



## Quality Characteristics of Raw Milk Samples Purchased from Automatic Milk Vending Machines in Niğde Province

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**Abstract:** This study aims to examine some physical, chemical and microbiological properties of a total of 40 raw milk samples sold in automatic vending machines and unpackaged, by regulations in Niğde province, and to compare the milk samples according to the retailer from which they were purchased. The study also aimed to quantitatively determine carbonate and hydrogen peroxide residues in milk samples and the presence of beta-lactam and tetracycline group antibiotics with a commercial kit. The average value of lactic acid (%) density, fat, protein, non-fat dry matter, lactose, freezing point, and pH values of the samples were determined as  $0.153\% \pm (0.022)$ ;  $1.028 \pm (0.03)$  g/ml;  $3.525\% \pm (0.656)$ ;  $3.5\% \pm (0.107)$ ;  $9\% \pm (0.277)$ ;  $5.134 \pm (0.152)$ ;  $-0.549^{\circ}\text{C} \pm (0.018)$  and  $6.55 \pm (0.102)$  respectively. As a result of microbiological analysis of milk, the average number of total aerobic mesophilic organisms, coliforms, fecal coliforms, yeast-molds and micrococci-staphylococci was determined as  $5.38 \pm (0.47)$ ;  $3.73 \pm (1.11)$ ;  $2.76 \pm (1.66)$ ;  $2.33 \pm (1.86)$  and  $4.29 \pm (1.20)$  log cfu/ml respectively. Sodium carbonate, hydrogen peroxide, beta-lactam, and tetracycline antibiotic residues could not be detected in the milk samples. The fact that some of the results obtained in the study were found to be outside the limits specified in the codexes and different from the study average reveals that these samples may cause public health problems, especially in terms of microbial quality, and therefore the milk sold in street milk retailers should be analyzed regularly.

**Keywords:** Milk vending machine, Niğde, raw milk quality

## Niğde İlinde Otomatik Süt Satış Makinelerinden Satın Alınan Çiğ Süt Örneklerinin Kalite Özellikleri

**Özet:** Bu çalışma Niğde ilinde yönetmeliğe uygun olarak, otomatik satış makinelerinde ve ambalajsız olarak satılan toplam 40 çiğ süt örneğinin bazı fiziksel, kimyasal ve mikrobiyolojik özelliklerinin incelenmesi ve süt örneklerini satın alındığı perakendeciye göre karşılaştırılmasını amaçlamaktadır. Çalışmada ayrıca süt örneklerinde karbonat ve hidrojen peroksit kalıntıları kantitatif olarak, beta-laktam ve tetrasiklin grubu antibiyotiklerin varlığının ticari kit ile belirlenmesi hedeflenmiştir. Çalışma sonunda örneklerin laktik asit (%), yoğunluk, yağ, protein, yağsız kuru madde, laktoz, donma noktası ve pH değerleri sırasıyla ortalama olarak  $0.153 \pm (0.022)$ ;  $1.028 \pm (0.03)$  g/ml;  $3.525 \pm (0.656)$ ;  $3.5 \pm (0.107)$ ;  $9.4 \pm (0.277)$ ;  $5.134 \pm (0.152)$ ;  $-0.549^{\circ}\text{C} \pm (0.018)$  ve  $6.55 \pm (0.102)$  olarak belirlendi. Sütlerin mikrobiyolojik analizleri sonucu, toplam aerobik mezofilik genel canlı, koliform, fekal koliform, maya-küf ve mikrokok-stafilokok sayısı sırasıyla ortalama  $5.38 \pm (0.47)$ ;  $3.73 \pm (1.11)$ ;  $2.76 \pm (1.66)$ ;  $2.33 \pm (1.86)$ ;  $4.29 \pm (1.20)$  log kob/ml olarak tespit edildi. Süt örneklerinde karbonat, hidrojen peroksit ve beta-laktam ile tetrasiklin antibiyotik kalıntısı tespit edilemedi. Çalışmada elde edilen bazı sonuçların kodekslerde belirlenen limitlerin dışında ve çalışma ortalamasından farklı bulunması, bu örneklerin özellikle mikrobiyal kalite yönünden halk sağlığı sorunlarına yol açabileceğini dolayısıyla sokak sütü satan perakendecilerde satılan sütün düzenli olarak analiz edilmesi gerektiğini ortaya koymaktadır.

**Anahtar Kelimeler:** Çiğ süt kalitesi, Niğde, otomatik süt satış makinesi

### 1.Introduction

Milk, consumed by consumers for both its organoleptic properties and positive effects on health, is an important component of nutrition all over the world, but it is also a suitable environment for the proliferation of many microorganisms, including pathogens (1). In order to adequately ensure its positive effects in nutrition, the physical, chemical and microbiological properties of milk

must be of good quality and these properties must be preserved during the sales process. The quality criteria of milk are determined by standard organizations Türkiye and around the world. According to these criteria, raw milk must be provided from herds officially free of brucellosis and tuberculosis, and the milk must not contain pathogenic microorganisms, toxic chemicals and physical contaminants such as antibiotics and preservatives, and must have a normal

chemical composition, good aroma and high microbial quality (2-5).

Raw milk, sold by small businesses (grocery stores and small markets) under the name of street milk in Türkiye, is consumed by many consumers considering it to be healthy. Various survey studies conducted on milk consumption habits in Türkiye reveal that consumers prefer street milk to other types of drinking milk (Pasteurized or UHT) depending on their socio-economic status. Among the reasons why consumers prefer street milk, they think that this product is healthier and more nutritious and does not contain preservatives (6, 7).

In the Turkish Food Codex Communiqué (TFCC) on the Supply of Raw Milk (4), raw milk is defined as "milk secreted from the mammary glands of farm animals, which has not been heated above 40 °C or has not undergone any treatment with an equivalent effect". In the same communiqué, it is stated that the temperature of raw milk should not exceed 4°C during sale, and that under these conditions, milk sales must be made through an automatic vending machine made of corrosion-resistant material, which has a temperature sensor and can be easily cleaned. The criteria specified in the communiqué in question control retail milk sales in order to preserve the quality characteristics of raw milk and ensure that the consumer has access to healthy milk. However, considering that raw milk production is generally carried out by small family businesses (8), this study was designed with the idea that the milk collected from the producer and delivered to the sales place and then put into automatic machines may show different quality characteristics depending on the milking and transportation conditions. In the literature review, it is revealed that the storage and sales conditions of the samples examined in studies investigating the quality characteristics of street milk samples in Türkiye are different from each other (9-12). The aim of this study was to determine the physico-chemical and microbial quality of raw milk samples taken from automatic milk vending machines of different retailers in Niğde province, to investigate the presence of preservatives and antibiotics, and to compare retailers in terms of these characteristics.

## 2. Materials and Methods

### 2.1. Materials

In this study, a sample was made from retailers selling unpackaged raw milk from automatic vending machines defined in article 10 of the 4th section of the TFCC on the Supply of Raw Milk (4) in the province of Niğde. Between September and November 2023, 5 retailers selling milk through automatic machines were visited in 10-day periods and a total of 40 milk samples were purchased, 8 from each. Milk samples were delivered to the laboratory within two hours via cold chain and analyzed.

## 2.2. Methods

### 2.2.1. Physico-chemical Analysis

In order to determine the physico-chemical properties of milk samples, pH measurement (Isolab, 422522), density determination with lactodensimeter, titratable acidity in % lactic acid (LA) (31), fat, protein, lactose, non-fat dry matter (NFD) and freezing point (FP) (Funke Gerber Lactostar 3510, Germany) values were determined.

### 2.2.2. Antibiotic Residue and Inhibitory Substances Analysis

A commercial kit (Kwinbon Biotechnology, KB02154Y) was used to detect beta-lactam and tetracycline group antibiotics. Drug detection sensitivity in the commercial kit for beta-lactam antibiotics were reported as 2 µg/L, 3 µg/L, 4 µg/L, 6 µg/L, 20 µg/L, 20 µg/L, 40 µg/L, 50 µg/L, 90 µg/L, 10 µg/L for benzyl penicillin, ampicillin, amoxicillin, oxacillin, nafcillin, cefquinom, ceftriaxone, ceftiofur, cephalonium, respectively. Drug detection sensitivity in the commercial kit for tetracycline antibiotics were reported as 80 µg/L for tetracycline, terramycin, fortimycin, duomycin. The presence of sodium carbonate and hydrogen peroxide in raw milk samples was determined according to Tekinşen et al (13).

### 2.3. Microbiological Analysis

Raw milk samples (10 mL) were taken and homogenized in 90 mL of ¼ Ringer's solution, and serial dilutions up to 10<sup>-5</sup> were prepared with Ringer's solution. All prepared dilution (1 mL) was used for total aerobic mesophilic organisms, coliforms and fecal coliforms, yeast-moulds and micrococci-staphylococci, respectively. To determine total aerobic mesophilic bacteria (TAMB) counts, petri dishes containing Plate Count Agar (PCA, Merck, Germany) were incubated for 48-72 hours in an aerobic environment at 30 °C, according to the ISO 4833 (14) technique. Violet Red Bile Agar (VRBL, Merck, Germany) was used to count coliform and fecal coliform bacteria and the petri dishes were incubated at 35°C for 24-48 hours for coliforms and 24-45 °C for fecal coliforms according to ISO 4832 (15). -Incubated for 48 hours. For yeast-mold enumeration, petri dishes containing pH 3.5 Potato Dextrose Agar (PDA, Merck, Germany) with 10% tartaric acid added were incubated at 28°C for 5 days (16). For micrococcus-Staphylococcus counting, after 24-48 hours of incubation at 37°C in Mannitol Salt Phenol-Red Agar (MSA Merck Germany), yellow colonies with a yellow zone around them were counted, and pink and red colonies were counted as staphylococci and micrococci (17). The number of colonies was calculated logarithmically, quantitatively the colony count of samples < 1 log cfu/mL below the limit of detection was set to log<sub>10</sub>.

## 2.4. Statistical analyzes

Statistical differences in the physicochemical and microbiological properties of street milk samples on a retailer basis were tested by analysis of variance, and Tukey, Welch and Man-Whitney U tests were applied according to normality distribution. For each group, the normality of the

## 3. Results

The milk samples examined in this study was determined as % LA values ranged from 0.081-0.207, with an average of average  $0.137 \pm (0.026)$ ; pH values ranged from 6.24 to 6.69, with an average of  $6.55 \pm (0.102)$ ; % fat ratio between 1.850-5.24, with an average of  $3.525 \pm (0.656)$ ; % protein between

distribution was examined with the Shapiro-Wilk test and the homogeneity of variances was examined with the Levene Test. One Way Anova test was used for group comparisons. The mean results were given with standard deviation. Statistical analyzes were performed using the IMB SPSS®v.24.Ink (SPSS, 2016) package program.  $P < 0.05$  and  $P < 0.005$  values were considered statistically significant.

3.3-3.8 with an average of  $3.5 \pm (0.107)$ ; % lactose between 4.81-5.44, with an average of  $5.133.5 \pm (0.152)$ ; density 1.022 -1.035 g/ml, with an average of  $1.028 \pm (0.03)$  g/mL; %NFD ranged from 8.9 to 10.0, with an average of  $9.4 \pm (0.277)$ ; FP  $-0.584^{\circ}\text{C} - (-0.510^{\circ}\text{C})$  with an average of  $-0.549^{\circ}\text{C} \pm (0.018)$ . It was found that there was a significant difference in terms of % protein value in retailers C and E ( $p < 0.005$ ) (Table 1).

**Table 1:** Some physicochemical properties of raw milk samples

| Physicochemical Properties | Retailers      | N | Min.   | Max.   | Mean± (SD)*     |
|----------------------------|----------------|---|--------|--------|-----------------|
| pH                         | A              | 8 | 6.49   | 6.68   | 6.578± (0.074)  |
|                            | B              | 8 | 6.43   | 6.64   | 6.534± (0.086)  |
|                            | C              | 8 | 6.45   | 6.63   | 6.58± (0.058)   |
|                            | D              | 8 | 6.24   | 6.69   | 6.533± (0.185)  |
|                            | E              | 8 | 6.46   | 6.62   | 6.545± (0.067)  |
| % Lactic acid              | A              | 8 | 0.117  | 0.158  | 0.136± (0.016)  |
|                            | B              | 8 | 0.081  | 0.176  | 0.139± (0.034)  |
|                            | C              | 8 | 0.090  | 0.153  | 0.124± (0.022)  |
|                            | D              | 8 | 0.081  | 0.171  | 0.135± (0.027)  |
|                            | E              | 8 | 0.126  | 0.207  | 0.149± (0.026)  |
| Density                    | A              | 8 | 1.022  | 1.029  | 1.026± (0.003)  |
|                            | B              | 8 | 1.027  | 1.031  | 1.029± (0.001)  |
|                            | C              | 8 | 1.026  | 1.035  | 1.029± (0.003)  |
|                            | D              | 8 | 1.025  | 1.035  | 1.030± (0.004)  |
|                            | E              | 8 | 1.026  | 1.035  | 1.029± (0.003)  |
| Freezing point             | A              | 8 | -0.560 | -0.513 | -0.537± (0.016) |
|                            | B              | 8 | -0.566 | -0.545 | -0.558± (0.007) |
|                            | C              | 8 | -0.584 | -0.510 | -0.537± (0.023) |
|                            | D              | 8 | -0.560 | -0.539 | -0.547± (0.007) |
|                            | E              | 8 | -0.580 | -0.550 | -0.566± (0.01)  |
| % Non-fat dry matter       | A              | 8 | 8.85   | 9.76   | 9.298± (0.309)  |
|                            | B              | 8 | 9.39   | 9.75   | 9.563± (0.117)  |
|                            | C              | 8 | 8.92   | 10.01  | 9.259± (0.344)  |
|                            | D              | 8 | 9.26   | 9.60   | 9.389± (0.119)  |
|                            | E              | 8 | 9.47   | 9.93   | 9.701± (0.167)  |
| % Fat                      | A              | 8 | 2.170  | 5.24   | 3.930± (1.042)  |
|                            | B              | 8 | 3.250  | 3.850  | 3.508± (0.209)  |
|                            | C              | 8 | 1.850  | 4.170  | 3.139± (0.818)  |
|                            | D              | 8 | 2.990  | 3.600  | 3.336± (0.222)  |
|                            | E              | 8 | 3.380  | 4.210  | 3.713± (0.331)  |
| % Protein                  | A              | 8 | 3.32   | 3.67   | 3.499± (0.119)  |
|                            | B              | 8 | 3.52   | 3.66   | 3.591± (0.044)  |
|                            | C <sup>1</sup> | 8 | 3.33   | 3.76   | 3.474± (0.134)  |
|                            | D              | 8 | 3.48   | 3.57   | 3.525± (0.046)  |
|                            | E <sup>1</sup> | 8 | 3.56   | 3.74   | 3.646± (0.066)  |
| % Lactose                  | A              | 8 | 4.82   | 5.31   | 5.05± (0.169)   |
|                            | B              | 8 | 5.11   | 5.30   | 5.2± (0.062)    |
|                            | C              | 8 | 4.81   | 5.44   | 5.033± (0.190)  |
|                            | D              | 8 | 5.03   | 5.18   | 5.113± (0.069)  |
|                            | E              | 8 | 5.15   | 5.40   | 5.275± (0.089)  |

\*SD: Standard deviation

<sup>1</sup> C and E There is a statistically significant difference.  $P < 0,05$  and  $p < 0.005$ , statistically significant difference in the physico-chemical properties of raw milk samples

The number of TAMB at 30 °C ranged from 4.85 to 6.77 log cfu/ml, with an average of 5.38 log cfu/ml $\pm$  (0.47); coliform count ranged from 0 to 4.74 log cfu/ml, with an average of 3.73 log cfu/ml $\pm$  (1.11); fecal coliform count ranged from 0 to 4.74 log cfu/ml, with an average of 2.76 log cfu/ml $\pm$  (1.66);

yeast-mold count ranged from 0 to 4.84 log cfu/ml, with an average of 2.33 log cfu/ml $\pm$  (1.86); micrococci-staphylococci count ranged from 0 to 5.91 log cfu/ml, with an average of 4.29 log cfu/ml  $\pm$  (1.20) were found (Table 2).

**Table 2:** Presence of microorganisms in raw milk samples (log<sub>10</sub> cfu/ml)

| Microorganisms             | Retailers | N | Min. | Max. | Mean $\pm$ (SD)*  |
|----------------------------|-----------|---|------|------|-------------------|
| Total aerobic mesophilic   | A         | 8 | 4.92 | 6.00 | 5.44 $\pm$ (0.29) |
|                            | B         | 8 | 4.88 | 6.50 | 5.66 $\pm$ (0.55) |
|                            | C         | 8 | 4.87 | 5.97 | 5.25 $\pm$ (0.46) |
|                            | D         | 8 | 4.85 | 5.75 | 5.11 $\pm$ (0.31) |
|                            | E         | 8 | 4.88 | 6.77 | 5.48 $\pm$ (0.58) |
| Micrococcus-Staphylococcus | A         | 8 | 3.74 | 5.89 | 4.62 $\pm$ (0.65) |
|                            | B         | 8 | 3.86 | 5.79 | 4.58 $\pm$ (0.79) |
|                            | C         | 8 | 0    | 5.26 | 3.35 $\pm$ (2.17) |
|                            | D         | 8 | 3.26 | 5.32 | 4.28 $\pm$ (0.67) |
|                            | E         | 8 | 3.97 | 5.91 | 4.63 $\pm$ (0.64) |
| Yeast-Mold                 | A         | 8 | 0    | 3.86 | 1.78 $\pm$ (1.92) |
|                            | B         | 8 | 0    | 4.84 | 1.90 $\pm$ (2.10) |
|                            | C         | 8 | 0    | 4.30 | 3.38 $\pm$ (1.43) |
|                            | D         | 8 | 0    | 4.21 | 1.85 $\pm$ (2.0)  |
|                            | E         | 8 | 0    | 4.16 | 2.74 $\pm$ (1.72) |
| Coliform                   | A         | 8 | 3.67 | 4.74 | 4.29 $\pm$ (0.45) |
|                            | B         | 8 | 3.74 | 4.74 | 4.21 $\pm$ (0.39) |
|                            | C         | 8 | 2.26 | 4.54 | 3.74 $\pm$ (0.90) |
|                            | D         | 8 | 0    | 4.16 | 2.49 $\pm$ (1.69) |
|                            | E         | 8 | 3.04 | 4.72 | 3.92 $\pm$ (0.66) |
| Fecal Coliform             | A         | 8 | 2.44 | 4.65 | 3.51 $\pm$ (0.64) |
|                            | B         | 8 | 0    | 4.74 | 2.62 $\pm$ (1.78) |
|                            | C         | 8 | 0    | 4.74 | 3.08 $\pm$ (1.6)  |
|                            | D         | 8 | 0    | 3.70 | 1.53 $\pm$ (1.71) |
|                            | E         | 8 | 0    | 4.68 | 3.03 $\pm$ (1.93) |

\*SD: Standard deviation

Residues of sodium carbonate, hydrogen peroxide and beta-lactam and tetracycline antibiotics could not be detected in any of the milk samples.

#### 4. Discussion and Conclusion

The milk samples analyzed in this study were purchased from automatic milk vending machines that have a temperature sensor and keep the milk at a maximum temperature of 4°C. In the literature research, no study was found in Türkiye in which only milk samples taken from automatic milk vending machines were analyzed. In studies conducted with street milk samples in Türkiye, it was determined that milk samples were obtained from grocery stores, markets and

##### 4.1. Physico-chemical Analysis

The total acidity of milk consists of natural acidity and developed acidity. The initial acidity in fresh milk is due to the proteins of the milk, especially casein, and the soluble phosphate, colloidal calcium phosphate, bicarbonate and, to a lesser extent, citrate and carbon dioxide in the milk; the developed acidity is due to lactic acid produced by bacteria

supermarkets, local milk delicatessens, milk collection center cooling tanks, milk producers and food bazaars (9, 11, 12, 18, 19). In some studies conducted abroad, unlike this study, milk samples were collected from milk distribution tankers of traditional farms (20); vendors selling milk in pickup trucks and donkey carts (21), or from the producer after milking (22). It is stated that in many European countries, raw milk is sold self-service in automatic milk vending machines. In these countries, there are studies to determine the quality of milk samples offered for sale in automatic milk vending machines in order to reveal the risks that may occur due to the increase in raw milk consumption among consumers (1, 23-26).

from lactose in milk (27). Titratable acidity and pH are physical parameters measured to evaluate the freshness of raw milk, its suitability for hygienic standards and heat treatment, and storage conditions (25). According to the TFCC on the Supply of Raw Milk (4), the acidity value in terms of LA should be between 0.135-0.200%. The LA value of the milk samples examined in this study was found to be

between 0.113-0.207%, with an average of  $0.153\% \pm (0.022)$ . The %LA value of 5 of the samples (12.5%) was detected below the range specified by TFC, and the %LA value of 2 (5%) was detected above the range specified by TFC. Unlike this study, in the study conducted in Erzurum, it was reported that the %LA value was between 0.157-0.178 and within the limits specified in the notification (18). There are studies reporting average % LA values in milk samples as between 0.180-0.270 and higher than this study (10, 12, 21). Alan et al. (11) reported the %LA value as lower than this study ( $0.16 \pm 0.01$ ). Consistent with the findings in this study, it has been reported in studies conducted in Türkiye that %LA values are outside the lower and upper limits of the TFC criteria (10-12). The low %LA value may be due to the addition of an alkaline preservative to the milk, the milk being milked from mastitis-affected breasts, and the large sample size or dilution in the titration method applied (11, 28). In this study, the average pH value was determined as  $6.55 \pm (0.102)$ , minimum 6.24 and maximum 6.69. The pH value of cow's milk at room temperature is reported to be between 6.5-6.7. The data obtained in this study show that the pH value of some analyzed milk is below average. In studies conducted in Türkiye and around the world, pH values of raw milk samples are reported to be in the range of 5-7.33. (9, 10-12, 18-20, 22). The pH of milk is affected by factors that affect the composition of milk, such as lactation time and mastitis. While the pH value of colostrum is measured around 6, the pH value of mastitis and post-lactation milk can reach up to 7.5 (29). It is accepted that the high level of lactic acid and low pH level in raw milk are largely caused by microorganism activity. Tremonte et al. (26) stated that the pH value of raw milk at refrigerator temperature decreased from 6.72 to 6.27 in 72 hours due to microorganism proliferation. Similarly, among the 40 raw milk samples examined in the study, samples with pH values lower than the average of this study were detected. The microorganism counts of these samples were also found to be higher than the study average (data not shown).

Density, which is one of the physical properties of milk and an important criterion in determining adulteration, is primarily affected by the amount of fat and other chemical compounds in milk. According to the TFCC on the Supply of Raw Milk (4), the density must be at least 1.028 g/ml. In this study, the density of milk samples was determined to be between 1.022-1.035 g/ml, with an average of  $1.028 \pm (0.03)$  g/ml. The density of 12 (30%) of the milk samples examined in the study was found to be below the limit set by TFC. It has been reported in various studies that the density value in raw cow milk samples was determined to be between 1.015-1.035 g/ml (9, 10-12, 19- 22). In this study, it was observed that the % fat values of 11 of the milk samples whose density values were below the TFC (4) limit were above 3.5. It is stated that there is a negative correlation between milk density and the fat rate of milk, and the fat rate is affected by

factors such as the genetic characteristics of the animal, feeding practice, seasonal changes and lactation period (30). In a milk sample analyzed in the study, the density and fat rate were found to be below the limit, and the freezing point value was above the limit. It is thought that water was added to this milk sample.

The chemical composition of raw milk, in addition to being an indicator of the chemical compounds that make up the nutritional value of milk, also affects the processability of milk and the quality of the final products (30). According to the TFCC on the Supply of Raw Milk (4), the fat, protein and non-fat dry matter contents in raw milk should be at least 3.4%, 2.8%, and 8.5% respectively. The % fat content in the milk samples examined in this study was determined to be between 1.850 and 5.24, with an average of  $3.525 \pm (0.656)$ . According to the Drinking Milk Communiqué (5), all milks to be offered for sale are based on their fat content: full fat (milk fat  $\geq 3.5$  g/100 ml), semi-skimmed ( $1.8 < \text{milk fat} \leq 1.5$  g/100 ml), skimmed (milk fat  $< 0.15$  g/100 ml). In the study, the fat content of 11 (27.5%) samples was found to be lower than 3.4%. It is stated that the % fat content of raw cow milk is between 1.4-7.59% in studies conducted in Türkiye and various countries (9, 11, 12, 18, 22, 24). In this study, the fat content in 11 milk samples was found to be lower than 3.4%, which shows that the milk sold in automatic milk vending machines does not always comply with the criteria set by the codex in terms of quality characteristics. The amount of milk fat is also used in the pricing of raw milk (4), and selling 11 milk samples with low fat content at the same price as other milk creates a negative situation for the consumer.

The NFD value of the milk samples examined in the study was determined to be between 8.9-10.0%, with an average of  $9.4\% \pm (0.277)$ , and all of the samples (100%) were found to be in accordance with the value determined in the notification. Studies have shown that the average NFD value is between 8.18-9.32% (9, 11, 12, 18, 19, 22, 24). The NFD value in raw milk is especially affected by the protein and lactose content of the milk. In the milk samples examined in this study, the % protein ratio was determined between 3.3-3.8, with an average of  $3.5 \pm (0.107)$ . All samples (100%) were found to meet the criteria, but a significant difference was found between retailers C and E in terms of protein values. Studies have reported that the average protein ratio is between 2.87-3.83 (9-11, 19, 24). In this study, the % lactose value was determined as  $5.134 \pm (0.152)$  between 4.81-5.44. There is no limit for lactose in TFC (4). It is stated that cow milk contains an average of 4.7% lactose, and the amount of lactose affects the freezing and boiling points of milk and its density. In other studies, it is reported that the lactose value was detected between 3.57-5.27% (9, 22, 24). In the milk samples examined in this study, it is seen that the % NFD and % protein and % lactose values affecting the %NFD value are

in accordance with the general chemical composition of milk according to the criteria specified in TFC and the literature.

Evaluation of the chemical quality of raw milk samples was done by determining adulteration with water added by determining the freezing point of milk. According to TS 1018 (31), the freezing point of raw milk is determined as  $-0.520^{\circ}\text{C}$  at most. In this study, the freezing point of milk samples was determined between  $-0.510$  -  $(-0.584)^{\circ}\text{C}$ , with an average of  $-0.549^{\circ}\text{C} \pm (0.018)$ . The freezing point values of 3 (7.5%) of the milk samples were found to be higher than the criteria specified in TS 1018 (31). In these 3 samples, fat ratios and NFD were determined as 2.9-8.9%, 1.8-8.9 and 2.1-8.9%, respectively, below the average values determined in this study. Freezing point values were found to be between  $-0.52$  -  $(-0.625)$  on average in other studies (9, 11, 22, 24). It is stated that adding water changes the composition of milk, reduces its specific gravity and nutritional value, and detecting the increase in freezing point is the most important method for detecting the presence of added water (28).

#### 4.2. Microbiological Analysis

Raw milk is an environment containing a diverse and complex microbial population. Factors such as animal cleanliness, milking equipment, conditions of the milking environment, post-milking holding and cold chain affect the microbiological quality of milk. TAMB is one of the important parameters of milk hygiene and is a good indicator for monitoring the hygiene conditions during the production and processing of dairy products. It is reported that the most important factors affecting the microbial behavior of raw milk in terms of TAMB are storage temperature and duration (25). According to the TFCC on the Supply of Raw Milk (4), the number of colonies per milliliter at  $30^{\circ}\text{C}$  is stated as  $\leq 100.000$  (5 log cfu/ml). In this study, it was found that 29 milk samples (72.5%) did not meet the specified criteria. Mean TAMB count was  $5.38$  log cfu/ml  $\pm (0.47)$ ; The minimum was determined as  $4.85$  log cfu /ml and the maximum was  $6.77$  log cfu /ml. Ertem and Çakmakçı (18) reported that the temperature of raw milk collected from various sales points at the time of purchase was between  $9.5$ - $19.0^{\circ}\text{C}$ , and the TAMB number varied between  $6.17$ - $8.40$  log cfu/ml. Also, in various studies where it was not reported that any cooler was used to keep milk cold until sale, the average TAMB number was found to be between  $6.93$  and  $7.36$  log cfu/ml (10, 11). However, Açık and Özdemir (9) reported the TAMB number in the milk obtained from cooler tanks in December as  $6.74$  log cfu/ml, higher than the result obtained in our study. It is stated that in raw milk samples taken from automatic milk vending machines in Europe, this number was found to be between  $4.24$ - $4.9$  on average, higher than the current study (23, 24, 25). Tremonte et al. (26), similar to this study, reported that the average TAMB count was determined to be approximately 5 log cfu/ml and above the criterion in 10 out of 30 samples.

Since coliform group microorganisms are widely found in nature, they can be transmitted to raw milk due to mastitis infection, wet udder milking, dirty milking containers, fecal contamination and environmental pollution. The presence of high numbers of coliforms in raw milk tanks is considered to be an indicator of improper hygienic condition and inadequate cooling during milk transportation and processing (3, 32). There is no criterion regarding the number of coliforms and fecal coliforms in the dairy and products legislation in Türkiye. In the European Union legislation, it is stated that for general raw milk production, common pathogens should not be detected in defined sample amounts and the coliform count should be  $<100/\text{ml}$  (2 log cfu/ml) (14). In this study, the coliform count of 37 (92.5%) milk samples was found to be higher than specified in the criteria. The average number of coliforms in the analyzed milk samples was  $3.73 \pm (1.11)$ ; The minimum was determined as 0 and the maximum was 4.74. The average fecal coliform count was  $2.76 \pm (1.66)$ ; The minimum was determined as 0 and the maximum was 4.74. The number of coliforms and fecal coliforms has been reported in various studies to range between 1 and 5.50 log cfu/ml, on average, higher and lower than the value we obtained current study (12, 18, 19, 26, 33)

It is stated that the psychrotrophic bacteria that multiply during the cold storage of raw milk compete with yeasts and molds in terms of sharing nutrients and limit the development of these microorganisms, thus the number of yeasts and molds should be expected to be low in raw milk obtained under hygienic conditions. Yeast and molds are responsible for spoilage in milk and dairy products, leading to economic losses. It has also been reported that many molds produce mycotoxins that threaten human health (26, 33). In our study, the average yeast-mold count in raw samples was  $2.33 \pm (1.86)$ ; The minimum was 0 and the maximum was  $4.84$  log cfu/ml. Yeast-mold counts were reported by Baran and Adıgüzel (12); Alan et al., (10) and Tremonte et al. (26) to average between 4.59, 4.55, and 5 log cfu/ml, respectively.

Micrococci and staphylococci are commonly found in the skin and mucosa of warm-blooded animals, and some species can be used as indicators of microbiological quality (35). In our study, the average micrococcus-staphylococcus count was  $4.29 \pm (1.20)$ ; The minimum was 0 and the maximum was  $5.91$  log cfu/ml. The number of micrococci-staphylococci was determined by Tasci (19), Baran and Adıgüzel (12), Alan et al. (11) as an average of 4.32 log cfu/ml; 4.38 log cfu/ml; 2.88 log cfu/ml respectively. Some types of staphylococcus are the causative agent of mastitis, and milk with mastitis is of low physico-chemical quality. Some staphylococcal species pose health risks when presenting raw milk to consumers with the thermostable toxins (36). It is stated that, in addition to mastitis, contamination from the milker and poor care lead to the high number of staphylococci in milk (35).

Although storage in refrigerated machines has been reported as an effective method to keep the microbial load of raw milk under control, in a study, the number of TAMB, coliform, fecal coliform and yeast in raw milk samples kept at refrigerator temperature was 10-25% after 24 hours; It is reported that it increases by 20-50% after 48 hours (26). In the Communiqué on the Supply of Raw Milk, it is stated that the supply of raw milk to the final consumer must be carried out within 24 hours after milking, that the first milking time will be taken as basis for raw milk with different milking times and that the last consumption time of raw milk cannot exceed 48 hours from the first milking time. The numbers of all microorganism groups detected in our study were lower than the

samples obtained from markets, delicatessens, food bazaar etc., but not kept in a cooler (10, 12, 18, 19). However, Açıık and Özdemir (9), who obtained raw milk samples from cooling tanks, reported the number of TAMB (average of  $5.5 \times 10^6$  cfu/mL) and Beykaya et al. (33) reported the number of TAMB (average of  $1.48 \times 10^7$  cfu/mL) and yeast mold (average of  $3.73 \times 10^5$  cfu/mL) to be higher than the values obtained in this study. This variability in values may be caused by factors such as animal milking hygiene, the time and temperature in which the milk is kept after milking, and the tools and equipment contacted after milking. Milk can be contaminated at different levels with many different microorganism groups in its environment. In addition, in our study, although it was lower than the literature, the TAMB result obtained from the milk of each company was above the limits given in the notification. This situation revealed that the milk initially had a hygiene quality problem and that it was important to be careful against conditions that would increase the number of microorganisms until it was transferred to the vending machine. Coolers will only help ensure that milk reaches consumers in good condition if its microbiological quality is initially high.

### 4.3. Antibiotic Residue and Inhibitory Substances

No beta-lactam or tetracycline antibiotic residues were detected in any of the raw milk samples. Unlike this study, Ertem and Çakmakçı (18) and Mortaş et al. (37) reported that they found beta-lactam and tetracycline group antibiotic residues in 6.7% and 30%, respectively. Some antibiotics used in veterinary medicine pass to the consumer through milk. It has been reported that various problems occur especially in the quality of fermented dairy products when milk containing antibiotics is presented to consumers, and health risks such as various toxic effects and antibiotic resistance occur in consumers (38).

No traces of sodium carbonate or hydrogen peroxide were found in any of the raw milk samples analyzed. Ertem and Çakmakçı (18); Alan et al. (11) stated that sodium carbonate was added to 66.7% and 20% of the milk they analyzed,

respectively. Baran and Adıgüzel (15) and Açıık and Özdemir (9) also stated that they could not find carbonate in any of the milk samples they analyzed in Erzurum. Alan et al. (11) also stated that there was no hydrogen peroxide in the milk samples they examined, as in this study. In the TFCC on the Supply of Raw Milk, it is stated that it will not contain any substance other than the component of milk. Despite food legislation, additives such as sodium carbonate and hydrogen peroxide are often used to mask the pH and acidity values of milk that has not been stored properly. It is reported that these additives are harmful to human health, and if consumed continuously, carbonates, for example, cause gastrointestinal problems such as stomach ulcers, diarrhea, and colon ulcers (39).

Although it was determined that the %NFD, %protein and %lactose rates in the street milk samples examined in this study met the criteria and that the samples did not contain carbonate, H<sub>2</sub>O<sub>2</sub> and antibiotics, it was revealed that they did not comply with the regulations in terms of other criteria. According to the results of this study, it was revealed that some of the street milk samples sold in Niğde province were insufficient to meet the targeted health effects of milk consumption and that there were quality differences in terms of physico-chemical and microbiological properties. This study can be used as a basis for future scientific studies as it is the first study to determine the quality characteristics of milk samples offered for sale in accordance with the codex in Turkey. In addition, the low microbial quality determined in some raw milk samples analyzed in the study shows that these milk samples pose a risk to consumer health, even if they are offered for sale in accordance with the codex. In this context, it was concluded that retailers selling street milk should be analyzed regularly in order to check the compliance of the milk samples offered for sale in automatic milk vending machines with the regulations.

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