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1st INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

17–18 March 2023

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -



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- PROCEEDINGS -

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PREFACE

“The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter - for the future. His duty is to lay the foundation for those who are to come, and point the way.”

Nikola Tesla

Agriculture is a primary and strategic activity that ensures food security and food market stability, and protects living standards for people. We have witnessed that, in crisis situations in the world, agriculture has responded to its task as the main support in supplying the market with food products. The production of sufficient quantities of safe food enables the development of the working-age population that actively participates in the economic development of society. The specific conditions in which agriculture develops require economic support measures and subsidies from the state to preserve domestic agriculture. Planned investments in agriculture through the introduction of modern technologies and efficient organization in both production and trade reduce economic and market risks, thus enabling stable business conditions. The development of agriculture must be based on a multifunctional connection with other activities (food industry, trade, tourism, etc.)

Agricultural science and agriculture as a profession monitor and study changes occurring in this area, point out problems in agricultural practice, and find solutions. The Faculty of Agronomy in Čačak, in addition to educating students, traditionally organizes the Symposium on Biotechnology every year. This year marks the 28th anniversary of the Symposium. The main goal of the Symposium is to acquaint the wider scientific and professional public with the results of the latest scientific research, and bring together domestic and foreign scientists in the fields of primary agricultural production, food processing, and environmental protection.

At the 1st International Symposium on Biotechnology, a total of 71 papers were presented in the fields of Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology and Applied Chemistry.

We owe great gratitude to the **Ministry of Science, Technological Development and Innovation of the Republic of Serbia** and the **City of Čačak** for their financial support and patronage to this Symposium, which they provide every year. We thank companies, entrepreneurs, stakeholders and all

long-time friends of the Faculty of Agriculture for their material and organizational support.

Doing agriculture, in addition to economy and business, is also a noble social activity, considering that it satisfies people's basic daily needs of food. Agricultural producers deserve reputation and respect in society and should be enabled to make a decent living from their work, and society should recognize this.

In Čačak, March 2023

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1st INTERNATIONAL SYMPOSIUM
ON BIOTECHNOLOGY
(28th SYMPOSIUM ON BIOTECHNOLOGY
with international participation)

Faculty of Agronomy in Čačak
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is organizing

1st International Symposium on Biotechnology

17–18 March 2023, Čačak, Republic of Serbia

in cooperation with



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MICROBIOLOGICAL ASSESSMENT OF ICE CREAM SOLD AT THE TERRITORY OF BELGRADE

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Jelena Aleksić Radojković¹

Abstract: The aim of this research was to evaluate the microbiological quality of ice cream sold at catering facilities-restaurants, cake shops and bakeries on the territory of Belgrade. The material consisted of 40 samples of ice cream collected during monitoring in 2021 and 2022. Microbiological analyses were carried out according to the Rulebook on General and Special Conditions of Food Hygiene at any Stage of Production, Processing and Trade ("*Official Gazette of the RS*", No. 72/2010, 62/2018). ISO standard methods for detection of microorganisms in ice cream samples were applied. Out of 40 samples, all were in accordance to the safety criteria, as *Salmonella* spp. were not detected in any of tested samples. Four samples (10%) did not meet the hygiene criteria in production process as more than 100 CFU/g Enterobacteriaceae were detected. Out of 4 samples, one sample of ice cream was produced in the small craft facility and 3 samples were from mobile ice cream vending machines. It can be concluded that examined samples of ice cream from catering facilities in the area of Belgrade were safe for consumption, since they met the safety criteria prescribed by the applicable legislative. The improvement of hygienic measures during the production process of ice cream in small craft facilities and vedom mobile mashines was ordered.

Keywords: Enterobaceteriaceae, ice cream, microbiology, safety, *Salmonellae* spp.

Introduction

Ice cream is a frozen product of milk and according to the legislative (Rulebook of Quaility of Milk Products and Starter Cultures, "*Official Gazette RS No 33/10, 69,10, 43,13, 34/14*") belongs to the group of frozen desserts. It contains not less than 2.5% milk and/or vegetable fat in dry matter and not less than 24% in total

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dry matter, and must contain milk and/or vegetable protein. The technological process of ice cream production can be divided into two phases: preparation of the ice cream mixture (mix) and freezing of the ice cream mixture. The production of ice cream includes totally eight steps (raw compounds, preparation of ice cream mixture, pasteurization and homogenization, ripening of mixture, freezing, packing, hardening and storage). Any ingredient used in the production of ice cream may represent a source of microorganisms that can affect the composition of the microbiota in the finished product (Kambamanoli-Dimou, 2014). Pasteurization, freezing and hardening are the main steps to eliminate the microbiological hazards. Ice cream can be sold in soft or hard consistency. It is a very popular dessert which is usually consumed during summer months in all age groups of consumers, especially in children (Mokbul et al., 2016). This dessert has a history that stretches across the globe, with different countries enjoying various versions such as *kulfi* in India, *gelato* in Italy, and *mocha* in Japan. It seems every country has its own spin on this delicious frozen product. New Zealand leads the world in ice cream consumption with a per capita consumption of 28.4 liters per year. This country is renowned for the high standard of its dairy products. Following, next on the list of highest consumers of ice cream in the world in 2020 were: United States of America (20.8 L/capita), Australia (18.0 L/capita), Finland (14.2 L/capita), Sweden (12.0 L/capita), Canada (10.6 L/capita), Denmark (9.8 L/capita), Ireland (8.4 L/capita), Italy (8.0 L/capita), United Kingdom (7.0 L/capita). The market demand is still considerable, as evidenced by the amounts of production, which reached over 2.9 billion liters in the E.U. and almost 5 billion liters in the U.S. in 2020 (Eurostat, 2022; Economic Research Service, 2022).

The production of ice cream in the Republic of Serbia has been growing in recent years, and according to the data of the Serbian Chamber of Commerce (SCC), a particularly large growth has been recorded since 2019, when 51 percent more ice cream was produced than a year earlier. According to data from Statistical Office of the Republic of Serbia in 2021, 426.744hL of ice cream (including sherbet and lollipops, excluding mixtures and ingredients for ice cream) were produced, and 416.346 hL were sold. In addition to the large ice cream factories, which produce the largest quantities, ice cream is produced by round a hundred small craft producers. Considering all this, the production of ice cream is important issue in the food industry. Since this product is a very popular ultimate old-fashioned treat, that is readily consumed nowadays, we decided to evaluate the microbiological quality of ice creams sold at the territory of Belgrade.

Materials and methods

The material represented 40 samples of ice creams collected during monitoring in summer 2021 and autumn 2022 at catering facilities-restaurants, cake shops and bakeries at the territory of Belgrade city. Samples were collected aseptically in sterile plastic jars, labelled and transported in frozen condition, in transport freege to the laboratory where bacteriological analyses were started immediately.

Microbiological analyses were performed according to the Rulebook on General and Special Conditions of Food Hygiene at any Stage of Production, Processing and Trade ("Official Gazette of RS", No. 72/2010, 62/2018). For detection of *Salmonella* spp. in ice cream samples SRPS EN ISO 6579-1: 2017 method was used. The SRPS ISO 21528-2: 2017 method was used to detect Enterobacteriaceae in aim to examine the hygiene criteria of ice cream production process.

Results and discussion

The obtained results showed that out of 40 samples from catering facilities, 4 (10%) did not meet the hygiene criteria of the production process, as the presence of Enterobacteriaceae greater than 100 CFU/g was detected in 4 samples (1 sample from a craft facility and 3 samples from mobile ice cream vending machines). In none of 40 samples was not detected the presence of *Salmonellae* spp.

Table 1. Results of bacteriological analysis of ice cream samples collected in the catering facilities at territory of Belgrade city

| Year | <i>Sallmonellae</i> spp. in 25 g | | | | Enterobacteriaceae log CFU/ ml | | | |
|-------|----------------------------------|---|----------|------|--------------------------------|----|----------|------|
| | positive | | negative | | positive | | negative | |
| | Number | % | Number | % | Number | % | Number | % |
| 2021 | 0 | 0 | 25 | 62.5 | 4 | 10 | 21 | 52.5 |
| 2022 | 0 | 0 | 15 | 37.5 | 0 | 0 | 15 | 37.5 |
| Total | 0 | 0 | 40 | 100 | 4 | 10 | 36 | 90 |

Ice cream is a complex food matrix and a good medium for growth of microorganisms due its high content of nutrient constituents, nearly neutral pH (6–7) (Nalbone et.al., 2022; Kanbakan et al., 2004) and long storage duration (Bell and Kyriakides, 1998) as well. It consists of a frozen multiphase mixture

containing ice crystals, air bubbles and partially coalesced fat globules within an unfrozen serum phase of dissolved proteins, sugars and mineral salts (Nalbone et al., 2022). Due to that, this food may be classified as a high risk potential hazard which has been implicated in outbreaks of food poisoning (Baraheem et al., 2007). Microbial contamination can be introduced at various stages of production line from different human and environmental sources (Chukuezi, 2010). Heat treatment by pasteurization destroys most of the pathogens that pose risk to public health. However, what makes ice cream worthy of attention from a microbiological point of view is the significant amount of processing carried out after the pasteurization step (Cook and Hartel, 2010). This raises great concern especially for foodborne pathogens, which can contaminate the ice cream after heat treatment from the working environments or through the addition of contaminated ingredients. Overall, only a few outbreaks of foodborne illness related to ice cream consumption have been recorded and these are linked to different pathogens, such as *Salmonella* spp., *Listeria monocytogenes*, verotoxin-producing *Escherichia coli* and coagulase-positive staphylococci (Hennessy et al., 1996; Seo, 2006; Pouillot et al., 2015; De Schrijver et al., 2008; Fetschet et al., 2014). Among these bacteria, the most severe outbreak was related to *Salmonella* Enteritidis, involving 224.000 cases of infections in the U.S., as the result of cross-contamination during the transport of a pasteurized mix into inadequately sanitized tanks in which contaminated liquid eggs were previously transported (Hennessy, 1996).

In the manufacturing processes, pasteurization was effective in destroying of pathogenic bacteria and freezing and hardening processes inhibited the microbial growth. Automatic machines that are widely used for ice-cream production in dairy industry minimize direct hand manipulation and possibility of cross contamination. Furthermore, low temperature (< 7°C) of ice-cream mix is unfavorable for the multiplication of bacteria.

Our results disagree from those of Mohammed et al. (2013) who examined 60 samples of ice cream and isolated *Salomonella* spp. in 6.6% samples from unpacked-street vendors. They identified *S. Typhimurium* and *S. paratyphi* B. These authors detected Enterobacteriaceae strains in 60% and 80% samples of ice cream from unpacked-shop and unpacked-street vendors respectively, which are much higher values than ours. Our results are similar to Zadre et al. (2010) who examined samples of ice cream in Dubrovnik city area during three calendar years and did not detect *Salmonella* spp. in any of samples. They detected Enterobacteriaceae in 2006 (81 samples/9.87%), 2007 (101/12.87%) and 2008 (74/8.1%), which is in accordance to our results. Higher levels of

Enterobacteriaceae findings were reported by Fadihl et al. (2019), who out of 70 samples of ice cream detected in 28%. Our results are not in accordance to El-Makarem (2017) who analysed 100 (50 packaged and 50 unpackaged) ice creams and detected high numbers of Enterobacteriaceae in 27 % of packaged ($2.1 \times 10^3 \pm 0.8 \times 10^3$ CFU/ml) and 48% of unpackaged ice cream samples ($1.9 \times 10^4 \pm 0.8 \times 10^4$ CFU/ml). Yan et al. (2022) reported that positive rate of *Salmonellae* spp. in ice cream was very low at 0.10% among 2887 samples of ice cream from different regions in China. Güçlü et al. (2022) detected Enterobacteriaceae out of 75 samples of ice cream in Turkey at the level of 40% (n=10) in plain ice creams (1.62×10^4 CFU/g), 12% (n=3) in fruits (1.13×10^4 CFU/g) and 40% (n=10) in nuts ice cream (7.3×10^3 CFU/g) which are much higher values than we reported. Also, according to these authors *Salmonella* was not found in any of 75 ice cream samples. The Food Inspection Agency's 2017-2020 routine monitoring programs found no *Salmonellae* spp. in 1186 samples of ice cream collected from retail locations in 11 cities across Canada which is accordance to our results. Adžić (2022) reported that out of 35 samples of ice cream collected in café pastry shops at Novi Sad, 5.71% were positive for Enterobacteriaceae and none for *Salmonellae* spp. Finally, results of Milutinović (2016) showed that all ice cream samples collected in Niš were free of *Salmonella* spp. and Enterobacteriaceae.

Conclusion

Ice cream is one of the many dairy products consumed around the world. During the summer season, consumption increases and can pose a risk to consumers due to high temperatures and lack of hygiene. The potential microbiological hazards in the final products may be introduced after pasteurization due to the addition of contaminated ingredients and improper handling procedures. The above study showed that the overall risk associated with the consumption of ice cream in the Belgrade area is low, as *Salmonellae* spp. were not detected in any of 40 samples tested. Our results showed that out of 40 ice cream samples, Enterobacteriaceae were detected in 4 (10%), indicating low hygiene during the production process. In aim to improve the microbiological quality of ice cream hygiene measures were ordered. It was also indicated that regular controls should be performed on ice cream produced in vending machines due to inadequate manufacturing practices and poor hygiene of the working environment.

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