

RESEARCH ARTICLE

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Staphylococcus aureus in locally produced white cheese in Tirana marketELVIRA BELI^{1*}, RENIS MAÇI², SONILA ÇOÇOLI¹, HALIT MEMOÇI²¹Agricultural University, Kamez, Albania²Food Safety and Veterinary Institute, Str. Aleksandër Moisiu nr. 82 Tirana

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Abstract:

Cheese has nutritional value, its consumption is very common in Albania, but is also excellent medium for bacterial growth, source of bacterial infection, particularly when it is produced from raw poor quality or unpasteurized milk. Microbial safety of cheeses may be enhanced by usage good quality raw milk, pasteurized milk, following GMP in aim to prevent cross-contamination. The aim of this study was to evaluate the presence and amount of *Staphylococcus aureus* in white cheeses, as an Albanian traditional product. Totally 120 samples of white cheese, produced in small big plant at different Albanian district, by raw milk or pasteurized milk, were collected from Tirana market. All samples were tested by phosphatase test to determine whether raw milk or pasteurized milk it was used for cheese production. 53/120 samples (44%) resulted produced by pasteurized milk, 67/120 samples (56 %) resulted produced by raw milk. The *S. aureus* was isolated in Baird Parker agar, and submitted to coagulase and API-staph test. Out of 120 cheese samples, 47 showed contamination by *S. aureus* coagulase-positive corresponding to 39.16%, otherwise 58 out of 120, 48.33 % of cheeses samples being contaminated with coagulase-negative strain of *S. aureus*. The occurrence *S. aureus* coagulase-positive in cheeses produced by pasteurized milk and raw milk it was respectively 7/53 (13.2 %) and 40/67 (59.7%.) 10% of the samples had high levels 10^5 - 10^6 cfu/g of *S. aureus* coagulase-positive, suggested that white cheese, may represent a health risk for the consumers

Keywords: raw milk produced white cheese, pasteurized milk produced white cheese, Albania, *Staphylococcus aureus* coagulase-positive.

1. Introduction

White cheese is widely produced in Albania, based on traditional or industrial technology, and its consumption is very common as a traditional nutritional product. Traditionally, it is produced by families for self-consumptions or by small scale plants for sale, and nowadays most of the products are manufactured on an industrial scale, and rigorous control of the production and maturation processes is essential for safe products. Its microbiological quality and the rate of growth and survival of pathogenic bacteria, depend on the factors such as A_w (water activity), pH, salt content, temperature and duration of maturation, within the cheese and/or brine. The quality of the raw milk, heat treatment of the milk, hygiene practices, activity of the starter culture and the salting process—together with the storage in brine are the most important factors influences the microbial status of the white cheeses during the manufacturing process [5]. Food poisonings are due to many factors. Bacteria are major causative agents of food borne diseases. *Staphylococcus aureus* is one of the main pathogen involved in food poisonings related to dairy products [17]. The presence of *S. aureus* in

food causes food poisoning by releasing enterotoxins into the food and it can also cause Toxic Shock Syndrome by release of super antigens into the blood stream [17,6].

Dairy products represented an important vehicle of gastrointestinal infection in the world. Many enteropathogenic species have been found in milk and cheese stored under refrigerated temperatures and consumed without heating. Post pasteurization contamination, manufacturing and handling process, equipment, temperature abuse during transport and storage conditions might result in high levels of pathogenic microorganisms in cheese [10,16]. Isolation of relevant bacterial pathogens and indicator organisms is used to evaluate microbiological safety and quality of food. The use of tests such as coliform and *Staphylococcus aureus* counts as an indicator of post process contamination by the dairy industry permits sanitary control [8].

Milk can be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the

contamination, as this bacteria colonizes the nasal pathways in human beings [2,6]. When found in milk, high levels of contamination can be reached quickly under favorable conditions

In Albania milk is produced mostly in small non-organized farms and usually it is being supplied to the processors without cold chain and in poor hygienic conditions. On the other hand, milk is an excellent media for growth of a wide variety of bacteria [6,10]

The cheese may be produced by using raw milk or pasteurized milk, eaten fresh or after storage in a cool store or in brine. About one kilogram of cheese will be obtained from five liters of milk. The food legislation [3] has foreseen that *S. aureus* enterotoxins must be absent to cheese, ready-to-eat. In addition, process hygiene criteria have been laid down for the number of coagulase-positive staphylococci (*S. aureus*) allowed in food products during the production process.

2. Material and Methods

2.1. Sample collection

Totally 120 samples of white cheese, produced in small capacity plant and industrial scale plant at different Albanian district were collected in the Tirana market for analyzes. Cheese samples were collected over a period of one year and were processed on the day of acquisition. The cheeses in the market it is founds generally in brine, not packed, no label. Because of the lack of the label the consumer has not possibility to take information about production, by raw milk or pasteurized milk.

The collected samples were immediately transported to the laboratory with refrigerated boxes, in retail packages.

2.2. Sample testing in the laboratory

In aim to determine the role of raw material in the microbiological quality of cheese, all samples were tested by phosphatase test to determine whether

raw milk or pasteurized milk it was used for cheese production.

Phosphatase tests it was performed refer to the Standard Methods for the Examination of Dairy Products. [18]

The samples for microbiological examination were prepared according to standard methods of preparations dairy products ISO 707: 2008 [2]. Twenty-five grams of soft cheese were aseptically transferred to a sterile beaker and homogenized in 225 ml of 0.1% peptone saline solution by blending for 2–3 min. Tenfold serial dilutions were prepared and inoculated to Baird-Parker agar (BPA, Oxoid) with added egg yolk tellurite, incubated at 37°C for 48 h. After growth, Staphylococcus colonies were counted and classified as typical for *S. aureus* (jet black to dark gray, smooth, convex, entire margins with an opaque zone, clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies, entire margin without a halo). For each sampling point, 5 typical and 5 atypical colonies on plates of BPA were randomly selected and plated on Nutrient agar (Oxoid,). The strains were preliminary screened by Gram staining and coagulase test (FDA BAM 2001). API Staph biochemical test kit (bioMérieux) were used for further biochemical identification.

The number of coagulase-positive *S. aureus* strains it was calculated for each white cheese samples, and it was faced with the Albanian legislation for cheese. Also the number of coagulase-negative *S. aureus* strains it was calculated for each white cheese samples.

3. Results and Discussion

3.1. Phosphatase tests

The results of phosphatase tests presented in table 1, indicated that most of the cheese samples 56 % it is produced by using raw milk, against 44% resulted produced by pasteurized milk.

Table 1. Results of testing cheese by phosphatase tests.

Results of phosphatase tests	Number of samples	Percentage of samples	Results of the pasteurization process	Type of samples
Positive	67	56%	No pasteurizations	Cheese produced by raw milk
Negative	53	44%	Pasteurizations	Cheese produced by pasteurized milk

The phosphatase test is applied to dairy products to determine whether pasteurization was done properly and also to detect the possible addition of raw milk to pasteurized milk. The declaration for the type of milk used for cheese production it is very important for consumer safety. Control by competent authority with phosphatase test it is unnecessary for microbiological criteria application [3].

3.2. *Staphylococcus aureus* isolation and identification

In this study, isolation and identification of *Staphylococcus* spp. was conducted out in the market white cheese, sold in brine, without label and declaration of the pasteurization process of milk used. The incidence and the distribution of *Staphylococcus* spp. isolates based on the result of the coagulase test given in Table 2.

Table 2. The incidence of *Staphylococcus aureus* coagulase-positive, coagulase-negative and other staphylococcus spp. in white cheese samples

Microorganisms	Total cheese samples 120	Cheese samples from raw milk 67	Cheese samples from pasteurized milk 53
<i>S. aureus</i> coagulase positive	47 - 39.16 %	40 - 59.7 %	7-13.2 %
<i>S. aureus</i> coagulase negative	58 - 48.33 %	54 - 80.6 %	4- 7.5 %
Staphylococcus spp.	15 - 12.5 %	11	4-7.5 %

From the total of 120 cheese samples, were contaminated with *S. aureus* 87.49 % of them. Such high percentage it was determinate from the other authors [14] identified *S. aureus* in 852 of 1036 cheese samples (82.2%). Günşen and Yörük (2003) reported that 64% of the vacuum packed fresh cheddar samples they analyzed did not fulfill the requirements of Turkish Food Codex in terms of *S. aureus*.

The results of the tables 2, indicate that the incidence of *S. aureus* is very high in the white cheese samples produced with raw milk 100 %, against the 20.7 % of cheese samples produced with pasteurized milk. It verified from the studies that raw milk is the source of contamination of cheese. Milk can be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the contamination, as this bacteria colonizes the nasal pathways in human beings [2,6].

S. aureus coagulase positive is very high in the white cheese samples produced with raw milk 59.7 %, against 13.2 % of the white cheese samples produced with pasteurized milk. Normanno et al (2005) established that 20.7 % of the 3097 milk and milk product samples they examined (631/3097) were contaminated with coagulase-positive staphylococcus.

It is seen that the incidence of coagulase- positive *Staphylococcus* isolates obtained from white cheese samples is lower than that of coagulase-negative isolates (Table 2). However, it is reported that, as well as coagulase- positive staphylococcus, some coagulase-negative staphylococcus spp. (*S. epidermidis*, *S. saprophyticus*, *S. sciuri*, *S. warneri*) have the capacity to generate enterotoxins [7,9,12].

The number of *S. aureus* coagulase positive it was calculated for each samples. The results of calculation, expressed as number and percentage of samples for every level, are introduced in table 3.

Table 3. The number of samples for each level of *S.aureus* coagulase positive

The level of <i>S. aureus</i>	Number of white cheese samples	Percentage of samples	Cheeses samples from raw milk	Cheeses samples from pasteurized milk
>10 ²	2\120	1.7 %	0\67	2\53
>10 ³	19\120	15.8 %	14\67	5\53
>10 ⁴	14\120	11.7%	14\67	-
>10 ⁵	9\120	7.5%	9\67	-
>10 ⁶	3\120	2.5%	3\67	-

As it is showed by the table 3, more frequently it was determinate the level of *S.aureus* 10^3 at 15.8 % of the samples. The level of *S. aureus* more than 10^5 , 10^6 it was occur to 12 samples or 10 %. The 12th samples belong to the cheeses samples produced by raw milk. Illness is caused by the toxin which *S. aureus* has produced in the foodstuff. In order to produce detectable levels of toxin, the number of organisms must be over 10^5 - 10^6 per gram of product. The time between ingestion of the toxin and the symptoms is only two to five hours and depends on the amount and type of food and the state of health of the person[4]. Refer to the national and European legislation [3] 10 % of the product did not comply with food hygiene criteria. If the criteria are not met, the batch of product is not to be placed on the market. Products that have been placed on the market must be withdrawn or recalled.

4. Conclusions

The study conducted regarding *S. aureus* in white cheese and the results obtained, underline the food safety risks in milk industry resulting from staphylococcus and thus from enterotoxins. The safety of cheese should consider priority from competent authority, and emphases would be placed in animal health, mastitis control, the hygiene of the milking, the proper cooling and storing. The staffs who work in dairy plant must be trained concerning hygienic applications. The equipment used in production must be cleaned and disinfected properly after the production. The pasteurization process of milk used in cheese production should be under control. If raw milk is used for cheese production, it should be of very high quality, and must be declare to the label. Food laboratories must examine for *S. aureus* coagulase-positive but also for it enterotoxin.

6. References

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