Staphylococcus aureus Growth and Enterotoxin Production during the Manufacture of Uncooked, Semihard Cheese from Cows’ Raw Milk

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ABSTRACT

Staphylococcus aureus growth and enterotoxin production during the manufacture of model Saint-Nectaire, Registered Designation of Origin Saint-Nectaire, and Registered Designation of Origin Salers cheeses, three types of uncooked, semihard, raw milk cheese, were investigated. Coagulase-positive staphylococci (SC+) grew rapidly during the first 6 h. Between 6 and 24 h, counts increased by less than 0.5 log CFU/ml. Raw milk counts ranged from undetectable (<10 CFU/ml) to 3.03 log CFU/ml. Maximal levels reached in cheese on day 1 ranged from 2.82 to 6.84 log CFU/g. The level of SC+ after 24 h was mainly influenced by the milk baseline SC+ level (correlation coefficient, r > 0.80) but pH at 6 h influenced the SC+ growth observed between 6 and 24 h (r > 0.70). Thus, the initial level of SC+ in raw milk should be maintained below 100 CFU/ml and best below 40 CFU/ml. To limit growth, acidification should be managed to obtain pH values around or below 5.8 at 6 h in Saint-Nectaire cheeses and around or below 6.3 at 6 h in Salers cheeses. Enterotoxins were only detected in two Salers cheeses whose SC+ counts on day 1 were 5.55 log CFU/g and 5.06 log CFU/g, respectively, and whose pH values at 6 h were high (approximately 6.6 and 6.5, respectively).

Staphylococcus aureus is a ubiquitous organism commonly isolated from bulk raw milk and milk of dairy cattle suffering from mastitis (12, 29). Its presence in raw milk is a major concern for the safety and quality of traditionally produced cheeses. S. aureus is reported in France as the most frequent pathogen involved in foodborne diseases associated with raw milk cheese (2). Some S. aureus strains among natural populations may produce staphylococcal enterotoxins (SEs), which have recently been responsible for food poisoning associated with reconstituted milk in Japan (10) and cheese consumption in European countries (6) and Brazil (27). Until 2005, risk assessment relied on coagulase-positive staphylococci (SC+) quantification in cheese at the time of production release. New European standards, gathered together in the so-called hygiene package, applicable as of 1 January 2006 (EC regulation no. 852-853/2004), require that control analyses be performed at the time when the S. aureus count is expected to be the highest, rather than upon production release. This change was based on the fact that SEs may be produced in cheese if sufficient levels of S. aureus were reached during the initial growth phase, even if the S. aureus levels decreased sharply during cheese ripening. The “M-value” above which cheese quality is considered to be defective is 5 log CFU/g. If values above 5 log CFU/g are detected, the cheese batch has to be tested for SEs. Although no simple relationship can be established between S. aureus counts and SE production (15), data on the peak levels of S. aureus reached in cheese are considered critical in assessing the risk of SE production.

The behavior of S. aureus in some cheese varieties has been investigated, most often by inoculating variable levels of S. aureus strains in milk, between 2 and 6 log CFU/ml (1, 9, 13, 18), sometimes focusing more specifically on the behavior during cheese ripening and storage of cheese (4, 5). The first 24 h of the cheese-making process appeared critical for S. aureus growth in a number of raw milk cheeses such as Moroccan traditional fresh cheese (9), semihard cheese (1, 26), and Camembert-type cheese from goats’ milk (18). Enterotoxin production in cheese was most often evaluated in cheese made from milk artificially contaminated with enterotoxin-producing strains (5, 18, 23, 26). SEs were detected in cheese only when the enterotoxigenic strains had reached 107 CFU/g. Very few reports are available on the actual production of SEs in cheese by S. aureus populations naturally present in raw milk.

For each cheese variety, different technological parameters (e.g., starter culture, acidification kinetics, and pressing) are applied during the cheese-making process. Many factors such as NaCl concentration, pH, and temperature affect the growth of S. aureus and enterotoxin production in culture media (7, 8, 30). Meyrand et al. (20) hypothesized that the lower pH recorded during production of lactic cheese in comparison to Camembert-type cheese could explain the disappearance of S. aureus from the former.

Guidelines for herd management and hygiene during milk production have led to reduced S. aureus prevalence.
in raw milk. To go a step further and meet new EU standard requirements, studies are necessary to characterize *S. aureus* growth in each cheese variety and so define the most suitable sampling time for control analyses. This study was aimed at characterizing the growth characteristics and potential for enterotoxin production of *S. aureus* during the manufacture of three types of uncooked, semihard cheeses made from raw milk, including Registered Designation of Origin (RDO) Saint-Nectaire, model Saint-Nectaire cheese, and RDO Salers cheeses.

**MATERIALS AND METHODS**

Cheese manufacturing. All milks used were cows’ raw milk from morning milking, produced in the mid-mountaneous area of Massif Central (France). The milk protein content was approximately 31 g/liter and the fat content ranged between 27.6 and 39.5 g/liter.

**RDO Saint-Nectaire (SN) farm cheese.** A total of eight cheese-making batches from four farm productions were sampled twice at a 3-month interval, in winter and spring, for analysis. Milk was inoculated immediately after milking with a commercial starter culture, varying according to the farm. Raw milk was coagulated by adding calf rennet (27.5 to 30 ml/100 liter). After coagulation, the curd was cut into small pieces (ca. 0.5 cm) and gently stirred, then it was gathered, filled into molds, and pressed for 10 min to remove whey. Salt (30 to 40 g/kg) was added on the surface of each cheese and pressing was completed at 23 to 26°C for 6 h. Farm cheeses were 1.7 kg. Cheeses were ripened at 10 to 12°C for 28 days.

**Model Saint-Nectaire (mSN) cheese.** Twelve model cheeses were prepared from raw milk originating from three farms sampled four times over a 1-month period in the winter. Raw milk was collected from the farms immediately after milking, cooled at 4°C, and transferred to the experimental cheese plant (INRA, URF, Aurillac) within 2 h. Milk was placed into 5-liter vats and reheated to 33°C. The commercial MY800 starter culture (*Streptococcus thermophilus, Lactobacillus delbruekii subsp. lactis, and L. delbruekii subsp. bulgaricus* [Texel, Dangé-Saint-Romain, France]) was used to inoculate each vat (0.6%, vol/vol). Rennet was added at 1.51 g/liter (Beaugel 520 mg/liter of chymosin). Coagulation proceeded for about 45 min and then the curd was cut, pressed, and filled into molds and draining was completed under 3-bar pressure. After 2 h of pressing, 20 g of salt was added to each cheese and pressing was continued for 13 h. Model cheeses were 500 g. Ripening took place at 10°C and 96% relative humidity for 28 days.

**RDO Salers (SA) farm cheese.** A total of 29 cheese-making batches were analyzed. They originated from three farms that were sampled three times over spring and summer and from 20 farms each sampled once during summer. Milk was processed immediately after milking. For 10 cheese-making batches, milk was inoculated with commercial starter culture, varying according to the farm. Raw milk was coagulated by adding calf rennet. After coagulation, the curd was cut into small pieces (ca. 1 cm) and then it was gathered and pressed under an increasing pressure starting from around 1.4 up to 8 bars over about 90 min to remove whey. The dry curd was matured for 18 to 24 h depending on the farm. The curd was milled and salted (2.2 to 2.3% of salt). Further maturation occurred during storage at 16 to 18°C for 2 to 4 h. For each cheese, 42 kg of salted curd was filled into a hoop for pressing. Cheeses were pressed at 12 to 15°C for 48 h and turned several times. Cheeses were ripened during 5 months at 10 to 13°C.

**Milk and cheese samples.** Samples were taken from raw milk, 6-hour cheese, and then from cheese after 1, 30, 90, and 150 days of ripening, with aseptic instruments. Subsamples were stored at 4°C and analyzed within 24 h for SCγ count and the remainder stored at −20°C for further microbiological tests and to investigate enterotoxin production.

**Physicochemical analyses of milk and cheese samples.** Milk and cheese pH were determined with a 926 VTV pH meter with an Ingold 406 MX penetration electrode (Mettler-Toledo S.A., Viroflay, France). Dry matter content was determined according to AFNOR standard NF V04-282 (12/95).

**Microbiological analyses of milk and cheese samples.** Cheese samples were emulsified in sterile phosphate buffer (2% wt/vol) and blended in a stomacher lab blender (Seward Medical, London, UK) for 4 min. The suspension was diluted with Ringer’s solution and examined for microbiological populations by the spread plate procedure. Coagulase positive staphylococci were enumerated on rabbit plasma fibrinogen agar (RPFA) (EN ISO 6888-2) incubated for 24 h at 37°C. Terzagli and Sandine M17 agar medium plates (31) were incubated either at 30°C (mesophilic bacterial populations) or at 42°C (thermophilic bacterial populations). Lactococci were enumerated on Turner-Sandine-Elliker (TSE) agar media with nalidixic acid (32), facultatively heterofermentative (FH) lactobacilli on agar described by Isolini et al. (11) incubated at 37°C for 3 days under anaerobic conditions, dextrane-producing leuconostocs on Mayeux-Sandine-Elliker (MSE) agar with 10% saccharose (16) agar incubated at 30°C for 48 h, enterococci on Slanetz-Barley (SB) agar (28) incubated at 42°C for 48 h, pseudomonads on cetrimide-fucidin-cephalosporin (CFC) agar (17), *Enterobacteriaceae* on violet red–bile–glucose (VRBG) agar (21), and yeasts and molds on oxytetracyclin-glucose agar (OGA) (22). All media were purchased from Biokar Diagnostics (Pantin, France).

**Analysis of enterotoxin production in cheese.** Cheese samples (25 g) were tested for the presence of enterotoxins (A to E) by using the Transia Plate Staphylococcal Enterotoxins (ST 0796, Differchamb, Lyon, France). The tests were performed by the Laboratoire Interprofessionnel d’Analyse Laitières (LIAL)–Massif Central (Aurillac, France) as recommended by the manufacturer. This kit has a detection limit of ca 0.1 ng/g as determined by LIAL. Samples found positive for enterotoxins by the Transia Plate Staphylococcal Enterotoxins were further analyzed by the Agence Française de Sécurité Sanitaire des Aliments (AFSSA, Maisons-Alfort, France) using the SET-RPLA assay (Staphylococcal Enterotoxin Test–Reversed Passive Latex Agglutination; Oxoid, Basingstoke, UK), to determine the type of enterotoxin produced (A to D). Detection limits of this kit were 0.12, 0.07, 0.17, and 0.1 ng/g of cheese for enterotoxins A, B, C, and D, respectively.

**Statistical analysis.** For each variable measured at the different cheese-making stages and ripening times, standard analyses of variance were performed to assess the effect of the cheese-making technology (SN, mSN, and SA cheeses) using Statistica software (Statsoft, version 6, Maisons-Alfort, France). When the differences were significant, a Newman-Keuls test was performed. Statistical correlations were carried out by the Pearson’s correlation coefficient. Considering the correlation (rs > 0.80) between levels of SCγ at 6 h and their initial level in raw milk, the analysis of covariance on repeated measures was performed to assess the
FIGURE 1. Growth of coagulase-positive staphylococci in three types of uncooked, semihard cheeses. Values are the mean of 8 cheese-making batches for farm Saint-Nectaire cheeses (SN, ∆), 12 batches for model Saint-Nectaire cheeses (mSN, *), and 29 batches for Salers cheeses (SA, △).

Effect of the cheese-making technology (SN, mSN, SA cheeses). Ripening times (6 h, 24 h, and 30 days) were used as repeated effect. The number of SC⁺ at the initial sampling time in milk (T₀) and pH were used as covariates in the model. When a significant effect was detected, the least-squares means test was performed to compare the adjusted means of time and cheese-making technology. The mixed procedure of SAS 8.01 was used for these analyses. At 6 h and 24 h of ripening time, a stepwise multiple linear regression was performed for each cheese-making technology to assess the parameters which can explain the number of SC⁺.

RESULTS

Changes in coagulase-positive staphylococci counts.
Cheeses were treated as three groups according to the cheese-making technology. A total of 49 cheese-making batches comprising 8 SN cheeses, 12 mSN cheeses, and 29 SA cheeses were analyzed. Figure 1 and Table 1 show the evolution of SC⁺ counts in the three types of cheeses during cheese making and ripening, as determined on RPF agar. Independent of the cheese type, SC⁺ grew rapidly during the first 6 h (Table 1, delta values), then more slowly up to 24 h, when the population reached a peak. Afterward, population levels either remained stable in SN cheeses or slightly decreased in mSN cheeses over the 30 days of ripening. In SA cheeses, population levels slightly decreased between day 1 and day 30 and markedly decreased further between day 30 and day 150 of ripening. So, the SC⁺ populations were almost at a peak in all 6-h-old cheeses.

Evolution of physicochemical parameters. The pH of all cheeses decreased sharply over the first 24 h (Table 1). At 6 h, the pH values were similar in SN cheeses (average, 5.56) and in mSN cheeses (average, 5.68), but they were significantly higher in SA cheeses with an average at 6.26 (Table 1 and Fig. 2, y axis values). In 24-h cheeses, pH values ranged between 5.10 and 5.42, independent of the cheese type, with average values of 5.24 and 5.19 in SN and SA cheeses, respectively. Similarly, the dry matter content of the cheeses increased rapidly over the first 24 h (Table 1). As early as the 6th hour, the dry matter contents were significantly higher in SA cheeses, ranging between 48.45 and 57.38% with an average of 52.52%, than in SN cheeses where values ranged between 46.43 and 49.58% with an average of 47.69%.

Changes in microbial counts. Principal component analysis of bacterial, yeast, and mold counts data (Fig. 3) showed that cheeses at 24 h were clustered according to

TABLE 1. Counts and growth of coagulase-positive staphylococci, pH values, and dry matter content in raw milk and in two different types of uncooked, semihard cheeses at different times of ripening

<table>
<thead>
<tr>
<th>Cheese type</th>
<th>Time, days</th>
<th>Staph (log)</th>
<th>pH</th>
<th>Dry weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x s t</td>
<td>x s t</td>
<td>x s t</td>
</tr>
<tr>
<td>mSN</td>
<td>0</td>
<td>1.54 0.78 NS</td>
<td>6.70 0.05 NS</td>
<td>12.25 0.28 B***</td>
</tr>
<tr>
<td>SN</td>
<td>0</td>
<td>2.14 0.56 NS</td>
<td>6.56 0.03 NS</td>
<td>11.91 0.43 AB***</td>
</tr>
<tr>
<td>SA</td>
<td>0</td>
<td>1.99 0.77 NS</td>
<td>6.67 0.05 NS</td>
<td>11.62 0.46 A***</td>
</tr>
<tr>
<td>mSN</td>
<td>0.25</td>
<td>4.69 0.62 A*</td>
<td>5.56 0.18 A***</td>
<td>47.69 1.12 A***</td>
</tr>
<tr>
<td>SN</td>
<td>0.25</td>
<td>4.84 0.60 A*</td>
<td>5.68 0.23 A***</td>
<td>47.69 1.12 A***</td>
</tr>
<tr>
<td>SA</td>
<td>0.25</td>
<td>5.49 0.86 B*</td>
<td>6.26 0.22 B***</td>
<td>52.52 1.94 B***</td>
</tr>
<tr>
<td>mSN</td>
<td>1</td>
<td>4.75 0.64 A*</td>
<td>5.20 0.06 A**</td>
<td>51.26 2.47 A**</td>
</tr>
<tr>
<td>SN</td>
<td>1</td>
<td>5.10 0.75 AB*</td>
<td>5.30 0.07 B**</td>
<td>50.85 1.12 A**</td>
</tr>
<tr>
<td>SA</td>
<td>1</td>
<td>5.59 0.87 B*</td>
<td>5.19 0.09 A**</td>
<td>54.40 2.16 B**</td>
</tr>
<tr>
<td>mSN</td>
<td>30</td>
<td>4.20 1.12 NS</td>
<td>5.12 0.03 A**</td>
<td>51.91 2.15 A**</td>
</tr>
<tr>
<td>SN</td>
<td>30</td>
<td>4.99 0.83 NS</td>
<td>5.21 0.09 B**</td>
<td>53.27 2.20 A**</td>
</tr>
<tr>
<td>SA</td>
<td>30</td>
<td>4.87 1.11 NS</td>
<td>5.22 0.06 B**</td>
<td>59.55 0.94 B**</td>
</tr>
<tr>
<td>SA</td>
<td>90</td>
<td>2.24 1.42 NS</td>
<td>5.34 0.06 NS</td>
<td>59.71 0.75 NS</td>
</tr>
<tr>
<td>SA</td>
<td>150</td>
<td>1.41 0.47 NS</td>
<td>5.37 0.08 NS</td>
<td>60.30 0.92 NS</td>
</tr>
</tbody>
</table>

a Values are the mean of 8 cheese-making batches for farm Saint-Nectaire cheeses (SN), 12 batches for model Saint-Nectaire cheeses (mSN), and 29 batches for Salers cheeses (SA). Counts in log CFU per milliliter for milk or log CFU per gram for cheese.

b Delta at 6 h: log count at 6 h − log count in milk (time 0); at day 1: log count at day 1 − log count at 6 h, etc.

c At each time, letters A, B, and C in the same column indicate homogeneous statistical processing groups that were significantly different according to the Newman-Keuls statistical test, with A < B < C, *, P < 0.05; **, P < 0.01; ***, P < 0.001; NS, not significant.
FIGURE 2. Relationship between growth of coagulase-positive staphylococci over the first 6 h and pH values in cheeses at 6 h. Growth of coagulase-positive staphylococci was expressed as log CFU per gram at 6 h and log CFU per milliliter in milk. Farm Saint-Nectaire cheeses (SN, ×), model Saint-Nectaire cheeses (mSN, ◦), Salers cheeses (SA, △).

the cheese type. Compared to SN and mSN cheeses, SA cheeses were characterized by high counts of mesophilic bacterial populations (M17 agar at 30°C), FH lactobacilli (FH agar), dextran-producing leuconostocs (MSE+), lactococci (TSE agar), and molds (OGA molds). Among the Saint-Nectaire cheeses, SN cheeses were characterized by higher counts in mesophilic bacterial populations (M17 agar at 30°C), enterococci (SB agar), and yeasts (OGA yeasts) compared to mSN cheeses which were characterized by higher counts in Enterobacteriaceae (VRBG agar) and pseudomonads (CFC agar).

Effects of parameters affecting levels of SC+. Coagulase-positive staphylococci numbers in raw milk ranged from undetectable (<10 CFU/ml) to 3.03 log CFU/ml. Within 6 h, curd counts increased by 2.4 to 4.2 log depending on the batch. The highest increase was observed in SA cheeses with an average value of 3.51 log (Table 1, delta values). Between 6 h and 24 h, counts increased by less than 0.5 log independent of the cheese type, except for two batches of mSN and SA cheeses, where increases were 0.7 log and 1.3 log, respectively. The maximal levels reached in cheese on day 1 ranged between 2.82 and 6.84 log CFU/g and were the highest in SA cheeses with an average value of 5.59 log CFU/g (Table 1). From day 30 to day 150, in long-ripened SA cheeses, SC+ counts decreased markedly and final levels ranged from undetectable (<10 CFU/ml) to 2.30 log CFU/ml.

Table 2 indicates that the level of SC+ reached during the first 24 h was greatly influenced by the initial level of SC+ in milk, followed by the cheese type. The level of SC+ in Salers cheeses and Saint-Nectaire cheeses (SN or mSN) differed most significantly at 6 h, slightly so at 24 h, and no longer differed on day 30.

The stepwise multiple regression analysis (Table 3) selected the initial level of SC+ in milk as sole relevant factor to explain the level at 6 h in all cheese types. A strong positive correlation ($r > 0.80, n = 49$) between levels of SC+ at 6 h and their baseline level in raw milk was ob-
served whatever the cheese type (Table 3). All cheeses exhibiting counts below 5 log CFU/g on day 1 were prepared from milk containing less than 1.61 log CFU/ml (41 CFU/ml), except for one milk batch which contained 2.39 log CFU/ml. The SC⁺ count reached in cheese on day 1 was best explained by the interactive effect of the SC⁺ counts and pH values at 6 h (Table 3). So, strong positive correlations (r > 0.70) between pH values in cheese at 6 h and further SC⁺ growth between 6 and 24 h were observed for all cheese types (Fig. 2). More than 80% of the cheeses for which no significant growth was observed between 6 and 24 h (delta ≤ 0.2 log CFU/g) had reached pH values at 6 h below 5.6 in SN and mSN cheeses or below 6.3 in SA cheeses. The higher threshold pH value associated to the slowing down of SC⁺ growth in SA cheeses suggested that other factors may affect SC⁺ growth in SA cheeses. However, no correlation was found between dry matter content of the cheeses and SC⁺ growth at any time (data not shown). In addition, no significant link was established between SC⁺ growth (during the first 6 h or further to 24 h) and the levels of any microbial group at any time (data not shown).

**Enterotoxin production in cheese.** All 49 cheeses on day 1 were analyzed using the Transia ELISA kit to check for the presence of staphylococcal enterotoxins. Two samples originating from SA cheeses and three from mSN cheeses gave positive results. Further analysis by AFSSA confirmed the presence of enterotoxins only in the two SA cheeses. SC⁺ counts on day 1 in the first cheese was 5.55 log CFU/g and it was 5.06 log CFU/g in the second cheese. No enterotoxin was detected in the other 33 SN, mSN, and SA cheeses that had SC⁺ counts above 5 log CFU/g and up to 6.84 log CFU/g. In the first cheese, enterotoxin C was detected below the quantification limit at 6 h and estimated at 3.45 ng/g in cheese on day 1. In the second cheese, no enterotoxin was detected up to 6 h but enterotoxin A was detected below the quantification limit in cheese on day 1. The pH at 6 h was 6.62 in the first cheese, the highest among all cheeses tested, and it was 6.50 in the second cheese.

**DISCUSSION**

Despite major differences in the cheese-making parameters (i.e., addition of starter cultures, pre-pressing, and maturation step), the general behaviour of SC⁺ was similar in the three groups of cheeses. SC⁺ counts dramatically increased from milk to reach their maximal values in 24-

h-old cheeses. Afterward, they either stabilized or decreased markedly in long-ripened SA cheeses. This result is consistent with other studies where *S. aureus* was found to peak within the first days in Saint-Nectaire cheese (19, 26) and within 24 h in lactic cheese, Camembert-like cheese (20), and Reblochon (19). Although part of this increase was due to a concentration effect resulting from the physical entrapment of cells in the curd, our results show that the critical phase of exponential growth of SC⁺ occurs mainly within the first 6 h. In the three cheese types, the initial level of SC⁺ in milk had the strongest influence on the level reached in 24-h-old cheese, but the pH values in cheese at 6 h also contributed significantly to this level. This result was in agreement with those of Lindqvist et al. (15) which showed that the level of *S. aureus* in unripened cheeses at the time of consumption was a function of the initial level in raw milk. The SC⁺ levels found in cows’ raw milk in this study were highly variable but still ranged below the levels most frequently applied in milk inoculation experiments inoculation between 10⁴ and 10⁶ CFU/ml (1, 5, 18). Meyrand et al. (18, 20) studied the development of SC⁺ in raw milk Camembert cheese with slow acidification kinetics. When starting from 4 log CFU/ml, SC⁺ reached 6.9 log after 6 h and 7.48 log after 22 h. When plotted with the data obtained from our results, data from Meyrand et al. (18, 20) at 0 and 6 h closely fitted the linear regression line. However, starting from a higher inoculum (above 5 log CFU/ml), slow growth was observed and the data did not fit in our results anymore (1, 18, 20).

SC⁺ growth was only affected after 6 h of cheese making. The positive correlation found between pH at 6 h and growth of SC⁺ between 6 and 24 h suggested that, in the varieties of cheeses studied with relatively slow acidification kinetics during the first 6 h, the pH did not have any effect on the initial growth phase of *S. aureus* before 6 h but may have had a modulating effect on subsequent growth up to 24 h. These results were in agreement with those of Lamprell (13) who indicated 6 h was the critical time for pH values for the semihard cheeses Cantal and Tomme de Savoie. Dry matter content and microbial counts in cheeses did not seem to influence the slowing down of SC⁺ associated with different pH values in the two cheese varieties. Nevertheless, microbial antagonistic effects on SC⁺ may lie in the diversity of species or strains found on the different culture media rather than in the total population counted on these media.

In the present work, despite the fact that a large frac-
tion of the cheeses contained levels of *S. aureus* above the M-value (5 log CFU/g), SEs were rarely detected. In fact, no simple relationship has been established yet between the bacterial count and the toxin concentration (15). In Saint-Nectaire cheese, after addition of an enterotoxigenic strain at 10^6 to 10^8 CFU/g in raw milk, no production of SE was observed until *S. aureus* levels exceeded 10^7 CFU/g (20, 26). In Manchego cheese, in spite of *S. aureus* populations above 7 log CFU/g, but in the two cases where SE production occurred, not only did they exceed 5 log but pH values of the cheeses at 6 h also exceeded 6.3. The production of enterotoxins is optimal in neutral pH and is usually inhibited at pH values below 5 (14). Three samples from mSN cheeses that gave positive results using the Transia Plate Staphylococcocal Enterotoxins were not confirmed positive by AFSSA using the SET-RPLA assay. False-positive results were noted when using the Transia Plate Staphylococcocal Enterotoxins by Vernozy-Rozand et al. (33) when testing some meats and seafood products and by Lamprell (13) in semihard cheeses.

Staphylococcocal enterotoxigenic (SE) C was produced in larger quantities than SEA and was detected as early as the 6th hour, suggesting it was formed during the exponential growth of the bacteria, while SEA started to be produced between the 6th and the 24th h only. In the surveys by Stephan et al. (29) and Villard et al. (34), SEC-producing strains were most frequently isolated from cows’ milk, followed by SEA and SE. However, SEA and SE would be the most frequently isolated from cheeses implicated in food poisoning (5, 10, 33).

In conclusion, these results provide useful information for the control of *S. aureus* in uncooked, semihard cheeses. They show that the first 6 h of cheese making are critical for SC⁺ development and that any tool to inhibit growth of *S. aureus* must be effective within this time interval. The initial level of SC⁺ in raw milk should be maintained below 100 CFU/ml and best below 40 CFU/ml. Moreover, managing the cheese-making process to obtain pH values within the range acceptable for each cheese type, around or below 5.8 at 6 h in Saint-Nectaire cheeses and around or below 6.3 at 6 h in Salers cheeses, would limit growth and may help controlling enterotoxin production. Inhibitory starters may also be used. Bacteriocin-producing strains of *Lactococcus lactis* were found to reduce levels of *S. aureus* in cheese during ripening (1, 25). However, until now, bacteriocin-producing strains have been of little help to inhibit growth of *S. aureus* in cheese between 0 and 24 h (9), except in an acid-coagulated cheese (24).

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