

POSITIVE COAGULASE STAPHYLOCOCCUS RESEARCH IN ARTISANAL CHEESE WITH THE ARTE SEAL SOLD IN VIRTUAL STORES

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Abstract: Cheese is one of the most consumed dairy products in the world. And, to guarantee the quality of artisanal cheeses produced in Brazil, the ARTE Seal was created. Therefore, the objective of this study was to research coagulase-positive *Staphylococcus* in artisanal cheeses with the ARTE Seal sold in online stores.

INTRODUCTION

Cheese is a food that has a significant historical milestone, and there is evidence that its history began years BC and was used in human food. Years later, cheese began to be produced all over the world with a variety of types of milk (BARBOSA, 2021). With the increase in production and the advancement of technology, new studies emerged that allowed the creation and implementation of more elaborate laws to guarantee better quality on the consumer's table (SILVA, 2022). Despite all the evolution, many producers have difficulties in marketing their products, resulting in artisanal products presented informally, that is, without sanitary inspection, which results in an impact on consumer health. Based on this principle, the ARTE Seal was created so that, through it, producers can sell their high-quality products nationwide under sanitary inspection in a less bureaucratic way (BRASIL, 2022).

The global crisis caused by the coronavirus in 2020 marked history and caused harm to thousands of people. It is believed that this situation will continue to be reflected in society in the future. Many areas were affected by this new reality and, consequently, people had their habits and behaviors transformed, a fact that is reflected in the market and, therefore, it becomes important to understand the changes in consumer behavior during the pandemic faced. (LOPES; LIMA; RIBEIRO, 2022).

According to Schneider et al. (2020),

the main transformation resulting from the pandemic in relation to food sales is the increase in virtual shopping. These new marketing dynamics have transformed delivery practices and the ways in which farmers offer their products. Therefore, food trade mediated by information technologies is growing.

In order for cheeses to be sold on a regular basis, they must be subjected to a microbiological evaluation, in order to certify the absence of possible pathogens that could pose risks to public health, among 25 of which the research on *Staphylococcus* coagulase stands out. positive because it is the main causative agent of food poisoning. *Staphylococcus aureus*, for example, is frequently researched in foods, with cheese being one of the main vehicles causing food poisoning, as its presence is associated with inadequate hygiene and handling practices (LOGUERCI; ALEIXO, 2001 apud FERREIRA et al., 2011).

GOAL

The objective of this study was to research coagulase-positive *Staphylococcus* in artisanal cheese with the ARTE Seal sold in online stores.

MATERIAL AND METHODS

In the months of June and July 2022, 15 samples of artisanal cheeses with the ARTE Seal sold in virtual stores were analyzed. The cheeses used were produced in the states of Minas Gerais and Santa Catarina. Purchases were strictly made on specific websites selling artisanal cheeses. Upon receiving the samples, information such as date, time and temperature was collected. Furthermore, the conditions of the packaging in which the product was placed were observed, including checking that the Cheeses were sent in isothermal boxes, but without cooling with

recyclable ice or any other means. Soon after, the samples were sent to the Microbiology Laboratory of `` Centro Universitário Barão de Mauá `` in their original packaging and in an isothermal box.

For the preparation of samples and serial dilutions, the recommendations of the Manual of Methods and Microbiological Analysis of Food and Water (SILVA et al., 2021) were followed. In the laboratory, before opening the packages, they were sanitized with cotton soaked in 70% alcohol. Then, near the Bunsen burner, 25 g of cheese were removed with the aid of previously sterilized tweezers and scalpels, transferred to Erlenmeyer flasks containing 225 mL of 0.1% peptone saline solution, corresponding to a 10⁻¹ dilution. The 10⁻² dilution was achieved by transferring 1 mL of the 10⁻¹ dilution to a test tube containing 9 mL of the diluent. Finally, subsequent dilutions were obtained in the same way, until reaching dilution 10⁻³ (serial dilution ratio 10).

To quantify coagulase-positive Staphylococcus in food, the American Public Health Association (APHA) 39.63:2015 protocol was followed. 0.1 mL of dilutions 10⁻¹ to 10⁻³ of each sample was inoculated onto the dry surface of Baird-Parker agar plates, in duplicate, with the aid of a Drigalski loop, then spreading the inoculum over the entire surface. from the medium until its complete absorption. The plates were incubated inverted in an oven at 35-37 °C for 48 hours.

To carry out the count, plates that presented between 25 and 250 typical and atypical colonies were selected, and the result was given in CFU/g. As a priority, we chose to select five typical colonies and, in their partial or total absence, atypical colonies were selected for the coagulase test.

Each colony was transferred to tubes with 5 mL of Brain Heart Infusion (BHI) Broth and incubated at 35-37 °C for 24 hours.

Afterwards, 0.1 mL of each culture obtained at BHI was transferred to a sterile 10 x 100 mm tube, where 0.3 mL of Coagulase Plasma-EDTA (rabbit plasma with EDTA) was added to 0.1 mL of culture.

The tubes were mixed with circular movements, without shaking to avoid any interference with coagulation. The tubes were incubated at 35-37 °C and clot formation was observed after 4 to 6 hours (Figure 1).

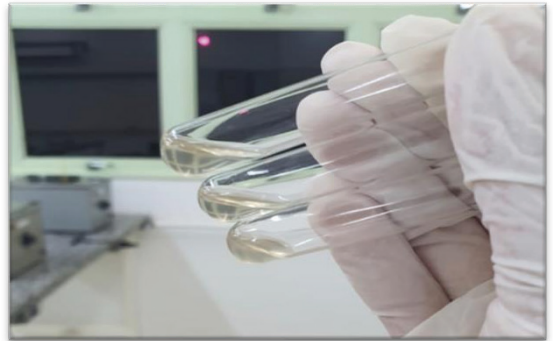


Figure 1 - Positive coagulase test in the first 4 – 6 hours.

Source: Personal archive, 2022.

In case of a negative test, the tubes were incubated for 24 hours to be examined and reevaluated (Figure 2). To consider a sample positive, it was necessary to show the formation of a clot occupying more than half of the original volume of liquid.



Figure 2 - Positive coagulase test after 24 hours.

Source: Personal archive, 2022.

RESULTS AND DISCUSSION

To count coagulase-positive Staphylococci, Normative Instruction No. 60, dated December 23, 2019, currently in force, was used as a parameter. It establishes the maximum permitted value of this microorganism in milk derivatives and, more specifically in cheeses, of up to 103 CFU/g (BRASIL, 2019).

In this present research, of the 15 analyzed samples of artisanal cheeses with the ARTE Seal sold in virtual stores from June to July 2022, one (6.67%) of the samples showed a positive Staphylococcus coagulase count of 4.0×10^4 CFU/g in the cheese samples, that is, it was not in accordance with what was established by legislation.

The presence of coagulase-positive staphylococci highlights the lack of hygienic-sanitary conditions during the production, processing, distribution, storage and commercialization stages (TIGRE; BORELLY, 2011). Furthermore, it must not be consumed by the population if the quantity of colony-forming units per gram of cheese is above legislation, as it poses risks to public health due to the possible production of enterotoxins (SILVA; FEITOSA; RODRIGUES, 2017).

On the other hand, in this research it was also found that 93.33% (14) of the samples presented coagulase-positive Staphylococcus counts lower than 103 CFU/g in the cheese samples, which means they are in accordance with what is established by legislation, that is, can be consumed by the population in terms of the presence of this microorganism.

Since to date no scientific publication has been found on the microbiological quality of artisanal cheeses with the ARTE Seal, which further highlights the importance of this work, work carried out with other types of cheese, but without the ARTE Seal, was used.

In a study where the objective was to evaluate the microbiological quality of industrialized and artisanal Minas

cheeses sold in Belo Horizonte – MG, 18 cheese samples were collected, nine of the fresh Minas type, industrialized sold in supermarkets, and nine of the artisanal Minas type, sold at the Municipal Market. Regarding coagulase-positive Staphylococcus counts, it was observed that 100% of the samples, both industrialized and artisanal cheese, presented counts higher than legal standards, with three (33%) of the industrialized samples presenting values ≥ 105 CFU/g and five (55%) of the handmade samples followed the same pattern. Therefore, it is concluded that the presence of this microorganism in the values presented becomes an epidemiological threat due to the probability of the existence of enterotoxins, which in significant quantities cause intoxication if the strain of Staphylococcus spp. is toxigenic (LOPES et al., 2020).

Unlike the present study, Souza et al. (2015), in order to understand the phenotypic and genotypic characteristics of strains of Staphylococcus aureus isolated from artisanal Minas cheese, produced in the region of Araxá – MG, they biochemically and molecularly analyzed 30 samples of a certain brand sold in the city of Sacramento-MG. Of these, 28 (93.3%) were outside the established standards, whose counts ranged from 3.0×10^2 to 2.0×10^7 CFU/g. Therefore, the analyzes carried out showed that 93.3% of the artisanal Minas cheese samples were in unsatisfactory hygienic and sanitary conditions.

In a study carried out by Martins and Reis (2012), the microbiological quality of Minas frescal cheeses sold in the municipality of Frutal - MG was evaluated, aiming to establish the quality in relation to the microorganisms evaluated. Therefore, regarding the presence of coagulase-positive Staphylococcus, it was observed that 18 samples (45.0%) disagreed with the legislation regarding their count.

The ingestion of food contaminated by microbial agents and/or toxins poses

a considerable risk to people. Foodborne illnesses not only cause disruption to the health and well-being of affected individuals, but are also associated with serious economic consequences for society. For example, one of the most common food poisonings is caused by *Staphylococcus aureus* and is due to the ingestion of preformed enterotoxins in food contaminated by the bacteria (PRADO et al., 2015). Cheese, according to Mendes et al. (2018), is one of the main carriers of *Staphylococcus aureus*, as its presence is associated with animal mastitis, inadequate hygiene practices for production and human handling. Therefore, to prevent contamination, good practices in food manufacturing are necessary.

If contamination occurs, in inadequate temperature conditions, staphylococci, which are mesophilic bacteria, can multiply at room temperature and still produce enterotoxins in the food within four to six hours (TIGRE; BORELLY, 2011). Therefore, since the samples analyzed in this research arrived in Ribeirão Preto – SP with temperatures varying between 19.9°C and 24.8°C, the lack of temperature control during transport may have been responsible for the increase in CFU/g of coagulase-positive staphylococci above legislation.

According to the Ordinance of the Mineiro Agricultural Institute n° 2,049, of April 7, 2021, it is extremely important that cheeses are stored and transported in appropriate conditions in order to avoid the multiplication of undesirable microorganisms. Therefore, they must be kept refrigerated at temperatures below 10 °C and must be transported, at a minimum, in a vehicle with an insulated

body or in a vehicle with a closed body, as long as they are in isothermal boxes that can be sanitized so that, upon delivery to retail stores, are not at a temperature exceeding 10°C (MINAS GERAIS, 2021).

The probable reason why the results of most of the samples in this research were below 10 UFC/g, that is, lower than the maximum established by legislation, it is likely that the producers of the cheese brands analyzed adopted and respected good production practices.

manufacturing, resulting in good quality products regarding the research of this pathogen.

CONCLUSION

In the present research, it was observed that the majority of the samples analyzed (93.33%) were within the limits established by legislation for the count of coagulase-positive *Staphylococcus*, making artisanal cheeses with the ARTE Seal sold in virtual stores suitable for consumption with a view to this microorganism. However, the presence of a sample (6.67%) above the established maximum limit draws attention to the need to control the temperature of the cheeses during transport, as if it had been refrigerated, it would possibly have presented a count below that established by legislation.

In view of this, it is suggested that further research be carried out to verify whether artisanal cheeses with the ARTE Seal sold in virtual stores meet the quality parameters of current legislation, so that they do not pose risks to consumer health and also serve as parameters of specific quality for these products.

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