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Short communication: psychrotrophic microorganism count in raw milk samples preserved with azidiol®

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ABSTRACT. The study aimed to evaluate the use of the azidiol® preservative for psychrotrophic microorganism count (PMC) in cooled raw milk. Two studies were carried out, one under controlled conditions (experiment 1) and the other under field conditions (experiment 2), in which samples of raw milk were taken with and without the use of the azidiol® preservative and analyzed at predefined times (0, 6, 12 and 24 hours - experiment 1) and at varying times (experiment 2). Analysis of variance and regression analysis using SAS were applied for data statistical analysis. Milk samples without azidiol® showed higher PMC with increasing time between sampling and analysis, while in samples preserved with azidiol®, this count remained constant. Samples of cooled raw milk intended for PMC should be collected in flasks containing the azidiol® preservative.

Keywords: chemical preservative; microbiology; milk quality.

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Introduction

Azidiol® is a bacteriostatic preservative broadly used in the dairy industry, and is formed by sodium azide and chloramphenicol. Its use has been widely studied for electronic analysis of total bacterial count (TBC), somatic cell count (SCC), and composition of raw milk (Cassoli, Machado, & Coldebella, 2010; Wentz et al.,2018); however, it has been little studied in psychrotrophic microorganism count (PMC). After instituting mandatory cooling of raw milk in dairy farms, these microorganisms gained space in discussions and research on the dairy chain, mainly because they produce thermostable proteolytic and lipolytic enzymes, which have already been associated with several sensory changes and a reduction in the shelf life of milk and its derivatives (Nörnberg, Tondo, & Brandelli, 2009; Santos, Cerqueira, Leite, & Souza, 2018; Marioto et al., 2020). In ultra-high temperature (UHT) and powdered milk, the residual activity of heat-stable psychrotrophic peptidases causes bitterness, the formation of suspended particles, creaming and gelation, as well as interfering with rennet clotting time and the clot firmness in the production of cheeses with pasteurized milk (Zhang et al., 2015; Paludetti, Kelly, & Gleeso, 2020). These enzymes also affect the percentage of proteins and pH of yogurt, causing a decrease in shelf life and bitter taste in these milk derivatives (Fagnani, Schuck, Botaro, & Santos, 2017).

Considering the negative effect on the quality of products and their economic impact, knowing the quality of the raw material regarding the PMC is of high importance so that preventive measures can be taken to minimize its effects at all stages of the milk production chain (Santana et al., 2001; Marioto et al., 2020; Arcuri et al., 2008). In addition, it is important to standardize the milk sample collection procedures and storage conditions of the sampled milk, so that the result obtained is not negatively influenced by these variables, especially for PMC in milk samples from cooling tanks. Usually, laboratory tests for PMC are not performed immediately after sample collection and these microorganisms continue to multiply even at low temperatures (Santana et al., 2001; Vithanage et al., 2017). Therefore, this study aimed to evaluate the effect of the azidiol® preservative and the time between sampling and analysis of PMC in cooled raw milk samples.

Material and methods

The study was carried out in two research, the first was carried out under controlled conditions and the second under field conditions. Samples of cooled raw milk (n = 524) were evaluated, half of which were preserved with the azidiol® bacteriostatic agent (4.79mg sodium azide and 0.2mg chloramphenicol) and the other half without preservative.

Research 1

The study was carried out using raw milk samples collected from three dairy farms in the Serrana Region of the state of Santa Catarina, Brazil, located near the milk quality laboratory at the State University of Santa Catarina in Lages, state of Santa Catarina. Five visits were made to each dairy farm; eight raw milk and preservative-free samples were taken in each visit using sterile 50 mL Falcon tubes. Samples were identified in duplicate as hour zero, six, twelve, and twenty-four. Arriving at the laboratory and in a laminar flow hood, one of the samples from the duplicates of each hour (0, 6, 12, and 24) was transferred to sterile bottles with the azidiol® preservative and named hereafter as 'samples with preservative'. Consequently, raw milk samples without conservative from each hour (0, 6, 12, and 24), were called 'samples without preservative'. Samples were transferred to a BOD incubator, which was programmed to remain at 7ºC, and, subsequently, the incubation and counting procedures for psychrotrophic microorganisms of the samples of hour 0 started. Six hours after arriving at the laboratory, the procedure was repeated with samples with and without preservative at hour six, and thus so on until samples at hour twenty-four.

Research 2

The study was carried out in partnership with a dairy industry in the Vale do Itajaí Region, state of Santa Catarina, Brazil. Among the routes for the collection and bulk transportation of milk set forth by this industry, six trucks were followed, at random, on nine trips on their daily milk collection routes. Two raw milk samples of each farm, with and without the azidiol® preservative, were collected from bulk tanks of 143 dairy farms (milk production ranging from 16 to 975 liters day^{-1}). Microbiological analysis of PMC of the milk samples with and without azidiol® were performed only when the vehicle returned to the dairy industry to unload the raw material. In addition to microbiological analysis, the volume collected in each farm was recorded, the time, in hours, between collections and the beginning of analysis, and the milk temperature was monitored at the time of collection using a digital skewer thermometer AKSO AK05.

Laboratory analysis

For PMC, milk samples preserved or not with Azidiol® were diluted in sterile peptide saline solution, from 10^{-1} to 10^{-4} and the count was done according to American Public Health Association standard methods, described by Frank & Yousef (2004). Plates (inoculated in duplicate) were inverted and incubated at 7ºC for ten days in a BOD bacteriological oven. After, the colonies formed were quantified with the help of a colony counter, the value obtained was multiplied by the reciprocal of the corresponding dilution, and the final results expressed in CFU mL^{-1}.

Statistical analysis

PMC data from the first and second studies were tested by analysis of variance and regression by the MIXED procedure of the SAS statistical package (Statistical Analysis Sofware [SAS], 2002). To achieve residual normality, PMC was log transformed (log10), and normality was tested by the Kolmogorov-Smirnov test. The statistical model included the effects of using or not the azidiol® preservative, the storage time of the samples, and the interaction between these factors. From the results, the regression coefficients of PMC values as a function of storage time were estimated.

Results and discussion

Mean values ± standard deviation of PMC were, respectively, 4.64 ± 1.21 (log10 CFU mL^{-1}) in the study under controlled conditions (research 1) and 5.45 ± 1.01 (log10 CFU mL^{-1}) in the study under field conditions (research 2). Under controlled conditions (research 1), the evolution of the PMC of milk samples from dairy farms as a function of hours of storage was affected by the use of the azidiol® preservative (Figure 1). The

sample storage time did not affect the PMC (p = 0.9012) in milk samples preserved with azidiol®, while milk samples not added with this preservative showed a linear increase in PMC with progressing time (hours) (p < 0.0001). Izidoro, Pereira, Soares, Spina and Pinto (2013) evaluated the PMC of milk samples at different storage times (12, 24, and 48 hours) and temperatures (4, 8, and 12°C) and concluded that, regardless of the storage temperature, PMC always increased with increasing storage time. Reche et al. (2015) observed higher PMC in bulk milk tanks of the model of 'four milkings', and related such results to the higher storage temperature compared to the bulk milk tanks of the model of 'two milkings' since the latter have a greater capacity for faster cooling of milk.

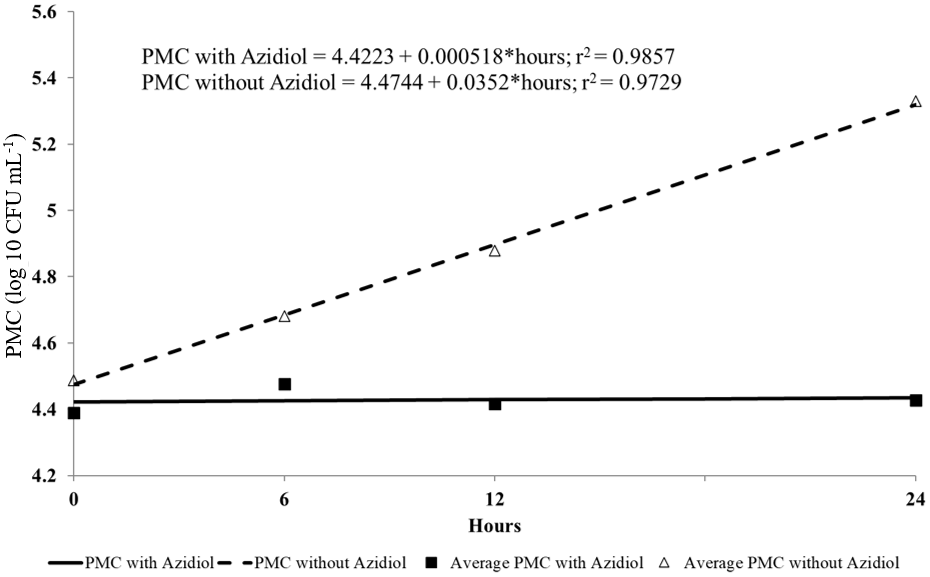

Figure 1. Psychrotrophic microorganisms count (PMC) of milk samples preserved or not with azidiol® according to the time under controlled conditions (laboratory).

The literature on the use of the azidiol® preservative in milk samples for PMC is very scarce. In a study on sheep milk stored in an industrial silo, Garnica, Santos and Gonzalo (2011) found higher PMC (p < 0.05) in milk samples without preservative compared to those preserved with azidiol®, which have remained constant over time.

With respect to the study under controlled conditions (research 1), similar results were observed in the study under field conditions (research 2; Figure 2). Despite the variable time between sampling and analysis (3 to 24 hours), PMC of milk samples added with azidiol® was not influenced by transportation time and consequently the storage time (p = 0.1016). Milk samples without the use of preservatives had a linear increase in PMC with increasing time between sampling and analysis of the samples (p = 0.0226).

In the first study, the same initial milk sample from each dairy farm was analyzed, with different storage times (0, 6, 12, and 24 hours), thus showing a greater homogeneity of results obtained, which is clearly illustrated in Figure 1. The mean points are very close to the regression lines and with a high coefficient of determination. In these controlled conditions, samples not added with azidiol® at time zero were not different (p > 0.05) from those with the azidiol® preservative, with linear increases over time.

Different from the study under controlled conditions (research 1), in the study under field conditions (research 2), paired-samples (with and without preservative) from 143 dairy farms were analyzed. These farms showed a large difference in distance from the dairy industry, which together with the variability in length of the milk collection routes, affected the transportation time and, consequently, the storage time of samples. Dairy farms also showed innumerable differences, for example, in structure, technification, number of animals in lactation, access to information, and the use of techniques to improve milk quality, education level of the farmers, among others. Therefore, PMC in the different sample transportation times refers to different dairy farms (Figure 2), while in the study under controlled conditions, they are aliquots of the same milk sample (Figure 1). However, higher PMC in each storage time in Figure 2 demonstrates that the use of preservative prevents the increase in the number of colony-forming units during transportation, which may enable the PMC in field studies or in the routine evaluation of milk quality in conditions where laboratory analysis is not possible immediately after sample collection.

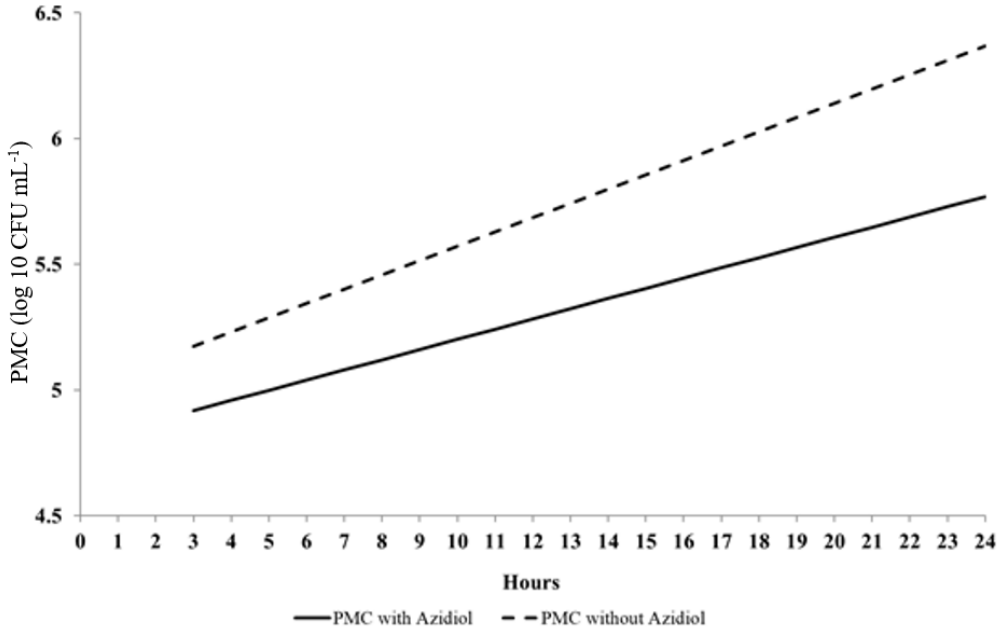

Figure 2. Psychrotrophic microorganisms count in samples of cooled raw milk from bulk tanks in dairy farms, preserved or not with azidiol® according to the time (hours).

Conclusion

Under similar conditions to those described in this experiment, samples of cooled raw milk for psychrotrophic microorganism count and which will not be analyzed immediately after collection have to be taken with the azidiol® preservative.

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