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RAW COW MILK QUALITY: PHYSICOCHEMICAL, MICROBIOLOGICAL, AND SEASONAL VARIATION

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ABSTRACT

Milk serves as a vital source of essential nutrients in human diets and must be safe for consumption. In the dairy industry, it is crucial to analyze the physicochemical and microbiological properties of raw cow milk (RCM) throughout the year to gather fundamental information, as milk quality significantly influences the final quality of dairy products. This study aimed to investigate the physicochemical and microbiological properties of 60 RCM samples collected from 15 primary milk collection centers in Qazvin, Iran, and assess the impact of seasonal variation on these properties. The study discovered that the RCM samples contained >7 Log_{10} cfu/mL of *Staphylococcus aureus*, total coliform count, aerobic mesophilic plate count, and mold and yeast in 15%, 30%, 37%, and 28% of the samples, respectively. The results indicated that aerobic mesophilic plate count, mold and yeast, total coliform count, and *Staphylococcus aureus* were higher in warmer seasons compared to colder seasons. Furthermore, the study revealed that the average pH, freezing point, solid-not-fat, and protein content were higher in warm seasons, while the average acidity, lactose, and fat content were higher in cold seasons. The physicochemical and microbiological properties of RCM in the Qazvin region were found to be below the acceptable quality value. Although seasonal variation throughout the year affected the microbial and physicochemical quality of RCM, the differences were not statistically significant ($p>0.05$), except for the solid-not-fat value in winter ($p<0.05$).

Keywords: Raw cow milk; Microbial quality; Physicochemical properties; Seasonal variation; Public health

INTRODUCTION

Milk is widely recognized as a highly nutritious food, containing fat, protein, sugar, minerals, and vitamins that make it a complete and healthy option. Consumption of milk during early life has been associated with a reduced risk of developing respiratory and skin diseases, such as pollen allergy, allergic rhinitis, asthma, atopic sensitization, and hay fever **(Loss *et al.*, 2011; Quigley, O'Sullivan, *et al.*, 2013)**. Despite the popularity of raw milk (RM) due to its perceived taste and potential health benefits, it is important to note that RM can serve as a favorable growth medium for a variety of potentially pathogenic and spoilage microbes. This is due to its neutral pH and high water activity **(Claeys *et al.*, 2013)**, which can support the growth of pathogenic microorganisms, including bacteria and fungi. As a result, RM carries an increased risk of contamination with harmful microorganisms. It is worth noting that pasteurized or UHT milk has undergone a thermal process that aims to eliminate pathogenic microorganisms, ensuring their safety and reducing the risk of foodborne illness **(Quigley, McCarthy, *et al.*, 2013)**.
Milk and dairy products are prone to contamination by various microorganisms, which can affect their nutritional value and pose a potential risk to public health **(Aali *et al.*, 2017; Júnior *et al.*, 2018)**. RM is particularly susceptible to contamination by foodborne pathogens such as *S. aureus*, yeasts, molds, and coliforms, which can thrive in RM due to its high moisture content and neutral pH **(Hummerjohann *et al.*, 2014)**. As a result, consuming RM can pose a significant microbiological risk to consumers **(Fadaei, 2014)**. In addition to microbial contamination, changes in the physicochemical properties (Ph-Ch-Ps) of RM can also have a significant impact on the quality of dairy products. For instance, changes in pH have been linked to weak heat resistance in skim milk powder **(Faka *et al.*, 2009)**, while variations in fat, protein, solid-not-fat (SNF), lactose, and freezing point (FP) can affect the composition and quality of milk and dairy products **(Boukria *et al.*, 2020)**. The present study aims to investigate the microbiological and physicochemical attributes of raw cow milk (RCM) over one year and evaluate the impact of seasonal variation on these properties. By examining these factors, this research aims to identify potential sources of contamination and develop strategies to enhance the safety and quality of RCM.

MATERIALS AND METHODS

Sample collection

For this experimental study, RCM samples were collected from fifteen milk collection centers (MCCs) located in the Qazvin region (Coordinates: 36°16′N 50°00′E) every season in the early morning. A total of 60 samples were collected over the course of a year, with 15 samples collected per season from each MCC. The sample collection period was from September 2016 to August 2017. The samples were collected in 50 mL sterile screw-cap tubes (ISOLAB, Germany) and were immediately stored in a cool box at 4°C to preserve their quality until they were transferred to the food hygiene laboratory at Qazvin University of Medical Sciences in Qazvin, Iran. In the laboratory, the samples were analyzed for microbiological and Ph-Ch-Ps.

Table 1 Culture media and procedures used for the microbiological analyses of RCM samples

Type of germ	Procedure	Method
APC	Plate count	Samples cultured on the nutrient agar medium were incubated at 30 °C for 72 hours **(Anonymous, 2003a)**.
M & Y	Plate count	Samples cultured on the yeast extract glucose chloramphenicol medium **(ISO, 2001, 2008)**.
TCC	Plate count	Samples were incubated for 3 days at 30 °C in an eosin methylene blue medium **(ISIRI, 2008)**.
S. aureus	Plate count and identification	Samples were cultured on the baird parker agar medium and incubated at 37 °C for 48 hours. Then coagulase test was used to confirm the suspected colonies **(Anonymous, 2003b)**.

Microbiological analyses

To assess the hygienic quality of the RCM samples, four microbial indicators were measured: aerobic mesophilic plate count (APC), *Staphylococcus aureus* (*S. aureus*), total coliform count (TCC), and mold and yeast (M & Y). For each milk sample, serial dilutions (10^{-1} to 10^{-6}) were prepared using a sterile Ringer's solution. The diluted samples were then incubated in plates, and the resulting colonies were counted between 30 and 300 colonies per plate **(Cowhx and Morisetti, 1969)**. The microbiological analyses were conducted using culture media and procedures detailed in Table 1. All culture media used in the study were prepared by Liofilchem Company, Italy.

Physicochemical analyses

The RCM samples underwent analysis using a milk scanner device (Lactostar/ Funke-Gerber, Germany). The Ph-Ch-Ps that were assessed during the analysis comprised the fat content, protein content, SNF (Solids-Not-Fat), lactose content, and FP.

Assessment of pH and acidity

The pH value of the RCM samples was measured using a digital pH meter (Mettler MP 220, Switzerland) following the ISIRI method **(ISIRI, 2006)**. To assess milk acidity, a 10 mL sample was mixed with 10 drops of 1% phenolphthalein in a beaker and titrated with 0.1 N NaOH until a clear pink color was obtained, indicating the endpoint of the reaction. The lactic acid value, which represents the amount of lactic acid produced as a result of lactose fermentation, was recorded following the ISIRI method **(ISIRI, 2006)**.

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

N: The amount of NaOH in ml of 0.1 normal consumed
V: Sample size

Statistical procedure

To ensure normal distribution, the microbiological values obtained from the RCM samples were transformed into logarithmic values. Statistical analyses were performed using SPSS ver. 20 for Windows and charts for the microbiological and Ph-Ch-Ps values were created using Excel ver. 2019 for Windows. The experiment was conducted in triplicate, and the data were analyzed using mean ± standard deviation (M±SD) and one-way analysis of variance (ANOVA). To identify significant differences between groups ($p<0.05$), the Tukey test was used.

RESULTS AND DISCUSSION

The RCM samples were thoroughly analyzed to observe changes in the milk compound over the course of a year. Milk was also processed under standard conditions to create a diversity of dairy products to survey how the selected quality characteristics of these products were affected by milk compounds. The amounts of microbial and Ph-Ch-Ps of RCM samples are presented in Tables 2 and 3. Pearson correlations among microbiological and Ph-Ch-Ps of RCM samples are shown in Table 4. It should be noted that not all significant correlations are cause and effect, some may be random or indirectly related to other parameters.

Table 2 Microbiological characteristics of RCM samples over one year

Season	**Analysis**	**Autumn**	**Winter**	**Spring**	**Summer**	**Total**	**P-value**
Properties	(N)	(15)	(15)	(15)	(15)	(60)	
APC ($\log_{10}$ cfu/mL)	Min	4.47	4.00	4.00	4.47	4.00	0.704[a]
	Max	8.30	8.08	8.17	8.63	8.63	
	Mean±SD	6.36±1.04	6.16±1.34	6.42±1.34	6.69±1.21	6.41±1.22	
M & Y ($\log_{10}$ cfu/mL)	Min	3.27	0.00	0.00	0.00	0.00	0.104[a]
	Max	7.51	7.77	8.53	8.44	8.53	
	Mean±SD	5.43±1.22	3.95±3.43	4.85±3.17	6.31±1.99	5.13±2.69	
S. aureus count ($\log_{10}$ cfu/mL)	Min	4.30	0.00	0.00	0.00	0.00	0.858[a]
	Max	6.65	8.43	8.61	7.55	8.61	
	Mean±SD	5.42±0.81	5.24±1.95	5.65±2.17	5.75±1.84	5.52±1.73	
TCC ($\log_{10}$ cfu/mL)	Min	3.84	4.00	0.00	4.00	0.00	0.758[a]
	Max	7.84	8.10	8.50	8.65	8.65	
	Mean±SD	5.99±1.03	6.14±1.27	6.15±2.03	6.56±1.49	6.21±1.48	

Legend: [a] ─ No significant difference ($p>0.05$), cfu ─ colony forming units.

Table 3 Physicochemical characteristics of RCM samples over one year

Season	**Analysis**	**Autumn**	**Winter**	**Spring**	**Summer**	**Total**	**Accept Limited**	**Standard range (%)**	**P-value**
Properties	(N)	(15)	(15)	(15)	(15)	(60)			
pH	Min	5.79	5.45	5.91	5.12	5.12	6.60 to 6.80	30.00	0.483[a]
	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	14 to 16	8.33	0.749[a]
	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			
Freezing point (°C)	Min	-0.53	-0.53	-0.53	-0.53	-0.53	-0.50 to -0.54	88.33	0.102[a]
	Max	-0.49	-0.49	-0.50	-0.49	-0.49			
	Mean±SD	-0.51±0.01	-0.50±0.01	-0.51±0.01	-0.51±0.01	-0.51±0.01			
Solid-not-fat (g/100g)	Min	7.64	7.39	8.15	7.88	7.39	more than 8	76.66	0.002[b]
	Max	8.99	8.63	8.67	8.68	8.99			
	Mean±SD	8.35±0.46	8.02±0.33	8.42±0.15	8.42±0.21	8.30±0.35			
Lactose (g/100g)	Min	4.69	4.59	4.74	4.48	4.48	more than 5	31.66	0.519[a]
	Max	5.12	5.16	5.07	5.09	5.16			
	Mean±SD	4.96±0.12	4.93±0.15	4.91±0.10	4.89±0.18	4.92±0.14			
Protein (g/100g)	Min	2.94	2.87	2.95	2.85	2.85	3 to 3.30	81.66	0.915[a]
	Max	3.16	3.16	3.16	3.16	3.16			
	Mean±SD	3.06±0.06	3.05±0.08	3.07±0.06	3.06±0.08	3.06±0.07			
Fat (g/100g)	Min	2.69	2.47	2.53	2.08	2.08	more than 3.20	45.00	0.089[a]
	Max	4.06	3.73	3.42	4.00	4.06			
	Mean±SD	3.27±0.34	3.20±0.32	3.11±0.27	2.95±0.44	3.13±0.36			

Legend: [a] ─ No significant difference ($p>0.05$), [b] ─ Statistically significant ($p<0.05$).

Table 4 Pearson correlation coefficients among microbial and Ph-Ch-Ps of RCM samples collected from Qazvin in Iran

properties	**APC**	**M & Y**	***S. aureus***	**TCC**	**pH**	**acidity**	**FP**	**SNF**	**Lac**	**Pro**
M & Y	0.757^a	-	-	-	-	-	-	-	-	-
S. aureus	0.725^a	0.724^a	-	-	-	-	-	-	-	-
TCC	0.801^a	0.727^a	0.682^a	-	-	-	-	-	-	-
pH	-0.360^a	-0.295^b	-0.368^a	-0.286^b	-	-	-	-	-	-
acidity	0.291^b	0.135	0.332^a	0.171	-0.515^a	-	-	-	-	-
FP	-0.066	-0.215	-0.116	-0.109	-0.182	0.244	-	-	-	-
SNF	0.086	0.167	0.144	0.020	-0.055	0.022	-0.460^a	-	-	-
Lac	0.142	0.190	0.120	0.143	0.119	-0.037	-0.520^a	0.547^a	-	-
Pro	0.076	0.192	0.135	0.138	0.166	-0.160	-0.671^a	0.518^a	0.767^a	-
Fat	-0.167	-0.137	-0.233	-0.144	0.398^a	-0.302^b	-0.094	0.013	0.127	0.310^b

Legend: Lac – Lactose, Pro – Protein, ^{c} – Correlation is significant in the 0.01 value, ^{b} – Correlation is significant in the 0.05 value.

Table 5 Average APC of RCM samples from MCCs in Iran and other regions of the world since 2004

Parameter **Area/continent**	**APC (Log$_{10}$ cfu/mL)**	**Ref/year**	**Parameter** **Area/continent**	**APC (Log$_{10}$ cfu/mL)**	**Ref/year**
Estonia/ Europe	4.54 ↓	**(Stulova *et al.*, 2010)**/ 2004	Kerman, Iran/ Asia	6.21 ~	**(Mansouri-Najand *et al.*, 2013)**/ 2013
Chile/ South America	3.80 ↓	**(Van Schaik *et al.*, 2005)**/ 2005	Semnan, Iran/ Asia	5.48 ↓	**(Hooshmand *et al.*, 2020)**/ 2013
Estonia/ Europe	5.00 ↓	**(Stulova *et al.*, 2010)**/ 2005	Rwanda/ Africa	5.50 ↓	**(Kamana *et al.*, 2014)**/ 2014
Jordan/ Asia	5.69 ↓	**(Riadh, 2005)**/ 2005	Belgium/ Europe	3.96 ↓	**(Piepers *et al.*, 2014)**/ 2014
Estonia/ Europe	4.73 ↓	**(Stulova *et al.*, 2010)**/ 2006	Czech/ Europe	4.30 ↓	**(Bogdanovièová *et al.*, 2016)**/ 2014
Fars, Iran/ Asia	6.90 ~	**(Hashemi and Shekarforosh, 2007)**/ 2007	Rwanda/ Africa	6.17 ~	**(Doyle *et al.*, 2015)**/ 2015
Estonia/ Europe	4.64 ↓	**(Stulova *et al.*, 2010)**/ 2007	Banaskantha District, India/ Asia	6.08 ~	**(Nalwaya *et al.*, 2018)**/ 2015
USA/ North America	3.69 ↓	**(D'AMICO *et al.*, 2008)**/ 2008	Sri Lanka/ Asia	7.08 ↑	**(De Silva *et al.*, 2016)**/ 2016
Canada/ North America	4.10 ↓	**(Elmoslemany *et al.*, 2009)**/ 2009	China/ Asia	5.10 ↓	**(Lan *et al.*, 2017)**/ 2017
USA/ North America	4.69 ↓	**(D'amico and Donnelly, 2010)**/ 2010	Tabriz, Iran/ Asia	7.43 ↑	**(Moosavi *et al.*, 2018)**/ 2018
Zimbabwe/ Africa	6.40 ~	**(Mhone *et al.*, 2011)**/ 2011	Norway/ Europe	4.27 ↓	**(Skeie *et al.*, 2019)**/ 2019
Brazil/ South America	4.86 ↓	**(Costa Sobrinho *et al.*, 2012)**/ 2012	Ethiopia/ Africa	7.11 ↑	**(Yeserah *et al.*, 2019)**/ 2019
USA/ North America	3.69 ↓	**(Gillespie *et al.*, 2012)**/ 2012	Brazil/ South America	3.63 ↓	**(Araújo *et al.*, 2020)**/ 2020
Morocco/ Africa	8.83 ↑	**(Hadrya *et al.*, 2012)**/ 2012	Hungary/ Europe	4.72 ↓	**(Petróczki *et al.*, 2020)**/ 2020
Tanzania/ Africa	7.17 ↑	**(Ngasala *et al.*, 2015)**/ 2012	Ethiopia/ Africa	6.20 ~	**(Berhanu *et al.*, 2021)**/ 2021
Markazi, Iran/ Asia	6.80 ~	**(Rezaei *et al.*, 2013)**/ 2013	Germany/ Europe	4.90 ↓	**(Böhnlein *et al.*, 2021)**/ 2021
Finland/ Europe	4.11 ↓	**(Ruusunen *et al.*, 2013)**/ 2013	Qazvin, Iran/ Asia	6.41	Present study

Legend: ↓, ↑, and ~ mean that these symptoms are less, higher, and somewhat equal to our results, respectively.

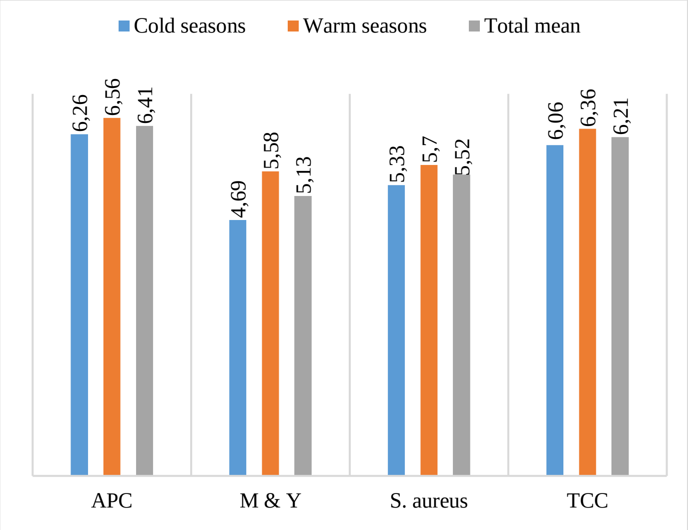

Figure 1 Microbial properties in CSs (autumn and winter), WSs (spring and summer), and the overall average across all seasons

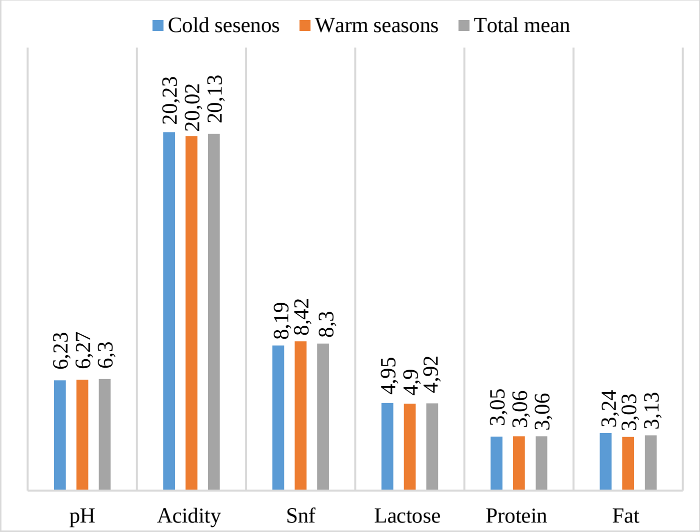

Figure 2 Ph-Ch-Ps in CSs (autumn and winter), WSs (spring and summer), and the overall average across all seasons

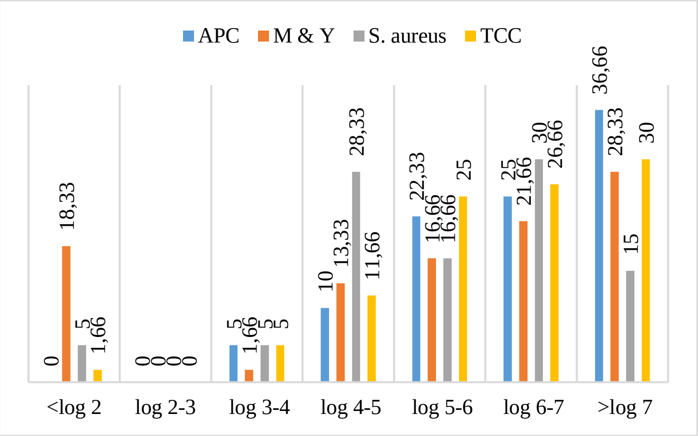

Figure 3 The results for APC, *S. aureus*, TCC, and M & Y in RCM samples at the MCCs

This study examines the changes in RCM composition over time and how milk components affect the quality of dairy products. The findings can be used to develop strategies for improving milk production efficiency and enhancing the quality of dairy products. The study also identifies potential sources of contamination by analyzing correlations among the microbiological and Ph-Ch-Ps of RCM, which can contribute to improving food safety. The study investigates seasonal variations in the properties of RCM, including microbiological parameters such as APC, *S. aureus* count, TCC, and M & Y count, as well as Ph-Ch-Ps such as pH, acidity, FP, SNF, lactose, protein, and fat content. The results show that seasonal variations can significantly influence both microbiological and Ph-Ch-Ps properties of RCM. For instance, the lowest values for APC, *S. aureus*, TCC, and M & Y were observed in winter, while the highest values were observed in summer. Similarly, the lowest values for pH, acidity, FP, SNF, lactose, protein, and fat were observed in summer, while the highest values were observed in different seasons. These findings underscore the importance of considering seasonal variations when evaluating the quality of RCM, as factors such as temperature, humidity, and changes in animal diet can significantly influence both microbiological and Ph-Ch-Ps properties of RCM **(Bjerg *et al.*, 2005)**.

APC is a measure of the total number of aerobic mesophilic bacteria present in RCM. A high APC value indicates the presence of bacteria, which could be due to cows being afflicted with mastitis or poor cow preparation, unsanitary milking and cleaning practices, or other sources of contamination. In our study, the APC values and rankings of the RCM samples were as follows: excellent degree (APC<3×10^4 cfu/mL) 6.66%, first-degree (APC=3×10^4 to <10^5 cfu/mL) 6.66%, second-degree (APC= 10^5 to <5×10^5 cfu/mL) 16.66%, third-degree (APC=5×10^5 to 10^6 cfu/mL) 8.33%, and non-standard (APC>10^6 cfu/mL) 61.66%. These results suggest that a significant proportion of the RCM samples collected in this study had high APC values, indicating the presence of bacterial contamination.

Microbial quality of RCM

In the Qazvin region, RCM is commonly used to produce traditional dairy products including soft cheese, cottage cheese, and traditional ice cream. However, there is a lack of literature addressing the occurrence of foodborne pathogens in the Qazvin province, despite the significant public health concern posed by pathogenic bacteria in RCM. Therefore, it is crucial to assess the quality of RCM in MCCs to improve the overall quality of RCM delivered to dairy factories. To evaluate the hygienic quality of RCM samples, four microbial indicators were measured: APC, which measures the total number of aerobic bacteria present in the milk; *S. aureus*, a pathogenic bacterium that can cause foodborne illness; TCC, an indicator of fecal contamination; and M & Y, an indicator of spoilage.

Previous studies conducted in Khuzestan, Iran by **Kamal zade *et al.*, (2010)** and Kerman, Iran by **Mansouri-Najand *et al.*, (2013)** reported that a high percentage of RCM samples from MCCs were below the acceptable quality value, with 88.30% (48 out of 54) and 76.10% (83 out of 109) of the samples below the acceptable quality value, respectively. In contrast, the present study found that only 66.61% of the samples were below the acceptable quality value, indicating that the quality of RCM samples in the Qazvin province is better than in the Khuzestan and Kerman regions. Similarly, a study assessing 248 RCM samples from MCCs in Markazi Province in Iran found that 90.90% of the samples were below the acceptable quality value, with 9.09% classified as 3rd grade in terms of microbial quality **(Rezaei *et al.*, 2013)**. Another study examining RCM samples from MCCs in East Azerbaijan province in Iran by **Safaei *et al.*, (2018)** reported that 73.60% of the samples were below the acceptable quality value, with 15.80% and 10% of the samples classified as 2nd and 3rd grade in terms of microbial quality, respectively. In comparison, only 8.33% of the samples in the present study were classified as 3rd grade, indicating a better microbiological quality of RCM in the Qazvin province compared to the Markazi and East Azerbaijan provinces in Iran. However, a study conducted in Rwanda by **Doyle *et al.*, (2015)** reported a significantly higher percentage of 3rd-grade milk samples, with 78% of the samples classified as 3rd-grade. The authors suggest that this high percentage may be due to various factors, including contamination before and after milking, transportation of untreated milk cans, and a lack of temperature control during transportation.

The present study found that the APC levels recorded in RCM samples from MMCs were excessive, indicating that blending such milk with higher-quality milk at MCCs would increase the overall APC of the milk at the MCC. High levels of APC in RCM can reduce milk quality and shelf life, ultimately leading to a decrease in the quality of dairy products. Therefore, it is crucial to prevent microbial growth from the MMC to the dairies, which requires the establishment of equipment and procedures to quickly segregate poor-quality milk in the milk chain or to create economic incentives for producers to produce milk with lower APC levels **(Kazeminia *et al.*, 2019)**.

Secondary bacterial contamination of RCM can occur at various stages of the milking process, including from ambient and air resources or through improper cleaning methods. Hygiene indicators such as *S. aureus*, TCC, and M & Y have been identified as useful measures at this stage. In Iran, RCM is typically boiled before consumption. Although boiling generally makes milk safer by killing most microorganisms, it still carries the risk of exposing the consumer to pathogenic bacteria due to possible re-contamination. *S. aureus*, for example, is sensitive to heat and does not compete well with other microorganisms, thus *S. aureus* infection usually occurs after boiling milk, when there is minor contact with other microorganisms **(Kamana *et al.*, 2014)**.

TCC in milk is an indicator of environmental and fecal contamination, which can be caused by various factors such as inadequate herd hygiene, unsanitary milking methods, contaminated water, improper washing, and unsuitable maintenance equipment. These factors can lead to an increase in the number of coliforms in RM. The high prevalence of *S. aureus* in RM may be due to the organism's prevalence in the udders of dairy cows, as reported by **Mhone *et al.*, (2011)**.

The presence of *S. aureus* in RCM can indicate the presence of mastitis in dairy herds, which can lead to inflammation of the mammary glands and the release of *S. aureus* cells into the milk. From a food safety perspective, *S. aureus* is an enterotoxin-producing pathogen, but its value must be >5 Log_{10} cfu/mL to produce a sufficient toxin to cause disease in humans. In this study, more than 62% of RCM samples had >5 Log_{10} cfu/mL for *S. aureus*, indicating a potential risk for human consumption. Additionally, TCC, APC, and M & Y values were above this threshold for 83%, 88%, and 68% of the samples, respectively, indicating inadequate conditions of milk production. A similar study in Zimbabwe by **Mhone *et al.*, (2011)** reported *S. aureus* and TCC values consistent with our study, indicating that milk production conditions in both areas may be suboptimal. However, in Norway, only a small percentage of samples (2 out of 135) showed >5 Log_{10} cfu/mL for *S. aureus*, indicating better milk production conditions. Comparing our APC values with other studies shows conflicting results, with studies from Northern China (43.75%), Italy (44.80%), Germany (37.60%), Switzerland (34.40%), and Vermont in the USA (7%) reporting different percentages of samples exceeding counts of >5 Log_{10} cfu/mL **(D'amico and Donnelly, 2010; Giacometti *et al.*, 2012; Lan *et al.*, 2017; Poether *et al.*, 2019; Zulauf *et al.*, 2018)**. According to a study conducted in Ethiopia, the values for APC, M & Y, *S. aureus*, and TCC were consistent with the results of our study, with values of 7.11, 4.12, 4.95, and 3.36 Log_{10} cfu/mL, respectively. This suggests that the conditions for milk production in both areas may be similar **(Yeserah *et al.*, 2019)**.

The APC values for all 60 RCM samples in this study ranged from 4.00 to 8.63 Log_{10} cfu/mL, with a mean of 6.41 Log_{10} cfu/mL. The M & Y values ranged from 0.00 to 8.53 Log_{10} cfu/mL, with a mean of 5.13 Log_{10} cfu/mL. The *S. aureus* count ranged from 0.00 to 8.61 Log_{10} cfu/mL, with a mean of 5.52 Log_{10} cfu/mL, and the TCC ranged from 0.00 to 8.65 Log_{10} cfu/mL, with a mean of 6.21 Log_{10} cfu/mL (Table 2). Comparing our results to other studies, the APC value of RCM in Lorestan province in Iran was consistent with our results, however, the recorded TCC value was higher **(Hashemi and Shekarforosh, 2007)**. In Tabriz City in Iran, the M & Y and APC values were somewhat consistent with our results **(Moosavi *et al.*, 2018)**. In Morocco, APC and TCC values were higher than in the present study, but *S. aureus* values were lower **(Hadrya *et al.*, 2012)**. In Sri Lanka and Rwanda, APC values were consistent with our results **(De Silva *et al.*, 2016; Doyle *et al.*, 2015)**, while in Jordan **(Riadh, 2005)** and Chile **(Van Schaik *et al.*, 2005)**, the values were better than in our study. In Vermont, the USA, Bangladesh, China, Finland, and Hungary, *S. aureus*, APC, and TCC values were lower than in our study **(D'amico and Donnelly, 2010; Islam *et al.*, 2018; Lan *et al.*, 2017; Petróczki *et al.*, 2020; Ruusunen *et al.*, 2013)**. In Belgium and Canada, the APC and TCC values were lower than our results **(Elmoslemany *et al.*, 2009; Piepers *et al.*, 2014)**. In Kerman province in Iran and Australia, the APC and *S. aureus* values were better than in our study **(Mansouri-Najand *et al.*, 2013; Mcauley *et al.*, 2014)**. Upon analyzing Table 5, it becomes evident that there is a stark contrast between the high microbial load of RCM samples collected in Qazvin, Iran, and those collected in other regions of Iran and other countries. The primary reason for this difference in milk microbial quality can be attributed to the multiple processing steps that occur before milk is delivered to MCCs or poor hygiene practices in MCCs. However, it is important to note that other factors can also influence the microbial quality of RM, including personal hygiene and health, sanitation and udder washing before milking, the animal's health status, mastitis, hygiene of milking utensils and tankers, the time elapsed between milking and milk transfer to the MCCs, milk storage, and ambient temperatures, among others, as pointed out in other studies **(de Almeida Júnior *et al.*, 2015; Mullen *et al.*, 2013)**.

Effects of seasonal variation on microbial quality of RCM

APC

Table 2 and Figure 1 demonstrate that the APC of RCM was higher during the warm seasons (WSs) compared to the cold seasons (CSs). However, no significant relationship was found among APC values in different seasons in the present experiment ($p>0.05$). Interestingly, there was a positive correlation between APC and acidity ($p<0.05$). In a study conducted in Burkina Faso, the APC value was recorded as 3.65 Log_{10} cfu/mL in the CSs and 4.52 Log_{10} cfu/mL in the WSs, indicating that the APC value in the WSs is higher than expected **(Millogo *et al.*, 2010)**. The increase in APC during the WSs could be due to delays in milk transfer to the MCC or the mixing of contaminated milk with milk from other livestock in the MCC, leading to an increase in the APC of the milk in the tank. Additionally, the rising ambient temperatures in the WSs could be a reason for an increased microbial load of milk. Conversely, RCM samples in the CSs, which contain the lowest levels of colonies, may be related to the inhibition of mesophilic microbial growth at temperatures below 8 ºC.

M & Y

In this study conducted by **Mennane *et al.*, (2007)**, it was observed that although the number of fungi is expected to increase in winter due to the use of stored forage by livestock, the number of M & Y colonies in RCM samples during the CSs was remarkably lower than the WSs. However, a positive correlation was found between M & Y and APC values ($p<0.01$). The M & Y values of RCM samples were higher in WSs than in CSs, as shown in Table 2 and Figure 1. However, there was no significant difference among M & Y counts in different seasons ($p>0.05$). The high temperature and dry weather in the WSs could stimulate the expansion of mold spores in the air, leading to higher M & Y values in RCM samples. These findings are consistent with a study conducted in Latvia, where the highest percentage of M & Y counts was reported in spring and autumn, and the lowest in winter and summer **(Gulbe and Valdovska, 2014)**.

TCC and *S. aureus*

The results presented in Table 2 and Figure 2 indicate that seasonal variations had an impact on the values of *S. aureus* and TCC. Specifically, the values were lower in autumn and winter and higher in spring and summer. However, no significant relationship was found between TCC and *S. aureus* values in different seasons ($p>0.05$). Interestingly, there was a positive correlation between *S. aureus* with M & Y and APC values ($p<0.01$), as well as a positive correlation between TCC with *S. aureus*, M & Y, and APC values ($p<0.01$). These findings suggest that the presence of *S. aureus* and TCC in milk samples may be influenced by various factors, seasonal changes, and other microbial populations. The results of this study are consistent with those of a previous study conducted in Bihar County, Hungary, which found that *S. aureus* and TCC counts were higher in samples collected in summer than in samples collected in winter. This suggests that seasonal variations may have a significant impact on the microbial populations present in milk samples. Additionally, the highest colony counts in RCM samples collected in WSs can be attributed to the heat stress experienced by cows during the spring and summer due to adverse weather conditions such as high humidity and temperature **(Petróczki *et al.*, 2020)**.

Effects of seasonal variation on of Ph-Ch-Ps of RCM

The fat content of milk is an essential quality factor that can affect the texture, taste, and nutritional value of dairy products. Similarly, the protein content is crucial because it can affect the formation of curds and the quality of cheese and yogurt. The SNF, which includes non-fat components such as proteins, lactose, and minerals, is an indicator of milk quality and suitability for processing. The lactose content is particularly important for lactose-intolerant individuals. The FP of milk can indicate whether it has tampered with water since the presence of added water reduces the FP. The pH and acidity of milk are also crucial factors that can significantly affect the production, shelf life, and taste of milk and dairy products. Proper regulation of pH and acidity levels is necessary to ensure the safety, quality, and flavor of these products **(Ahmad *et al.*, 2008; B. Chen *et al.*, 2017; S. Chen *et al.*, 2004; Christiansen *et al.*, 2020; Gai *et al.*, 2021; McCarthy and Singh, 2009)**. Table 3 and Figure 3 demonstrate that all milk samples have acceptable quality values in terms of Ph-Ch-Ps above 50%, except for fat, lactose, pH, and acidity. The pH, acidity, FP, lactose, protein, and fat values in CSs are higher than in WSs, while SNF values in WSs are higher than in CSs, indicating that the major components of RCM are affected by seasonal variations. It can be concluded that there is a direct relationship between ambient temperature and SNF values, as well as an inverse relationship among pH, acidity, FP, lactose, protein, and fat values with the ambient temperature. Among the milk Ph-Ch-Ps, only the SNF value was significant ($p<0.05$). According to the Tukey test for SNF, there is a significant relationship between SNF and winter out of all the seasons.

pH and acidity

Upon analysis of the RCM samples, it was observed that the pH contents and acidity values did not vary significantly during the study period. There were no significant differences between pH contents and acidity values in different seasons ($p>0.05$). However, the pH contents were slightly lower in CSs compared to WSs, which could be attributed to the increased growth of psychrotrophic microorganisms at low ambient temperatures and the production of acidic metabolites. The study found a positive correlation between acidity with *S. aureus* ($p<0.01$), as well as APC ($p<0.05$), and a negative correlation between acidity and pH. Similarly, there was a negative correlation between pH content with M & Y, TCC ($p<0.05$), APC, and *S. aureus* ($p<0.01$). These findings are consistent with a study conducted in Turkey by **Tasci (2011)**, where the pH content of most samples was reported to be between 5.00 and 7.00 with an average of 6.74. In Tabriz City, Iran, the SNF, protein, fat, lactose, FP, and pH levels of the samples were recorded as 6.75, 8.56%, 3.15%, 3.38%, 4.65%, and -0.53 °C, respectively. The physicochemical factors, except for lactose and pH, did not change significantly in different seasons ($p>0.05$) **(Moosavi *et al.*, 2018)**. Another study conducted on MCCs in Iran by **Kazeminia *et al.*, (2019)** reported that seasonal variations had a significant effect on psychrotrophic microorganism values ($p<0.05$), but did not affect the pH and acidity values ($p>0.05$).

Freezing point

The study observed slight changes in the levels of FP and lactose in milk samples, with changes in FP ranging from -0.50 ºC to -0.51 ºC and changes in lactose levels ranging from 4.89% to 4.96%. However, there were no significant differences in FP and lactose levels among different seasons ($p>0.05$). This is consistent with previous research that has shown FP and lactose to be consistent physical and chemical variables in milk components **(Henno *et al.*, 2008)**. The study found that the range of FP was relatively narrow, which is expected as it is a result of an osmotic balance between blood and milk. However, the FP levels were slightly higher in winter compared to other seasons, which may be due to temperature and dietary variations. Another study suggested that the increase in milk FP was possibly due to increased water consumption resulting from rising temperatures and sunny hours **(Bjerg *et al.*, 2005)**. The study also found that FP was the most stable feature of the milk samples in different seasons, making it a potential criterion for detecting milk fraud and detecting excess water. The researchers noted that the relative stability of milk lactose, as reported by other studies, is also noteworthy **(Fox *et al.*, 2015)**.

Lactose

The main carbohydrate present in milk is lactose, although small amounts of other carbohydrates are also present. The lactose content in milk samples fluctuated slightly during the study period. The study found a negative correlation between lactose and FP, and a positive correlation between lactose and SNF ($p<0.01$). On average, the lactose content in the samples was measured at 4.92 (g/100g), with a range from 4.48% to 5.16%. In similar studies conducted on RCM samples in Kerman province in Iran, the average lactose levels in the samples were reported at 4.33%, 4.00%, and 4.51%, respectively **(Mansouri-Najand *et al.*, 2013)**. In a study conducted on Dutch bovine RM by **Heck *et al.*, (2009)**, the values of protein, fat, lactose, and FP were reported at 3.48%, 4.38%, 4.51%, and -0.51 °C, respectively. The lactose values reported in this study were lower than the lactose values in the current study. The present study did not find any significant differences in lactose levels among seasons, unlike a study conducted in Tabriz City, Iran, by **Moosavi *et al.*, (2018)** which reported a significant difference between the mean milk lactose in spring and summer. The highest amount of lactose was observed in autumn samples in the current study, while a study conducted in the sub-Mediterranean area by **Matutinovic *et al.*, (2011)** reported the highest lactose levels in winter samples. It is known that lactose levels decrease with increasing ambient temperature and that increasing forage consumption during the summer can negatively impact milk lactose concentration. These findings suggest that lactose levels in milk may be influenced by various factors, including geographic location, breed of animal, seasonal variations, and dietary factors.

Solid-not-fat

The study measured the average SNF content in milk samples at 8.30 (g/100g), with a range from 7.39% to 8.99%. There was a negative correlation between SNF and FP ($p<0.01$), and the SNF content showed slight fluctuations during the study period. The study found significant seasonal differences only in winter ($p<0.05$), while other studies reported no significant relationship between SNF content and different seasons **(Nguyen *et al.*, 2020; Rao and Mishra, 2010)**. One study conducted in Okayama, Japan by **Nguyen *et al.*, (2020)** reported lower SNF content in summer than in winter, while the present study found lower SNF content in winter than in summer. These differences may be due to variations in geographical conditions, climate, livestock breed, and nutrition type. The findings suggest that SNF content in milk may be influenced by various factors, and seasonal variations in SNF content may vary depending on the location and other environmental factors.

Protein

In this study, the average protein content in the RCM samples was measured at 3.06 (g/100g), with a range from 2.85% to 3.16%. The protein content showed a similar seasonal trend with fat content, being remarkably higher in spring compared to winter and summer. However, the protein content did not show any remarkable seasonal trend, but it was positively correlated with fat ($p<0.05$) and showed a negative correlation with FP ($p<0.01$), while being positively correlated with lactose and SNF ($p<0.01$). Studies conducted in Romania by **Pavel and Gavan, (2011)** on milk samples in the three seasons of spring, summer, and autumn did not find any statistically significant differences in protein content. Nonetheless, a study consistent with the present study reported higher protein and fat content in the CSs than in the WSs milk **(Chen *et al.*, 2014)**. Similarly, studies conducted in Ethiopia by **D Gemechu (2016)** and Yazd province in Iran by **Shokoohmand *et al.*, (2012)** reported higher fat levels in the CSs than in the WSs. Overall, these findings suggest that the protein content in milk may be influenced by various factors, including season, breed of animal, and environmental factors. The positive correlation between protein and fat content in milk suggests that the two components may be influenced by similar factors.

Fat

In this research, the milk samples were analyzed for their protein content, which was found to have an average of 3.13 (g/100g), with a range from 2.08% to 4.06%. The study revealed a negative correlation between fat and acidity ($p<0.05$) and a positive correlation between fat and pH ($p<0.01$). However, the fat content in RCM produced during autumn was significantly higher than in other seasons, while the fat content in spring and summer was less than the Iranian standard. This could be attributed to reduced forage consumption during heat stress, which can lead to a decrease in milk fat percentage **(Quist *et al.*, 2008)**. A study conducted in Burkina Faso by **Millogo *et al.*, (2010)** reported fat, lactose, and protein values in CSs of 4.36%, 3.50%, and 4.92%, respectively, and in WSs of 3.92%, 3.33%, and 4.69%, respectively. The study found an inverse relationship between fat and lactose values and ambient temperature, which supports the findings of the present study. Overall, these findings suggest that the fat content in milk may be influenced by various factors, including season, forage consumption, and environmental factors such as ambient temperature. The correlations observed between fat content and other milk components such as pH and acidity suggest that these components may also be influenced by similar factors.

The trend of Ph-Ch-Ps of RCM in the dairy industry

The dairy industry faces both challenges and opportunities due to the observed seasonal variations in milk composition. These variations are likely caused by a combination of factors, including increased prolactin secretion in the summer and changes in the animal's diet and environment **(Pacarrynak and Danyk, 2012)**. While the higher ratio of unsaturated fatty acids in summer milk can be advantageous for butter expansion during production, it can also create challenges in the production of cheese and other dairy products due to variations in clotting time, which can lead to disruptions in production programs or coagulation failure in cheese production **(McSweeney and Fox, 2003)**. Seasonal variations in milk composition can also affect the production of milk powder, casein powder, and cream. Therefore, it is crucial for the dairy industry to consider these variations when planning production and processing strategies to ensure consistent quality and optimal product outcomes **(Chen *et al.*, 2014)**.

CONCLUSION

The aim of this study was to investigate the rate of change in milk composition and Ph-Ch-Ps of milk samples from MMCs in the Qazvin province throughout the year. The study found no significant seasonal variations in Ph-Ch-Ps, except for SNF. However, correlations between some Ph-Ch-Ps and milk composition were observed. The study revealed that most of the RCM samples collected from MMCs in the Qazvin region had poor quality and undesirable conditions. This poor hygienic quality in RCM can be a potential source of pathogenic microorganisms, particularly enteropathogens, making RM without heat treatment unsafe for human consumption. Therefore, it is crucial to monitor pathogenic bacteria in RM and dairy products to prevent potential health risks to consumers. The primary goal of RM production should be to minimize the presence of germs to reduce the risk of pathogenic bacteria to human health.

The study highlights the importance of regularly monitoring the microbiological characteristics of RCM samples, especially during summer and on farms with larger herds. This is essential in devising strategies to reduce the risk of bacterial contamination and enhance the safety of RCM for human consumption. The study also suggests that the dairy industry should focus on predicting pasteurization and sterilization processes, as well as standardizing milk solids according to the season, to maintain RCM's quality. However, it is important to note that the study has limitations since all RCM samples were collected from the Qazvin region only, and the findings cannot be generalized to other regions. Therefore, future research studies should include larger and more diverse sample sizes from different countries or regions worldwide to obtain more comprehensive results.

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