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MICROBIOLOGICAL CRITERIA IN THE MANUFACTURE OF PASTEURIZED MILK**

Bojan GOLIĆ[1]*, Milijana GOLIĆ[1], Tanja ILIĆ[1]

[1] Dr. Sci. Bojan Golic Spec. Dr. Vet., MSc. Aleksandra Babic Dr Vet., MA Milijana Golic BSc. Technology Engineer, Public Institution Veterinary Institute of the Republic of Srpska „Dr. Vaso Butozan“ Banja Luka, Branka Radicevica 18
* Corresponding author: Dr. Sci. Bojan Golic Spec. Dr. Vet., bojan.golic@virs-vb.com

Abstract: The process hygiene criterion is a microbiological criterion that applies to the process of food production and processing and indicates the proper functioning of the production process by representing the value of the contamination above which corrective measures are taken to maintain the process hygiene. Pasteurized milk is a product obtained by heat treatment of raw milk at a temperature of 63° C for 30 minutes or 72° C for 15 seconds. Pasteurization has two purposes, the first one is to elimination of all pathogenic microorganisms, and the second is to reduce the number of saprophytic microorganisms, which prolongs the shelf life without changing the nutritional and biological value of the milk.
The aim of the study is to examine the safety of pasteurized milk and the conditions of hygiene in the production process based on the results of the examination of pasteurized milk on the criteria of hygiene in the production process, as well as to develop a proposal for the recommended microorganisms to be tested in the process of the production of pasteurized milk.
Samples of pasteurized milk come from a pasteurizing plant that purchases milk from the territory of Republika Srpska, and were sampled in the six-month period (January-June), within self-control and official control. For microbiological testing of raw milk, standard BAS ISO methods were used.
The results of pasteurized milk testing in relation to the hygiene criteria in the production process are satisfactory in relation to the Rulebook on Microbiological Criteria for Food. The results of self-control on the recommended microbiological criteria in the process of obtaining pasteurized milk, which are given in the Guideline on the Application of Microbiological Criteria for Food, are satisfactory in relation to the finding of Listeria monocytogenes, Salmonella spp. and coagulase positive staphylococci, and unsatisfactory for a number of microorganisms.
The results of official controls in the production process of pasteurized milk are unsatisfactory due to the increased number of microorganisms in 12.50% of the samples. 22.20% of pasteurized milk samples had the number of microorganisms larger than 10^5CFU/ml within self-control and official controls. Examination of pasteurized milk in the self-control of the production process should be performed on enterobacteria and the total count of microorganisms, and in the interpretation of the results the recommended limit values from the Guideline on the application of microbiological criteria for food should be used.
Keywords: pasteurized milk, microbiological criteria, process hygiene

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INTRODUCTION

Rulebook on Microbiological Criteria for Food (2012; 2013) prescribes general and special conditions of food hygiene at any stage of production, processing and trade (microbiological criteria for food) as well as rules which food business operators have to respect when applying general and special hygienic measure based on risk analysis of critical control points. Food safety criteria define the acceptability of a product and is applied to products in the market. Food safety criteria and their limit values apply to food from the dispatch phase until the expiration date. In cases where food is in the dispatch phase and transport documents have already been drawn up, food safety criteria are considered to be applied (Guide, 2013). The process hygiene criterion is a criterion that applies to food production and processing and indicates the proper functioning of the production process by representing the value of the contamination above which corrective measures are taken to maintain the process hygiene (Rulebook, 2012; Rulebook, 2013).

Guideline on Microbiological Criteria for Food (2013) is primarily intended for food business operators, with the aim of clarifying the application of the Rulebook on microbiological criteria for food including the mandatory criteria, as well as providing review of other mandatory microbiological requirements. Recommended microorganisms, with the category of food they refer to, are applied primarily at the end of the production process, but their limit values are recommended throughout the shelf life of the product, and can be used in the definition of microbiological criteria in the preparation of the manufacturer's raw material specifications.

Food business operators decide on the frequency of sampling, except in cases for which the sampling frequency is specified in the Rulebook on Microbiological Food Criteria (2012; 2013), and in such cases the sampling frequency should be at least equal to the sampling frequency specified in the Rulebook. The frequency of sampling can be adjusted to the nature and extent of food business, provided that food safety is not compromised.

In case of obtaining only one unsatisfactory result, the food business operator must take appropriate corrective measures (Guide, 2013). Measures taken by the operator to determine the cause of unsatisfactory results in order to prevent the recurrence of unacceptable microbiological contamination may include changes to procedures based on HACCP principles or other existing measures to control food hygiene (Ordinance, 2012; Ordinance, 2013). The food safety criterion for heat-treated milk is applied to products placed on the market during the product's lifetime and to the stage before the food business operator who has produced the food ceases to be directly responsible for it. Heat-treated milk is examined for the presence of Listeria monocytogenes before it ceases to be under the direct control of the producer and when a food business operator can not satisfactorily demonstrate to the competent authority that the product will not exceed the limit of 100CFU / ml during shelf life. The test method is BAS EN / ISO 11290-1 / A1 (Microbiology of Foods and Animal Feed, 2005b), and the limit value is absence in 25ml (M = m, n = 5, c = 0).If the food business operator can prove to the competent authority that the product does not exceed the limit of 100CFU / ml during the shelf life, then heat-treated milk is tested by the test method BAS EN / ISO 11290-2 / A1 (Microbiology of food and animal feed, 2005.v), and the limit value is 100CFU / ml (n = 5, c = 0).The hygiene criterion in the process of pasteurized milk production is applied at the end of the production process. It is tested using the method BAS ISO 21528-2 (Microbiology of food and animal feed, 2013), which determines the number of enterobacteria

and the limit value is M = 10CFU / ml (n = 5, c = 0).

Recommended microbiological criteria for pasteurized milk (Guide, 2013):

- absence of Salmonella spp. in 25 ml (M = m, n = 5, c = 0),
- absence of Listeria monocytogenes in 25 ml (M = m, n = 5, c = 0),
- number of coagulase positive staphylococci M = 10CFU / ml (n = 5, c = 0),
- number of Enterobacteriaceae m <1CFU / ml, M = 10CFU / ml (n = 5, c = 2),
- number of microorganisms m = 103CFU / ml, M = 104CFU / ml (n = 5, c = 1).

Pasteurized milk is a product obtained by the thermal processing of raw milk during the pasteurization process. Pasteurization is a heat treatment process that is applied in food processing, and is used for two purposes: the first is the destruction of all pathogenic microorganisms, the second is to reduce the number of saprophytic microorganisms, which prolongs the shelf life without altering the nutritional and biological value of milk. There are two pasteurization processes, LTH (Low Temperature Holding) at 62.8 ° C for 30 minutes and HTST (High Temperature Short Time) at 71.7 °C for 15 seconds (Adams and Moss, 2008). Minimum combinations of time and temperature for pasteurized milk are 63 ° C for 30 minutes or 72 ° C for 15 seconds (Burton, 1986; Ordinance, 2011a). The definition of the International Diary Federation (IDF) also includes requirements for the product to be cooled down without delay after heat treatment, to be packed with minimal delay to reduce contamination and to have a negative phosphatase test immediately after heat treatment (Cerf, 1986).

The aim of the paper is to assess the safety of pasteurized milk and hygiene conditions in the production process based on the results of the testing of pasteurized milk on the criteria of hygiene in the production process, as well as to develop a proposal for the recommended microorganisms to be tested in the pasteurized milk production.

MATERIAL AND METHODS

Material

Samples of pasteurized milk originate from the pasteurizing plant that purchases milk from the territory of Republika Srpska, and were sampled in the six-month period (January-June), within the framework of self-control and official control. Pasteurized milk was sampled within the official control at least once a month and three times in February. Eight samples were sampled with 5 units each. Within the framework of self-control, one sample (five units) of pasteurized milk was sampled in June. Samples were sampled from the collection tank, immediately after the process of pasteurization was completed. Laboratory testing of samples was carried out at the JU Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Banja Luka.

Methods

For the microbiological examination of pasteurized milk, the following standard test methods were used:

-for the enumeration of microorganisms BAS EN ISO 4833: 2006 (Microbiology of food and animal feed, 2006)

- for the enumeration of Enterobacteriaceae BAS ISO 21528-2: 2008 (Microbiology of food and animal feed, 2013)
- for the enumeration of coagulase positive staphylococci and Staphylococcus aureus. BAS EN ISO 6888-1 / Amd1: 2005 (Microbiology of food and animal feed, 2005a)
- for the detection of Listeria monocytogenes BAS EN ISO 11290-1 / A1: 2005 (Microbiology of food and animal feed, 2005b)

- for the detection of Salmonella spp. BAS EN ISO 6579 / Cor2: 2010 (Microbiology of food and animal feed, 2010).

We used descriptive statistical parameters as basic statistical methods in our research and in the statistical analysis of the obtained results. The results of the research are shown in the table.

RESULTS AND DISCUSSION

Raw milk is a suitable medium for the growth of microorganisms due to high aw values, moderate pH (6.4-6.6) and sufficient nutrient content. This requires high standards of hygiene in the production of milk and milk products.

Milk obtained from healthy animals, aseptically taken, is generally sterile or contains very few microorganisms, usually less than 102-103 CFU / ml. Microorganisms mature in milk in the case of mastitis when their number may be about 105 CFU / ml or by subsequent contamination from the outside environment. In fresh milk, heat-resistant microorganisms that survive the pasteurization process can be present (Adams and Moss, 2008). These are mainly Gram-positive bacteria that form spores, as well as members of the genus Micobacterium, Micrococcus, Enterococcus and Lactobacillus. Most Gram-negative psychrotrophic bacteria do not survive the pasteurization temperature, but there are some microorganisms resistant to these temperatures, which lead to the spoilage of pasteurized milk. These are psychrotrophic Gram- negative bacteria Pseudomonas, Alcaligenes and Acinetobacter, but the spoilage can also occur due to the growth of Bacillus spp. resistant to pasteurization temperature, also referred to as postpasterization contaminants.

Within the microbiological criteria for pasteurized milk, mandatory hygiene control in the production process is envisaged by the Rulebook on Microbiological Criteria for Food (2012; 2013) This criterion envisages the enumeration of Enterobacteriaceae in pasteurized milk during the production process. Self-control carried out by a food business operator in the pasteurized milk production was done according to the recommendations set out in the Guidelines on Microbiological Food Criteria (2013), as well as on the basis of its own self-control plan. Within the control of the hygiene criterion in the production process, it is recommended to study Salmonella spp., Listeria monocytogenes, coagulase positive staphylococci, Enterobacteriaceae and the number of aerobic mesophilic microorganisms. The food business operator in the plan of self-control for the microbiological criterion number of microorganisms in pasteurized milk determined a limit value of M = 105CFU / ml.

Based on the recommended microbiological criteria in the Guideline (2013), in a period of six months the food business operator did one self-control (june) on the hygiene criterion in the process of pasteurization.

The results of the pasteurized milk test according to the criteria set out in the Guideline (2013) are given in Table 1.

Table 1. Results of pasteurized milk testing according to recommended criteria

Microorganisms	Sampling plan		Interpretation of the results
	n	c	
Salmonella spp.	5	0	satisfies
Listeria monocytogenes	5	0	satisfies
Coagulase Positive Staphylococci	5	0	satisfies
Enterobacteriaceae	5	2	satisfies
Aerobic mesophilic bacteria	5	1	doesn't satisfy

The results of self-control for one sample in the process of pasteurization, according to the recommended criteria, are satisfactory for Salmonella spp. and Listeria monocytogenes due to the absence in 25 ml, coagulase positive staphylococci due to a fixed number less than 10CFU /ml, Enterobacteriaceae due to a fixed number less than 1CFU /ml, and unsatisfactory due to an increased number of microorganisms, which is in three units greater than 105CFU /ml, and between 103CFU / ml and 104CFU /ml in two units.

The official control of the production process in the six-month period, according to the plan given by the operator, sampled five units of pasteurized milk samples eight times. In the official control, according to the food business operator's self-control plan the number of microorganisms in the samples of pasteurized milk was determined. The results of the official control of pasteurized milk in the production process are shown in Table 2.

Table 2. Results of official control of pasteurized milk in the production process

Sampling period/ Number of samples	Parameter	Sampling plan		Limit value м=M	Results
		n	c		
January / 1	Number of microorganisms	5	0	10^5CFU/ml	doesn't satisfy
February					satisfies
March / 1					satisfies
April / 1					satisfies
May / 1					satisfies
June/ 1					satisfies

The results of the official control in the process of pasteurization for eight samples are satisfactory for seven samples (87.50%) due to the determined number of microorganisms, with the number of microorganisms less than 105CFU /ml in all five units, and unsatisfactory for one sample (12, 50%) due to the increased number of microorganisms that was in all five units greater than 105CFU / ml in all five units. From the results shown in Table 1 and 2, there is a discrepancy in relation to the recommended criteria. Namely, the Guideline (2013) set more stringent requirements for the production process control (m = 103CFU / ml, M = 104CFU /ml), while the food business operator in the self-control plan listed a limit value for a number of microorganisms that is too high (M = 105CFU /ml). This is the limit value set for raw milk (Rulebook, 2011.b; Rulebook, 2015), whereas for pasteurized milk it should be significantly lower. The sustainability of pasteurized milk in traffic depends directly on the number of survived microorganisms after heat treatment. The application of heat treatment during which pasteurization takes place is sufficient to reduce 99.99% of microorganisms from raw milk. D value ie. decimal reduction time for Listeria monocytogenes at 71.1°C is 0.17 minutes (June 2003). D value for Salmonella spp. at 65°C is 0.02-0.25 minutes, for Staphylococcus aureus at 65°C is 0.02-2 minutes, and for Escherichia coli at 65°C is 0.1 min (Adams and Moss, 2008).It is realistic to expect that after the heat treatment the number of microorganisms in pasteurized milk does not exceed 103CFU / ml, and in some cases when contamination of raw milk with microorganisms was higher, the value of 104CFU / ml. Microorganisms that have survived thermal processing during the storage of pasteurized milk may multiply and increase in

number, causing the spoilage of pasteurized milk (Adams and Moss, 2008).

In the process of pasteurized milk control, the food business operator decided on the number of microorganisms which is 105CFU / ml, which is an indicator of poor raw material, raw materials with a significantly higher number of microorganisms than the number allowed by the Rulebook (2011b; 2015), or bad hygiene in the process of pasteurization. This is confirmed by the results of self-control and official control of pasteurized milk, where 2 samples (22.20%), out of a total of 9, had a number of microorganisms greater than 105CFU / ml.

By analyzing the recommended microbiological criteria (Guide, 2013), there is no explanation for controlling salmonella and listeria in thermally processed milk. These two microorganisms can not survive the process of heat treatment applied in pasteurization (Adams and Moss, 2008, June 2003) and their finding in pasteurized milk would mean that pasteurization was not performed.

The European Commission (2003) proposed that products made from raw milk should not contain Staphylococcus aureus above a certain number (105), because if raw milk contains Staphylococcus aureus in a greater number of these values, enterotoxin may be synthesized in the process of production. If intense growth of enterotoxigenic staphylococci is enabled, during the first 48 hours of production sufficient amount of enterotoxin is induced to cause food-borne diseases. Usually it is considered that enterotoxigenic staphylococci must reach a level of at least 105-106 CFU / g or ml to produce a detectable amount of enterotoxin. The recommended criterion for coagulase positive staphylococci (Guide, 2013) does not reflect the real risk assessment status when it comes to coagulase positive staphylococci because the limit value given is not significant for enterotoxin findings (European Commission, 2003) and staphylococci in pasteurized milk are also indicative of subsequent contamination (Adams and Moss, 2008).

The recommended control of enterobacteria is well formulated (Guide, 2013) and if it is determined that two sample units have enterobacteria, timely corrective measures may be taken to ensure that the final product is within the limits prescribed by the Rulebook on Microbiological Criteria for Food (2012; 2013).

CONCLUSION

Based on the obtained results, the following conclusions are drawn:

1. The results of the pasteurized milk test in relation to the hygiene criterion in the production process are satisfactory in relation to the Rulebook on Microbiological Criteria for Food.
2. The results of self-control on the recommended microbiological criteria in the process of obtaining pasteurized milk, as given in the Guideline on the Application of Microbiological Criteria for Food, are satisfactory with respect to the findings of Listeria monocytogenes, Salmonella spp. And Coagulase of Positive Staphylococci and unsatisfactory for the number of microorganisms.
3. The results of official control in the process of pasteurization are unsatisfactory due to the increased number of microorganisms in 12,50% of samples.
4. 22.20% of pasteurized milk samples had the number of microorganisms greater than 105CFU / ml within their own and official control.
5. It is suggested that the examination of pasteurized milk, in the self-control of the production process, is carried out on enterobacteria and the number of microorganisms, and that the recommended limit values from the Guideline on the

Application of Microbiological Criteria for Food are used in the interpretation of the results.

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