Microbiological quality of fresh cow's milk cheese sold at Sarajevo and Zagreb farmers' markets

Viktor Landeka, Mufida Aljičević, Ana Sesar and Lidija Kozačinski*

Abstract

The paper compares the specific microbiological parameters of fresh cow's milk cheese produced in the Zagreb and Sarajevo areas and sold at local farmers' markets. The research was conducted on 60 samples of fresh cheese, sampled from the most frequented farmers' markets in each city, over a one-year period. The testing of microbiological quality of fresh cheese sold in both cities revealed no presence of the bacterial species *Salmonella* spp. and *Listeria monocytogenes*. Of all the analysed samples of fresh cow's milk cheese sold at farmers' markets in both cities, nine samples (15.0%) did not meet the microbiological criteria, due to the presence of *Escherichia coli* in eight samples (*n*=30; 26.6%) from Zagreb and coagulase-positive staphylococci (*Staphylococcus aureus*) found in one sample (*n*=30; 6/6%) from Sarajevo. The microbiological analysis results indicate the need to improve hygiene practices involved in cheese production.

Key words: *microbiological quality; fresh cow's milk cheese; Escherichia coli; Staphylococcus aureus; Zagreb; Sarajevo*

Introduction

Cheese is a food that belongs to the group of ready-to eat products that are consumed fresh. From the aspect of food poisoning, it may be considered dangerous to human health and could cause significant public health problems. Fresh (young) cheese is probably the oldest and most popular type of cheese produced in households. It is characterised by a high water content, low milk fat content and high acidity. It has a characteristic taste, smell, colour and consistency. Traditional fresh cheese is usually produced from raw milk left to naturally become acidified at room temperature (22 °C). The acidification process, also called curdling, naturally lasts 1 to 2, but no more than 3 days

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(Barukčić et al., 2015). Sour cream is skimmed from the surface of this acidified and curdled milk. The skimmed soured milk is poured into a container, which is heated for 2 to 3 hours at 40 °C. The curdled milk is then poured into a cheesecloth or strainer bag (Kirin, 2009; Valkaj et al., 2013). It can be cut into cubes or sheets that are then cooled and placed into moulds or other type of appropriate packaging.

Raw milk, however, provides an ideal medium for the growth of microorganisms. The microbiological contamination of milk and dairy products should primarily be attributed to the poor hygienic quality of raw milk. However, both inappropriate equipment and its improper use also affect this quality, particularly during the heat treatment of milk, in addition to inadequate hygiene maintained on the premises and insufficient care for hygiene by staff (Kozačinski et al., 2003; Dizdarević, 2009).

Cheese is a favourable medium for the development of pathogenic microorganisms. Microbial species and their initial number, the presence of other microflora, the physiological state of pathogenic bacteria and pH, and the type and composition of cheese will determine the ability of pathogens to survive in the cheese. Of all the pathogenic bacterial species that may potentially be present in cheese, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. are the most commonly isolated species (Havranek et al., 2014).

The determination of the amount and type of microorganisms present in milk and dairy products gives the best insight into the production quality of a particular producer (Kozačinski et al., 2003), though no technological process can guarantee perfect product safety.

However, the pasteurisation of milk represents the most effective measure for the prevention of growth of pathogenic bacteria. Measures such as monitoring the health status of dairy cows and maintaining good hygiene are necessary to sustain health and safety standards of milk, as well as to produce milk free of mastitis pathogens (Havranek et al., 2014).

The aim of this study was to investigate and determine the level of health and safety associated with the consumption of fresh cheese sold at the largest farmers' markets in Sarajevo and Zagreb. Although fresh cheese produced in households belongs to the same group of products, there are subtle differences in the production and packaging methods. The differences in cheese production processes between households and family farms in the Republic of Croatia and Bosnia and Herzegovina were also compared.

Materials and methods

Samples

Fresh cheese was produced as a part of domestic production on family farms in the Križevci, Varaždin, Zagreb and Bjelovar areas, *i.e.* in north-western Croatia, and on farms located throughout Bosnia and Herzegovina, where it is referred to as young cheese.

Fresh cow's milk cheese was sampled from farmers' markets in Zagreb and Sarajevo during two seasons (summer and late winter/early spring). The most common packaging types are PVC containers holding 250-300 grams or PVC containers with a capacity of 500 grams that are exclusive to the Croatian market. In Bosnia and Herzegovina, cheese is usually stored in specially manufactured oval enamel pots that hold 3-5 kg of cheese, which is then cut and weighed for sale on the spot. This represents a novelty in the sale of this type of cheese that is unique to Bosnia.

We analysed a total of 60 cheese samples, with 30 samples collected from each city: 15 in summer and 15 in the transition period between winter and

spring. Analyses were carried out at the Federal Institute for Agriculture in Sarajevo and the ZIN-LAB Laboratory for Foodstuffs of Animal Origin at the Veterinary Station in Zagreb. In Croatia, fresh cheese samples were collected at busy Zagreb farmers' markets (Dolac, Kvatrić, Trešnjevka, Utrine, Branimirova). The sampled cheeses were produced by registered family farms. Cheese was stored in refrigerated display cases with a temperature control regime ranging from 4 to 8 °C. Fresh cheese from Sarajevo was also sampled at busy city farmers' markets (Markale, Ciglane, Grbavica, Dobrinja, Ilidža). Cheese samples produced in Bosnia and Herzegovina also originated from agricultural farms and was stored in refrigerated display cases with a controlled temperature ranging from 4 to 8 °C. A portion of the samples collected during the summer season (outdoor temperature about 22 °C), were taken from an outdoor farmers' market, where conditions were not controlled (open market without refrigerated cases).

Microbiological analysis

Microbiological analysis focused on detecting the presence of *Escherichia coli, Listeria monocytogenes*, *Salmonella* spp. and coagulase-positive staphylococci (*Staphylococcus aureus*). Analyses were performed in accordance with the procedures described in ISO methods:

- EN ISO 16649-2 Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* - Part 2;
- EN ISO 11290-1 and EN ISO 11290-2 Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1 and 2 with corresponding amendments
- EN ISO 6579 Horizontal method for the detection of *Salmonella* spp. with corresponding amendments;
- EN ISO 6888-1 and EN ISO 6888-2 Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1 and 2 with corresponding amendments;
- Detection of staphylococcal enterotoxins types SEA to SEE using VIDAS® SET2 method. European screening method of the EU RL, Version 5, September 2010.

In the interpretation of bacteriological analysis, we referred to the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (Anonymous, 2005) and the Guidelines on microbiological criteria for foodstuffs (Anonymous, 2011a). Bosnian regulations are identical to the Commission Regulation, i.e. the provisions have been transposed into the the Ordinance on microbiological criteria for foodstuffs of Bosnia and Herzegovina (Official Gazette of Bosnia and Herzegovina 11/13; Anonymous, 2013) and the Guidelines on microbiological criteria for foodstuffs of Bosnia and Herzegovina (Anonymous, 2011b).

The Commission Regulation on microbiological criteria for foodstuffs (Anonymous, 2005) allows the presence of *L. monocytogenes* in ready-to-eat food in the quantity of 10^2 cfu/g (for products placed on the market during their shelf life). If values of coagulase-positive staphylococci (*S. aureus*) exceed 10^5 cfu/g, the cheese must be tested for staphylococcal enterotoxins (food safety criteria; products placed on the market during their shelf life). The criterion for the absence of *Salmonella* spp. in 25 g is applied for products placed on the market during their shelf life (Anonymous, 2005). According to the recommended criteria in the Guidelines (Anonymous 2011a,b), the number of *E. coli* may not exceed 10^3 cfu/g in raw milk cheeses.

Statistical analysis

The results were statistically analysed using the IBM SPSS Statistics 20 statistical software. The level of significance of

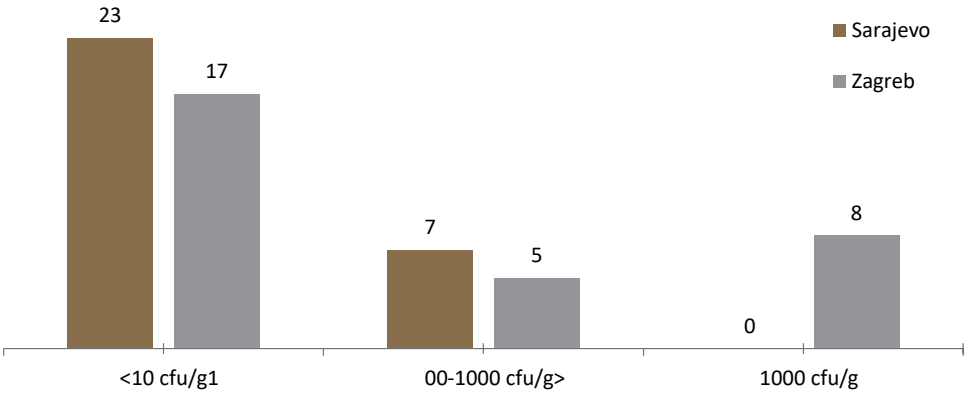

Figure 1. Results of *E. coli* findings in samples of fresh cheese from Zagreb (*n*=30) and Sarajevo (*n*=30)

difference was determined at $P<0.05$. First, the distribution of the outcome variables *E. coli* and *S. aureus* was determined. Since these variables did not have a normal distribution, they were converted using logarithmic (10) functions. Descriptive statistics were used to present the relationship between the amounts of microorganisms and risk factors (average, standard deviation and variability coefficient for normally distributed variables; median and interquartile range for variables that were not distributed normally). Furthermore, we tested differences in the amounts of microorganisms with regard to the city of origin of the cheese. For this purpose, we used the t-test (Mann-Whitney U test) and simple linear regression. ANOVA test (Kruskal-Wallis) and linear regression were used to determine differences in the amounts of microorganisms in cheese with regard to origin. We then compared unacceptable and acceptable amounts of microorganisms for each city. Comparisons for smaller samples were made using the chi-squared test (Fisher's test) and simple exact logistic regression.

Results and Discussion

All fresh cheese samples from both cities (total n = 60) tested negative for *Salmonella* spp. and *L. monocytogenes*. However, the bacterial species *E. coli* and coagulase-positive staphylococci (*S. aureus*) were detected. Because these bacterial species were found in a large number of the analysed samples, the results of positive findings of *E. coli* are presented in three categories: negative (< 10 cfu/g), acceptable (100-1.000 cfu/g) and unacceptable (> 1000 cfu/g). Positive findings of *S. aureus* are shown in categories as negative (< 10 cfu/g), acceptable (between 10-10,000 cfu/g and 10,000-100,000 cfu/g) and unacceptable (> 100,000 cfu/g).

Table 1. Statistical indicators of *E. coli* in samples of fresh cheese with regard to the city of sampling.

Indicator	City	*n*	Average	Standard deviation
E. coli, log10 cfu/g	Zagreb	30	1.52	1.81
	Sarajevo	30	0.74	1.17

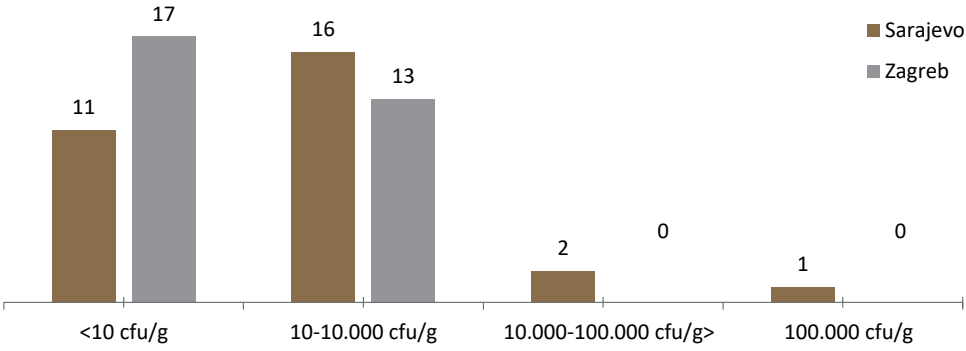

Figure 2. Results of *S. aureus* findings in samples of fresh cheese from Zagreb (*n*=30) and Sarajevo (*n*=30)

Figure 1 shows the results of cheese analysis for the presence of *E. coli*. The results indicate the presence of eight unacceptable cheese samples, with the amount of *E. coli* ranging from 3.15 to 4.45 $\log_{10}$ cfu/g, collected from Zagreb farmers' markets. Although 17 cheese samples collected from Zagreb farmers' markets tested < 10 cfu/g for this bacterial species, five samples were collected that contained amounts of bacteria within the permitted limits. Thus, 16.66% of the total number of tested samples with regard to origin (*P*=0.05) tested positive for *E. coli*. In contrast, in Sarajevo, bacteria were not found in 23 cheese samples. All cheese samples from Sarajevo farmers' markets complied with the Guidelines on microbiological criteria (Anonymous 2011a, 2011b) concerning the microorganism in question. However, seven samples tested positive for bacteria in amounts below 10^3 cfu/g. The amount of *E. coli* considered within the permissible limits for both cities ranged, on average, between 1.6 and 3.0 $\log_{10}$ cfu/g.

Table 1 demonstrates that the average amount of *E. coli* was 0.74 ± 1.17 $\log_{10}$ cfu/g in Sarajevo and 1.52 ± 1.81 $\log_{10}$ cfu/g in Zagreb.

Figure 2 presents the results of fresh cheese analysis for bacteria *S. aureus* carried out in both cities.

The results presented in Figure 2 indicate that one Sarajevo sample tested positive for *S. aureus* in the amount > 100,000 cfu/g (*P* = 0.04). Even though the amount of *S. aureus* was higher than the reference value in only one sample where it totalled 3.14 x 10^5 cfu/g (5.50 $\log_{10}$ cfu/g), no enterotoxins were detected.

Table 2 shows that the average amount of *S. aureus* in samples of fresh cheese collected in Zagreb was 1.13 ± 1.37 $\log_{10}$ cfu/g, while the average amount found in Sarajevo was 1.94 ± 1.59 $\log_{10}$ cfu/g.

When the results are exampled as a whole, of the 60 analysed samples

Table 2. Statistical indicators of the amount of *S. aureus* in samples of fresh cheese with regard to the city of sampling.

Indicator	City	*n*	Average	Standard deviation
S. aureus, log10 cfu/g	Zagreb	30	1.13	1.37
	Sarajevo	30	1.94	1.59

collected in both cities, 9 samples (15.0%) did not meet the compliance criteria of the Guidelines (Anonymous 2011a, b) due to the presence of *S. aureus* and *E. coli*. The presence of these bacteria in fresh cheese has also been reported by other authors. Živković et al. (1992) indicated the problem of coagulase-positive staphylococci findings in fresh cheese from domestic production, with 64.5% of samples sold at farmers markets containing these bacteria. Kozačinski et al. (2003) analysed dairy products from the Križevci area and north-western Croatia and found that 26.77% of fresh cheese samples did not meet the prescribed microbiological safety standards due to excessive amounts of *Enterobacteriaceae*, yeasts and moulds, aerobic mesophilic bacteria, and *E. coli* and *S. aureus*.

The same bacterial species were detected in the present study, whereby 15.0% of samples from both cities did not meet the criteria due to *E. coli* and *S. aureus* findings. In comparing these results with the evaluation of microbiological safety of fresh cheese from the Bjelovar farmers' market (Kirin, 2009), similar microbiological parameters were obtained. Namely, Kirin (2009) detected *E. coli* in 21.43% of samples, and *S. aureus* in two samples or 14.48%. In the present study, cheese sold on the Sarajevo farmers' markets contained *E. coli* in 30% of tested samples, though their numbers did not exceed the limit of 1000 cfu/g. In an analysis of fresh cheese, Kalaba et al. (2008) detected *E. coli* in 33.33% and coagulase-positive staphylococci in 26.66% of tested samples.

Little et al. (2008) also determined the contamination of fresh cheese produced from raw or heat-treated milk caused by bacterial species *S. aureus* and *E. coli*. Rosengren et al. (2010) detected the amount of *S. aureus* over 4 $\log_{10}$ cfu/g in fresh and soft cheese, and found *E. coli* in 34% of raw milk cheese samples. The highest amount of *E. coli*, of > 6.62 $\log_{10}$ cfu/g, was detected in fresh cheese made from raw milk. In the present study, a positive find of *S. aureus* over 5 $\log_{10}$ cfu/g was detected in only one sample, while *E. coli* was found in 15% of the analysed cheeses.

O'Brien et al. (2009) reported finding *S. aureus* in 96% of tested fresh cheese samples. All positive findings of *E. coli* were present in a very small number of samples in amounts less than 10^3 cfu/g, which is consistent with results of microbiological analysis of cheese samples from the Zagreb farmers' markets.

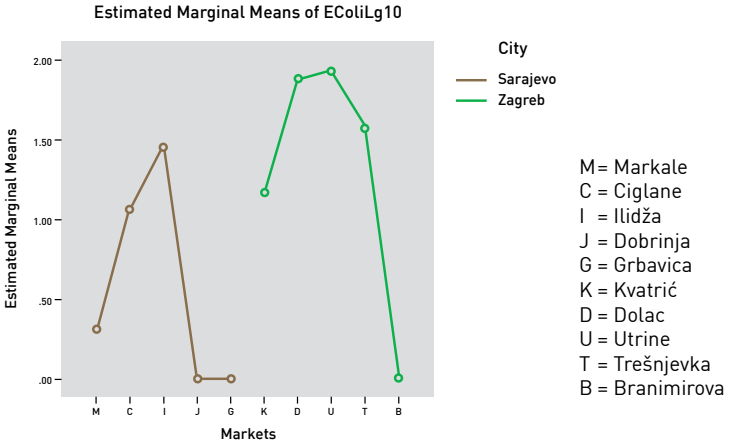

Figure 3. Comparison of *E. coli* findings detected in samples of fresh cheese by city and farmers' market

We assume that inadequate sale conditions could be associated with the higher number of *E. coli* and coagulase positive staphylococci found in cheeses. Cheese is a good medium for the growth of pathogenic microorganisms, particularly if stored under inadequate conditions. Cheeses sampled from open markets which are not sold in refrigerated display cases with a controlled temperature ranging from 4 to 8 °C could be better matrix for the growth of these bacteria.

Our analysis, like many other studies, did not detect *L. monocytogenes* and *Salmonella* spp. in any of the samples (Kozačinski et al., 2003; Kalaba et al., 2008; Kirin, 2009; Markov et al., 2009; Bojanić-Rašović et al., 2010; Rosengren et al., 2010). Humski et al. (2011) detected *L. monocytogenes* in one sample (3.3%) and *L. innocua* in two samples of fresh cow's milk cheese. Little et al. (2008) also detected *Listeria* spp. (including *L. monocytogenes*) contamination in 3.1% of cheese samples made from raw milk and 2.5% of cheese samples made from pasteurised milk. Similarly, O'Brien et al. (2009) reported findings of *L. monocytogenes* in fresh cheese (3% of samples), which was not the case here. *Salmonella* spp. was also not detected in fresh cheese tested in the study carried out by Sabljak et al. (2013).

Findings of bacteria *E. coli* and *S. aureus* in fresh cheese with regard to the city of origin and farmers' market of sampling is shown in Figures 3 and 4.

The amounts of *E. coli* were greater at Zagreb farmers' markets than Sarajevo farmers' markets, which is particularly evident at the Dolac and Utrine farmers' markets.

The number of *S. aureus* detections was higher in samples of fresh cheese collected from the Sarajevo farmer's markets than Zagreb farmers' markets, which was particularly evident at the Grbavica farmers' market.

Tables 3 and 4 show the results of linear and logistic regression analysis.

Linear regression analysis was applied to evaluate the correlation of risk factors with the amount of bacterial species *E. coli* and *S. aureus* in cheese samples (Table 3). A statistically significant correlation was found between the selling place of cheese (city and farmers' market) and excess amounts of *E. coli* and *S. aureus*. A significantly higher amount of *E. coli* (β=0.178; CI: 0.00; 1.57; *P*=0.05) and a significantly smaller amount of *S. au-*

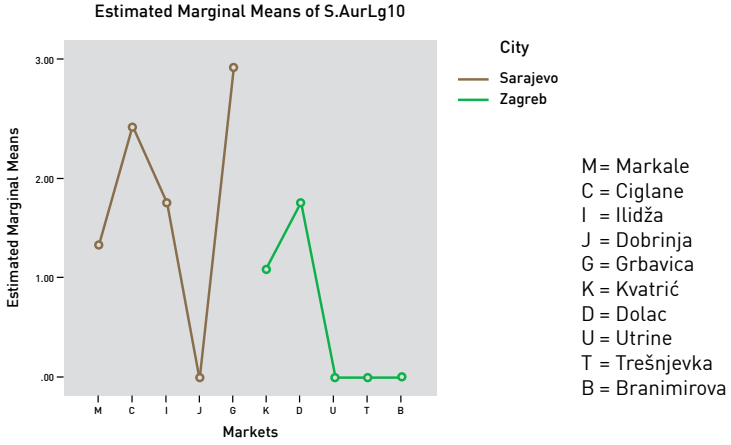

Figure 4. Comparison of *S. aureus* findings with regard to the city and farmers' market

reus was detected in cheese samples from Zagreb, as compared to cheese samples from Sarajevo (β= -0.30; CI: -0.99; 0.39; *P*=0.04).

A simple logistic regression was applied to assess the impact of risk factors on the likelihood that cheese would test positive for a greater, unacceptable amount of the bacterial species *E. coli* and *S. aureus*. As indicated in Table 4, only the city model was statistically significant. Such a model demonstrates the difference in excessive amounts of *E. coli* between cheeses sold in Zagreb and Sarajevo. Fresh cheese produced in Zagreb contained 8.52 times more *E. coli* than cheese sampled in Sarajevo (OR=8.52; CI: 0.98; 74.39; *P*=0.05).

Conclusions

The results of the study confirm that the bacterial species *S. aureus* and *E. coli* are frequent contaminants of fresh cheese. Their presence may indicate the level of hygiene during cheese production and in procedures involving milk after milking and its processing, not only the implementation of good hygiene and production practices. In addition, the results indicate the need to provide better conditions during the sale of cheese, especially regarding the sale at outdoor farmers' markets in the Sarajevo area, where refrigerated display cases should be used as in indoor farmers' markets, which is the case at the Zagreb farmers' markets. From the point of view of health and safety of fresh cheese, it is very important to note that *L. monocytogenes* and *Salmonella* spp. were not detected.

Because fresh cheese is considered a healthy domestic product, which is accepted among consumers, and most commonly consumed "fresh", consumers need to be educated and informed about the process of cheese production and microbiological risks that accompany such natural production. They should also be aware that fresh cheese, especially cheese

Table 3. Correlation of microorganisms found in fresh cheese samples (*n*=60) and risk factors – simple linear regression.

	Escherichia coli log10 cfu/g**		*Staphylococcus aureus* log10 cfu/g**	
Risk factors	β (95% CI)*	*P*	β (95% CI)*	*P*
City	0.178 (0.00; 1.57)	0.05	-0.30 (-0.99; 0.39)	0.04
Farmers' market	0.13 (-0.03; 0.29)	0.10	-0.14 (-0.29; 0.01)	0.06
Registered producers	-0.49 (-1.47; 0.49)	0.33	0.75 (-0.20; 1.70)	0.12

* Confidence interval
** Values defined in the Guidelines (Anonymous, 2011a,b)

Table 4. Correlation of microorganisms found in fresh cheese samples (*n*=60) and risk factors – simple logistic regression.

	Escherichia coli log10 cfu/g***		*Staphylococcus aureus* log10 cfu/g***	
Risk factors	OR *(95% CI)**	*P*	OR* (95% CI)**	*P*
City ~	8.52 (0.98; 74.39)	0.05	0.97 (0.91; 1.03)	0.52
Farmers' market	1.41 (0.98; 2.203)	0.08	1.05 (0.47; 2.31)	0.91
Unregistered producers	0.52 (0.06; 4.63)	0.56	1.08 (0.93; 1.27)	0.23

* Odds ratio
** Confidence interval
** Values defined in Guidance (Anonymous, 2011a,b)
~ Reference of Zagreb – Sarajevo relationship

produced from raw milk, could present a significant public health problem.

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Mikrobiološka kakvoća svježeg kravljeg sira u prodaji na gradskim tržnicama Sarajeva i Zagreba

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U radu je usporedno istražena higijenska ispravnost svježih kravljih sireva proizvedenih u okolici Zagreba i Sarajeva koji se nalaze u prodaji na gradskim tržnicama. Istraživanje je obuhvatilo ukupno 60 uzoraka svježeg sira uzorkovanih s najfrekventnijih tržnica u oba grada tijekom jedne godine. Ispitivanjem mikrobiološke kakvoće svježeg sira u oba grada nije utvrđeno prisustvo bakterija *Salmonella* spp. i *Listeria monocytogenes*. Od ukupno 60 analiziranih uzoraka svježeg kravljeg sira, deset uzoraka ili 15 % nije zadovoljilo mikrobiološke kriterije zbog prisustva bakterije *Escherichia coli* u osam uzoraka sira (*n*=30) sa zagrebačkih tržnica (26,6 %), odnosno koagulaza pozitivnih stafilokoka (*Staphylococcus aureus*) u jednom uzorku sira (*n*=30) uzorkovanih sa sarajevskih tržnica (6,6 %). Rezultati mikrobiološke pretrage ukazuju na potrebu poboljšanja higijene proizvodnje sira.

Ključne riječi: *mikrobiološka kakvoća, svježi kravlji sir, Escherichia coli, Staphylococcus aureus, Zagreb, Sarajevo*