The growth of *Staphylococcus aureus* and enterotoxin SEA production in meat products

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Abstract

The aim of this study was to determine the number of coagulase-positive staphylococci (*Staphylococcus aureus*) using the automated TEMPO STA system on samples of the Vysočina-type of heat-treated dry salami stored at 2 different temperatures and to detect the presence of SEA with the MiniVIDAS device. These samples were inoculated with a known strain of *S. aureus* that produces SEA (representing a potential secondary contamination of the meat product). A first sensory change (change of smell) was recorded for the samples stored at room temperature on day 5 of incubation. The production of SEA was recorded on day 4, the same day on which the highest number of *S. aureus* ($3.1x10^8$ CFU·g^{-1}) was determined. The *S. aureus* count in samples stored at a deliberately chosen higher refrigerator temperature (around 11 ºC) showed no significant changes. The highest number was recorded on days 3 and 4; production of the toxin was not recorded during the entire storage period. In addition, the samples of salami stored at cooler temperatures showed no sensory changes. Our results show that meat products, especially in cases of improper storage, can be a good medium for the growth of *S. aureus* and toxin production, which can cause food-borne intoxication in consumers.

TEMPO STA, VIDAS, ELFA, salami, food

Introduction

Staphylococcus aureus is a major pathogen that produces a whole range of toxic substances capable of causing a variety of diseases. From a food microbiological point of view, the most important characteristic of *S. aureus* is its production of thermostable enterotoxins (SE) that cause food-borne intoxication. At the present time, *S. aureus* is one of the most frequent agents of food-borne intoxication (Martín et al. 2003; Normano et al. 2005). Among foods that are most often contaminated are a range of meats and meat products (Balaban and Rasooly 2000). There is a particular risk of SE production when these food products are improperly stored, as toxins are not produced at temperatures below 10 °C. This also depends, of course, on the particular composition of the product, especially its pH a_w and concentration of salt (Roberts et al. 1996).

The amount of SE necessary to cause illness in people is not exactly known. Balaban and Rasooly (2000) and Omoe (2002) have indicated 100 mg of enterotoxin type A (SEA) as a minimum toxic dose. Nevertheless, it also depends on an individual's sensitivity and body weight. SEA is considered to be the most frequently occurring SE responsible for causing staphylococcal enterotoxicosis. An *S. aureus* count higher than 10^5 CFU/g^{-1} or·ml^{-1} of food is able to produce a quantity of enterotoxins that may cause foodborne intoxication (Balaban and Rasooly 2000; Ercolini et al. 2004). Staphylococcal enterotoxicosis has a very rapid onset and course. The first symptoms of intoxication (vomiting, headache and stomach ache and sometimes diarrhoea) appear as early as 1 to 6 hours after consuming

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food containing SE (Atanassova et al. 2001; Loir et al. 2003). The course of the disease is more serious in older people and only in rare cases it results in death from associated complications (Balaban and Rasooly 2000).

The goal of this work was to determine the coagulase-positive staphylococci count (*Staphylococcus aureus*) using the automated TEMPO STA system in samples of sliced Vysočina-type salami. The samples were inoculated with a known *S. aureus* strain that produces SEA (representing a possible secondary contamination of the meat product) and were then subsequently stored at 2 different temperatures. Along with determining the count, the presence of SEA was also detected in the samples using the ELFA (MiniVIDAS) method.

Materials and Methods

Samples of sliced salami obtained from the market were prepared for the actual experiment in the following manner: samples (n =16, weighing 10 g each) were made from a composite sample. These samples were inoculated with a suspension prepared from a known SEA production strain of *S. aureus* so that the resulting sample contained approximately 10^2 CFU/g. A control sample was concurrently prepared in order to study possible contamination before inoculation. The strain of *S. aureus* used was initially isolated from a meat product and was kept in the micro-organism collection of the National Institute of Public Health in Brno at a temperature of -75 °C in a glycerin medium. After a period of 7 days, half the samples (n=8) were kept at a refrigerator temperature ($t_{min.}$ = 10.8; t_{max} = 12.5 ºC) and the second half (n=8) at room temperature (t_{min} = 22.5; t_{max} = 25.9 ºC). The refrigerator temperature was deliberately chosen higher in order to imitate possible inappropriate storage within a household. The *S. aureus* count was determined every day in one of the samples and the presence of SEA was monitored as well. All of the samples were prepared and examined in duplicate. Selected physical parameters were established in the sample of salami, which were a_w = 0.926, pH = 6.12, dry matter content = 37.52% and salt content 3.8%.

To determine the *S. aureus* count, a fast modern method was used – TEMPO STA using the TEMPO testing system (bioMérieux, France). The TEMPO STA test is an automated test based on the enzymatic principle. It is used for the direct determination of the coagulase-positive staphylococci count (*S. aureus*) in food. The result is obtained on the principle of the most probable number method (MPN). The presence of SEA was detected with the aid of the MiniVIDAS testing unit (bioMérieux, France). It is a fully automated system based on the enzyme-linked fluorescent assay (ELFA).

Results and Discussion

On the basis of established *S. aureus* counts it was possible to create the growth curves provided in Figure 1.

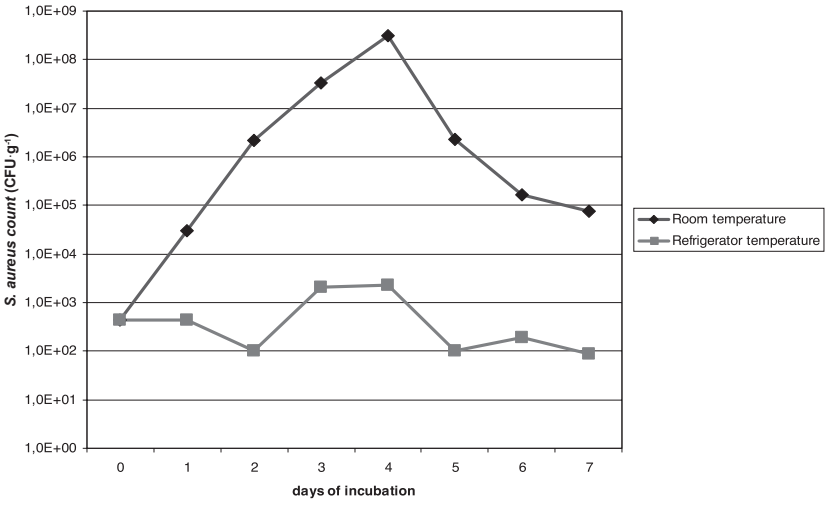

Fig. 1. Growth of *S. aureus* at room temperature and refrigerator temperature

The samples that were stored at room temperature showed their first sensory changes (change of smell) on day 5. The production of SEA, however, was recorded as early as day 4, which was also when the highest *S. aureus* count was recorded ($3.1x10^8$ CFU·g^1); i.e. on the day before there were any sensory changes in the product. In the following days the *S. aureus* count decreased, though the presence of enterotoxins was recorded. This is consistent with findings showing that the determined *S. aureus* count in the final product is only indicative information, which does not necessarily correlate with the quantity of staphylococcal enterotoxins. The *S. aureus* count at refrigerator temperature did not show any significant changes. The highest counts were recorded on day 3 ($2.1x10^3$ CFU·g^{-1}) and day 4 ($2.3x10^3$ CFU·g^{-1}). The production of toxin was not recorded during the storage period and no sensory changes presented themselves during that time. On the basis of the results, it can be confirmed that though *S. aureus* is capable of slow growth even under refrigeration, the production of SE is significantly limited, and not only at temperatures of around 7-10 °C, which have been confirmed by previously published studies (Schmitt et al. 1990; Roberts et al. 1996), but also at higher temperatures; as in our case when temperatures reached a high of 12.5 °C. At proper temperatures of storage there should not be any growth of *S. aureus* nor production of enterotoxins recorded at all (at temperatures around 4 °C), as has been shown by a study by Hallpin-Dohnalek and Marth (1989), not even with an initial inoculation density of 10^6 CFU·ml^{-1} (Anunciacao et al. 1995).

The bacteria *S. aureus* that contaminate food can originate from raw materials, the environment, or food workers (Varnam and Evans 1991). Another potential source is through the inappropriate handling of the products at their place of sale (cutting, packaging and similar activities) (Karpíšková and Gelbíčová 2009). With heat-treated meat products it is assumed that the *S. aureus* contained in the raw material should be devitalized by heat treatment. The presence of this strain of bacteria in finished products sold in retail shops at staffed fresh meat counters (not products packaged directly by the manufacturer) constitutes contamination during the distribution, storage, cutting, portioning and sale of the products. The source of this contamination is primarily the staff and the environment of the shop. According to our results, there is a low risk for the consumer when meat products are properly stored, even in the case of secondary contamination. This risk, however, increases in proportion to the higher temperature of the stored product. According to our results, the sensory changes appeared after the presence of SEA was recorded in the product. This finding is serious because sensory changes do not necessarily alert consumers in time to the fact that the consumption of the product can be dangerous.

Conclusions

Current European legislation does not include any requirements for identifying *S. aureus* in meat products. Our results, however, have shown that meat products may be a good medium for the growth of *S. aureus* and the production of toxins, especially when the products are improperly stored, and may subsequently contribute to health risks to the consumer.

Acknowledgement

The present study was supported by grants VZ MŠMT MSM 6215712402 and project MZe-NAZVA no. QH81111.

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