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Investigation of Tetracycline Residues in Plain and Flavored UHT Milk on Sale

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ABSTRACT

In this study, tetracycline group antibiotic residue was investigated in plain and flavored UHT milk of different brands. A total of 90 UHT milk samples (62 plain and 28 flavored) were analyzed by ELISA method. Tetracycline residues were detected in all samples as a result of the analysis. The mean level of tetracycline was determined as 6.98±0.38 ppb in plain milk and 6.53±0.48 ppb in flavored milk. Residue levels in analyzed UHT milk were found to comply with the legal limits announced by the European Union (EU) commission and Turkish Food Codex Communiqué.

Keywords: UHT milk, tetracycline, ELISA, residue

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Introduction

Ultra-high temperature (UHT) is drinking milk produced by filling in sealed packages under aseptic conditions after applying high temperature (over 135°C) process according to the Turkish Food Codex Communique on Drinking Milk (TGK, 2019). UHT process extends the shelf life of milk by protecting it from bacteria and external contamination, it has advantages such as not needing a cooler in its distribution, not losing its flavor and nutritional values (Alves, 2001; Scott, 2008).

Tetracyclines are in the broad-spectrum antibiotic group that slows down or inhibits the growth and reproduction of bacteria. Due to its pharmaceutical properties, it is included in the drugs that cause residue (Yarsan, 2013). They are effective against gram-positive and gram-negative bacteria, chlamydia, spirochetes, actinomyces, mycoplasma and rickettsia species. It is highly preferred in veterinary medicine due to its wide area of influence. There are many antibiotic groups such as tetracycline derivative oxytetracycline, chlortetracycline and tetracycline. However, oxytetracycline is more preferred (Kaya et al., 2000).

The European Union (EU) commission has determined the Maximum Residue Limit (MRL) of tetracycline group drugs for milk as 100 ppb (EU, 2010). In Turkey, legal regulations regarding antibiotic residues in animal foods have been determined in the "Regulation on Classification of Pharmacological Active Substances that May Be Found in Animal Foods and Maximum Residue Limits" and the same MRL values have been given for tetracycline group antibiotics (chlortetracycline, oxytetracycline) (TGK, 2017).

This study was carried out to evaluate the residues of tetracycline group antibiotics in plain and flavored UHT milk sold.

Material and Methods

Material

In this study, tetracycline groups of antibiotic residues were detected in plain and flavored (strawberry, banana, cocoa) UHT milk of different brands sold in markets in Sivas. For this purpose, a total of 90 UHT milk samples, 62 of which are UHT plain milk and 28 of which are flavored milk, were used as material. Samples were collected periodically from various sales points in Sivas province. ELISA analyzes of milk were performed in the laboratory.

Methods

Tetracycline group antibiotic levels in UHT plain and flavored milk were determined by ELISA (Enzyme Linked Immunosorbent Assay) method. Commercial ELISA assay kit (WISHERKON Lot: WE19032421B) was used for analysis. The procedures were performed in accordance

with the kit procedure. The descriptive statistics of arithmetic mean, minimum, maximum, standard error values of tetracycline levels investigated in the samples were determined in SPSS 22.00 program (SPSS, 2014).

Preparation of samples for analysis

The homogenized milk samples were taken into test tubes of 200 µL. Volume of 3800 µL sample extraction was added to the samples and mixed for 1 minute. This prepared mixture was transferred to an eppendorf tube of 100 µL and used in the ELISA step.

Test procedure

Amount of 50 µL each standard and samples were added to wells of the microplate. By added 50 µL each of conjugate and antibody, it is incubated at 25oC and in the dark for 30 minutes. After the wells were washed 5 times, substrate A and substrate B were added to each well at the amount of 50 µL. Covered the microplate and incubated at 25oC for 15 minutes in the dark. Lastly, 50 µL of stop solution was added to the wells and the changing of blue color to yellow was observed. Finally, the absorbances of the standards and samples were measured in an ELISA device at a wavelength of 450 nm. A calibration curve was created and the absorbance of the samples versus the tetracycline group antibiotic levels were calculated as ppb.

Results and Discussion

Tetracycline group antibiotic levels in UHT plain and flavored milk are given in Table 1. According to the results, the mean value was found as 6.98±0.38 ppb in 62 UHT plain milks, and 6.53±0.48 ppb in 28 UHT flavored milks.

Table 1. Tetracycline group antibiotic levels in UHT plain and flavored milk

	Tetracycline Concentration (ppb)					
	n	**n1**	**Min**	**Max**	**Mean± SE**	**Exceeding maximum residue levels**
UHT plain milk	62	62	1	13.41	6.98±0.38	-
UHT flavored milk	28	28	2	12.14	6.53±0.48	-

n1: positive sample

Antibiotic contamination of milk is increasing because of inadequate management of antibiotic therapy of mastitis in dairy cattle. However, insensitivity to antibiotic treatments and sensitivity to antibiotic use are formed in people who consume it. It also causes production losses in the dairy industry (Larocque and Neville, 1985). Tetracyclines, beta-lactams, and aminoglycosides are the most used antibiotics in dairy cattle systems today in the treatment of mastitis in dairy cows, and accordingly they are the most common type of residues in milk (Kabrite et al., 2019; Kaya and Filazi, 2010).

Geçer (2006), because of the analysis performed with HPLC method in 100 pasteurized milk and 100 UHT milk samples offered for sale in Ankara, reported that they detected oxytetracycline in 1 UHT milk sample (1%), penicillin in 7 UHT milk samples (7%), oxytetracycline+penicillin residues in 2 UHT milk samples (2%). The lowest and highest residue limits were found to be 4.28-33.86 ppb for penicillin, 111.3-318.51 ppb for oxytetracycline, and 116.26-225.40 ppb for tetracycline. Kaya and Filazi (2010), in the analysis of a total of 240 milk samples (raw and pasteurized) taken from the markets in Ankara, found antibiotic residues in 1.25% of the samples via Thin Layer Chromatography/Bioautography. One sample of oxytetracycline (150.4 µg/L) and 1 sample of penicillin G (33.5 µg/L) were found positive in pasteurized milk, and 1 sample was positive for neomycin (7688.4 µg/L) in raw milk. Unusan et al. (2009) investigated the antibiotic residue levels of tetracycline, streptomycin, and chloramphenicol in 60 UHT milk samples taken from markets in Konya, Turkey, by ELISA method. Chloramphenicol and tetracycline found 28 milk samples (46.8%) and 40 milk samples (66.8%), respectively. Mean residue levels of chloramphenicol, streptomycin, and tetracycline were 806 ng/l, 360 ng/l and 602 ng/l respectively. Saraç (2015) determined antibiotic residues in 16 raw milk, 14 pasteurized and 30 UHT milk samples by LC/MS/MS in the analysis of 26 raw milk, 30 pasteurized milk of different brands and 93 UHT milk samples taken from various sales outlets in Istanbul. The most common antibiotic in all milk samples was oxytetracycline and the highest level was determined as 11.19 µg/kg. The highest amount of 13.76 µg/kg was determined for doxycycline in all milk samples. The tetracycline group antibiotic levels obtained in this study were lower than the findings of these researchers.

Fritz and Zuo (2007) examined the antibiotic residue levels in the market milk samples purchased from New Bedford, USA, using the HPLC analysis method. They reported that they detected oxytetracycline was 13-106 µg/l, 4-epitetracycline concentration was 18-65 µg/l, tetracycline concentration was 44 µg/l. Rassouli et al. (2010) analyzed pasteurized milk samples from 90 supermarkets in Tehran in terms of tetracycline and oxytetracycline residues using HPLC method and reported that 7 milk samples (7.8%) contained tetracycline residues. While oxytetracycline and tetracycline were below 100 ppb in six of them, they found it as 138.8 ppb in one. Han et al. (2013), tetracycline, sulfonamide, sulfamethazine, and quinolone residue analyzes were performed by ELISA method on 180 UHT milk samples collected from 25 different cities in China. Percentage of tetracycline, sulfonamide, sulfamethazine, and quinolones 0, 16.7, 40.6, and 100% were found in the samples, respectively. The maximum concentrations of tetracycline, sulfonamide, sulfamethazine, and quinolone are <1.5, 26.2, 22.6, 58.8 µg/ kg, respectively. Zhang et al. (2014) analyzed 94 UHT milk and 26 pasteurized milk

samples sold in markets in China in terms of tetracyclines, sulfonamides, sulfamethazine, and quinolones by ELISA method. According to the analysis results, levels of tetracycline, sulfonamide, sulfamethazine, and quinolone in UHT milk samples were 0%, 20.2%, 7.4%, 95.7%, and 7.7%, 15.4%, 0%, 61.5% in pasteurized milk samples, respectively. The maximum concentrations of tetracycline, sulfonamide, sulfamethazine, and quinolone residues were 47.7 mg/kg, 20.24 mg/kg, 14.62 mg/kg, 20.49 mg/kg, respectively. Novaes et al. (2017) analyzed 961 milk samples, which are 470 of UHT, 275 of milk powder and 216 of pasteurized milk, in terms of β-lactam, tetracycline, aminoglycoside, quinolone, sulfonamide, amphenicol and ivermectin's, via LC-MS, HPLC-UV/Vis methods. They reported that β-lactam in 1 UHT and 2 pasteurized milk, enrofloxacin in 170 (17%) milk, ciprofloxacin in 238 (25%) milk, norfloxacin in 134 (15%) milk, chlortetracycline in 93 (12%) milk, oxytetracycline 231 (31%) in milk and tetracycline in 104 (14%) milk contains residues. Du et al. (2019) evaluated the residue analyzes of tetracycline, quinolone, lincomycin and streptomycin in 148 UHT and 50 pasteurized milk samples

Conclusion

In this study, tetracycline group antibiotic residues were found in plain or flavored milks analyzed. Tetracyclines are one of the most preferred antibiotic groups in bacterial infections due to their broad spectrum in veterinary medicine. However, when applying treatment protocols, attention should be paid to drug properties such as the diagnosis of the disease, the appropriate antibiotic selection, dosing, duration of use, pharmacokinetic properties of the drug, and washout time. The veterinarian should decide on the necessity of antibiotic use, and use should be avoided in cases where there is no need.

Conflict of Interest

There is no conflict of interest between the authors.

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