Growth and Survival of Staphylococcus aureus in Egyptian Domiati Cheese

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ABSTRACT

Domiati cheese was prepared from unsalted raw milk and from raw milk with 5 or 10% added sodium chloride. Milks were inoculated with enterotoxigenic Staphylococcus aureus strain 100 (produces enterotoxin A) before addition of salt and rennet. Cheeses were stored in whey containing 15% sodium chloride; were held at 30°C and were examined periodically for S. aureus count, aerobic plate count, DNase and moisture and salt contents. Numbers of S. aureus increased rapidly during preparation of cheese. There was a rapid decrease in number of viable S. aureus during storage of cheese made from unsalted milk and cheese made from milk with 5% added salt. In cheese from milk with 10% added salt, S. aureus survived until the fourth week. An increase in salt content and a decrease in the pH value of all cheeses occurred during storage in salted whey. DNase was detected only in cheese made from salted milk, but these samples did not contain a detectable amount of staphylococcal enterotoxin either after cheeses were made or after they were stored for 1 week.

Domiati cheese is one of the pickled cheeses which is characterized by addition of salt at the very first step in its manufacture. It is traditionally prepared from raw milk without addition of lactic culture or colorant, and can be consumed fresh, but usually is eaten after maturation in a salt solution (pickle), which is prepared from whey by increasing its salt content to 15%. This pickle improves the flavor of cheese and acts as a preservative. Since Domiati cheese is one of the most popular varieties of cheese in Egypt, it could, if contaminated, be a major cause of foodborne disease. In spite of advances in food processing and hygienic measures, staphylococcal food poisoning is sometimes associated with certain dairy products. Cases of food poisoning caused by consumption of cheese heavily contaminated with staphylococci (and enterotoxin) have been documented (9). S. aureus was the major cause of food poisoning in U.S. during 1973-1975 (19). In 1977, 15 cases of staphylococcal intoxication occurred in Canada, and were traced to 59 lots of Swiss-type cheese infected with S. aureus (19). Data concerning the incidence of staphylococcal food poisoning in Egypt are not available, although existence of enterotoxigenic S. aureus in dairy products is recognized (2,3). Therefore, it is important from a public health point of view to study growth and survival of enterotoxigenic S. aureus in Domiati cheese and also the possibility of enterotoxin production in this food.

This paper provides results of such a study.

MATERIALS AND METHODS

Strain of S. aureus

S. aureus strain 100, which produces enterotoxin A, was obtained from Merlin S. Bergdoll, Food Research Institute, University of Wisconsin.

Preparation of S. aureus culture

The S. aureus culture was prepared as described by Minor and Marth (13).

Preparation of Domiati cheese

Cheese was made from unsalted raw milk and from raw milk containing 5 or 10% added sodium chloride. Raw milk was inoculated with S. aureus to give the desired number of the bacterium per ml. A sample was taken after inoculation to determine the S. aureus count, aerobic plate count and pH value. The inoculated raw milk was divided into three equal portions. Two portions were salted by addition of sodium chloride to give concentrations of 5 or 10%, while the other portion was left without salting. The procedure described by Fahmi and Sharara (7) was used to manufacture Domiati cheese. Three control blocks of Domiati cheese were prepared from raw milk and they contained the test amounts of salt, but S. aureus was not added. Raw milk for these experiments was obtained from the Dairy Plant of the Department of Food Science, University of Wisconsin-Madison.

Samples of the curd and finished product were tested for S. aureus count, aerobic plate count and pH value. Finished fresh cheeses were examined for fat, moisture and salt contents. Cheeses with their controls were stored at 30°C in whey containing 15% salt, and were tested after the 1st and 2nd and 4th week for S. aureus count, aerobic plate count, pH value, DNase and for contents of salt, moisture and total solids. The samples positive by the DNase test were examined for presence of enterotoxin A.

Preparation of cheese samples for microbiological examination

Cheese samples were prepared according to the "alternative" method described in Standard Methods for the Examination of Dairy Products (12).

Aerobic plate count

Serial dilutions of cheese emulsions were prepared using 0.1% sterile peptone water. Standard Plate Counts (SPC) were determined with duplicate plates of Plate Count Agar (Difco), as described in Standard Methods (12).

Staphylococcus aureus count

Numbers of S. aureus were determined by using Baird-Parker agar...
plates (Difco). Duplicate plates were prepared and incubated 48 h at 37°C. Some of the colonies were randomly selected for confirmation as *S. aureus*, and were tested for their ability to produce heat-stable deoxyribonuclease, as described by Lachica et al. (11).

**Assay of thermostable DNase in cheese**

Cheese samples and their controls were periodically assayed for DNase. The procedure of Cords and Tatini (6) was used with the modification described by Ibrahim and Baldock (10). Twenty grams of cheese were suspended in 30 ml of deionized water (60-65°C) and blended thoroughly in an Omnimixer (Sorval) operating at maximum speed for 3 min. The pH was then adjusted to 4.5, using 3 N HCl, and the suspension was centrifuged at 4°C for 30 min at 32000 × g. The supernatant fluid was treated with 5% (v/v) of cold 3 N HCl and then was reconstituted as mentioned before. The precipitate was adjusted to pH 8.5 with 1 N NaOH and the final volume was made up to 2.0 ml, using 0.05% (hydroxyethyl) aminomethan (Tris) buffer at pH 9.0. The extract was steamed at 100°C for 15 min and cooled rapidly before assay. Toluidine blue DNA agar was prepared according to the methods of Lachica et al. (11). Five-ml quantities of melted toluidine blue DNA agar were pipetted into 15 × 60-mm plastic petri plates. Wells of 2 mm (diameter) were cut into the agar after it was solidified by cooling for 1 h at 4°C. Plates were tempered at 37°C, and each well was filled with the previously prepared extract. Plates were then covered tightly, incubated at 37°C for 4 h and examined for heat-stable DNase, presence of which was indicated by a bright pink zone of DNA hydrolysis.

**pH**

The pH value of cheese was determined according to Standard Methods (12) with a pH meter (Corning Model 10) equipped with a standard combination electrode.

**Detection of enterotoxin A**

Samples of cheese that were positive with the DNase test and their controls were examined for the presence of enterotoxin A. This was done at the Food Research Institute, University of Wisconsin where the Enzyme-Linked Immunosorbent Assay technique (ELISA) described by Freed et al. (8) was used.

**Composition of cheese**

Cheese was dried in a vacuum oven at 100°C for 5 h with a minimum of 26 in. of vacuum, as described in Standard Methods (12). The moisture content was determined from the difference in weight before and after drying. The salt content in cheese was measured with the test described by Atherton and Newlander (5). The fat content of the finished product was measured with the modified Babcock method as described in Standard Methods (12).

**RESULTS**

**Chemical composition of cheese**

Results in Table 1 indicate that the fat content of fresh cheese was 22.1, 21.5 and 19.5% while the moisture content was 53.7, 60.2 and 64.2% in cheese prepared from unsalted milk and from milk with 5 or 10% added salt, respectively. The salt content (in the water phase) was 2.3 and 4.6%, respectively, in cheese made from milk containing 5 or 10% sodium chloride. The results also indicate a gradual increase in the salt content of cheese (water phase) during the first 15 d of storage; the change thereafter was not consistent, but there was a gradual decrease in the moisture content of cheese.

**Microbiological assay**

**Cheese from unsalted milk.** Results in Fig. 1 show that *S. aureus* grew rapidly during preparation of unsalted cheese. Growth continued slowly until the end of the first week that cheese was stored. A sharp drop in number of *S. aureus* occurred by the end of the second week when the count was log10 of 2 cells/g. *S. aureus* could not be recovered from this cheese by the end of the fourth week. The number of aerobic bacteria increased substantially during preparation of cheese and thereafter. A high aerobic plate

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**TABLE 1. Changes in chemical composition of cheese during storage.**

<table>
<thead>
<tr>
<th>Time of storage (weeks)</th>
<th>Cheese from unsalted milk</th>
<th>Cheese from milk with 5% added salt</th>
<th>Cheese from milk with 10% added salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat(%) T.S.(%) Moisture (%) Salt(%)</td>
<td>Fat(%) T.S.(%) Moisture (%) Salt(%)</td>
<td>Fat(%) T.S.(%) Moisture (%) Salt(%)</td>
</tr>
<tr>
<td>0</td>
<td>27.1 40.3 53.7  -</td>
<td>21.5 39.8 60.2 2.3</td>
<td>19.5 36.7 64.3 4.6</td>
</tr>
<tr>
<td>1</td>
<td>- 52.3 47.7 1.8</td>
<td>- 41.2 58.8 2.4</td>
<td>- 40.9 59.1 4.9</td>
</tr>
<tr>
<td>2</td>
<td>- 53.6 46.4 6.3</td>
<td>- 45.2 54.8 6.6</td>
<td>- 44.7 55.3 7.6</td>
</tr>
<tr>
<td>4</td>
<td>- 54.5 45.5 6.3</td>
<td>- 50.2 45.5 6.3</td>
<td>- 48.8 51.2 7.4</td>
</tr>
</tbody>
</table>

*S. = Total solids.
Salt content in water phase of the cheese.

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**Figure 1. Count of *S. aureus* strain 100 and aerobic plate count of Domiati cheese prepared from unsalted raw milk during preparation and storage in whey containing 15% salt. R = Inoculated raw milk, C = Curd, F = Finished cheese.**
count was found by the end of the fourth week. The pH value of cheese was 6.6 when the curd was made and dropped sharply by the end of manufacture at which time it was 5.1. At the end of the fourth week of storage cheese had a pH value 4.2. No DNase could be detected in any of our samples.

![Figure 2](image)

**Figure 2.** Counts of *S. aureus* strain 100 and the aerobic plate count of Domiati cheese prepared from raw milk with 5% salt and stored at 30°C in whey containing 15% salt. R = Inoculated raw milk, C = Curd, F = Finished cheese.

**DISCUSSION**

**Chemical composition of cheese**

The variation in chemical composition that occurred in fat, moisture and salt contents of cheeses made by the several treatments resulted from the different amounts of salt added to milk before it was coagulated with rennet. It was found that with an increase in amount of added salt, coagulation of milk by rennet was retarded, production of cheese with high moisture content resulted, and fat losses in whey increased.

Our results showed the moisture content of cheese decreased during its storage. This was comparable to results obtained by Amer et al. (4), but they produced cheese with more moisture than was in ours. This may be the result of differences in pressure applied on curd or differences in techniques of cheese preparation. Data in Table I reveal an increase in salt content of cheese (water phase) occurred during the first 15 d of storage. This was also noted by Saleem et al. (15).

**Microbiological assay**

Results in Fig. 1, 2 and 3 verify that there was substantial multiplication of *S. aureus* during preparation of the three types of cheese. *S. aureus* generally achieved its maximum population by the end of cheese manufacture. These findings are parallel to those of Takahashi and Johns (16) and Walker et al. (21), who stated that presence of *S. aureus* in significant number in raw milk results in an increase in number of the bacterium during manufacture of Cheddar cheese. From data in Fig. 1, it is evident that *S. aureus* rapidly lost its viability in cheese made from unsalted milk after the first week of storage, and the organism was completely inactivated by the fourth week. This may be the result of competition from the huge...
number of bacteria other than *S. aureus*, including lactic acid types, and also the result of lactic acid production and a consequent rapid decrease of pH to 5.1 by the end of cheese preparation. These results are in agreement with those of Tatini (17).

Minor and Marth (13) mentioned that the antimicrobial activity of an acidic medium causes inactivation of *S. aureus*. The data in Fig. 2 and 3 show that addition of 5 and 10% salt to raw milk induced inhibition of growth of microorganisms other than *S. aureus*, and minimized the decrease in pH value during cheese making. This inhibition of the competitive microflora, including the lactic acid bacteria, and the resultant favorable pH of the cheese medium provided an opportunity for *S. aureus* to grow rapidly. The increased salt content of cheese prepared from milk with 5% salt and the decrease of the pH value during storage had a negative effect on survival of *S. aureus*. This probably resulted from the combined effects of salt and acid, as described by Minor and Marth (14). It is evident from data in Fig. 3 that the slow decrease of pH (range of 6.2 to 5) during storage of cheese prepared from milk with 10% salt allowed survival of *S. aureus*. The increase in salt content of cheese inhibited growth of bacteria other than *S. aureus*. Furthermore, data in Fig. 2 and 3 show both good growth of *S. aureus* and its survival at pH values which ranged from 5.9 to 6.2. This is in good agreement with data of Todd et al. (21), who observed maximum growth of *S. aureus* at pH values of 5.95-6.16.

Enterotoxin A could not be detected in spite of the presence of DNase in cheese samples having a high population of *S. aureus*. These results differ from those of Tatini et al. (18), who stated a good correlation exists among staphylococcal count, presence of DNase and enterotoxin production. Our observation may have resulted because of natural inhibitors in milk, variation in the oxidation-reduction potential of the medium and/or competitive growth of other organisms since these factors influence enterotoxin production (17). Another explanation for these results is the conclusion of Ibrahim and Baldock (10) that there is not always a good correlation between the amount of *S. aureus* growth and the amount of enterotoxin produced. Moreover, in the presence of normal food microorganisms, good growth of *S. aureus* can occur without formation of detectable levels of enterotoxin. Inhibition of competing microorganisms by addition of salt to the milk before cheese making usually was accompanied by good growth of *S. aureus*. This active growth of *S. aureus* could be accompanied by enterotoxin production in cheese curd, which would persist in cheese during storage and so become a public health hazard.

It is noteworthy that in this study *S. aureus* grew to a high level during cheese manufacture. The increased amount of salt served to enhance survival of *S. aureus*. Salt may retard growth of *S. aureus* and reduce its survival only when accompanied by a pH value lower than 5. Salted Domiat cheese could become a public health hazard if the milk is contaminated with *S. aureus* and if good growth of *S. aureus* occurs after addition of salt and during curdling of milk and preparation of cheese. This growth could be accompanied by production of enterotoxin, which is not affected by storage of the cheese. Strict hygienic measures should be imposed during milk production, handling and cheese processing to prevent contamination with *S. aureus*.

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milk samples exposed to light decreased as the pasteurization temperature was increased from 73 to 90°C. This criticism was the predominant one in milk pasteurized at 90°C after only 14 d under the fluorescent light. These data indicate that cooked flavor either inhibits or masks oxidized flavor.

By observing changes in peak heights of C-2, 3, 5 and 6 saturated aliphatic straight chain aldehydes, it is obvious that these carbonyl compounds increase as a function of the time of light exposure. With regard to pasteurization temperatures, acetaldehyde and n-pentanal concentrations were greater in milk pasteurized at 90°C throughout most of the light exposure times (Fig. 2). On the other hand, propanal and n-hexanal concentrations were greater in milk pasteurized at the lowest temperature (73°C) throughout 14 d under fluorescent light. Previous research with light-exposed skim milk resulted in relatively greater increases in acetaldehyde and n-pentanal than in propanal and n-hexanal (2). This would implicate the role of nonfat milk components (most likely protein) in increases in acetaldehyde and n-pentanal. Higher pasteurization temperatures may have accommodated some of those changes. However, relatively lower concentrations of propanal and n-hexanal in light-exposed milk at higher pasteurization temperatures may occur as a result of reducing substances generated by higher temperature that inhibit lipid oxidation.

There appears to be an advantage to using higher pasteurization temperatures in minimizing light-activated flavors. However, more work is needed to establish if there is an optimum combination of temperatures and times that will limit the light-activated flavor while controlling the heated flavor.

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11. Ahmed et al., cont. from p. 415