Behavior of *Staphylococcus aureus* in Cheddar Cheese Made with Sodium Chloride or a Mixture of Sodium Chloride and Potassium Chloride

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**ABSTRACT**

Stirred-curd Cheddar cheese was manufactured from milk artificially contaminated with <1000 *Staphylococcus aureus* cells/ml. Lactic starter culture was added to the milk at the rate of 1.0 or 0.5% (v/v). Curds were divided and salted with either NaCl or a mixture of KCl/NaCl to achieve final salt concentrations of approximately 2.4 or 1.2%. Some portions of curd remained unsalted. Cheeses were analyzed for moisture and salt content and were stored at 4 or 10°C for 8 weeks. Bacterial counts and pH values were determined during manufacture and storage of cheeses. Unsalted cheeses had the lowest and the 2.4%-salted cheese had the highest *S. aureus* counts. Cheeses salted with KCl/NaCl had considerably lower *S. aureus* counts than did cheeses salted with NaCl. All cheeses made with 1.0% starter culture had appreciably lower counts of *S. aureus* than did cheeses made with 0.5% starter culture. Low levels (0.05 to 0.52 ng/g) of enterotoxin A were found in 16 of 17 samples tested with the radio immunoassay procedure. Presence of enterotoxin was not directly associated with the kind or amount of salt used to produce the cheese.

Salt is thought to be a necessary functional component in cheese and some other fermented foods. In addition to influencing flavor, texture and moisture content, the proper salt concentration controls the fermentation, inhibits some spoilage and hazardous microorganisms, and provides a selective environment for desirable microorganisms.

Consumers are becoming more aware of the amount of sodium in their diets. Elevated sodium intake has been clearly linked to increased incidence of hypertension in certain populations. The U.S. Select Committee on Nutrition has also recommended decreasing the amount of sodium chloride in processed foods. The cheese industry is responding to the needs of some consumers by manufacturing some cheeses with no sodium chloride or a substitute for sodium chloride. Lindsay et al. used mixtures of KCl and NaCl to prepare cheese with less than the usual amount of sodium. This cheese rated well in sensory analysis. However, not much has been published on the microbiological safety of such cheeses. This study was undertaken to determine the behavior of *Staphylococcus aureus* in Cheddar cheese made in ways to reduce its content of NaCl.

**MATERIALS AND METHODS**

* Cultures

Three strains of *S. aureus*, A-100, 361, and 196-E, were obtained from M. S. Bergdoll, Food Research Institute, University of Wisconsin-Madison. All strains produce enterotoxin. Cultures were maintained in Brain Heart Infusion (BHI) broth (Difco), and were transferred daily 3 d before use in an experiment. Incubation time was 24 h at 37°C. A sufficient amount of 24-h-old culture of *S. aureus* in BHI broth was added to pasteurized milk to provide a concentration of <1000 cells/ml of milk.

A commercial lactic starter culture (OD) was used to make cheese, and was obtained from the Marschall Division of Miles Laboratories, Inc., Madison, Wisconsin. The starter culture was incubated in previously steamed (100°C, 45 min) reconstituted nonfat dry milk at 37°C for 16-18 h before cheesemaking. A sufficient amount of the resulting starter was used to provide an inoculum of 0.5 or 1.0%.

* Manufacture and sampling of cheese

Stirred-curd Cheddar cheese was made according to the procedure described by Kosikowski. One hundred and ten lb of whole milk (3.5% fat) were pasteurized in a pilot plant-size vat at 62.8°C for 30 min, cooled to 31°C, and inoculated with the lactic starter culture. Five min later the broth culture of *S. aureus*, which had been mixed with approximately 1 L of pasteurized milk, was added to the vat of milk. Marzamie Single Strength Microbial Rennet (Marschall Division-Miles Inc., Madison, WI) was used as the clotting agent. After curd was cut, cooked and drained, it was divided into two or three equal parts. To these portions was added (a) NaCl, (b) a mixture of KCl/NaCl, 1:1 molar basis (Lite Salt®), Morton's Salt Division, Morton-Norwich Products, Chicago, IL) or (c) no salt of either type. Sufficient NaCl or NaCl/KCl was added to appropriate portions of curd to achieve final concentrations of ca. 1.2 or 2.4%. Curd receiving either 1.2% NaCl or NaCl/KCl or no salt was cooked and stirred slightly longer than was curd that received 2.4% NaCl or NaCl/KCl to help control the moisture content of the finished cheese. The curds were hooped and pressed overnight in cylindrical 2-lb hoops placed in a small press equipped with a hydraulic pump. Cheeses were wrapped with plastic film and aluminum foil to prevent...
drying. Duplicate cheeses were ripened at 4 and 10°C. Samples for bacterial enumeration were taken according to the following scheme: (a) milk after pasteurization, (b) milk after addition of lactic starter and S. aureus (0 h), (c) curd after cutting (2 h), (d) whey after cutting of curd, (e) curd after draining (4 h), (f) curd at hooping (6 h), (g) cheese after pressing (0 week) and (h) cheese after 2, 4, 6, and 8 weeks of ripening.

**Enumeration of bacteria**

Samples of cheese used to enumerate bacteria were prepared according to the "alternative" method described in *Standard Methods for the Examination of Dairy Products* (16). This procedure involved weighing 1 g ± 10 mg of cheese into a sterile 6-oz Whirl-Pak bag (Nasco, Fort Atkinson, WI), macerating the contents to a fine paste and adding 9 ml of a sterile aqueous solution of 2% sodium citrate at 40°C. Further mixing of the contents of the plastic bag produced a uniform emulsion, which was subsequently plated or serially diluted in sterile water containing 0.1% peptone. Standard Plate Counts (SPC) were determined with duplicate plates of Plate Count Agar (Difco), as specified in *Standard Methods* (16). Counts of *S. aureus* were determined with duplicate plates using the direct-plating method on Baird-Parker Medium (Difco) as described by Minor and Marth (18). Black shiny colonies were counted after 48 h at 37°C, and some were randomly selected for confirmation as *S. aureus*, using the catalase test (10) and the tube coagulase test (20).

**Determination of moisture, salt, pH and enterotoxin levels**

Duplicate moisture determinations were made on 2-3-g cheese samples by calculating the percentage of weight difference in cheese before and after drying in a forced air draft oven for 16 h at 40°C. Further mixing of the contents of the plastic bag produced a uniform emulsion, which was subsequently plated or serially diluted in sterile water containing 0.1% peptone. Standard Plate Counts (SPC) were determined with duplicate plates of Plate Count Agar (Difco), as specified in *Standard Methods* (16). Counts of *S. aureus* were determined with duplicate plates using the direct-plating method on Baird-Parker Medium (Difco) as described by Minor and Marth (18). Black shiny colonies were counted after 48 h at 37°C, and some were randomly selected for confirmation as *S. aureus*, using the catalase test (10) and the tube coagulase test (20).

**RESULTS**

**Salt and moisture content of cheese**

Results of salt and moisture determinations made in nine cheese trials are in Table 1. Analyses were done on 3-d-old cheeses. Of salted cheese (SC) made from curd with 2.4 or 1.2% added salt, the KCl/NaCl-SC had slightly higher final salt concentrations and slightly lower moisture contents than did the corresponding NaCl-SC. Unsalted cheeses (USC) had an average salt content of 0.21%, presumably from the inherent salts of milk and from unintentional exposure to salted curds during hoop-pressing. The U.S. legal limit for moisture content of Cheddar cheese is 39.00% (13). All cheeses made in these experiments had less than the permitted maximum amount of moisture except for the 1.2% NaCl-SC, which exceeded the limit by an average of 0.04%.

**Changes in pH during manufacture and storage of cheese**

Table 1 also gives the mean pH values after pressing for cheeses in each salt category. In all trials, USC had pH values ranging from 0.11-0.33 pH unit lower than did SC. These low pH values can be attributed to lactic acid produced by an uninhibited population of lactic acid bacteria, whereas SC would have a smaller, salt-inhibited population of lactic acid bacteria and thus a somewhat smaller amount of lactic acid was produced, resulting in a correspondingly higher pH. Accordingly, pH values should decrease as the concentration of salt decreases. Results of preliminary trials in this study and those from other investigators (21,26) show this to be true.

Average pH values of selected cheeses during manufacture and storage are given in the lower portions of Fig. 1-5. The change in pH during manufacture of cheese was essentially normal (Fig. 1-5). Different storage temperatures did not significantly affect pH values of cheese until after the fourth week when, at 10°C, there was a definite increase in pH from 5.19 (week 4) to 5.68 (week 8) (Fig. 1). During this same interval, pH values of cheeses stored at 4°C were nearly stable at about 5.1. The concentration of starter used to make cheese did not seem to affect pH, as the pH curves for cheeses made with either 1.0 or 0.5% starter were similar (Fig. 2). Figure 3 gives the pH values of cheeses made with (a) NaCl, (b) KCl/NaCl or (c) no salt. NaCl-SC had somewhat higher average pH values than did the other cheeses for the time period between 0 and 6 weeks. During this interval, KCl/NaCl-SC and USC had pH values averaging 0.07 ± .02 and 0.15 ± .08 pH unit, respectively, below those of NaCl-SC. These differences narrowed by the eighth week when all three cheese types had pH values in the range of 5.48 to 5.52. Figure 4 gives the average pH values for cheeses representing the three different salt concentrations: 2.4, 1.2 and 0%. Because results of different trials were averaged for each of the three salt concentrations, the pH appears to vary before salt actually becomes a variable (at 6 h). These early deviations are negligible. The 2.4%-SC had pH values near 5.40 throughout the storage period, while pH values of 1.2%-SC and USC were in the ranges of 5.26-5.54 and 5.16-5.51, respectively.

| Table 1. Mean values ± S.D. of salt concentration, moisture content, and pH of experimental 3-day-old Cheddar cheese. |
|---|---|---|---|
| Salt type and amount added to curd | Actual salt concentration (%) | Moisture content (%) | pH after pressing |
| 2.4% NaCl | 1.95 ± 0.11 | 38.25 ± 2.21 | 5.34 ± 0.17 |
| 2.4% KCl/NaCl | 2.08 ± 0.18 | 37.53 ± 1.83 | 5.35 ± 0.13 |
| 1.2% NaCl | 1.90 ± 0.07 | 39.01 ± 0.40 | 5.38 ± 0.13 |
| 1.2% KCl/NaCl | 1.22 ± 0.06 | 38.97 ± 0.52 | 5.30 ± 0.05 |
| 0% | 0.21 ± 0.15 | 36.39 ± 1.15 | 5.17 ± 0.02 |

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Effect of amount of lactic acid starter culture on bacterial growth

Figure 2 shows the difference in average *S. aureus* counts and SPC for cheeses made with 1.0 or 0.5% starter culture. The curves for the SPC were similar regardless of amount of starter that was used. However, in cheeses made with 1.0% starter, the population of non-*S. aureus* organisms (SPC minus *S. aureus* count) was larger by a mean log value of 0.31, as compared to cheeses made with 0.5% starter. *S. aureus* counts obtained during storage from cheeses made with 0.5% starter were nearly a full log cycle greater than were the counts from cheeses made with 1.0% starter. The difference caused by starter concentration was most profound in cheeses that received no salt (Fig. 5). *S. aureus* counts in USC made with 1.0% starter decreased rapidly from a mean log value of 5.73 after pressing to 2.23 after 8 weeks of storage. During this same interval, mean log values of *S. aureus* counts in USC made with 0.5% starter decreased more gradually from 6.68 to 4.46. The overall difference in *S. au-

![Figure 1. Mean changes in pH, SPC, and numbers of *S. aureus* during the manufacture and ripening of artificially contaminated Cheddar cheese as affected by storage at 4 and 10°C.](image)

![Figure 2. Mean changes in pH, SPC, and numbers of *S. aureus* during the manufacture and ripening of artificially contaminated Cheddar cheese as affected by amount of lactic starter culture used to make cheese.](image)
Counts between the two types of USC during storage amounted to a mean log difference of 2.02 ± .63; the USC made with 1.0% starter always had the lower values. KCl/NaCl-SC and NaCl-SC made with 1.0% starter had mean log counts of S. aureus during storage that were 0.90 ± .15 and 0.92 ± .36, respectively, lower than those of S. aureus in corresponding cheeses made with 0.5% starter. This apparent inhibitory effect of increased starter concentrations has been attributed to various properties of the lactic acid bacteria which will be mentioned later.

Response of different S. aureus strains
S. aureus strains 196-E, 361 and 100-A all had the same growth patterns when subjected to the various treatments of these experiments; best survival resulted when NaCl rather than KCl/NaCl was used, the poorest survival occurred when no salt was used. Strains 196-E and 361 were inoculated into milk at a mean log concentration of 2.83 ± .17 and had final (8-week) mean log values in cheese of 6.58 ± .14 for NaCl-SC, 6.04 ± .27 for KCl/NaCl-SC and 4.38 ± .43 for USC. Strain 100-A was used as the inoculum for two trials at a low

Figure 3. Mean changes in pH, SPC, and numbers of S. aureus during the manufacture and ripening of artificially contaminated Cheddar cheese as affected by salt treatment.

Figure 4. Mean changes in pH, SPC, and numbers of S. aureus during the manufacture and ripening of artificially contaminated Cheddar cheese as affected by amount of salt in cheese.
Effect of storage temperature

Figure 1 shows the differences in average bacterial counts between cheeses stored at 4 or 10°C. At 10°C, the SPC peaked after 2 weeks of storage and decreased to a final mean log count of 6.93. The SPC from cheeses stored at 4°C peaked after 6 weeks of storage, and decreased slightly to a final mean log count of 6.97. S. aureus counts were similar at both temperatures until week 4 when counts from cheeses stored at 4°C decreased slightly to a final mean log value of 6.18, and counts from cheeses stored at 10°C decreased to a final mean log value of 5.92.

Effect of different salt types

During the storage period of 2-8 weeks, SPCs of NaCl-SC and KCl/NaCl-SC differed by a mean log value of 0.74 ± .09, with KCl/NaCl-SC always having the lower counts (Fig. 3). S. aureus counts of the two types of salted cheeses did not vary as greatly (mean log differences of 0.28 ± .23 during 2-8 weeks of storage), but again the KCl/NaCl-SC had the lower counts. USC, during the same storage period, had S. aureus counts averaging 1.46 ± .40 log cycles lower than salted cheeses.

Effect of salt concentration

From hooping until 8 weeks of storage, average SPCs of 1.2%-SC were higher than those of 2.4%-SC by a mean log value of 0.49 ± .24 (Fig. 4). S. aureus counts at hooping in 2.4%-SC were lower than were corresponding counts from 1.2%-SC and USC. By week 4 of storage, 2.4%-SC had the highest S. aureus counts of any cheeses tested. During the storage period of 4-8 weeks, 1.2%-SC and USC had mean log S. aureus counts which were 0.29 ± .09 and 1.77 ± .35, respectively, lower than 2.4%-SC.

Detection of enterotoxin A in cheese

Results obtained when cheeses made from milk that was inoculated with S. aureus were tested for enterotoxin A are in Table 2. The type of salt used did not appear to be as important in affecting enterotoxin production as did the salt concentration. The average enterotoxin level for NaCl-SC was 0.24 ng/g, while those salted at the 2.4% concentration had an average enterotoxin level of 0.34 ng/g, which was the level in both the KCl/NaCl-SC and USC. Cheeses salted at 2.4% concentration had an average enterotoxin level of 0.34 ng/g, while those salted at the 1.2% concentration had 0.12 ng/g. Cheeses stored at 10 or 4°C had average enterotoxin levels of 0.30 ng/g, and 0.21 ng/g, respectively. Cheeses made with 1.0% starter culture had an average enterotoxin level of 0.25 ng/g, while cheeses made with 0.5% starter culture had an average enterotoxin level of 0.24 ng/g.

**DISCUSSION**

Results of preliminary trials showed that the moisture contents and pH values of cheese after pressing varied greatly among 2.4%-SC, 1.2%-SC and USC when all were made from the same vat of milk. Accordingly, for trials described in this report, 1.2%-SC and USC were manufactured from the same vat of milk, and curds were cooked slightly longer (than for 2.4%-SC) to reduce the moisture content. The 2.4%-SC was made separately using the recommended cheesemaking schedule (3).
TABLE 2. Production of enterotoxin A by strain 196-E in 8-week-old contaminated Cheddar cheese.

<table>
<thead>
<tr>
<th>Trial</th>
<th>S. aureus inoculum (log)</th>
<th>Salt type</th>
<th>Salt concentration (%)</th>
<th>Storage temp. (°C)</th>
<th>Enterotoxin A (ng/g of cheese)</th>
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<td></td>
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<td>no salt</td>
<td>0.10</td>
<td>10</td>
<td>0.27</td>
</tr>
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</table>

*None detected.

*Range maximum S. aureus/g, 9.6 x 10⁶ - 4.4 x 10⁹.

*Range maximum S. aureus/g, 8.1 x 10⁵ - 1.6 x 10⁹.

*Range maximum S. aureus/g, 1.3 x 10⁶ - 4.7 x 10⁹.

*Range maximum S. aureus/g, 6.7 x 10⁸ - 1.2 x 10⁹.

The KCl/NaCl-SC consistently had slightly higher salt concentrations than did the NaCl-SC, even though curds were salted at the same rate. From these data, the KCl/NaCl mixture appears to be retained in the curd more than does the NaCl, but the salt content of the whey was not measured. The KCI/NaCI-SC had slightly lower moisture contents than did the NaCl-SC. This difference reflects the slightly higher retention rate of KCI/NaCl in curd. While 2.4%-SC had lower moisture contents than 1.2%-SC, which was expected, USC had the lowest average moisture level, which differs from other published data (11, 12, 25). The USC also had the lowest pH values, probably because lactic acid and other bacteria were not inhibited by salt.

The S. aureus counts in all trials increased approximately 3.5 log cycles from the time of inoculation to after pressing of cheese. Along with growth, this rapid increase reflects the concentration of S. aureus through curd formation, as very few cells were detected in the whey. During the storage period of 0 to 8 weeks, the S. aureus count decreased by approximately 0.5 log cycle. This survival pattern was observed regardless of the size of initial inoculum, so larger inocula gave rise to larger final populations of S. aureus. Other investigators have reported similar results (15, 23).

Results of this study showed that USC and 1.2%-SC had lower S. aureus counts than did 2.4%-SC (Fig. 4). The reduction of salt content led to greater survival of non-S. aureus bacteria (SPC minus S. aureus count), and because staphylococci are poor competitors, the S. aureus count decreased. The USC had the lowest S. aureus counts of all cheeses, which is consistent with the results of other investigators (9, 11).

The KCI/NaCl-SC mean log counts of S. aureus and non-S. aureus bacteria that were 0.60 and 0.70, respectively, lower than were corresponding counts from NaCl-SC. The differences in pH values, salt concentration and moisture content between KCI/NaCl-SC and NaCl-SC were negligible. It is possible that the K⁺ ion was partly responsible for increased bacteriostatic activity of KCl/NaCl mixture over NaCl. The K⁺ ion is required by nearly all bacterial cells. It is usually the most abundant cytoplasmic cation, and is found in their intracellular fluid even though their environment contains it in high concentration (6). Hence, it is possible that bacterial cell membranes are selectively permeable to potassium ions and that the cells have special transport systems to pump K⁺ ions in and Na⁺ ions out. In a recent experiment, Hueting et al. (8) used an actively growing culture of Klebsiella aerogenes, and, while the cells were growing in a medium containing 1 mM K⁺, enough KCl was added to the mixture to raise the extracellular K⁺ concentration to 20 mM. A substantial decrease in bacterial respiratory activity was observed. This reduction was attributed to lowering of the transmembrane K⁺ gradient because all other conditions had been kept constant. Although Hueting et al. experimented with K. aerogenes only, it seems likely that an abundance of K⁺ ions could have a similar effect on other genera of bacteria, and could have been responsible for the observed inhibitory effect of the KCI/NaCl mixture as compared to NaCl.

As discussed earlier, S. aureus counts from cheeses made with 1.0% starter were nearly a full log cycle lower than were those from cheeses made with 0.5% starter, even though the pH curves for both types of cheese were similar. The apparent inhibitory action against S. aureus could, in part, be caused by the slightly larger population of non-S. aureus.
bacteria in cheeses made with 1.0% starter as compared to those made with 0.5% starter. This alone cannot account for all the inhibition that was noted. Other factors that were not measured, such as \( \text{H}_2\text{O}_2 \) level and antibiotic production, could have been involved (5).

The RIA method for measuring enterotoxin is from 10- to 100-fold more sensitive than is the older microslide method which commonly was used in earlier studies to assay cheese for enterotoxin. Had our cheeses been tested by the microslide method they probably would have appeared to be free of enterotoxin. Nevertheless, with the more sensitive test, we demonstrated the presence of some enterotoxin in cheese made from milk contaminated with \( \text{S. aureus} \), even though acid production during cheesemaking appeared to be normal. Furthermore, none of the salting procedures we studied appreciably affected presence or amount of enterotoxin in the cheese.

Since the presence of any amount of enterotoxin in cheese is clearly unacceptable, it is essential that cheese be made from staphylococcus-free milk and that contamination of milk with staphylococci be prevented during the cheesemaking process. In this regard, our data suggest that results of earlier investigations based on less-sensitive methods for measurement of enterotoxin must be interpreted with caution. In fact, further studies appear to be warranted on relationships between presence of enterotoxigenic staphylococci in milk, cheesemaking practices and occurrence in cheese of enterotoxin at concentrations detectable by the RIA method.

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