PMs are able to produce thermostable enzymes such as proteases [9] and lipases as a by-product [10] in raw milk. The appropriate temperature for PMs to produce enzymes is lower than the appropriate temperature for cell growth. Thus, the organoleptic changes by proteases and lipases have been found in refrigerated milk with the existence of the lower number of PMs at low temperatures [11]. Many enzymes such as proteases and lipases in heat treatment retain significant activities at temperatures 72 to 75°C for a period 15 to 20s (pasteurization treatment) and even at temperatures 130 to 150°C for a period 2 to 4s (UHT treatment) [7]. The proteolytic activity cause coagulation and unclean and bitter flavor of raw milk or thermal treatment milk [13] and lipolytic activity cause off-flavor particularly pronounced in raw milk, cheese, butter, cream, pasteurization, and UHT treatment milk [14]. Previous studies indicated that the decrease of storage temperature from 7°C to 2°C significantly reduces the PMs growth rate and reduces the production of thermostable enzymes leading to the reduction of proteolytic and lipolytic activities [15]. Although total counts one of the adequate indicators of the raw milk hygienic quality, the of psychrotrophic microorganisms count (PMsC) as a supplementary indicator can contribute to the explanation of seasonal problems in milk processing [16]. In other ways, PMs are the most customarily isolated germs which cause the spoilage of the heat treated milk include pasteurization, UHT treatment, and dairy products as the result of post-pasteurization contamination of the products [14]. The PMsC value (PMsCV) is more significant than the total count value because each germ has its own multiplication and enzyme production rates [17]. PMs are generally a trace of insufficient refrigeration temperature or long storage time [18]. In the end, contaminated milk and dairy product by PMs cause the poor quality of them and even their rejection [19]. Thus, PMsC of milk is used as an the indicator of its hygienic quality [20]. That is a method for evalauting PMs, Ph, and acidity levels in raw cow milk samples of main MCCs during four seasons from Qazvin province, Iran. Moreover, the possible effect of seasons on milk quality was examined.

MATERIALS AND METHODS

Studied area

This study was conducted in different regions of Qazvin province located in the north of Iran.

Sampling method

In this experimental study, the raw cow milk samples were collected from MCCs in 15 main milk collection center (MCC) of Qazvin, Iran in each season (autumn, winter, spring, and summer) in the early morning. These samples were kindly provided by the commercial dairy processor in Qazvin province and other regions of Iran. A total of 60 samples (15 samples per season and 1 from each MCC) were collected during four seasons from September 2015 to August 2016. Milk samples were collected in 50 mL sterile screw-cap tubes (ISOLAB, Germany) and kept in a cool box at 4°C and then transferred to food hygiene laboratory, Qazvin University of medical sciences, Qazvin, Iran.

Sample preparation and analysis of the chemical and microbial quality

Psychrotrophic microorganisms count

The samples were delivered to the laboratory as soon as they were processed. For sample dilution, sterile physiological saline was applied with peptone. Plate count agar (PCA) medium with tempered 45°C was added to 1 ml of the inoculum of the respective dilution (pour plate method). The plate count agar (Liofilchem Company, Italy) was used to determine PMsC. Incubation was performed at 7°C for 10 days. The plates with the number of colonies 10 to 300 were accepted [21].

Assessment of pH

The amount of pH for samples was measured using a digital pH meter (Mettler MP 220, Switzerland) and calibrated routinely with fresh pH 4.01 and 6.86 standard buffers [22].

Assessment of acidity

In order to measure the titratable acidity of milk, 10 ml of the sample was casted into the beaker and 10 drops of the phenol-phthalin 1% were added to it, and with a NaOH of 0.1 normal, the titration was carried out to achieve a vivid pink color. The amount of lactic acid was reported as a result of lactose fermentation [22].

$$\text{Acidity (\%)} = (N \times 0.009 \times 100)/V$$

Where N= NaOH ml value 0.1 normal consume

V= Sample size

Statistical Analysis

PMsCV were transformed logarithmically for providing normal distribution. In order to evaluate the relations among the studied parameters, correlation coefficients were computed by SPSS19 for windows. PMs, Ph, and acidity values of raw milk in relation to the seasonal variation was evaluated by the Tukey test. The chart of the PMsC, Ph, and acidity values was drawn by Excel 2013 for windows.

RESULTS AND DISCUSSION

The minimum, maximum, and mean±standard deviation values as well as the P-value of PMs and chemical characteristics of raw cow milk samples collected in different seasons were presented in Table 1. The viability of PMsCV within each season including autumn, winter, spring, and summer was detected in the range of 4.41 - 5.54, 4.44 - 5.37, 3.95 - 5.43 and 3.77 - 5.25 Log_{10} CFU/ml respectively.

Table 1. Microbiological and chemical analysis of raw cow milk.

Parameters (N)	Analysis	Autumn (15)	Winter (15)	Spring (15)	Summer (15)	Total (60)	P-value
PMsC (log_{10} CFU/mL)	Min	4.41	4.44	3.95	3.77	3.77	0.000[a]
	Max	5.54	5.37	5.43	5.25	5.54	
	Mean±SD	5.09±0.38	5.03±0.24	4.64±0.41	4.58±0.44	4.83±0.43	
pH	Min	5.79	5.45	5.91	5.12	5.12	0.483[b]
	Max	6.79	6.73	6.66	6.83	6.83	
	Mean±SD	6.36±0.35	6.28±0.43	6.38±0.26	6.16±0.56	6.30±0.41	
Acidity (°D)	Min	16.90	15.00	16.50	14.70	14.70	0.749[b]
	Max	24.40	24.10	28.10	24.80	28.10	
	Mean±SD	20.49±2.74	19.97±3.35	20.61±3.76	19.44±3.11	20.13±3.21	

[a]Statistically significant (P<0.05), [b]No significant difference (p>0.05)

The viability of pH levels in cold season and warm season was detected in the range of 5.45 to 6.79 and 5.12 to 6.86 and acidity levels in the cold season (autumn and winter) and the warm season (spring and summer) was detected in the range of 15.00 to 24.40 and 14.70 to 28.10. Samples in terms of pH and acidity levels were significantly higher (P<0.05) but in terms of PMsC , they were a significantly lower (P<0.05).

Evaluating the effect of seasonal variation on PMsC, pH, and acidity indicated that only PMsC was significant (P<0.05). Thus, Tukey test was carried out for PMsC in different seasons, indicating that there is no significant relationship between PMsC and other seasons including autumn, winter, spring, and summer (Table 2).

Based on the results in Table 3, a negative significant correlation was found between acidity and pH levels measurements of raw milk samples (p<0.01) and no significant correlation was found among PMsCV with acidity and pH measurements of raw milk samples.

Table 2. Tukey test for PMsC of raw cow milk samples in different seasons.

(I) season	(J) season	Mean Difference (I-J)	Sig.
autumn	winter	0.06000	0.973
	spring	0.44467[a]	0.012
	summer	.050933[a]	0.003
winter	autumn	-0.06000	0.973
	spring	0.38467[a]	0.037
	summer	0.44933[a]	0.010
spring	autumn	-0.44467[a]	0.012
	winter	-0.38467[a]	0.037
	summer	0.06467	0.966
summer	autumn	-0.50933[a]	0.003
	winter	-0.44933[a]	0.010
	spring	-0.06467	0.966

[a]The mean difference is significant at the 0.05 level

Table 3. Pearson correlation coefficients between PMsC, pH, and acidity of raw cow milk samples.

	PMsC	pH
pH	0.076	-
Acidity	0.189	-0.515[a]

[a]Correlation is significant at the 0.01 level

Figure 1 indicates the mean values of PMs and chemical characteristics of raw cow milk samples being collected in cold and warm seasons. Based on Figurer 1, PMsC, pH and acidity levels in cold seasons (autumn and winter) were greater than warm seasons (spring and summer). It may be concluded that there is a direct relationship between pH and acidity levels with the presence of PMsCV but was not found a significant relationship (P>0.05) between PMs with pH and acidity.

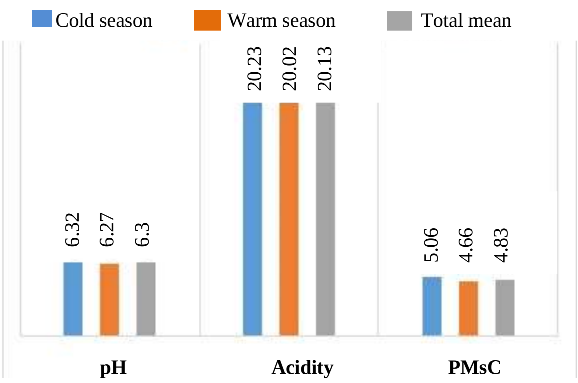

Figure 1. PMsCV and pH and acidity levels in cold seasons (autumn and winter) compare to warm seasons (spring and summer).

Figure 2 showed the accept limited PMsCV in different seasons. Out of the 60 bulk milk samples screened, PMsCV was obtained in 33 (55%) samples presumably of good quality while 24 (45%) samples scored weak quality. The outcome of the present study revealed that all of the milk samples were contaminated with PMs.

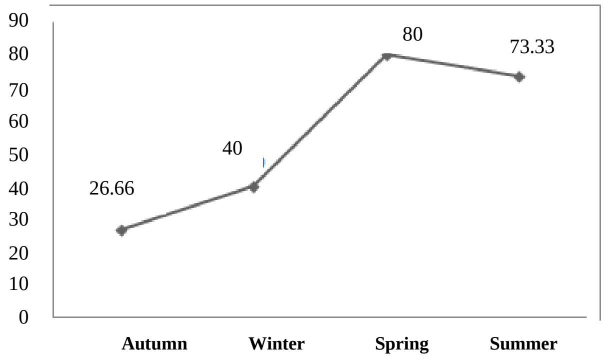

Figure 2. Acceptable limit (%) of PMsC in different seasons.

This study evaluated the microbiological and chemical qualities of raw cow milk currently produced on farms located in Qazvin, Iran. PMs in milk has been a major problem for milk quality. Thus, the survey of raw milk quality in MCC as one of the main focus is essential in increasing the quality of raw milk delivered to dairy factories.

The European Union (EU) maximum limit for PMsC in raw milk was set at 5 Log_{10} CFU/ml equivalent 100000 CFU/ml [23]. Alterations in milk and dairy product become appreciable when the PMsCV above 6 Log_{10} CFU/ml [11]. According to the EU limit, the PMsCV in the present study in cold seasons (5.06 Log_{10} CFU/ml) was higher than the EU limit, but in warm seasons (4.66 Log_{10} CFU/ml) and in total mean (4.83 Log_{10} CFU/ml) it was less than the EU limit. As expected, the growth rate of PMs in cold seasons better than warm seasons. In several studies performed in Greece [24], Belgium [25], and south-east Victoria, Australia [21] it was stated that the microbial populations of raw milk can be affected by seasonal variation. Based on the results of the present study, cold seasons prepare good condition for PMs than warm seasons.

Due to the non-pathogenic majority of PMs, few studies dealt with such a subject, but it should not be overlooked that PMs produce destructive enzymes such as protease and lipase which can reduce the quality of milk ,shelf life, and organoleptic characteristics and most importantly can make raw milk unsuitable for dairy products process in dairy industries [26].

PMsCV in cold seasons greater than warm seasons, a study in contradiction with the present study conducted in Canada reported bacterial counts with the lowest counts tending to occur in winter,. The reason for this issue expressed different methods of premilking udder preparation or milking and storage conditions [27]. A study [25] which matched to the present study recorded PMsC in winter and summer as 3.66 and 3.13 Log_{10} CFU/ml, indicating that PMsCV in cold seasons was greater than warm seasons.

PMsCV in milk from Tunisia [5], Malaysia [28], Italy [29], Brazil [30] was recorded as 2.81, 3.87, 3.60, 4.04 Log_{10} CFU/ml, respectively. PMsCV in Czech [16] was recorded from 2.54 to 4.81 Log_{10} CFU/ml. The result of previous studies was lower than the present study Probably because their samples were collected from MCCs with high quality and hygienic condition.

In a study performed on bulk milk tank samples which matched to the present study, the level of PMsCV and pH was reported as 4.83 Log_{10} CFU/ml and 6.74[15].

In India, PMsCV recorded for raw milk from dairy farms, vendors, and dairy processors 3.66, 4.96 and 5.03 Log_{10} CFU/ml,[31]. In a study in Brazil, PMsCV was recorded between 1.70 and 6.10 Log_{10} CFU/ml for the samples being collected at dairy farmers individual as 2.10 and 5.40 Log_{10} CFU/ml at collective milk containers and as 4.90 and 5.80 Log_{10} CFU/ml at milk plant silo [3]. Another study recorded PMsCV in Brazil as 4.90 and 7.80 Log_{10} CFU/ml for refrigerating raw milk samples collected from the thermal container of the milk truck and as 5.30 to 7.20 Log_{10} CFU/ml for milk storage silo [7]. The results obtained from three previous studies were greater than the present study.

In a study conducted in the Midwest, USA an average of mesophile and thermophile values for raw milk was recorded in winter and summer period as 2.61 and 2.76

Log_{10} CFU/ml,. As a result, a better growth rate of mesophile and thermophile bacteria was indicated in high-temperature conditions [32]. However, teh present study indicated a better growth rate of PMs in low-temperature conditions. The relationship between PMs value of raw milk and temperature variation in the current study was similar to a study conducted in Western Europe [33].

Table 4 indicates the studies on the PMsCV on different continents. As can be observed in this table, different continents display somewhat similar values in terms of PMsC. The PMsCV in Europe, Asia, Australia, and Africa have been lower than the EU-approved level (less than 5 Log_{10} CFU/ml) but PMsCV in South America was higher than the EU-approved level. Africa and Asia had the lowest PMsCV compared to Europe and America.

Table 4. Psychrotrophic microorganisms count in the different area of the world since 2000 till now.

Area	PMsCVLog_{10} CFU/ml	Method	Source samples	Reference
Czech/Europe	2.54 to 4.81	Pour plate	MCCs	[16]
Australia	3.30 to 4.40 (A) 3.40 to 4.20 (B)	Pour plate	Two farms (A and B)	[40]
Brazil/South America	5.08	Pour plate	Bulk milk tank	[11]
Malaysia/Asia	3.87	Enumeration of total plate count	MCCs	[28]
Slovak/Europe	4.09	Pour plate	MCCs	[41]
Israel/Europe	4.83	Standard plate count	MCCs	[15]
Belgium/Europe	3.66 to 3.13	Pour plate	MCCs	[25]
Czech/Europe	3.46	Pour plate	MCCs	[42]
Brazil/South America	4.90 to 7.80 (A) 5.30 to 7.20 (B)	Pour plate	the container of the milk truck (A) Milk storage silo (B)	[7]
Italy/Europe	3.60	Surface plate count	Bulk milk tank	[29]
Tunisia/Africa	2.81	Pour plate	MCCs	[5]
Czech/Europe	3.76	Pour plate	MCCs	[21]
Chine/Asia	4.10	Pour plate	Farms	[43]
Victoria/Australia	3.37	Drop plate	Farms	[44]
Korea/Asia	2.56	Standard plate count	Bulk milk tank	[45]
Spain/Europe	1.60 to 3.00	Surface plate count	Bulk milk tank	[39]
Brazil/South America	4.04	Pour plate	Refrigerate raw milk	[30]
Qazvin area, Iran/Asia	4.83	Pour plate	MCCs	our work

As it can be observed, various studies cited different PMsCV [34]. Various reports justified dairy locations, feed, milking season, bedding type, change the bacterial populations in cow teats, dust, and air in the milking room, altitude of the pasture where milk was produced [35], soiling value on the teats before the udder preparation, handy cleaning method of the bulk tanks, usage of a specific type of detergent (antimicrobial effect or not antimicrobial effect) [27], variety in technological factories including milking practices, milk storage conditions, milking hygienic quality [36], hygienic condition and cleaning procedure practices of milking equipment [29], inadequate alternation of acid wash in cleaning in place (CIP) system [27], and washing water quality in dairy farms or MCCs [37]. Enhancement in plate counts value was found at the transfer of raw milk from farm tanks to dairy industries bulk tanks [38]. Thus, the sampling location (farm or MCC) can affect the recorded values in different studies. As well as the storage times of raw milk affects its quality. In a study performed in Spain [39] PMsCV was recorded for a period of 24h and 48h storing 4.43 and 5.45 Log_{10} CFU/ml. Thus, it can be concluded that reducing the storage time leads to an increase in the raw milk quality. Finally, it contributes to the difference between raw milk microbial quality and physicochemical properties.

CONCLUSIONS

This study revealed that Qazvin raw cow milk samples of MCCs are contaminated with PMs which consequently affect the shelf life of raw milk. Raw cow milk quality is affected by thermostable enzymes being secreted by PMs before heat treatment or during cold storage. Ultimately, off-flavor, off-odor, bitter and soapy taste, and rancidity issues during the shelf life of pasteurized or UHT milk. Thus, good quality dairy products require the high quality of raw milk.

Most cases of poor quality raw milk are the sources of bacterial origin and it isimperative to observe the sanitation principles practices and control the temperature until the raw milk from MCC or farm is transferred to the milk processing companies for reducing the growth rate of microbial load. However, combined small transportation distances with short storage times and low temperature (keep cold chain) as much as necessary are considered as a means for controlling PMs growth rate and preventing the undesirable effects of PMs.

The findings of the current study could raise efforts to inform and engage producers about appropriate food safety strategies. The different approach for the management and control of the raw cow milk chain could be performed through the following steps such as the accomplishment of good dairy farming practices, control, supervision of low temperature in the transport chain, and sanitation of handling procedures. Thus, an accomplishment and sanitation in these steps can reduce PMs growth rate present in raw milk and increase the sanitary and quality characteristic of raw cow milk.

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Conflict of interests

The authors declare no conflict of interests.

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