MONITORING THE PRESENCE OF STAPHYLOCOCCUS COAGULASO POSITIVE IN SHARRI CHEESE DURING THE TRADITIONAL RIPENING

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Abstract

Sharri cheese is a farming traditional product of Sharra region. Sharri cheese is prepared from sheep milk. While the chemical and physical aspect of this type of cheese is already completed the aspect of safety is much less studied. The safety of Sharra cheese may be compromised because it is produced from unpasteurized sheep's milk. *Staphylococcal* food poisoning is one of the most common food-borne diseases worldwide resulting from the ingestion of *Staphylococcal enterotoxins* preformed in food by enterotoxigenic strains of coagulase positive Staphylococci, mainly *S. aureus. Staphylococcus coagulase positive* is considered one of the most problematic bacteria presented in sheep milk. If it is presented in milk in a certain level has the ability to produce *Staphylococcal enterotoxins* (SE). The milk contaminated with these enterotoxina can cause foodborne intoxication, in consummators. Taking in consideration the lack of this information in my country is considered of great value the conclusion released from this study. The study was performed on cheese and not on the raw milk. The test for the thermostable thermonuclease (TNase) was conducted to detect the potential presence of thermostable thermonucleases (TNase). The data performed that *Staphylococcus coagulase positive* was not presented in cheese. Although the results and conclusions achieved from this study are of great importance not only for this scientific research but also for public health. Taken together, this study should lead to better control and a subsequent reduction of Staphylococcal food poisoning outbreaks.

Key words: traditional cheese, unpasteurized milk, sheep's milk, *Staphylococcus coagulaso positive.*

1. Introduction

*Staphylococcal foodborne intoxica*tion, occurs after ingestion of food contaminated with Staphylococcal *enterotoxins* (SE). This intoxication involve some typical symptoms such as vomiting and diarrhea, caused mainly from enterotoxinogenic coagulase positive strains *of S. aureus.* Staphylococcal foodborne intoxication is reported to be one of the most common bacterial foodborne outbreak in many countries. Dairy products are considered very important in charged food because they constitute 1 – 9 % (mean 4.8 %) of S. *aureus* outbreaks in Europe. SE is considered slightly, inactivated during cheese processing, storage, or during cooking the cheese in the kitchen. Therefore, enterotoxinogenic Staphylococci strains are capable to grow in cheese at a high level (more then 10^5 to 10^6 cfu/g or /ml). The Community legislation in force for milk and milk products (Council Directive 92/46/EEC) lays down criteria's for *S. au*reus in raw milk, cheese, milk powder and frozen milk products. Cheese is a good substrate for growth of *S. aureus*. Such product is involved in foodborne diseases due to: the occurrence of coagulase-positive staphylococci in raw milk; cross-contamination during the process; the possible cross-contamination thereafter. However, the number of *S. aureus* is not always a good indicator for the presence of Staphylococcal enterotoxins in milk product. As *Staphylococcal enterotoxins* are heat stable, they may be present in food when *S. aureus* are absent [2]. Moreover, not all strains of *S. aureus* are enterotoxigenic. Therefore, a conclusive staphylococcal food poisoning diagnosis is mainly based on the detection of *Staphylococcal enterotoxins* in food. Unpasteurized milk and cheese are typical dairy products often charged as the cause of foodborne outbreaks from *Staphylococcal enterotoxins* (SE). The symptoms for SE intoxication include nausea, vomiting, abdominal pain and diarrhea, but not rare they are accompanied with headache and blood pressure drop. The processing of foods such as heating, can reduce the presence of *S. aureus* but it is necessary to take in consideration that

the number of S. aureus colony count is not a real indicator hazard because the toxins are much more heat tolerant than *S. aureus*. Such situations may require testing for SE, which is expensive. While this may be justifiable in cases of foodborne intoxication, it may be not essential for routine quality control purposes. Screening food for thermonuclease (TNase) is an indicator of Staphylococcal growth at high levels.

Table 1: Factors affecting growth and enterotoxin production by *S. aureus*.

	Organism growth		SE production	
Factor	**Optimum**	**Range**	**Optimum**	**Range**
Temperature	37	7-48	40-45	10-48
pH	6-7	4-10	7-8	4-9.6
Water activity (a_w)	0.98	0.83- >0.991	0.98	0.85->0.992
NaCl (%)	0	0-20	0	0-10
Atmosphere	Aerobic	Anaerobic-aerobic	Aerobic (5-20% dissolved O_2)	Anaerobic – aerobic

1 Aerobic (anaerobic 0.90 – > 0.99)
2 Anaerobic (anaerobic 0.92 – > 0.99)

2. Material and methods

20 samples were taken from the cheese produced in traditional terms. Analysis were conducted at the Institute of Public Health in Skopje and Faculty of Food Technology and Nutrition in Gostivar. Parameters analyzed were:

Microbiological – *Aerobic mesophylic bacteria* (ISO 4833); *Staphylococcus coagulaso positive* (ISO 6888-1; TNase test; SE test Vidas)

Physical- Chemical: Acidity SH; pH, Moohr method- %NaCl, %NaCl in brine, water activity – a_w meter Testo 650, temperature and humidity of environment – dataloger Testo – 157-H2.

The first group consisted of 5 samples from lots of cheese produced in traditional terms. The performed analyses were microbiological and physical-chimical tests. The above analysis were performed every two week from the same lot of cheese in order to evaluate the reaction of physical and chemical parameters (such as pH,% NaCl, a_w, temperature, etc) over *Aerobic mesophilic bacteria* and potential *Staphylococcus coagulaso positive* strains in cheese. In each term were analysed 5 samples. The samples were sent to the above mentioned laboratories, maintaining aseptic situation and cooling condition.

In our study we took in consideration that enterotoxinogenic staphylococci must reach levels of at least 10^5 to 10^6 cfu/g or ml to produce detectable amounts of SE. Under optimum conditions (incubation in Brain Heart Infusion broth in pure culture) found SE when *S. aureus* was grown to populations of $\geq 5 \times 10^6$/ml. [1] Thus, it can be concluded that a minimum of 10^6 cfu *enterotoxinogenic S. aureus*/g or ml are needed to produce detectable amounts of SE. Since SE are more stable compared to *S. aureus* bacterial cells, it is possible to test a product with negative results for *S. aureus* counts although SE exists in the products.

3. Results and discussion

The results were presented in table 2 were presented the data performed from microbiological analysis, while in table 2 were performed the data of physical-chemical analysis.

Sharri cheese is a fast fermented cheese reaching pH of 5.5 within 3-4 h and pH 4.6 within 72 h.

The average pH of our samples at the first test was 4, 72 (27.09.2011). As it can be see from the first test the acidulant has an influence on the minimum pH not allowing growth (table no 2) of *Staphylococcus coagulaso positive*. Most staphylococcal strains grow at pH values between 4 and 10, with the optimum being 6 – 7 (Table 1).

With regard to *staphylococci* the water activity (a_w) is of great importance because these bacteria are able to grow over a much wider a_w range than other pathogens. As it can be seen from the table 3 the bacteria cannot grow at a_w of 0.880 (average value on table no 3). The a_w conditions for SE production are somewhat different than that for growth depending on the type of toxin. Important factors affecting growth and SE production are also the humectant used to lower the a_w, the pH, the atmospheric composition as well as the incubation temperature. Related to our results (table 2) it is evident that even in the first samples there is not suspected colony's of *Staphylococcus coagulaso positive*. In a study [2] resulted that inoculation of ripened feta cheese with *S.*

aureus did not result in enterotoxin production and *S. aureus* counts fell rapidly.

S. aureus grows between 7 and 48°C, temperature being optimal at around 37°C (Tab 1). The effect of temperature depends on the strain tested and on the type of the growth medium. In an extensive study [3] using 77 strains isolated from different foods the optimum growth temperature was generally without much deviation within the range of 35 to 40°C. The minimum temperatures for SE production varied quite irregularly over a broad range within 14 and 38°C, and the maximum temperatures from 35 to 38°C and 45°C. The results of our study that there is not suspected colony's of *Staphylococcus coagulaso positive (table* 2) are in good agreement with data from the literature compiled by [3].The Sharri cheese lots were maintained in rooms with temperature between 4-8^{0}C, deemed inappropriate for increasing *Staphylococcus coagulaso positive* strains.

Thermal stability of SE is influenced by the nature of the food, pH, presence of NaCl etc, and the type of toxin. If SE is not completely inactivated by heat reactivation may occur under certain circumstances like cooking, storage or incubation [4]. Samples do not have presence of *Staphylococcus coagulaso positive* for all the dates of measurements.

Table 2: Results of Microbiological analysis for aerobe mesophilic bacteria in different dates

Sample	**27.09. 2011**	**12. 10. 2011**	**27. 10. 2011**	**12. 11. 2011**
Dilution	10^{-1}	10^{-1}	10^{-1}	10^{-1}
X_1 CFU/g	52	34	30	17
X_2 CFU/g	45	26	26	15
X_3 CFU/g	56	30	16	20
X_4 CFU/g	64	44	28	18
X_5 CFU/g	78	49	32	25
Average	59	37	26	19

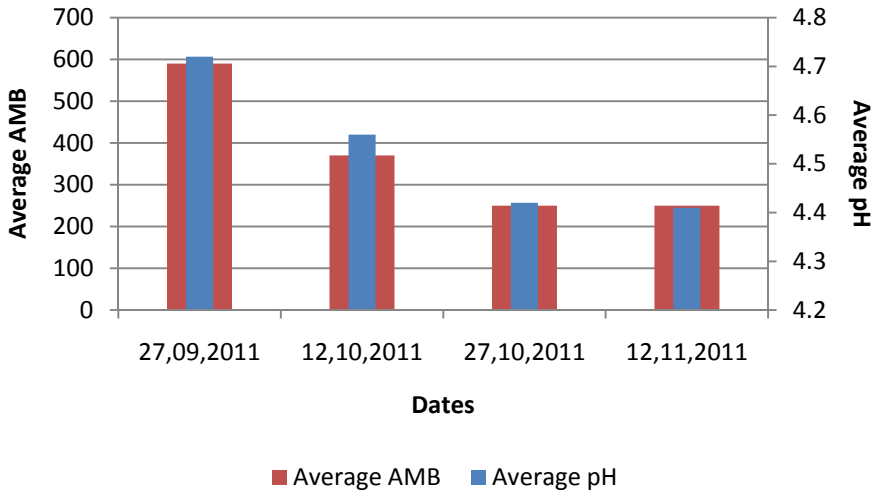

Figure 1: The average values of pH and of aerobe *mesophilic* bacteria during the repining stage of Sharri cheese

Table 3: Average values of physical - chemical parameters of 4 measurements

pH	**Acidity of cheese °SH**	**a_w**
4,52	99,26	0,86

There were slight differences in physical-chemical parameters, pH values were decreased, acidity increased and a_w decreased. In Table 3 are presented the average values of physical - chemical parameters of 4 measurements.

We have presented in two graphs the obtained results. In the following figures (Figure no 1 and figure no 2) is presented the change of the pH values and the number of *aerobe mesophilic bacteria* during the repining stage of Sharri cheese.

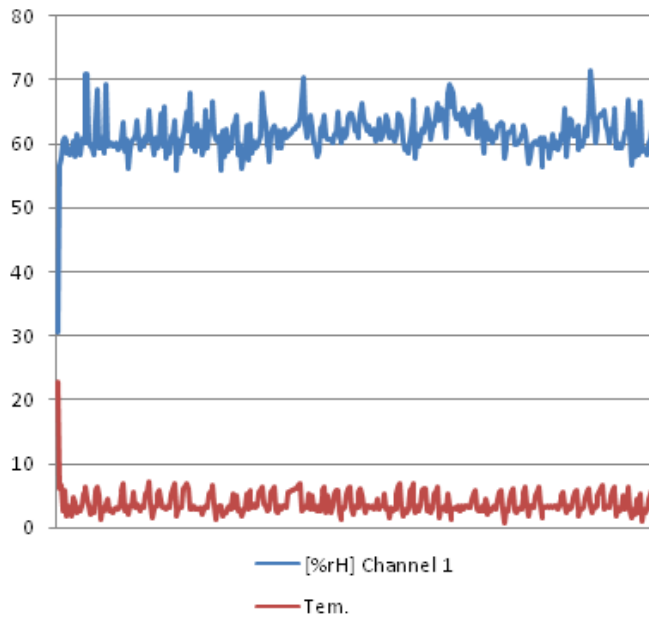

Figure 2: Graphical representation of temperature and relative humidity during ripening of cheese

Data logger for measuring humidity and temperatures in the ripening environment of cheese is set from 27.09.2011 to 12.11.2011. The measuring were programmed in every 3 hours.

4. Conclusion

Based on the identification of Sharri cheese processing conditions, analytical methods available and evaluation of current criteria we can conclude:

1. Sharri cheese meets the microbiological criteria for the analyzed microorganisms (*Aerobe mesophilic bacteria* and *Staphylococcus coagulaso positive)*;
2. The stage of Sharri cheese ripening ends in 45 days;
3. The number of *Aerobic mesophilic bacteria* has a small decrease change after 45 days;
4. In Sharri cheese produced in traditional way due to the extrinsic and intrinsic factors doesn't exist *Staphylococcus coagulaso positive* strains growth.
5. NaCl concentration at the final product, (4,93%) is higher than the Regulation requirements (3%).

6. Literature

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