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The significance of *Enterobacteriaceae* as a process hygiene criterion in yogurt production

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Abstract. Yogurt is one of the most popular fermented dairy products with a worldwide acceptance. There are many types of yogurt differing in flavor, physical and chemical properties. Yogurt is produced by adding bacterial culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to milk and cream products. During the period from January 2017 to December 2020, a total of 202 yogurts from different small and medium sized dairy plants were analyzed as part of HACCP self-control programs. The determination of *Enterobacteriaceae* was performed as an alternative indicator of good hygiene practice. The results showed that 21.29% of analyzed yogurts contained more than 10 CFU/g *Enterobacteriaceae*, which is the evidence of poor hygiene or inadequate processing, process failure and post-process contamination. Generally, dairy products are potential vehicles for microorganisms from the *Enterobacteriaceae* family. Good manufacturing practices and good hygiene practices must be followed throughout the production line thoroughly. The absence of classic foodborne pathogens does not indicate that the yogurt is fit for consumption, since other potentially pathogenic bacteria of the *Enterobacteriaceae* family could be present. Thus, rather than pathogen testing, using *Enterobacteriaceae* to monitor the effectiveness of implemented preventive prerequisite measures could offer a better view of the quality, sanitary conditions, and safety of yogurt products.

1. Introduction

Yogurt is one of the most popular fermented dairy products with a worldwide acceptance [1]. There are no available records regarding the origin of yogurt, but it is believed that milk fermentation dates to 10,000-15,000 years ago, together with beginnings of animal domestications. Ancient civilizations such as the Sumerian, Babylonian, Pharonic, Indian, Greek and Roman used to make yogurt in order to preserve milk and extend its shelf-life [2, 3].

At present there are many types of yogurt produced worldwide, which differ in flavor, physical and chemical properties [4]. According to the current requirements of the Serbian Rulebook on milk products [5], yogurt is defined as a fermented dairy product produced by adding characteristic bacterial cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to milk and cream products.

This easily digestible dairy product is highly nutritious. Although the nutritional composition of yogurt can vary, depending on type of milk used, animal species the milk is obtained from, strains of starter culture used in the fermentation, adding and length of fermentation, it is, generally, a rich source of proteins, carbohydrate, minerals such as calcium and phosphorous, and vitamins such as riboflavin (B2), thiamin (B1), cobalamin (B12), folate (B9), niacin (B3) and vitamin A [6]. However, highly nutritious yogurt is suitable for bacterial growth. During its manufacturing, processing, storage, distribution, and marketing, this dairy product can be subject to inadequate hygiene conditions, which can promote spoilage and contamination with pathogenic microorganisms, including *Enterobacteriaceae* [7].

The *Enterobacteriaceae* family comprises a large group of Gram-negative non-spore-forming bacteria. These facultative anaerobes include some harmless commensal species as well as important human and animal pathogens. Their ubiquitous distribution means that it is inevitable that some members of the *Enterobacteriaceae* will enter the food chain. This family includes a large number of organisms (*Escherichia*, *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus*, *Edwardsiella*, *Erwinia*, *Morganella* and *Providencia*) [8, 9]. So, as an alternative indicator of good hygiene practice in the yogurt production line, *Enterobacteriaceae* can be used.

The purpose of this paper was to present the occurrence of *Enterobacteriaceae* in yogurt in small and medium sized dairy plants from Vojvodina, Serbia and to emphasize the importance of good hygiene practices along the yogurt production line.

2. Materials and methods

During the period from January 2017 to December 2020, a total of 202 yogurts from different small and medium sized dairy plants were collected. All samples were part of HACCP self-control programs. The yogurt samples were transported from the dairy plants to the laboratory of the Scientific Veterinary Institute Novi Sad in cooling transport boxes at ≤ 4 °C in their original packages, and analyzed for the presence of the *Enterobacteriaceae* following the standard method ISO 21528-2 [10, 11] within 24 hours. After incubation on violet red bile glucose agar (VRBG) (Biokar Diagnostics, France), characteristic pink to red or purple colonies were selected for biochemical confirmation tests. For oxidase reaction, commercially available disks were used (Himedia, India). All oxidase negative colonies were further analyzed for glucose (Biokar Diagnostics, France) fermentation. Results of the microbiological analyses were expressed as number of bacteria per milliliter (CFU/mL).

3. Results and discussion

The *Enterobacteriaceae* incidence in yogurt is shown in Table 1. In total, 21.29% of analyzed yogurts contained more than 10 CFU/mL *Enterobacteriaceae*. The Serbian Rulebook on food hygiene requirements [12] limits the number of *Enterobacteriaceae* in pasteurized milk and other pasteurized liquid dairy products to no more than 10 CFU/mL throughout the shelf-life. The popularity of yogurt as a fermented dairy product recommended for both children and adults has led many microbiologists to focus on its quality and safety.

Table 1. The number of *Enterobacteriaceae* (CFU/mL) detected in yogurt samples

Enterobacteriaceae (CFU/mL)	No of yogurt samples	% of yogurt samples
< 10	159	78.71
11 – 50	15	7.43
51 – 100	8	3.96
101 – 300	9	4.45
> 300	11	5.45
Total	**202**	**100.00**

Generally, *Enterobacteriaceae* are considered as process hygiene indicators. In the study conducted by N'Guessan et al. [13] similar results were presented, where the *Enterobacteriaceae* was above the limit in 21% of yogurt samples. In our study, 5.45% of yogurt samples contained > 300 CFU/mL of *Enterobacteriaceae*. In a study conducted in Albania 34.58% of collected samples counted > 300 CFU/mL of *Enterobacteriaceae* [14].

Enterobacteriaceae in yogurt indicate evidence of poor hygiene or inadequate processing (especially heat-treatment), process failure and post-process contamination. The failure to respect hygiene rules can take place in different parts of the production line. The need to improve microbiology, biochemistry and food engineering has yogurt production a complex activity. The generalized process of yogurt manufacture is comprised of standardization of milk, homogenization, pasteurization, cooling to incubation temperature, fermentation, cooling, packaging and storage [15].

The first step in safe fermented dairy products surely is the primary production of milk. Namely, dairy farms can be important reservoir of *Enterobacteriaceae*. They are ubiquitous in the environment from which they contaminate the cow, equipment, water, milkers' hands and milking machines. Contamination of milk can be due to excretion from the udder of an infected animal during the milking process [16]. These bacteria can also appear during collection and transportation of milk. Further manipulation of milk is equally important. The heat treatment of milk has a number of beneficial effects. One of them is to reduce the number of microorganisms present in milk [1]. Various heat treatments can be applied. Usually, the milk mixture is pasteurized at 85 °C for 30 minutes or at 95 °C for 10 minutes. A high heat treatment is used to denature the whey proteins and allows the proteins to form a more stable gel [17]. The high temperature further reduces the number of microorganisms in the milk to provide a better environment for the starter cultures to grow. Milk intended for yogurt production is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yogurt after fermentation to act as probiotics. However, bacteria from water and air in the filling equipment or immediate surroundings can recontaminate the product [18]. Post-pasteurization contaminations of yogurt are mainly due to the presence of biofilms on contact surfaces of filling machines [19]. Biofilms are matrix-enclosed bacterial populations [20]. Due to their resistance, they are difficult to eradicate with conventional cleaning and disinfection regimens [19]. Yogurt packaging ensures its hygienic condition is maintained and protected during distribution.

For years, coliform testing has been used to indicate the hygienic condition of dairy products. Studies between 2001 and 2010 have shown post-processing contamination with coliforms in 7.6–26.6% of tested U.S. fluid milk samples [21]. Generally, coliforms are Gram-negative, aerobic or facultative anaerobic, non-spore-forming rod shaped bacteria. Coliforms are capable of fermenting lactose, resulting in gas and acid production [22]. A majority of the yogurt samples collected in Cameroon from 2012 to 2013 had coliform counts higher than 10^2 CFU/mL [23]. Thereof, from 72 bacterial isolates previously considered as coliforms, 21 *Enterobacteriaceae* species were identified. The carbohydrate specified in the coliform test is lactose. Lactose is not fermented by *Salmonella*, *Shigella*, or *Yersinia*, so their presence would not be detected by the test. However, substituting glucose for the lactose in the test would allow detection of all members of the *Enterobacteriaceae*, including the pathogens. *Enterobacteriaceae* have greater resistance to environmental conditions than the coliforms, so they may be better indicators of sanitation [24].

Also, older starter culture can be a source of contamination. Survival of *Salmonella* spp. was noticed in 4% of commercial collected yogurt samples in the study conducted by Motawee et al. [25]. With testing of 211 bacterial isolates, Hervert et al. [26] demonstrated that testing for *Enterobacteriaceae* offers a more comprehensive indicator for the hygienic status of dairy products and processing environments when compared with coliform organisms.

4. Conclusion

Dairy products are potential vehicles for microorganisms from the *Enterobacteriaceae* family. Good manufacturing practices and good hygiene practices must be followed thoroughly throughout the yogurt production line. The absence of classic foodborne pathogens does not indicate that the yogurt is fit for

consumption, since other potentially pathogenic bacteria of the *Enterobacteriaceae* family could be present in the food. Thus, using *Enterobacteriaceae* to monitor the effectiveness of implemented preventive prerequisite measures could offer a better view of the quality, sanitary conditions, and safety of yogurt products. Testing for *Enterobacteriaceae* is useful to verify that the hygiene measures in a manufacturing facility are working as intended.

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