Growth and Enterotoxin Production by *Staphylococcus aureus* in Whey from the Manufacture of Domiati Cheese

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(Received for publication September 13, 1982)

**ABSTRACT**

*Staphylococcus aureus* able to produce enterotoxin A was inoculated into Domiati cheese whey treated four different ways. Included were raw and pasteurized whey with 15% salt and unsalted raw and pasteurized whey. All wheys and their controls were kept at 30°C, and tested after 6, 12, 24, 48 and 72 h. There was a substantial loss in viability of *S. aureus* after 6 h of incubation in unsalted raw whey. Substantial loss of viability by *S. aureus* occurred in unsalted heat-treated whey after 24 h of incubation. *S. aureus* decreased in number slowly after 24 and 48 h of incubation in both salted raw and salted pasteurized whey. Thermonuclease and enterotoxin A were detected in unsalted incubation in both salted raw and salted pasteurized whey. Thermonuclease and enterotoxin A were detected in unsalted heat-treated whey at and after 24 h when the number of *S. aureus* was 1.6 x 10⁹/ml. Thermonuclease could not be detected after 6 h of incubation of unsalted or salted raw whey and after 48 h of incubation of salted whey.

Domiati-cheese whey differs from other types of whey by its salty taste, which results from the amount of salt added to the milk before adding rennet in the cheese-making process. Most whey from cheese-making is used as a pickling solution for cheese after increasing the salt content of whey to 15%, which concentration was found to be best for preserving and ripening of Domiati cheese (9). At the end of the ripening period (2-8 months), there is an increase in the nutritional value of whey (\(I\)).

The importance of Domiati cheese whey does not come from its being used as a pickle for preserving cheese, but rather from its consumption by a large number of people in Egypt as a dairy food or an appetizer. Contamination of whey by different microorganisms, including *Staphylococcus aureus*, is possible and probably is common when hygienic measures are inadequate during cheese manufacturing. Presence of *S. aureus* in whey may be from an endogenous source, i.e. transferred from curd into whey during cheese making, or from an exogenous source, i.e. a result of handling and inadequate hygiene. The possibility of enterotoxin production in whey is an important consideration in the safety of this product. Therefore this study was initiated to determine the fate of enterotoxigenic *S. aureus* in Domiati cheese whey, and to study the effect of some treatments of whey on survival and enterotoxin production by *S. aureus*.

**MATERIALS AND METHODS**

**Strain of *S. aureus***

Coagulase-positive enterotoxigenic *S. aureus* strain 100, which produces enterotoxin A, was obtained from Merlin S. Bergdoll, Food Research Institute, University of Wisconsin-Madison.

**Preparation of *S. aureus* culture**

*S. aureus* was grown in Brain Heart Infusion broth for 48 h at 37°C. Subcultures were prepared on Brain Heart Infusion agar slants, which were incubated 24 h at 37°C and then were refrigerated until they were used. Before the experiment *S. aureus* was transferred from a stock slant to 10 ml of Trypticase Soy (TS) broth, which was then incubated at 37°C for 24 h. Finally, a loopful of broth culture was transferred to 125 x 16-mm screw-capped tubes each containing 10 ml of TS broth; these tubes were incubated 18 h at 37°C. This resulted in a population of approximately 10⁸ cells per ml (7).

**Preparation of whey**

Wheys given four different treatments were prepared; included were salted (15%) raw and pasteurized whey and unsalted raw and pasteurized whey. Raw whey was obtained by complete draining of Domiati-cheese curd, which was prepared in the laboratory from raw milk without addition of salt. The raw milk was obtained from the Dairy Plant of the Department of Food Science, University of Wisconsin-Madison. Whey was placed into a clean, dry and sterile container, and was divided into two equal portions. One portion was pasteurized in the laboratory at 65°C for 30 min, as described in Standard Methods (5), and then was left to cool. Raw and pasteurized wheys were inoculated with an equivalent amount of *S. aureus* obtained from the 18-h-old TS broth culture, to give the desired level of *S. aureus*/ml of whey. The inoculated wheys were tested directly for their staphylococcal count. Both inoculated raw and pasteurized wheys were divided into two equal volumes and placed in clean, dry and sterile flasks. Sodium chloride was added to one volume of both inoculated raw and pasteurized whey to give a 15% salt concentration. The other two volumes were left without salting. All portions of whey were incubated at 30°C, and samples for microbiological and other analyses were removed from each flask after 6, 12, 24, 48 and 72 h.

**Microbiological methods**

Aerobic plate count. The aerobic plate count (APC) of whey samples was made according to Standard Methods (5).
TABLE 1. Growth of S. aureus in salted wheys at 30°C.

<table>
<thead>
<tr>
<th>Time of Incubation (h)</th>
<th>Salted (15%) raw whey</th>
<th>Salted (15%) pasteurized whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>APC</td>
</tr>
<tr>
<td>0</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>5.8</td>
<td>7 x 10^4</td>
</tr>
<tr>
<td>12</td>
<td>5.5</td>
<td>3.3 x 10^6</td>
</tr>
<tr>
<td>24</td>
<td>5.7</td>
<td>6 x 10^6</td>
</tr>
<tr>
<td>48</td>
<td>5.1</td>
<td>3.9 x 10^8</td>
</tr>
<tr>
<td>72</td>
<td>5.35</td>
<td>12 x 10^9</td>
</tr>
</tbody>
</table>

*APC = Aerobic plate count.

TABLE 2. Growth of S. aureus in unsalted whey at 30°C.

<table>
<thead>
<tr>
<th>Time of Incubation (h)</th>
<th>Unsalted raw whey</th>
<th>Unsalted pasteurized whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>APC</td>
</tr>
<tr>
<td>0</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.6</td>
<td>2.7 x 10^8</td>
</tr>
<tr>
<td>12</td>
<td>6.2</td>
<td>&gt;10^9</td>
</tr>
<tr>
<td>24</td>
<td>4.2</td>
<td>&gt;10^9</td>
</tr>
<tr>
<td>48</td>
<td>4.0</td>
<td>&gt;10^9</td>
</tr>
<tr>
<td>72</td>
<td>4.0</td>
<td>&gt;10^9</td>
</tr>
</tbody>
</table>

*APC = Aerobic plate count.

S. aureus count. The staphylococcal count was determined by surface plating 0.1 ml of diluted samples on Baird-Parker agar (Difco). Duplicate plates were prepared for each dilution, and were incubated 48 h at 37°C. Confirmation of colonies suspected to be S. aureus was accomplished by the DNase test of Lachica et al. (4).

Thermonuclease assay and DNase test

Whey samples were boiled for 15 min and left to cool. Toluidine blue DNA agar of Lachica et al. (4) was prepared, and 5-ml quantities were pipetted into petri dishes (15 x 60 mm). Wells 2 mm in diameter were cut in the agar after solidification and filled with a previously boiled whey sample. Heat-stable nuclease activity was indicated by bright pink zones of DNA hydrolysis.

pH

The pH was determined with a pH meter (Corning Model 10) equipped with a standard combination electrode.

Enterotoxin A detection

Thermonuclease-positive samples accompanied by their control, were tested for enterotoxin A at the Food Research Institute, University of Wisconsin, by using the Enzyme-Linked Immunosorbenent Assay technique (ELISA) (2).

RESULTS

It is evident from results in Table 1, that the number of S. aureus increased slightly in 15% salted raw whey during the first 6 h of incubation; the population at that time was 5 x 10^5/ml. The S. aureus count then began to decrease while the total number of aerobic bacteria increased. A higher count of S. aureus was obtained in salted pasteurized whey than in salted raw whey after 12 h of incubation. Furthermore, salted pasteurized whey seemed better for survival of S. aureus through 72 h of incubation than was salted raw whey. The count of aerobic bacteria did not change appreciably in salted pasteurized whey. The pH values of both wheys decreased slightly early during the incubation time and were 5.8 and 5.75 in salted raw and pasteurized whey, respectively, after 6 h. Thereafter, the pH of salted raw whey continued to decrease but not that of salted pasteurized whey. Thermonuclease could not be detected in the whey samples.

Data summarized in Table 2 indicate a rapid decrease in staphylococcal count in unsalted raw whey but not in unsalted pasteurized whey after 6 h of incubation. After 72 h S. aureus could not be recovered from the raw whey. There was a high count of aerobic bacteria after 6 h, in raw whey, which then was 2.7 x 10^9/ml, and continued to increase thereafter. S. aureus in unsalted pasteurized whey grew rapidly during the first 6 h of incubation and achieved a population of 1.6 x 10^9/ml after 24 h. After that the population began to decrease gradually. The total number of aerobic bacteria was very low at the beginning of incubation, i.e. 1.8 x 10^5/ml, and started to increase gradually after 24 h of incubation. The pH value of both types of whey decreased rapidly after the first 12 h of incubation; the values were 4.0 and 4.35 after 72 h of incubation of unsalted raw and pasteurized whey, respectively.

Thermonuclease was detected only in unsalted pasteurized whey at and after 24 h of incubation when the S. aureus count was 1.6 x 10^8 ml. Analysis of these samples revealed the presence of enterotoxin.
DISCUSSION

From results obtained in our study, it is evident that the viability of S. aureus decreased rapidly in raw wheys after a few hours of incubation. This may have resulted from competition with lactic acid bacteria and the microflora of raw milk that was in whey. The viability of S. aureus in salted raw whey decreased more slowly than in unsalted raw whey, and this may have happened because of the inhibitory effect of salt on the other types of bacteria present. Our results are in agreement with those of Miller and Ledford (6), who observed the competitive effect of lactic acid bacteria on loss of viability by S. aureus inoculated into Cheddar whey. Also, data of Minor and Marth (8) support our results when they concluded that there are two factors responsible for inactivation of staphylococci in an acidic environment, the extended period of exposure and the antimicrobial activity of acids. There is some difference between our results and those of Santos and Genigeorgis (10), who found that there was no loss of viability by S. aureus inoculated in Minase cheese whey. This difference in results may have been caused by difference in the amount of inoculum or in the nature of whey which resulted from Minase cheese made from pasteurized milk.

The increased number of S. aureus in unsalted heat-treated whey gives excellent proof for the lack of competition from the small number of bacteria that survived pasteurization. In salted pasteurized whey, salt seemed to be detrimental to growth of S. aureus. This was demonstrated by Minor and Marth (8), who noted that salt appears to be particularly inhibitory to staphylococcal growth at lower pH values.

Our results differ from those of Miller and Ledford (6), who detected thermonuclease but not enterotoxin at 1.7 x 10^7 S. aureus/ml after 24 h at 37°C. This difference may have resulted because methods of different sensitivity were used to detect enterotoxin. Santos and Genigeorgis (10) detected thermonuclease when the number of S. aureus was at log_{10} of 7, while Tatini et al. (11) observed a detectable level of thermonuclease when the S. aureus count was at log_{10} of 5. These differences between our findings and those of others can be attributed to sensitivity of S. aureus strain, use of starter cultures or type of whey substrate.

Results of our study suggest that S. aureus lost its viability in raw Domiati cheese whey because of the increase in the number of competing microorganisms and also the decrease in pH value of whey during the incubation period. Sodium chloride may enhance staphylococcal growth in raw whey because of its inhibitory effect on the competing microorganisms present in the whey. However, because of the decrease in pH, eventually sodium chloride also appeared to become inhibitory to S. aureus. Contamination of unsalted heat-treated whey by S. aureus and the absence of proper cooling may provide an opportunity for production of enterotoxin, which is likely to survive subsequent processing and storage of the whey.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, and by the American-Mideast Educational and Training Services, Inc. who supported through peace fellowships A.A-H.A. and M.K.M. while they were on leave from the Department of Hygiene and Food Control, Faculty of Veterinary Medicine, University of Assiut, Assiut, Egypt.

REFERENCES


JOURNAL OF FOOD PROTECTION, VOL. 46, MARCH 1983