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Identification of microbiological, physical, and chemical quality of milk from milk collection centers in Kastamonu Province

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Abstract: The aim of this study is to perform microbiological, physical, and chemical analyses of the samples from producers who continuously bring milk to 30 actively operating milk collection centers throughout the spring, summer, autumn, and winter seasons in Kastamonu Province and determine whether the identified values are within legal limits. For microbiological properties of milk included in the research, total aerobic mesophilic bacteria (TAMB) and coliform group bacteria counts were identified. For the determination of physical and chemical properties, dry matter, fat, nonfat dry matter, lactose, protein, ash, pH, titratable acidity, refractive index, and specific gravity values were identified and the presence of carbonate, peroxidase, and antibiotics was researched. As a result of the research, in the 240 investigated raw milk samples in all seasons, the majority of mean dry matter, fat, nonfat dry matter, lactose, protein, ash, pH, titratable acidity, refractive index, specific gravity, carbonate, peroxidase, and antibiotic values were found to comply with the Turkish Food Codex Communiqué on Raw Milk and Heat Processed Drinking Milk. However, the TAMB and coliform group bacteria counts were not found to comply with these criteria. The main sources of variation in the study of milk collection center, season (apart from lactose), and milk collection center × season interaction were identified to have very significant effects ($P < 0.01$) on the microbiological, physical, and chemical properties of the milk samples.

Key words: Kastamonu, milk, milk collection center, milk analysis, milk quality

1. Introduction

Milk is a porcelain-white fluid with unique flavor and odor containing all important nutritional elements used by female mammals to feed their young [1,2]. In the legislation, raw milk is defined as "secretions from the mammary glands, apart from colostrum, obtained by milking one or more cows, goats, sheep or water buffalo, which has not been heated above 40 °C or undergone any equivalent processing" [3]. According to the Turkish Standards Institution TS.1018 Cow Milk - Raw Standard, milk is defined as "a white or cream colored fluid, secreted by the mammary glands of cows, sheep, goats and water buffalo, with its own unique flavor and consistency, with no other material mixed with it or removed from it" [4]. Milk used as food material has a great importance for the nutrition of all people from young to old. Additionally, milk is the main source of protein, carbohydrate, fat, and mineral matter necessary for healthy growth and is the sole source of nutrition for feeding newborn organisms [5].

One of the most important properties affecting the quality and nutritional value of raw milk is the composition of the milk [6]. The Food and Agriculture Organization of the United Nations (FAO) reported the mean composition of cow's milk as 11.9%–12.7% total dry matter, 8.60%–9.60% nonfat dry matter, 3.10%–3.30% fat, 4.50%–5.10% lactose, 3.20%–3.40% protein, and 0.70% ash [7]. The composition of milk varies linked to the type of animal it is obtained from. One of the most important factors affecting the yield and composition of milk is animal care and nutrition, and the feed used [8]. Additionally, disease, time and form of milking, age of the animal, age of first breeding, breeding season, initial age of lactation, and environmental factors affect milk composition. Furthermore, season, fatty acid proportions, and pH provide information about the quality and composition of milk [8–10]. Milk, with an important place in nutrition, is an ideal medium for proliferation of microorganisms beneficial for humans, due to the fat, protein, carbohydrate, vitamins, and minerals it contains [1,11]. Raw milk may be contaminated with microorganisms from the animal, cowshed, humans, milking machines, air, and tools and equipment used, and

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these microorganisms may rapidly proliferate if milk is not stored under appropriate conditions [12].

High-quality milk does not contain bacteria, pathogens, antibiotics, or toxic material above the legal limits; it is produced from healthy animals in clean and hygienic conditions, and stored in likewise manner. Quality milk has fat amounts of at least 3.5%, high dry matter (12.8%), and is a product with no bad odor and unique color, flavor, structure, and composition [2,13]. Milk with a bacterial load above the limits in the legislation and low-quality milk rapidly lose their attributes. Thus, such milk may transform into a raw material that is risky for health and may also not be processable. As a result, it is mandatory to produce quality milk [14]. The bacterial count in raw milk being between certain values is important for determination of the quality of the milk. As a result, the milk should immediately enter the cold chain after milking with temperature lowered to 4 °C and be transferred to the processing facility [15]. Currently, with the increase in modern milking techniques due to rapidly developing technology and an understanding of the importance of the cold chain, improvement has been observed in the microbiological quality of milk; however, the microbiological quality has still not reached the desired standards in Turkey and other countries [16,17].

Lack of communication between dairy farms and the milk industry in our country is a problem for food safety in the sector. To overcome this problem, cooperatives or producer organizations founded "milk collection centers" to collect milk from shareholders with better quality and to increase the marketing power in the industry. Additionally, the market has ensured a demand for quality milk. In this way, milk collected illegally and with poor quality is prevented from entering the market and an important step was taken for food safety [18].

The aim of this study is to perform microbiological, physical, and chemical analyses of the samples from producers who continuously bring milk to 30 actively operating milk collection centers throughout the spring, summer, autumn, and winter seasons in Kastamonu Province and determine whether the identified values are within legal limits.

2. Materials and methods

The raw milk used in the research was obtained from producers who continuously brought milk to 30 actively operating milk collection centers located in Kastamonu. Samples were collected on the 15th day of the second month in the spring (April), summer (July), autumn (October), and winter (January) seasons. The milk was taken from two different intake vats and placed in 100 mL sterile containers in ice jackets to preserve the cold chain during transfer to Kastamonu University's Food Engineering Laboratory. The following analyses were performed for investigation of microbiological, physical and chemical properties. All the chemicals used in this study were of analytical purity and were obtained from Sigma.

2.1. Microbiological analyses

After mixing raw milk samples well, 1 mL was taken and dilutions with 9 mL of peptone water were prepared for use in microbiological analyses [19]. Total aerobic mesophilic bacteria (TAMB) count and coliform group bacteria count were determined according to the methods used by Kesenkaş and Akbulut [16].

2.2. Physical and chemical analyses

Dry matter was determined according to the standard methods [20]. Fat, lactose, ash, refractive index specific gravity, carbonate test, and peroxidase test were determined according to the methods used by Kurt et al. [19], while nonfat dry matter was determined according to the methods used by Metin [21]. Protein was determined according to the standard methods [22]. pH and titratable acidity (lactic acid, %) were determined according to the methods used by Oysun [23]. Antibiotic test was determined as described by Reybroeck et al. [24].

2.3. Statistical analyses

The research trial pattern was 30 (actively operating milk collection centers in Kastamonu) × 4 (seasons: spring, summer, autumn, winter) × 2 (repeats) with the research completed with a fully chance-linked factorial trial pattern. Data obtained from laboratory analyses of a total of 240 samples in parallel are given in the tables. For statistical assessment of the analysis results, variance analysis was used in SPSS 17.0 [25].

3. Results

3.1. Microbiological analysis

3.1.1. TAMB counts

The numbers found in our analyses are given in Table 1. All of the 240 milk samples analyzed were above the 5 log cfu/mL value in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk, and did not abide by the legislative criteria. For the main sources of variation, the milk collecting center, season, and milk collecting center × season interaction were found to have statistically very significant ($P < 0.01$) effects on the TAMB counts in the milk samples (Table 1). As seen in Table 1, the differences in mean TAMB counts for the milk collection center variable were generally found not to be statistically different. According to mean TAMB counts for the season variable, the highest TAMB count was determined in the summer, while the lowest TAMB count was determined in the winter. The TAMB counts determined in all seasons were statistically significantly different (Table 1).

Table 1. The effect of milk collection center and season on microbiological properties of the milk samples.

		TAMB (log cfu/mL)	Coliform bacteria (log cfu/mL)
Milk collection centers			
Minimum		6.87 ± 0.62	5.16 ± 0.25
Maximum		7.32 ± 0.46	5.99 ± 0.60
Mean		7.08 ± 0.44	5.60 ± 0.45
Season			
Spring		7.16 ± 0.27[c]	5.70 ± 0.46[c]
Summer		7.45 ± 0.28[d]	5.80 ± 0.43[d]
Autumn		7.08 ± 0.34[b]	5.50 ± 0.39[b]
Winter		6.63 ± 0.40[a]	5.38 ± 0.40[a]
Source	DF		
A	29	**	**
B	3	**	**
A × B	87	**	**
Error	120		
Total	239		

A: Milk collection center, B: Season. Means followed with the same superscript letter within each column are not significantly different at P < 0.01 probability levels; *, **: Significant at 0.05 and 0.01 probability levels, respectively.

3.1.2. Coliform group bacteria counts

The results of our analyses are given in Table 1. Of the 240 analyzed milk samples, 193 (80.4% of samples) were below the 6.0 log cfu/mL value stated in the literature and comply with values given in the literature. The means for the milk collection center variable affecting coliform group bacteria counts in milk samples are given in Table 1. As can be seen from Table 1, the differences in coliform group bacteria counts were generally statistically significant. The results for coliform group bacteria counts for the season variable are given in Table 1, with the highest coliform group bacteria counts in the summer and the lowest coliform group bacteria count determined in the winter. The coliform group bacteria counts determined in all seasons were statistically different from each other.

3.2. Physical and chemical analysis

3.2.1. Dry matter

The values found in our analyses are given in Table 2. As can be seen from Table 2, considering mean values, the lowest value of 12.74% was identified in summer, while the highest value of 13.40% was identified in winter. In our analyses, the mean dry matter amounts were 13.40%, 13.10%, 12.74%, and 12.90%, respectively. As can be seen in Table 2, the effect of the main variation sources of milk collection center, season, and milk collection center × season interaction on dry matter amounts in milk samples was statistically very significant (P < 0.01). The means for the milk collection center variable affecting dry matter amounts in milk samples are given in Table 2. The differences between the dry matter amounts were generally found to be statistically different from one another. According to the results for dry matter means belonging to the season variable, the highest dry matter amounts were determined in the winter, with the lowest dry matter amounts determined in the summer season. The dry matter amounts determined in all seasons were statistically different from each other (Table 2).

3.2.2. Fat

The variance analysis results for fat amounts determined in milk samples are given in Table 2. As seen in Table 2, the main sources of variation of milk collection center, season, and milk collection center × season interaction had significant effects on fat amounts in milk samples (P < 0.01). The means for the milk collection center variable, affecting fat amounts in milk samples, are given in Table 2. As seen from Table 2, there were generally statistical differences between the fat amounts. According to results for mean fat for the season variable, the highest fat amounts

Table 2. The effect of milk collection center and season on dry matter, fat, and nonfat dry matter of the milk samples.

		Dry matter (%)	Fat (%)	Nonfat dry matter (%)
Milk collection centers				
Minimum		12.10 ± 0.37	3.18 ± 0.25	8.77 ± 0.22
Maximum		13.68 ± 0.66	4.26 ± 0.44	9.70 ± 0.16
Mean		13.03 ± 0.63	3.72 ± 0.39	9.31 ± 0.41
Season				
Spring		13.10 ± 0.69^{c}	3.80 ± 0.35^{c}	9.29 ± 0.38^{c}
Summer		12.74 ± 0.45^{a}	3.55 ± 0.23^{a}	9.19 ± 0.45^{a}
Autumn		12.90 ± 0.60^{b}	3.66 ± 0.39^{b}	9.24 ± 0.40^{b}
Winter		13.40 ± 0.54^{d}	3.88 ± 0.45^{d}	9.51 ± 0.36^{d}
Source	DF			
A	29	**	**	**
B	3	**	**	**
A × B	87	**	**	**
Error	120			
Total	239			

A: Milk collection center, B: Season, Means followed with the same superscript letter within each column are not significantly different at P < 0.01 probability levels; *, **: Significant at 0.05 and 0.01 probability levels, respectively.

were determined in the winter season, with the lowest fat amounts determined in the summer. There were statistical differences for the fat amounts determined in all seasons (Table 2).

3.2.3. Nonfat dry matter

The lowest and highest nonfat dry matter values determined for milk analyzed in this study are shown in Table 2. As can be seen from Table 2, considering mean values, the lowest value of 9.19% was identified in summer, while the highest value of 9.51% was identified in winter. The variance analysis results for nonfat dry matter amounts in milk samples are given in Table 2. As seen in Table 2, the main variation sources of milk collection center, season, and milk collection center × season interaction were found to have statistically very significant effects on the nonfat dry matter amounts in milk samples (P < 0.01). The mean values for the milk collection center affecting the nonfat dry matter amounts in milk samples are given in Table 2. The differences between nonfat dry matter amounts were generally found to be statistically different. According to the mean results for nonfat dry matter belonging to the season variable, the highest nonfat dry matter amount was determined in the winter, with the lowest nonfat dry matter amounts determined in the summer. The nonfat dry matter amounts determined in all seasons were statistically different from each other (Table 2).

3.2.4. Lactose

The mean values found in this research are given in Table 3. Based on the mean values, the lowest value was identified in spring milk at 4.45%, while the highest value of 4.54% was identified in winter milk. The variance analysis results for the lactose amounts determined in milk samples are given in Table 3. The main variation sources of milk collection center and milk collection center × season interaction had a very significant effect on the lactose amounts in milk samples (P < 0.01), while season had a significant effect on the lactose amount (P < 0.05). The amounts of lactose determined in the summer and autumn seasons were not statistically different from the lactose amounts determined in the spring and winter seasons. The means for the milk collection center variable affecting the lactose amounts in milk samples are given in Table 3. As seen in Table 3, the differences in the lactose amounts were generally found to be statistically different.

3.2.5. Protein

The values found in this research are given in Table 3. Based on the mean values, the lowest value of 3.16% was identified in summer milk, while the highest value of 3.38% was identified in winter milk. The variance analysis results for protein amounts determined in milk samples are given in Table 3. As seen in Table 3, the main variation sources of milk collection center, season, and milk collection center

Table 3. The effect of milk collection center and season on lactose, protein and ash of the milk samples.

		Lactose (%)	Protein (%)	Ash (%)
Milk collection centers				
Minimum		4.08 ± 0.24	3.13 ± 0.19	0.62 ± 0.02
Maximum		4.98 ± 0.21	3.38 ± 0.17	0.89 ± 0.13
Mean		4.64 ± 0.42	3.26 ± 0.14	0.73 ± 0.10
Season				
Spring		4.45 ± 0.38^{a}	3.26 ± 0.09^{c}	0.75 ± 0.09^{c}
Summer		4.50 ± 0.42^{ab}	3.16 ± 0.11^{a}	0.69 ± 0.10^{a}
Autumn		4.49 ± 0.35^{ab}	3.23 ± 0.10^{b}	0.70 ± 0.07^{b}
Winter		4.54 ± 0.38^{b}	3.38 ± 0.19^{d}	0.79 ± 0.11^{d}
Source	DF			
A	29	**	**	**
B	3	*	**	**
A × B	87	**	**	**
Error	120			
Total	239			

A: Milk collection center, B: Season. Means followed with the same superscript letter within each column are not significantly different at P < 0.01 probability levels; *, **: Significant at 0.05 and 0.01 probability levels, respectively.

× season interaction were found to have very significant statistical effects on the protein amounts in milk samples (P < 0.01). The means for the milk collection center variable affecting the protein amounts in milk samples are given in Table 3. As seen in Table 3, the differences between protein amounts were generally found to be statistically insignificant. The protein amounts determined in all seasons were statistically different (Table 3).

3.2.6. Ash

Considering mean values, the lowest ash amount was identified in summer (0.69%) and the highest ash amount was identified in winter (0.79%) (Table 3). The variance analysis results for ash amounts determined in milk samples are given in Table 3. The effect of the main variation sources of milk collection center, season, and milk collection center × season interaction was found to be very significant on the ash amounts in milk samples (P < 0.01). The means for the milk collection center affecting the ash amounts in milk samples are given in Table 3. The differences between the ash amounts were generally found to be significant. The ash amounts determined in all seasons were statistically different from each other.

3.2.7. pH

The pH values of milk samples are given in Table 4. As can be seen, the lowest value of 6.54 was from the summer, while the highest value of 6.66 was from the winter. The variance analysis results for pH values determined in milk samples are given in Table 4. As seen in Table 4, the main variation sources of milk collection center, season, and milk collection center × season interaction were found to have very significant effects on pH values of milk samples (P < 0.01). The means for the milk collection center variable affecting the pH values of milk samples are given in Table 4. As seen in Table 4, differences between the pH values were generally found to be statistically significant. The pH values determined in all seasons were statistically different.

3.2.8. Titratable acidity

The values found as a result of analyses are given in Table 4. As seen in Table 4, the lowest mean value was identified in winter (0.178%), with the highest value identified in the summer (0.191%). The variance analysis results for titratable acidity determined in milk samples are given in Table 4. As seen in the table, the main variation sources of milk collection center, season, and milk collection center × season interaction had very significant effects on the titratable acidity of milk samples (P < 0.01). The means for the milk collection center variable affecting the titratable acidity of milk samples are given in Table 4. As seen in Table 4, the differences between the titratable acidity

Table 4. The effect of milk collection center and season on pH and titration acidity of the milk samples.

		pH	Titration acidity (lactic acid %)
Milk collection centers			
Minumum		6.36 ± 0.09	0.175 ± 0.01
Maximum		6.75 ± 0.06	0.199 ± 0.01
Mean		6.61 ± 0.13	0.184 ± 0.01
Season			
Spring		6.60 ± 0.13[b]	0.188 ± 0.00[c]
Summer		6.54 ± 0.11[a]	0.191 ± 0.01[d]
Autumn		6.64 ± 0.16[c]	0.180 ± 0.01[b]
Winter		6.66 ± 0.06[d]	0.178 ± 0.01[a]
Source	DF		
A	29	**	**
B	3	**	**
A × B	87	**	**
Error	120		
Total	239		

A: Milk collection center, B: Season. Means followed with the same superscript letter within each column are not significantly different at P < 0.01 probability levels; *, ** significant at 0.05 and 0.01 probability levels, respectively.

values were generally found to be statistically different. For all seasons, the titratable acidity values determined were statistically different.

3.2.9. Refractive index

As seen in Table 5, the lowest values were identified in summer and autumn milk (1.345), while the highest values were identified in winter and spring season (1.346) based on the mean refractive index values. The variance analysis results for refractive index values determined for milk samples are given in Table 5. The main variance sources of milk collection center, season, and milk collection center × season interaction were found to have a very statistically significant effect on the refractive index values of milk samples ($P < 0.01$). The means for the milk collection center variable affecting the refractive index values of milk samples are given in Table 5. As seen in the table, the differences between refractive index values are generally statistically significant. The refractive index values for all seasons were statistically different from each other.

3.2.10. Specific gravity

As seen in Table 5, based on the mean values, the lowest value was identified in winter as 1.0313 with the highest value of 1.0325 identified in summer. The variance analysis results for specific gravity determined for milk samples are given in Table 5. As seen in the table, the main variance sources of milk collection center, season, and milk collection center × season interaction were found to have very significant effects on the specific gravity of milk samples ($P < 0.01$). The means for the milk collection center variable affecting the specific gravity of milk samples are given in Table 5. As seen in the table, the differences between specific gravity values were generally found to be statistically different. The specific gravities determined in all seasons were statistically different.

3.2.11. Antibiotic, carbonate, and peroxidase tests

In our research, none of the samples had any antibiotic or carbonate findings identified. All milk samples undergoing the peroxidase test in our study were positive for peroxidase and it was identified that the milk had not been boiled. In all of the 240 milk samples analyzed, antibiotic and carbonate tests were negative; in other words, none was found in the milk, while the peroxidase test results were positive; in other words, it was present in the milk samples.

4. Discussion

According to the Turkish Food Codex Communiqué on Raw Milk and Heat Processed Drinking Milk (Communiqué No: 2009/14), the TAMB count in raw cow's milk should be at most <100,000 cfu/mL (5 log cfu/mL), and also, the checks of raw cow's milk with random sampling should find total

Table 5. The effect of milk collection center and season on refractive index and specific gravity of the milk samples.

		Refractive index	Specific gravity
Milk collection centers			
Minimum		1.3445 ± 0.00	1.0298 ± 0.00
Maximum		1.3487 ± 0.00	1.0343 ± 0.00
Mean		1.3461 ± 0.00	1.0319 ± 0.00
Season			
Spring		1.3465 ± 0.00^{c}	1.0316 ± 0.00^{b}
Summer		1.3455 ± 0.00^{a}	1.0325 ± 0.00^{d}
Autumn		1.3458 ± 0.00^{b}	1.0322 ± 0.00^{c}
Winter		1.3468 ± 0.00^{d}	1.0313 ± 0.00^{a}
Source	DF		
A	29	**	**
B	3	**	**
A × B	87	**	**
Error	120		
Total	239		

A: Milk collection center, B: Season. Means followed with the same superscript letter within each column are not significantly different at P < 0.01 probability levels; *, **: Significant at 0.05 and 0.01 probability levels, respectively.

live bacterial counts of less than 100,000/mL at 30 °C [26]. It is important to determine standards for bacterial counts developing in mesophilic and aerobic conditions in food with microbiological analyses. Raw milk has the property of being a very appropriate medium for these bacteria, especially. The numbers of these bacteria are important for determination of the quality in milk and determination of the hygiene standards [27]. Research by Eser and Bilgücü [28] determined the TAMB counts in 410 raw cow's milk samples and reported that the values did not comply with the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk. Diler and Baran [14] identified the total bacteria counts varied from 2.8–6.8 log cfu/mL with a mean of 5.29 log cfu/mL in samples taken from tanks in small-scale family operations located in the district of Hınıs (Erzurum, Turkey). They reported that only 36.7% complied with the criteria stated in the Turkish Food Codex. Some studies have reported the following TAMB counts: Kesenkaş and Akbulut [16], 4.2-7.4 log cfu/mL; Beykaya et al. [29], 1.48×10^7 cfu/mL; and Dede [30], 7.38 log cfu/mL. Generally, the studies performed have found that the TAMB counts were above the legislative criteria. The values in this research comply with those reported in the literature. Akın et al. [31] reported that the TAMB count in farm milks was 5.24–5.74 log cfu/mL in winter and summer seasons, respectively, while it was 6.45–7.01 log cfu/mL for milk samples obtained from milk collecting transporters. The bacteria counts in milks taken from milk collecting transporters comply with our findings. The higher bacteria counts in blended milks can be said to be due to an increase in bacterial load of milk during transport. Akın et al. [31] examined a total of 24 farm milk samples taken from different points in the winter, spring, summer and autumn seasons in Adıyaman and identified that 5 samples (20.8% of samples) abided by the 5 log cfu/mL value in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk. Statistical analyses in the research found that the effect of season was very significant for the TAMB counts in farm milks (P < 0.01). Our analysis results revealed that the effect of season was very significant among the main sources of variance (P < 0.01) and this complies with the statistical analysis by Akın et al. [31].

There is no standard related to the coliform group bacteria count values for raw milk in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk. In our literature search, coliform group bacterial counts were reported to be between 0.75 and 6.0 log cfu/mL [16]. The coliform bacteria are the group found mostly in fresh milk. They are not resistant to the pasteurization temperature (72 °C for 15–20 s). In places where these bacteria are found, the chances of finding other pathogenic bacteria (*Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., *Streptococcus agalactiae*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium perfringens, Brucella* spp., *Mycobacterium tuberculosis*, etc.) increase. The high coliform group bacteria counts in foods are a marker of deficiencies in terms of hygiene [32]. A variety of studies have reported coliform group bacteria counts as follows; Atasoy et al. [33], 8.50×10^2 to 2.25×10^5 cfu/mL; Kesenkaş and Akbulut [16], 0.75–6.0 log cfu/mL; and Güllüce et al. [34], 5.0×10^3 to 1.0×10^6 cfu/mL. Uraz and Yücel [35] reported that the mean coliform group bacteria count was 3.2×10^8 cfu/mL in raw milk samples taken from 169 milk processing facilities in Ankara, while the mean coliform group bacteria count was 2.9×10^8 cfu/mL in raw milk samples obtained from 42 street sellers. Diler and Baran [14] identified the coliform group bacteria counts as 3.03 log cfu/mL. They found that the highest coliform group bacteria count was 5.9 cfu/mL. The high levels of coliform group bacteria in raw milk was stated to indicate that hygiene precautions were not taken during and after milking, and the tools and equipment used for milking were not sufficiently cleaned and necessary care was not taken about cleaning [19,29].

There is no standard for dry matter amounts included in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk. However, it was stated that the fat amount in raw milk should be at least 3.5%, while the dry matter amount was determined as 8.5%. Accordingly, it can be said that the dry matter amounts in milk should be at least 12%. Kurt et al. [36] investigated the composition of milk obtained at one-week intervals from 10 different places with sales in the summer months in the market of Erzurum and found that the mean dry matter amounts were from 7.97% to 16.98%, with a mean of 12.43%. The mean dry matter value found in the summer months in our research was 12.74%. The mean value found by Kurt et al. [36] and the mean values found in our study comply with each other. The dry matter values from previous studies were from 12.33% to 13.28% in a study of farm milk in summer and winter seasons, respectively, and 10.05% to 11.53% in a study of street milk by Akın et al. [31], while they were from 10.66% to 13.19% in the study of Yaylak et al. [37]. In our analyses the lowest and highest values were 12.74% and 13.40%, respectively. Though the milk with these values identified in our study is not farm milk, the values show similarities to the farm milks of Akın et al. [31]. Measurement of dry matter amounts in milk has additional importance for identifying fraud. Additionally, factors like animal race, animal age, nutrition, temperature, and lactation duration are stated to be important among the reasons for differences in the dry matter amounts [2,38]. Tokur [39] identified mean total dry matter amounts of 10.79%, 11.43%, 11.24%, and 11.24% for samples obtained from street sellers in Ankara in the winter, spring, summer, and autumn months, respectively. Karakoç et al. [40] found that the mean dry matter amounts identified in summer and winter seasons in milk samples obtained from a private processing operation in the district of Silvan in Batman were 9.93 ± 0.03% and 10.4 ± 0.14%. They reported that the highest amount of dry matter was identified in the winter months. In our analyses, the highest dry matter amounts were identified in winter. Ateş [41] identified that the nutrition of animals had a great effect on dry matter amounts, i.e. dry matter amounts increased in the winter and autumn seasons while this rate reduced in the summer and spring seasons.

The Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk states the amount of fat in raw milk should be at least 3.5% [26]. According to TSE (Turkish Standards Institution) TS 1018 Cow Milk - Raw Standard, Class I milk should contain 3.0% fat, Class II milk should contain 2.5%, and Super milk should contain at least 3.5% fat [4]. The mean fat amounts for raw cow's milk for some years in a variety of studies were reported as 3.61% by Aslan et al. [42], 3.75% by Gayretli [43], and 3.64% (Simental cattle) and 3.72% (Holstein cattle) by Özkan [44]. Ateş [41] reported that in the spring when animals are sent out to pasture, the fat amount in milk is reduced. Our analysis results found that fat rates were higher in the winter, which complies with other studies [40,43,45]. In addition to the fat amount being important for quality standards, it leads to the values taken as a basis for pricing. Additionally, milk fat has great importance in terms of the milk processing industry. As premiums paid to producers are based on fat amounts, the amount of fat in milk is more important than the other quality criteria. The quality of raw milk is determined according to the fat amount, and there are many factors affecting the amount of fat [38]. Especially in the spring when animals are sent out to pasture, feeding with green pasture plants with high water content leads to a reduction in the fat amount in milk, while the amount of milk increases. In the summer months, the amount of fat in milk from cows fed with meadow pasture herbs with high cellulose reduces, while the fat amounts increase for cows fed with concentrated feed in the winter months (crushed grain, pulp, factory feed, etc.). The amount of fat is inversely proportional to the temperature. Additionally, as cows become stressed as the temperature increases, the fat amounts reduce in summer [2,46]. In addition to the seasonal effect on fat amounts, factors like animal breed, age and hereditary characteristics, animal husbandry, care and nutrition, time of milking, lactation period, and shelter properties are effective as well [2,38].

For raw cow's milk, the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk states the amount of nonfat dry matter should be at least 8.50% [26]. As there is a narrow interval between minimum and maximum amounts of the nonfat dry matter in milk, it is important for identification of interventions with fraudulent aims made to milk. The nonfat dry matter amounts are especially important to obtain information about the amount of water in milk [29]. Önal [47] obtained and investigated a total of 36 raw milk samples from 18, 10, and 8 milk collection tanks in Edirne, Tekirdağ, and Kırklareli, respectively. This research found that the mean nonfat dry matter amounts, in turn, were 8.34%, 8.50%, and 8.39% for Edirne, Tekirdağ, and Kırklareli. Research by Tuncer [48] found that the mean nonfat dry matter value for analysis of milk obtained from the provinces of Kırıkkale, Aksaray, Niğde, Nevşehir, and Kırşehir was 8.32%. At the end of our analysis, the values identified for the same months were found to be above the standards. These values were similar to the values found by Önal [47] and Tuncer [48].

The FAO states that the lactose proportion in raw cow's milk should be between 4.50% and 5.10% [7]. Lactose, the unique carbohydrate of milk, is found only in milk in nature and is a disaccharide consisting of glucose and galactose. The amount of cow's milk is around 3.60%–5.50% and it constitutes about 1/3 of the milk's dry matter [2]. Kurt et al. [36] reported a mean lactose rate of 4.45%. The mean lactose values in a variety of studies about raw cow's milk from some years were identified as 4.28% by Salman et al. [49] and 4.43% by Gemechu et al. [50]. When lactose amounts decrease, it should be remembered that animals may have mastitis. Additionally, milking and environmental conditions may cause different microorganisms to contaminate milk. The enzymes secreted by these bacteria transform lactose into lactic acid and cause an increase in the acidity of milk. This situation negatively affects the ability to process milk [51].

The protein amount in raw cow's milk is reported to be at least 2.8% in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk [26]. Kurt et al. [36] reported that the mean protein amount was 3.49% as a result of analyses applied to the raw milk obtained from 10 separate locations selling milk in the summer months on the market in Erzurum. In our analyses, the mean protein amount for the summer months was identified was 3.16%. The study by Ateş [41] found that mean protein amount was 3.39%, and they reported that this abided by the legislation. In our analyses, all samples were identified to have protein amounts above the legislative criteria. Protein amounts in studies of street milk from different regions in Turkey through the years were reported to be 3.19% by Yaylak et al. [37], 3.18% by Kesenkaş and Akbulut [16], and 3.11% by Diler and Baran [14]. As with many yield properties, the amount of protein in raw cow's milk displays variations with the breed of the animal, age, nutrition, and environmental conditions. Weather conditions directly affect the protein amounts. In winter, the protein amounts in raw cow's milk increase, while they decrease in summer. Heat stress clearly lowers the amount of protein in raw milk. Good ventilation and physical conditions in cowsheds positively affect the protein amounts [52]. Yurt and Uluçay [53] found that the protein amounts in raw milk samples they analyzed were between 2.50% and 3.80% with a mean of 3.19 ± 0.35%. Akın et al. [31] identified that the highest mean protein amount after analysis of farm milk was 3.51% in the winter, with the lowest protein amount in the summer of 3.25%. The same study identified the highest protein amount in street milks of 3.14% in the winter and the lowest protein amount of 2.65% in the summer. In our analyses, the highest mean protein amount of 3.70% was obtained in the winter, while the lowest mean protein amount of 3.16% was identified in the summer, which appears to comply with other studies.

There is no standard related to the ash amounts in raw milk in the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk. However, some references have reported % ash amounts in raw cow's milk from 0.70% to 0.90% [19]. The mean ash amounts in a variety of studies of raw cow's milk from certain years were reported as 0.72% by Salman et al. [49] and 0.74% by Gemechu et al. [50]. Analysis of raw milk samples obtained from Iğdır and its surroundings by Yurt and Uluçay [53] found that ash amounts varied from 0.31% to 1.23%, with a mean value of 0.77 ± 0.17%. They reported that very low amounts of ash in raw milk may be a result of adding water to milk for fraudulent purposes. In our study, the mean ash amount varied from 0.69% to 0.79%. The ash amounts in raw milk are low; however, it strengthens the nutritional value and processing features of the milk. Ash in food is the white inorganic portion remaining after the burning of organic matter. The ash amount in raw milk may increase or decrease due to different reasons. Milk from mastitic cows or milk with chemicals added to remove acidity have ash amounts above the mean, and this may provide information related to the microbial stability. The ash amount in raw milk varies according to the breed of the animal milked [19].

The Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk, providing important information about milk quality, does not state any value for pH. However, it is known that milk from healthy animals should have pH values from 6.6 to 6.8 [19]. The first milk from a healthy animal should be between 6.6 and 6.8. If the pH value is above 6.8, the animal may have teat infection (mastitis) or material to reduce acidity may have been added to the milk. If the pH value is below 6.5, the acidity is high and the animal can be said to have newly given birth and the milk may be colostrum [54]. Ceylan et al. [55], in research to determine the pH level in milk obtained from cows in every season of the year, identified the mean pH value in the winter and autumn seasons as 6.70, while it was 6.75 in the summer and 6.76 in the spring. In conclusion, they reported that the differences between these values were insignificant. In our analyses, the mean values for the winter, autumn, summer, and spring seasons were 6.66, 6.64, 6.54, and 6.60, respectively, and contrary to Ceylan et al. [55], the seasonal difference was identified to be very significant. The lowest and highest pH values in a variety of studies about raw cow's milk from certain years were identified as 5.80–6.05 by Diler and Baran [14], 6.40–7.00 by Kesenkaş and Akbulut [16], and 6.41–6.63 by Akın et al. [31], while the mean pH value was reported as 6.45 by Tuncer [48]. Tokur [39] investigated some physical and chemical properties of 58 milk samples obtained from street milk sold in the winter, spring, summer, and autumn seasons in Ankara. This research reported that the mean pH values for winter, spring, summer, and autumn were 6.65, 6.67, 6.68, and 6.63, respectively. In our analysis, the winter, spring, summer, and autumn milks had mean pH of 6.66, 6.60, 6.54, and 6.64, respectively, and appear to comply with the values reported by Tokur [39]. After milking, the milk should be cooled to 4 °C and stored. If not, the bacteria in milk transform lactose to lactic acid, increasing the acidity and lowering the pH. As this situation causes milk to clot, it makes it difficult or even impossible to obtain products from raw milk [29].

For raw milk acidity, the Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk reports that % lactic acid should be between 0.135% and 0.200% [56]. There is compliance between the pH values and acidity values of milk samples. The milk samples with low pH values are stated to have high titratable acidity values [29]. The acidity levels in milk are important to determine the quality. High acidity is an indicator of high numbers of microorganisms in milk and indicates that appropriate storage conditions were not ensured [2]. Problems are experienced with highly acidic milk during production and product quality decreases because clotting of highly acidic milk is known to create some problems like decrease in yield and changes in the odor of milk [43]. The lowest and highest titratable acidity for raw cow's milk in a variety of studies in different years were identified as 0.161% and 0.220% by Akın et al. [31], while the mean titratable acidity value was reported as 0.157% by Kesenkaş and Akbulut [16].

The Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk does not state any standard related to the refractive index of raw milk. The refractive index and nonfat dry matter amounts change in direct proportion and the refractive index of raw cow's milk is between 1.344 and 1.348. When water is added to the milk, the sugar density in milk reduces and the refractive index of milk decreases. As a result, the refractive index values provide an idea for identification of whether water has been added to the milk or not [19].

The Turkish Food Codex Communiqué on Raw Milk and Heat Treated Drinking Milk gives the specific gravity of raw cow's milk as 1.028 [3]. The Cow Milk - Raw Standard (TS 1018) states the specific gravity for cow's milk should be between 1.028 and 1.039 [4]. The specific gravity of milk is one of the important criteria in terms of providing an idea about fraudulent practices related to milk. The specific gravity of milk shows variations with the effect of all matter included in the composition. The specific gravity reduces with the increase in fat amount and increases with the increase in the amounts of other components. Additionally, temperature increases negatively affect the specific gravity [37]. Variation in the specific gravity of milk is affected by factors such as the breed of the animal, age, disease, season, and time of milking. Additionally, the specific gravity provides an idea for identification of fraud in relation to milk [19]. The mean specific gravity for milk sold in different provinces in Turkey was identified as 1.0287 by Diler and Baran [14], 1.0315 by Kurt et al. [36], and 1.0296 by Tokur [39]. Beykaya et al. [29] analyzed 50 milk samples obtained from the storage tanks in 5 milk factories in Sivas and reported that the specific gravity varied from 1.0230 to 1.0312 with the mean value of 1.0282. In our analysis, the lowest specific gravity was 1.0313 in winter, with the highest specific gravity of 1.0325 for summer. The temperature increase in the summer reduces the fat proportion, and this is thought to affect the high specific gravity values in the summer in this study. Research by Akın et al. [31] dealt with seasonal means for farm milk in the winter, spring, summer, and autumn seasons in Adıyaman, and they identified the lowest specific gravity of 1.0311 in the summer and the highest specific gravity of 1.0328 in the winter. In our analysis, contrary to Akın et al. [31], the lowest specific gravity was identified in the winter with the highest specific gravity identified in the summer. This situation is thought to be related to the temperature increase and the fat amount in the milk.

Some veterinary medications pass into raw milk through blood and negatively affect the quality of milk. For residue amounts of pharmacologically active matter, the Turkish Food Codex Regulation on the classification of pharmacologically active substances and maximum residue limits in foodstuffs of animal origin states which antibiotic groups and proportions pass into milk. Abiding by the limits carries great importance for consumer health [57,58]. Some veterinary medications administered to lactating animals to treat some diseases are injected into muscle and pass into milk through blood, while some applied to the teats pass into milk through the milk ducts and negatively affect the quality of milk. The half-life of these antibiotics in milk varies from 3 to 7 days depending on the degree of effect. Within this time period, if milk brought to milk collection centers and milk processing plants is accepted without analysis, it may cause product losses in the milk industry and lead to economic loss [59]. Additionally, antibiotics in milk create a danger in terms of human health. Allergic reactions and intoxication, and even accumulation in the body when taken frequently at low amounts, can cause reduction in efficacy for the treatment of humans with antibiotics [58,59]. If milk containing antibiotics is brought to milk collection centers and identified, there are severe penalties [60]. Fortunately, no antibiotic residues were encountered in any of our samples of milk obtained from milk collection centers. Known publicly as baking soda, the addition of sodium bicarbonate ($NaHCO_3$) to milk to reduce the acidity of milk and microbial load can be identified with the carbonate test. Peroxidase is a natural enzyme found in milk. This test identifies whether the stability of milk has been increased or not by boiling. As milk processing plants cannot obtain any products from cooked milk, it is important to perform the peroxidase test when accepting milk [19].

In conclusion, raw milk samples were generally identified to be above the standards for physical and chemical properties. This result occurred due to the awareness of producers about the need for good physical and chemical properties in order to market milk. However, if only physical and chemical features are good, it does not mean that the milk has a good quality. The values stated in legislation about raw milk in terms of microbiology are also important. However, in this study the raw milk samples did not abide by the microbiological criteria stated in the legislation. Considering our results, it appears that the physical and chemical quality properties of milk were good, while microbiological quality features were poor. The reason for this is that the producers of the raw milk samples were not professional operations, but produced milk from small family operations. Due to the differences in animal care, nutrition, milking conditions, milking hygiene, and cleaning habits in these operations, the bacterial load in raw milk increases. It was determined that seasonal differences significantly affected the analyses, and higher milk quality was observed in the winter months when air temperature was low. As temperature increased in the summer months, the quality of milk was identified to decrease. Especially due to problems with the transport of milk to milk collection centers in the summer months, and the increase in other agricultural activities of producers in the summer season, a lack of care about the cleaning of milking machines caused severe problems with the microbiological quality of milk. As a result, the milk should immediately enter the cold chain after milking with temperature lowered to 4 °C and be transferred to the processing facility.

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Conflict of Interest
The authors declare that there are no conflicts of interest in connection with this paper.

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