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SHORT COMMUNICATION

Staphylococcal enterotoxin production in model samples of milk and fresh cheese

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Summary

Goats' milk, ewes' milk, cows' milk and fresh cheese made from different milk types was inoculated with a *Staphylococcus aureus* strain. Growth of *Staph. aureus* was quantified by plate method on Baird-Parker agar and staphylococcal enterotoxin C production was determined by enzyme-linked immunofluorescent assay (ELFA) using an automated miniVIDAS instrument (BioMérieux, Marcy l'Étoile, France). The influence of inappropriate storage conditions (15 °C, 22 °C) and milk type on growth of *Staph. aureus* in dairy products were evaluated. Results of the model experiments linked the dependence of *Staph. aureus* multiplication and subsequent production of staphylococcal enterotoxins to the storage temperature and type of milk. In fresh cheese and in raw milk stored at 15 °C, no enterotoxin production was detected during the entire storage period. It is noteworthy that, in raw milk and fresh cheese, *Staph. aureus* growth and production of enterotoxins can be suppressed by competitive microflora. With the other samples, enterotoxin was detected within the first 24 h or 48 h of storage. Results of our study underscore the importance of cold chain maintenance from production through the retail sale to ensure safety of dairy products.

Keywords

Staphylococcus aureus; raw milk; pasteurized milk; fresh cheese

Staphylococcus aureus is considered the world's third most important cause of food-borne illnesses and it is the major causative agent of mastitis in cows [1]. Therefore, milk and dairy products may pose a risk to consumers. The products contaminated by *Staph. aureu*s are often the cause of food-borne intoxications due to the production of enterotoxins by the bacterium. Twenty-two types of staphylococcal enterotoxins (SE) designated with letters A–V are currently known [2]. Staphylococcal enterotoxicosis is a food-borne illness caused by SE, which is characterized by a rapid onset and course. The first symptoms of intoxication, such as vomiting, headache, abdominal pain and diarrhoea, develop as early as one to six hours after the consumption of food contaminated with SE. The symptoms resolve spontaneously within 24–48 h [3].

To ensure the food safety, to protect the consumers' health and to reduce as much as possible the risk of staphylococcal enterotoxicosis, Commission Regulation (EC) No 2073/2005 [4] lays down the requirements for the detection and enumeration of coagulase-positive staphylococci in cheeses (including ewes' milk and goats' milk cheeses). Staphylococcal enterotoxins are taken as prevented when the counts of coagulase-positive staphylococci in such products are lower than 10^5 CFU·g^{-1}. NECIDOVÁ et al. [5] found *Staph. aureus* in the range from 10^3 CFU·g^{-1} to 10^5 CFU·g^{-1} to be able to produce enterotoxins in quantities that may pose a health risk to the consumer.

The study objectives were to detect staphylococcal enterotoxins in raw and pasteurized goats' milk, ewes' milk, cows' milk and in fresh cheeses made from these types of milk in various storage conditions.

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MATERIAL AND METHODS

The *Staph. aureus* strain producing enterotoxin C (CCM 5971) was obtained from the Czech Collection of Microorganisms (Brno, Czech Republic). This strain was inoculated to raw and pasteurized goats', ewes' and cows' milk at levels from 4.78 log CFU·ml^{-1} to 5.00 log CFU·ml^{-1} after ruling out the presence of *Staph. aureus* in these samples. This strain was also inoculated to goats', ewes' and cows' fresh cheeses at levels from 5.23 log CFU·g^{-1} to 5.56 log CFU·g^{-1}. Inoculation was carried out in the form of a suspension. The inoculated model samples were stored at 15 °C (a temperature previously reported to be suitable for the production of SE). According to Valík et al. [6], this temperature was selected for its correspondence with the ewes' cheese ripening temperature. Another used storage temperature was at 22 °C to simulate poor temperature conditions during storage and transport. The enumeration of *Staph. aureus* and detection of enterotoxin during the storage were performed in 24-hour intervals for 7 days for milk, and on days 3, 7, 10 and 16 for cheeses. The plate method using Baird-Parker agar according to ČSN EN ISO 6888-1 [7] was used. The plates were incubated at 37 °C for 24 h. The automated mini-VIDAS instrument (BioMérieux, Marcy l'Étoile, France) was used for the detection of SE by enzyme-linked immunofluorescent assay (ELFA). This instrument was able to detect the sum of staphylococcal enterotoxins A–E with the detection limits of 0.5 ng·g^{-1} or per millilitre of food for staphylococcal enterotoxins A and B, and of 1.0 ng·g^{-1} or 1.0 ng·ml^{-1} of food for staphylococcal enterotoxins C–E. As the ELFA technique does not facilitate quantification of SE, the results are expressed as either positive or negative.

RESULTS AND DISCUSSION

Milk

Raw and pasteurized milk was inoculated with *Staph. aureus* at levels from 4.86 log CFU·ml^{-1} to 5.00 log CFU·ml^{-1}, and at levels from 4.78 log CFU·ml^{-1} to 4.98 log CFU·ml^{-1}, and incubated at 15 °C and 22 °C, respectively (Tab. 1 and Tab. 2).

In raw milk of any species stored at 15 °C, no enterotoxin production was detected during the whole 7-day storage period (Tab. 1). In raw ewes' milk samples stored at 22 °C, enterotoxin production was detected within 48 h, while in raw goats' and cows' milk, enterotoxin production was detected already after 24 h of incubation.

Pasteurized milk was apparently a good substrate for enterotoxin production. At 15 °C, staphylococcal enterotoxin C (SEC) was detected within 48 h of incubation in ewes' and goats'milk, and within 24 h of incubation in cows' milk. At 22 °C, enterotoxin production was detected as ear-

Tab. 1. Growth dynamics of *Staph. aureus* and staphylococcal enterotoxin C production in milk during storage at 15 °C.

Milk storage time [h]	*Staph. aureus* counts [log CFU·ml^{-1}]					
	Raw milk			Pasteurized milk		
	Cows'	Goats'	Ewes'	Cows'	Goats'	Ewes'
0	4.86	4.86	5.00	4.98	4.78	4.96
24	5.28	4.98	4.98	6.68*	5.34	4.84
48	5.08	5.11	5.04	7.65*	5.89*	5.62*

* – staphylococcal enterotoxin C was positively detected during storage.

Tab. 2. Growth dynamics of *Staph. aureus* and staphylococcal enterotoxin C production in milk during storage at 22 °C.

Milk storage time [h]	*Staph. aureus* counts [log CFU·ml^{-1}]					
	Raw milk			Pasteurized milk		
	Cows'	Goats'	Ewes'	Cows'	Goats'	Ewes'
0	4.86	4.86	5.00	4.98	4.78	4.96
24	6.41*	7.23*	6.18	9.70*	7.08*	6.40*
48	6.32*	6.61*	7.23*	8.52*	7.04*	7.36*

* – staphylococcal enterotoxin C was positively detected during storage.

Tab. 3. Growth dynamics of *Staph. aureus* and staphylococcal enterotoxin C production in fresh cheeses during storage at 15 °C and 22 °C.

Storage time [d]	*Staph. aureus* counts [log CFU.ml^{-1}]					
	Storage temperature 15 °C			Storage temperature 22 °C		
	Cows' cheese	Goats' cheese	Ewes' cheese	Cows' cheese	Goats' cheese	Ewes' cheese
0	5.56	5.36	5.23	5.54	5.36	5.23
3	5.56	5.41	5.36	5.53	5.45	5.73
7	5.34	4.48	5.16	3.70	4.79	4.77
10	4.08	4.66	5.23	2.26	4.46	4.34
16	2.48	4.57	4.89	2.26	5.53	5.95

No staphylococcal enterotoxin C was detected during storage.

ly as within 24 h of incubation.

Results presented in Tab. 1 and Tab. 2 give *Staph. aureus* counts and presence of SEC at the time of the first detection of SEC. Only the first 48 h were recorded, because SEC production began during this period and SEC remained present through the end of our experiment. In both raw and pasteurized milk, these counts were always higher than 5.00 log CFU·ml^{-1}.

Trnčíková et al. [8] isolated *Staph. aureus* strains from ewes' milk products and another food produced in Slovakia and Slovenia, and they found that 37% of the examined products contained at least one of the genes encoding for staphylococcal enterotoxins A–D. In bryndza cheese, SEC was found. Medveďová et al. [9] studied growth of *Staph. aureus* and staphylococcal enterotoxin D (SED) production in milk at temperatures from 12 °C to 21 °C. SED was detected at the level of *Staph. aureus* 10^6 CFU·ml^{-1} at the lower temperature of 12 °C. At the higher temperatures of 18 °C and 21 °C, SED was detected when *Staph. aureus* reached the counts of 10^7 CFU·ml^{-1}. The presence of *Staph. aureus* in raw goats' milk and goats' cheese was also studied by Valihrach et al. [10]. Positive detection of *Staph. aureus* in pasteurized goats' milk was rare. SEC producers were described as the most prevalent enterotoxin-producing *Staph. aureus* isolates from raw goats' milk cheese and the goat udder skin, teats and milk [11].

The fact that the growth of *Staph. aureus* in raw milk was clearly lower in comparison with pasteurized milk, and that no SE production was detected in it, can be explained, according to Charlier et al. [12], by the natural microflora of raw milk that can inhibit *Staph. aureus* growth and thus also SE production. An important part of this natural microflora are lactic acid bacteria that reduce pH of milk. Another basis for this effect may be the fact that several strains of *Lb. lactis* produce the bacteriocin nisin, which is known to be inhibitory against *Staph. aureus* [13].

Fresh cheese

Production of SEC in fresh (goats', ewes', cows') cheeses inoculated with *Staph. aureus* was not detected in our experiment during 16 days (Tab. 3).

In the production of fresh cheese, starter cultures are crucial for proper fermentation and provide a competitive environment for pathogens. Study of Rola et al. [14] revealed that domestically produced cheese made from raw milk did not always meet the production hygiene criteria. *Staph. aureus* was detected in 77.8% of the raw milk cheeses. Over a half (56%) of the final products tested in their study were contaminated with *Staph. aureus* at levels ≥ 10^5 CFU·g^{-1}, with the highest counts of 2.6×10^7 CFU·g^{-1}. However, no SE were detected.

Results of our experiment, which used the same methodology as Medveďová et al. [15], confirmed the influence of the addition of a starter culture on the growth of *Staph. aureus* and on enterotoxin production. This effect may based on the production of bacteriocins by lactic acid bacteria, some of which may have anti-staphylococcal activity [16].

CONCLUSION

With food-borne intoxication risk heightened by the production of staphylococcal enterotoxins, monitoring of *Staph. aureus* and staphylococcal enterotoxins is a crucial step in food quality and safety control. The results of our model experiments indicate that *Staph. aureus* multiplication and subsequent staphylococcal enterotoxin pro-

duction depend on the storage temperature and type of milk. With fresh cheese, SEC was not detected during 16 days of storage at 15 °C or 22 °C. It is noteworthy that, in raw milk, *Staph. aureus* growth and SEC production were suppressed by competitive microflora. Nevertheless, this finding should not be used as an argument to encourage consumption of raw, unpasterized milk. A limit count of 10^5 CFU·g^{-1}, laid down by regulation EC No. 2073/2005 [4] as a prerequisite for the production of SE, was confirmed by our experiments. The results of our study re-inforce the importance of maintaining the cold chain (at a safe temperature below 8 °C, which is the maximum temperature for storage, transport and retail display of fresh cheese pursuant to Regulation No 77/2003 [17]) from production to retail sale, in order to ensure safety of milk and dairy products.

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