

Research Article

Investigation of Antibiotic Residue and Quality Parameters in Sterilized Milks (UHT)

Sterilize Sütlerde (UHT) Antibiyotik Kalıntısı ve Kalite Parametrelerinin Araştırılması

Nabaa Ahmed Mahmood AL-GBURI¹, Recep KARA²

¹Department of Food Hygiene and Technology, Institute of Health Sciences, Afyon Kocatepe University, Afyonkarahisar, Türkiye.

²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye.

Corresponding Author:

Recep KARA

recepkara@aku.edu.tr

ORCID:

NAMAG: 0000-0003-1983-6185

RK:0000-0002-9257-7506

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Abstract

This study aimed to investigate the residual presence of antibiotics used in animal health and their impact on milk quality in Tetra Pak packaged UHT sterile milk (plain, strawberry, and chocolate). Within the scope of the study planned for this purpose, the presence of antibiotic residues and milk quality parameters was investigated using an LC-MS-MS device in UHT plain, strawberry, and chocolate milk from 50 different brands and producers, all offered for sale in their original packaging in the provinces and districts of Afyonkarahisar and its surrounding provinces. In the analysed samples, plain milk had an average pH of 6.49, milk fat of 2.29%, protein of 2.90%, lactose of 4.56%, non-fat dry matter of 8.16%, and malondialdehyde of 373.92 µmol/L. The average pH was 6.40, milk fat 1.02%, protein 2.38%, lactose 8.33%, non-fat dry matter 11.41%, and malondialdehyde 368.69 µmol/L were found in strawberry milk samples. In chocolate milk, the mean pH was 6.53, milk fat 2.47%, protein 2.91%, lactose 4.57%, non-fat dry matter 8.18%, and malondialdehyde 376.22 µmol/L. It was determined that antibiotic residues in UHT sterile milk (plain, strawberry, and chocolate) were below the Maximum Residue Limit (MRL). Consequently, it is recommended not to offer foods containing antibiotic residues for consumption, to minimise unnecessary antibiotic use in animal health, to comply with the legal withdrawal period in cases where antibiotic use is mandatory, and to monitor the quality parameters of milk periodically.

Key Words: Antibiotic Residue, Quality Parameters, UHT Milk

Öz

Bu çalışma, hayvan sağlığında kullanılan antibiyotiklerin Tetra Pak ambalajlı UHT steril sütte (sade, çilekli ve çikolatalı) kalıntılarını ve bunların süt kalitesine etkisini araştırmayı amaçlanmıştır. Bu amaçla planlanan çalışma kapsamında Afyonkarahisar il-ilçeleri ile çevre illerde orijinal ambalajında satışa sunulan 50 adet farklı marka ve üretilere sahip, UHT sade, çilekli ve çikolatalı sütlerde LC-MS-MS cihazı ile antibiyotik kalıntı varlığı ve süt kalite parametreleri araştırıldı. Analize alınan örneklerden sade sütlerde ortalama pH 6.49, süt yağı %2.29, protein %2.90, laktoz %4.56, yağsız kuru madde %8.16, malondialdehit 373.92 olarak tespit edilmiştir. Diğer bir örnek grubu olan çilekli sütlerde ortalama pH 6.40, süt yağı %1.02, protein %2.38, laktoz %8.33, yağsız kuru madde % 11.41, malondialdehit 368,69 olarak bulunmuştur. Çikolatalı sütlerde ise ortalama pH 6.53, süt yağı %2.47, protein %2.91, laktoz %4.57, yağsız kuru madde %8.18, malondialdehit 376.22 olarak saptanmıştır. UHT steril sütlerde (sade, çilekli, çikolatalı) antibiyotik kalıntısının Maksimum Kalıntı Limiti (MRL) altında olduğu tespit edilmiştir. Sonuç olarak antibiyotik kalıntısı içeren gıdaların tüketime sunulmaması, hayvan sağlığında bilinçsiz ve gereksiz antibiyotik kullanılmaması, antibiyotik kullanımı zorunlu hallerde yasal arınma süresine uyulması, sütün kalite parametrelerinin periyodik kontrol edilmesi önerilmektedir.

Anahtar Kelimeler: Antibiyotik Kalıntısı, Kalite Parametreleri, UHT Süt

Introduction

Milk is an essential nutrient in human nutrition. Milk produced by mammary glands is found in the udder. The milk secreted in the first days after birth is called colostrum (Kebchaoui, 2012). Cow's milk is a source of protein and energy for humans (Uzundumlu et al., 2018). Milk and dairy products hold a prominent place among animal-based foods in terms of their impact on human health. Milk is a nutrient-rich food containing all essential amino acids and high-quality proteins, as well as being rich in calcium, phosphorus, and riboflavin (Petti et al., 1997; Tekinsen, 2000; Metin, 2001; Sahni et al., 2013). Cow's milk, like breast milk, strengthens the human immune system and increases resistance to infections (Labbok et al., 2004).

Milk is considered nature's most complete and unique foodstuff, making it one of the most valuable and regularly consumed foods today. At the same time, milk is highly susceptible to bacterial contamination. Therefore, it can spoil easily. Furthermore, in many countries, safety issues with milk and dairy products are widespread. Furthermore, as milk and dairy products are one of the primary food sources for humans, they often contain residues of veterinary drugs, pesticides, mycotoxins, heavy metals, and similar chemical substances (Jahed, 2007).

Veterinary drugs used to treat and prevent diseases in animals are widely used in the UK and Europe to protect animal health and treat diseases. Feed additives that promote growth are added to feed for animals raised for breeding purposes to increase their growth rates. Animals cannot completely metabolise these compounds, and a large portion is excreted unchanged in faeces and urine (Özgüven, 2020). They cause numerous adverse effects on animals and consumers. For example, microorganisms may develop resistance to veterinary drugs. Food drug residues may reach the final consumer in edible animal products. After systemic drug administration, the highest residue concentrations have been observed in the animal's muscles, kidneys, and liver (Farre & Barcelo, 2013).

The study investigated the presence of antibiotic residues and milk quality parameters in UHT plain milk, strawberry milk, and chocolate milk from different brands and manufacturers sold in their original packaging.

Materials and Methods

Material

UHT sterilized milk, sold in its original packaging in Afyonkarahisar province and its districts, as well as in neighbouring provinces (20 plain, 15 strawberry-flavoured, and 15 chocolate-flavoured), formed the material for the study. A total of 50 UHT-sterilized milk samples, packaged in Tetra Pak cartons, were collected. The collected samples were brought to the laboratory and analysed.

Method

Antibiotic residue analysis

Antibiotic residue analysis was performed by outsourcing the service according to the method reported by Marilena et al. (2015) using an LC/MS/MS (Liquid chromatography–mass spectrometry/ mass spectrometry, Agilent Technologies, Waldbronn, Germany) device to determine 50 antibiotic residues in milk samples.

Preparation of antibiotic standards

Approximately 10 mg of each standard of the analysed antibiotic groups was weighed and placed in a 10 mL volumetric glass flask. Penicillins and cephalosporins (present in our results) were dissolved in MilliQ water, while all other analytes were dissolved in methanol. In the quinolone standard solution, 100 mL of formic acid was added to increase solubility. Stock solutions were prepared at a concentration of 1000 $\mu\text{g mL}^{-1}$ for each compound and stored in brown glass at $-20\text{ }^{\circ}\text{C}$ to prevent photodegradation. Four intermediate standard solutions containing several analytes were grouped according to their classifications and stabilities. They were prepared by diluting the stock solutions with methanol. The final concentration of these multi-component solutions was 10 $\mu\text{g mL}^{-1}$, and they were also stored at $-20\text{ }^{\circ}\text{C}$. All working solutions and calibration standards were obtained by gradient dilution of intermediate solutions at concentrations ranging from 1 $\mu\text{g mL}^{-1}$ to 1 ng mL^{-1} . They were stored at $-20\text{ }^{\circ}\text{C}$.

Preparation of milk samples

1 mL of milk was taken from the samples and placed in a 15 mL polypropylene centrifuge tube. All added samples were left to stand for 10-15 minutes before the process began. To remove drug residues and precipitate proteins, 2 mL of H_2O containing 0.1% formic acid (w/v) and 0.1% EDTA (Ethylene diamine tetra acetic acid) (w/v) was added to the samples, followed by 2 mL of MeOH and 2 mL of ACN. After each addition of solvent, the tube was vortexed for 30 seconds. The sample group was placed in an ultrasonic bath at 60°C for 20 minutes to facilitate ultrasonically assisted extraction. Subsequently, the samples were centrifuged at 4000 rpm for 10 minutes, and the supernatant was decanted into a new polypropylene centrifuge tube. The tubes were left at $23\text{ }^{\circ}\text{C}$ for 12 hours to precipitate lipids and remaining proteins. The samples were centrifuged again, and the precipitate was discarded. The supernatant was then transferred to another tube. The extracts were deoiled using 5 mL of hexane. They were vortexed for one minute and then centrifuged at 4000 rpm for 10 minutes. The hexane layer was aspirated, and the extracts were evaporated to dryness under a nitrogen stream at a temperature not exceeding 45°C . The resulting residues were resuspended in 1 mL of solution and filtered through a 0.22 mm RC filter. After vortexing for 10 seconds, each extract was transferred to a vial, and 10 μL was injected into the LC-MS/MS system for analytical measurement.

pH analysis

The pH measurements of the samples were determined using an Inolab pH meter (model pH-7110, WTW, Germany). The pH meter was calibrated with 4.00 and 7.00 standard solutions before measurement.

Somatic cell count

The somatic cell count (SCC) of milk samples was determined using a SHS Somatic Cell Counter (Chemometec SCC 100) device. Equal amounts of milk samples and solution (Chemometec Reagent C) were mixed. The mixture was injected into the SCC-Cassette. The cassette was inserted into the device, and the reading was taken.

Malondialdehyde (MDA) analysis

0.5 mL was taken from the milk samples, and 2.5 mL of 10% TAC solution was added and mixed. It was then kept in boiling water for 15 minutes. Immediately afterwards, the tubes were immersed in cold water to cool, and then centrifuged at 5000 rpm for 10 minutes at $4\text{ }^{\circ}\text{C}$. After centrifugation, 2 mL of supernatant was taken and mixed thoroughly with 1 mL of 0.67% TBA (thiobarbituric acid). It was then boiled for 15 minutes, cooled immediately, and measured against distilled water at 532 nm.

Chemical composition analyses of milk

The chemical properties of the milk, including fat, protein, and lactose levels, were determined using the Milk Analyser MID-Infrared (MIRIS, Uppsala, Sweden).

Results

The findings regarding the pH, fat, protein, lactose, fat-free dry matter, somatic cell count and MDA values of the milk samples are presented in Figure 1-6. Accordingly, the pH results for plain, strawberry and chocolate milk were found to be 6.49, 6.40 and 6.53 on average, respectively. The fat results of the milk samples were found to be 2.29, 1.02, and 2.47 on average for plain, strawberry, and chocolate milk, respectively. The protein results were found to be 2.90, 2.38, and 2.91 on average, respectively. The lactose value results of the study were 4.56, 8.33, and 4.57, respectively, for plain, strawberry, and chocolate milk. In the study, the somatic cell counts of milk samples were determined to be 41,250, 23,428, and 65,733 cells/mL on average for plain, strawberry, and chocolate milk, respectively; the MDA values were determined to be 373.92, 368.69, and 376.22 $\mu\text{mol/L}$ on average, respectively.

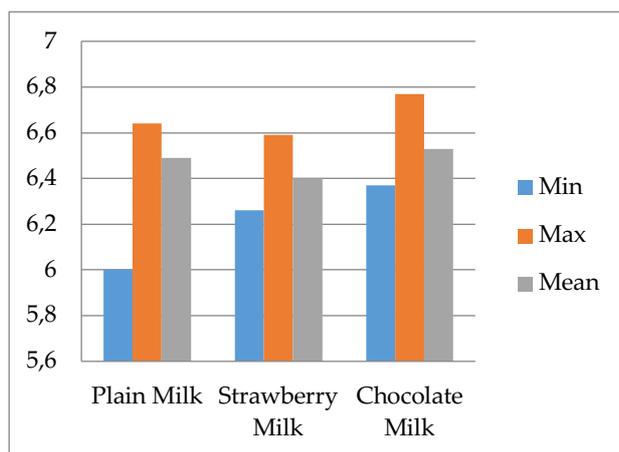


Figure 1. pH Analysis Results of Milk Samples

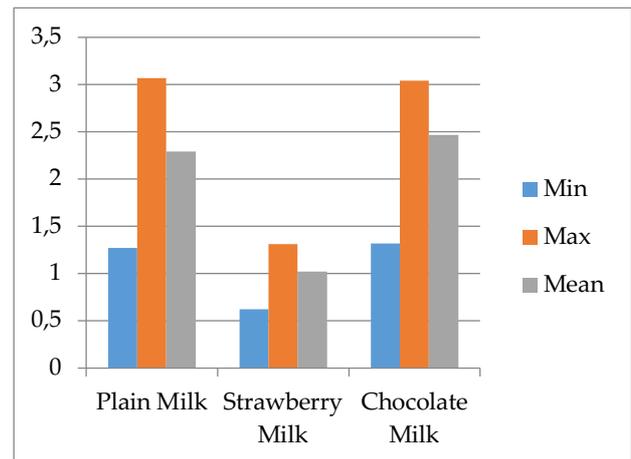


Figure 2. Fat Analysis Results of Milk Samples

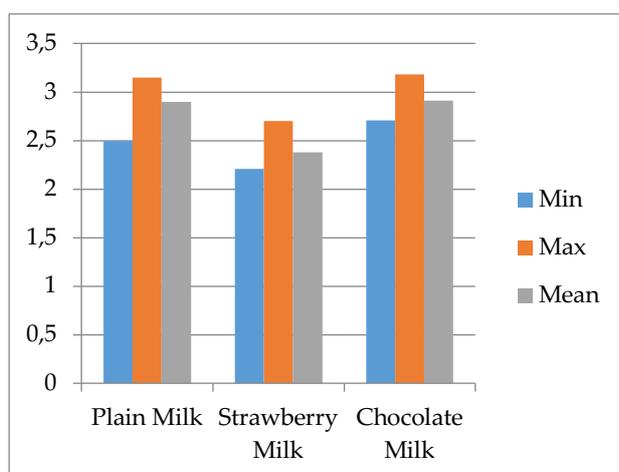


Figure 3. Protein Analysis Results of Milk Samples

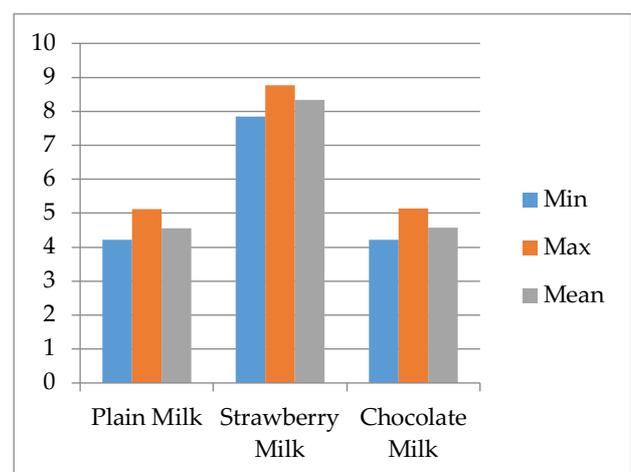


Figure 4. Lactose Analysis Results of Milk Samples

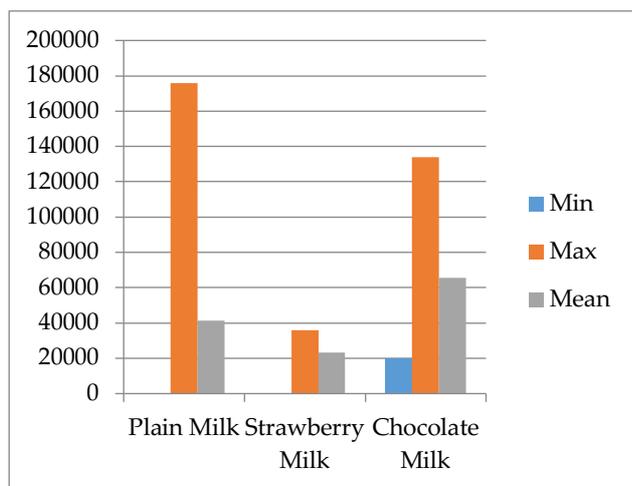


Figure 5. Somatic Cell Count Results of Milk Samples

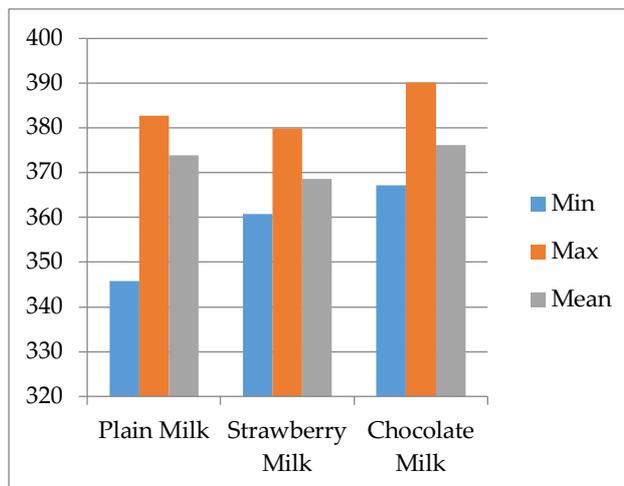


Figure 6. MDA Analysis Results of Milk Samples

The antibiotic residue levels analysed in milk samples were found to be below the MRL for all antibiotics tested. As a quinolone group antibiotic residue, Sufloxacin was highest in strawberry milk (6.306 ng/mL) (Table 1); as a sulphonamide group antibiotic residue, sulfadoxine was highest in plain, chocolate and strawberry milk (1.373 ng/mL) (Table 2); As a macrolide group antibiotic residue, Tilmicosin was highest (14,756 ng/mL) in strawberry milk and Tylosin A was lowest (0.115 ng/mL) in strawberry milk (Table 3); As beta-lactam group antibiotic residues, nafcillin, cefalexin and oxacillin was determined in milks (Figure 7); Lincomycin, as a lincomycin group antibiotic residue, was highest in both plain and chocolate milk (3.832 ng/mL) ; As an aminocyclitol group antibiotic residue, florfenicol was highest in chocolate milk (1,016 ng/mL) and lowest in plain milk (0.941 ng/mL); As a tetracycline group antibiotic residue, the highest level of tetracycline was found in chocolate milk (3,647 ng/mL) and the lowest level of oxytetracycline was found in plain milk (0.029 ng/mL); Rifaximin was detected at the highest level (0.725 ng/mL) in plain milk (Figure 8) among the rifamycin group of antibiotic residues.

Table 1. Quinolone Group Antibiotic Residues in Milk Samples (ng/mL)

		Plain Milk (n:20)	Strawberry Milk (n:15)	Chocolate Milk (n:15)
Olaquinox	m	0.612	0.610	0.616
	M	1,122	1,117	1,104
Marbofloxacin	m	0.643	0.643	0.644
	M	1.361	1.378	1.369
Norfloxacin	m	1,528	2,049	1,545
	M	1,961	2,448	1,950
Danofloxacin	m	2,049	2,055	2,056
	M	2,448	2,467	2,764
Enrofloxacin	m	0.786	0.786	0.810
	M	1.445	1.442	1.485
Serafloxacin	m	0.183	0.184	0.188
	M	6.145	6.306	0.896
Nalidixic acid	m	0.420	0.402	0.455
	M	1.410	1.895	1.460

Table 2: Sulfonamide Group Antibiotic Residues in Milk Samples (ng/mL)

		Plain Milk n:20	Strawberry Milk n:15	Chocolate Milk n:15
Sulfadiazine	m	0.445	0.446	0.445
	M	0.662	0.469	0.541
Sulfameter	m	0.368	0.369	0.368
	M	0.392	0.416	0.415
Sulfamethoxazole	m	0.916	0.916	0.916
	M	1.005	0.944	0.920
Sulfachlorpridazine	m	0.003	0.231	0.232
	M	0.236	0.264	0.239
Sulfamethoxazole	m	0.696	0.695	0.695
	M	0.704	0.720	0.721
Sulfadoxine	m	0.554	0.554	0.555
	M	1.373	1.373	1.373
Sulfadimethoxine	m	1,145	1,145	1,145
	M	1,149	1,149	1,149

Table 3: Macrolide Group Antibiotic Residues in Milk Samples (ng/mL)

		Plain Milk n:20	Strawberry Milk n:15	Chocolate Milk n:15
Spiramycin	m	3,628	5,554	3,631
	M	8,345	3,635	8,345
Tilmicosin	m	4,335	4,406	4,342
	M	10.94	14,756	9,352
Tilosin A	m	0.191	0.115	0.196
	M	1.751	6.845	7.092
Erythromycin A	m	4,589	4,630	4,595
	M	5,345	5,120	5,144
Roxithromycin	m	0.749	0.750	0.750
	M	1.495	1.501	1,500
Virginamisin	m	0.446	0.447	0.447
	M	1.099	2.557	2,603

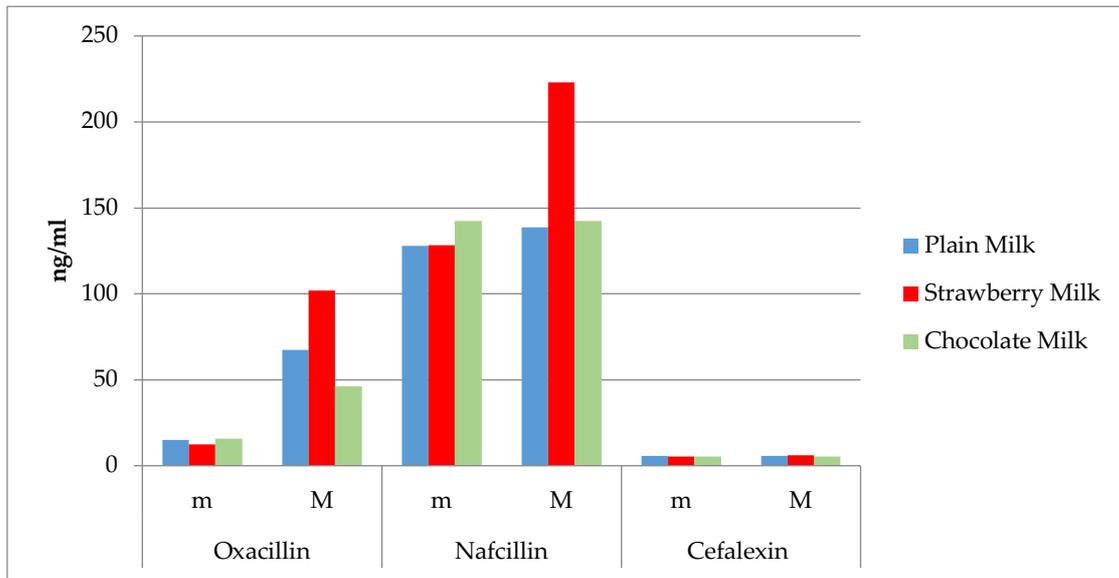


Figure 7. Beta-lactam Group Antibiotic Residues in Milk Samples (ng/mL)

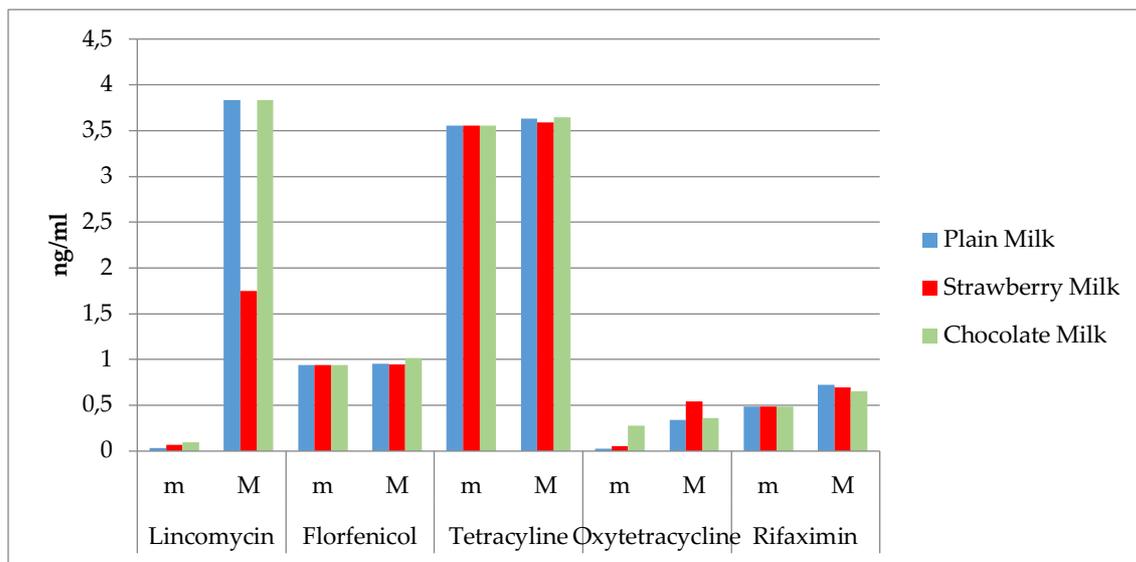


Figure 8. Residues of Lincomycin, Amphenicol, Tetracycline, and Rifamycin Group Antibiotics (ng/mL)

Discussion

Value of pH measurement is of great importance in the dairy industry. pH is the first parameter measured to obtain information about milk quality (Isildak & Gones, 2018; Metin, 2001). In the present study, the pH results for plain, strawberry, and chocolate milk were found to be 6.49, 6.40, and 6.53, respectively. Baran and Adıgüzel (2020) found the pH result of raw milk to be 6.89 on average in their study. Çelikel Güngör et al. (2020) reported an average pH of 6.56. In their study, the researchers determined the pH of raw milk obtained from small-scale businesses in the Hınıs district of Erzurum Province to be 5.93 (Diler & Baran, 2014). The pH findings of this study are lower than those of other studies but higher than those of Diler and Baran (2014). This may be due to the time of sample collection, storage conditions, and whether heat treatment was applied.

In recent years, somatic cell count has become an essential criterion for determining milk quality. The presence of somatic cells in milk is used as an indicator of both udder health and milk quality (Liu et al., 2012; Olechnowicz & Jaskowski, 2012; Hunt et al., 2013). In the study, the SCC results in plain, strawberry, and chocolate milk were determined to be 41,250, 23,428, and 65,733 cells/mL respectively. In their research, Şahin and Kaşıkçı (2014) determined the SCC level in raw milk samples from 128 cows to be between 10,444 cells/mL and 10,820 cells/mL on average. Patır et al. (2010) determined that the somatic cell count in all (100%) of 150 milk samples taken in Elazığ was above 100,000 cells/mL. In this study, SCC findings were lower than the limit of $\leq 400,000$ reported by Patır et al. (2010) and the Turkish Food Codex (TFC, 2011). In this study, SCC findings were higher than those reported in the survey by Şahin and Kaşıkçı (2014).

Protein analysis provides valuable information about milk quality and nutritional properties and is a crucial parameter in the dairy industry (Kucheryavskiy et al., 2014). Several factors, such as the breed of animal, lactation period, and animal nutrition, influence the protein content in milk (Şekerden & Öztürk, 1995; Campbell & Marshall, 2016). In the UHT milk samples examined, the protein content was found to be 2.90%, 2.38%, and 2.91% on average for plain, strawberry, and chocolate milk, respectively. According to the results of the present study, the protein content of strawberry milk was found to be below the 2.90% protein content specified in the TFC. Önal and Özder (2007) determined the protein content of raw milk from the provinces of Edirne, Tekirdağ and Kırklareli to be 3.05%, 3.09% and 3.05%, respectively. Çelikel Güngör et al. (2020) found that the average protein content of raw milk sold in Mardin was 3.07%. In their study, Diler and Baran (2014) determined that the protein content of raw milk obtained from small-scale businesses was 3.11%. The protein content results obtained in the study were found to be lower compared to those in other studies.

In the UHT milk samples examined, the lactose results for plain, strawberry and chocolate milk were 4.56%, 8.33% and 4.57% on average, respectively. The highest lactose content was found in strawberry milk. Kurt et al. (1981) found that the average lactose content in raw milk samples was 4.55% in winter and 4.45% in summer. Şahin and Kaşıkçı (2014) found that the lactose content in raw milk samples was 4.7% in the first group and 4.1% in the second group. The results obtained in the present study regarding the lactose content were found to be higher than those obtained in other studies.

Milk fat analysis is of great importance for milk and dairy products. Milk fat concentration can vary depending on several factors, including the breed, sex, nutrition, lactation period, season, and ambient temperature of the animal (Zhu et al., 2015). At the same time, milk fat is one of the key components in determining the market price of milk. In the study, the milk fat content of plain, strawberry, and chocolate UHT milk was determined to be 2.29, 1.02, and 2.47 on average, respectively. Diler and Baran (2014) determined the average fat ratio of milk samples collected in Erzurum to be 3.60. Ürkek and Şengül (2018) determined the average fat value of milk samples collected from conventional farms to be 3.67. Baran and Adıgüzel (2020) found the average fat value of the collected milk samples to be 3.89. The findings of this study were lower than those of other studies. Furthermore, the milk fat values obtained in the study were found to be below the desired value (3.5% milk fat) as specified in the Turkish Food Codex Raw and Heat-Treated Drinking Milk Regulation. This difference may be attributed to factors such as animal breed, sex, nutrition, and stage of lactation.

A positive correlation exists between somatic cell count and MDA level in milk (Kesenkaş et al., 2018). In the study, the malondialdehyde levels in UHT milk samples were found to be 373.92, 368.69, and 376.22 µmol/L on average for plain, strawberry, and chocolate-flavoured milk, respectively. The highest level was detected among the samples examined in chocolate milk.

When foods obtained from animals treated with antibiotics are consumed, there is a risk to public health due to the development of antibiotic resistance (Yarsan, 2012). The study investigated the presence of antibiotic residues in the collected milk using a LC/MS/MS device. In the survey, Nafcillin, a penicillin group antibiotic residue, was detected at the highest level in strawberry milk among UHT milk samples. Among the UHT milk samples analyzed, nafcillin, a penicillin-group antibiotic, was detected at the highest level in strawberry milk; however, its concentration did not exceed the maximum residue limit (MRL). Aycan (2016) investigated the presence of beta-lactam (penicillin) antibiotic residues in raw milk offered for consumption in Afyonkarahisar province using the ELISA method. As a result, no residues were found in 38 of the 80 milk samples, while 35 samples contained beta-lactam antibiotic residues within the maximum residue limits. Additionally, seven milk samples contained residues exceeding the maximum residue limits.

In the study, cefalexin was detected as the highest cephalosporin group antibiotic residue in strawberry milk (5,908 ng/mL) among UHT milk samples, and this value was found to be compliant with the TFC (Turkish Food Codex; maximum residue limit: 100 µg/kg). Furthermore, in UHT milk samples, the highest level of the quinolone group antibiotic residue, ciprofloxacin, was detected in strawberry milk (6.306 ng/mL), and this value was found to be below the maximum residue limit (30 µg/kg) required by the Turkish Food Codex. In their study, Bohm et al. (2009) investigated antibiotic residues from the tetracyclines, quinolones, macrolides, sulfonamides, diamino-pyrimidine and lincosamides derivative groups in fresh and UHT milk samples using an LC-MS/MS device. According to the results obtained, the following concentrations were detected: lincomycin 187 mg/kg, tilmicosin 66 mg/kg, sulfadoxine 111 mg/kg, tetracycline 113 mg/kg, and sarafloxacin 7.4 mg/kg. Çancılar (2017) investigated the presence of quinolone group antibiotic residues in raw milk offered for consumption in the city of Afyonkarahisar using the ELISA method. As a result, they found that 70 of the 80 milk samples did not contain residue, while 10 samples contained residue below the maximum residue limits. Chung et al. (2009) detected 4 out of 269 samples of cow's milk and goat's milk as positive in their study on sulphonamides and quinolones. In their research, Nizamlioğlu and Aydın (2012) found quinolone antibiotic residues in 17 (34%) of 50 chicken liver samples and reported that one sample (147.88 µg/kg) exceeded the MRL. The researchers detected no quinolone residues in any of the milk samples.

In the study, lincomycin was detected as the highest antibiotic residue from the lincosamide group in both plain and chocolate milk (3,832 ng/mL) in UHT milk samples; florfenicol was detected as the highest antibiotic residue from the amphenicol group in chocolate milk (1,016 ng/mL). These values were found to be below the MRL according to the TFC (lincomycin 150 µg/kg; florfenicol 150 µg/kg). Roca et al. (2013) detected sulphonamide compounds in UHT and pasteurised milk in their study. In the study, sulfadoxine was detected as the highest sulfonamide group antibiotic residue in plain and strawberry milk (1.373 ng/L) in UHT milk samples. This value was found to be below the maximum residue limit of 100 mg/kg for sulfadoxine, as specified in the Turkish Food Codex Regulation.

Geçer (2006) detected residues of oxytetracycline, tetracycline, chlortetracycline, and penicillin in 100 pasteurised milk and 100 UHT milk samples from different companies sold in Ankara province. He reported that antibiotic residues were detected in 36 milk samples, with a residue rate of 26% in pasteurised milk and 10% in UHT milk. In the study, Tetracycline was detected as the highest antibiotic residue in the tetracycline group in chocolate milk (3.647 ng/mL) among the UHT milk samples. Tilmicosin, a macrolide group antibiotic residue, was detected at the highest level in strawberry milk (14.756 ng/mL). Rifaximin, a rifamycin group antibiotic residue, was detected at the highest level in plain milk (0.725 ng/mL). According to the Turkish Food Codex Regulation, the maximum residue limits for tetracycline, tilmicosin, and rifaximin are 100, 50, and 60 µg/kg, respectively (Official Gazette of the Republic of Turkey, March 9, 2007, issue 26457). All residue levels detected in the UHT milk samples were below the maximum residue limits specified in the TDC.

Conclusion

The averages obtained for the plain UHT milk in the present study were as follows: pH 6.49, milk fat 2.29%, protein 2.90%, lactose 4.56%, non-fat matter 8.16%, and malondialdehyde 373.92 µmol/L. The averages obtained for strawberry UHT milk in the study were pH 6.40, milk fat 1.02%, protein 2.38%, lactose 8.33%, non-fat dry matter 11.41%, and malondialdehyde 368.69 µmol/L. The averages obtained in the study for chocolate milk, which is UHT milk, were as follows: pH 6.53, milk fat 2.47%, protein 2.91%, lactose 4.57%, non-fat dry matter 8.18%, and malondialdehyde 376.22 µmol/L. In this study, it was determined that antibiotic residues in UHT sterilized milk (plain, strawberry, chocolate) sold in its original packaging in Afyonkarahisar province and its districts, as well as in neighbouring provinces, were below the MRL. To prevent antibiotic or drug residues, consumers should be educated through training provided by veterinarians, organisations, and government agencies. Unnecessary use of antibiotics on farms should be avoided. It is recommended that milk from animals treated with antibiotics or other drugs should only be used after the legal withdrawal period; that antibiotics should not be used unnecessarily or indiscriminately in animal health; and that the quality characteristics of milk should be routinely checked.

Conflict of interest: The authors declared that there is no conflict of interest.

Authors' Contributions: **NAMAG:** contributed to the project idea and acquisition of data, drafted and wrote the manuscript, reviewed the manuscript critically **RK:** contributed to the project idea, design and execution of the study and acquisition of data, analysed the data, drafted and wrote the manuscript. All authors have read and approved the finalized manuscript.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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