BEHAVIOR OF VIRULENT YERSINIA ENTEROCOLITICA IN DAMIETTA CHEESE
(With Two Tables)

By
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(Received at 7/1/1989)

SUMMARY
A virulent strain of Yersinia enterocolitica was inoculated into pasteurized milks. The inoculated milks salted with 5 or 10% sodium chloride, were used for making Damietta cheese. The cheeses were stored in their whey at 30±1ºC, and were examined periodically for Yersinia counts, pH value, moisture and salt contents. A slight increase in number, of Y. enterocolitica occurred during preparation of cheeses. However, the growth rate of the organism in cheese made from milk with 5% added salt, was higher than that recorded in cheese made from milk with 10% added salt. Numbers of Y. enterocolitica sharply decreased in both types of cheeses during storage period, and could not be detected by the end of the second week.
INTRODUCTION


Although, there are no documented outbreaks of foodborne illness caused by Y. enterocolitica and associated with cheese, this study was planned to evaluate the safety of Damietta cheese artificially contaminated with virulent Y. enterocolitica strain.

MATERIAL and METHODS

Cultures:

Y. enterocolitica strain (2635 serotype 0:8) that caused the chocolate milk outbreak (BLACK, et al. 1978), was used in this study. The strain was obtained from the Food Research Institute, University of Wisconsin, Madison, USA. Culture of Y. enterocolitica was grown in Trypticase Soy broth (Difco) at 23 ± 1°C for 18 hours before inoculation into milk.

Preparation of Damietta cheese:

Raw milk obtained from the dairy farm of the faculty of Agriculture, Assiut Univ., was locally pasteurized at 63°C for 30 min. Pasteurized milk was inoculated with Y. enterocolitica to give the desired number of bacteria per ml. (1x10⁶). The inoculated pasteurized milk was divided into two equal portions, and were salted by addition of sodium chloride to give concentrations of 5 and 10%. The procedure described by FAHMY and SHARARA (1950) was used to manufacture the cheese. Two control blocks of Damietta cheese were prepared from pasteurized milk containing the same percentages of salt without addition of Yersinia culture. Contaminated cheeses as well as controls were kept at 30 ± 1°C, and tested periodically.

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Sampling:
Samples for detection of Yersinia counts and determination of pH value were taken from milk before and after addition of the inoculum and immediately after curdling. Samples were taken from the finished cheeses and controls after preparation and weekly thereafter for pH determination, Yersinia counts, moisture and salt contents.

Preparation of cheese for examination:
Cheese samples were prepared for examination according to Standard methods (MARTH, 1978).

Enumeration of Y. enterocolitica:
Surface plating onto cefsulodin-Irgasan Novobiocin (CIN) agar (SCHIEMANN, 1979) was done for each sample and its dilutions. The plates were incubated at 27°C for 48 hours, and typical colonies presumed to be Y. enterocolitica were counted. Confirmatory tests were done on each isolate suspected to be Y. enterocolitica as described by FEELEY, et al. (1976).

pH determination:
The pH values of milk and cheese were determined by using a pH meter (an orion, Model 701) equipped with standard combination electrode.

Moisture and salt content:
Moisture was determined according to Standard Methods (MARTH, 1978). The salt content was determined by the method described by ATHERTON and NEWLANDER (1977).

RESULTS
The obtained results were recorded in Tables (1 & 2).

DISCUSSION
As recorded in Table (1), an increase in salt (water phase) and a decrease in moisture contents of cheeses during storage period. These findings were comparable to the results obtained by AMER, et al. (1979) and AHMED, et al. (1983).

Data in Table (2), reveal that a slight increase in numbers of Y. enterocolitica occurred during manufacturing of cheese made from milk with 5% added salt. The organism achieved its maximum population (1x10^7 cells/g) by the end of cheese making Y. enterocolitica began to lose its viability rapidly and could not be detected (\_ 100 cells/g) by the end of the second week of storage. The pH value of cheese after preparation was 6.2 and slowly decreased to reach a low value of 4.6 by the end of storage.

Concerning the results obtained from cheese made from milk with 10% added salt (Table 2), no change could be observed in the number of \textit{Y. enterocolitica} during manufacture of cheese. The number of organisms decreased rapidly during the storage period of cheese, and could not be detected (/ 100 cells/g) by the end of the second week.

According to the data obtained by HANNA, et al. (1977 b) and STERN, et al. (1980) \textit{Y. enterocolitica} could grow and survive at pH value ranging from 4.6-9, it is obvious from the obtained results (Table 2) that pH values of both types of cheese were favorable for growth and survival of \textit{Y. enterocolitica} during manufacturing and storage of the product. However, the growth rate of the organism was lower than that obtained by MOUTTAFI, et al. (1983 b) who reported that \textit{Y. enterocolitica} increased rapidly 1000-fold to about 1x10^7/g of curd during the manufacturing process of Colby-like cheese. This probably resulted from the high percentage of salt content of Damietta cheese, which had a negative effect on growth and multiplication of \textit{Y. enterocolitica} during cheese making. Furthermore, the gradual increase in salt content of cheese during storage was accompanied with extensive reduction in numbers of \textit{Y. enterocolitica} (Table 1 & 2), which could not recovered from cheese by the end of the second week. Different results were recorded by SCHIEMANN (1978), who reported that \textit{Y. enterocolitica} disappeared from cheese curd samples stored at 40°C after 8 weeks. Also in another study, \textit{Y. enterocolitica} serotype 0:8 could persist in Colby-like cheese at numbers in excess of 200/g after 8 weeks of storage at 3±1°C (MOUTTAFI, et al. 1983). These differences could be attributed to the type of cheese used, amount of added salt and the variation in storage temperatures.

The results obtained by MORRIS and FEELEY (1976) have suggested that about 3.9x10^6 \textit{Y. enterocolitica} must be consumed to cause illness in human. If an average person consumes 200 g of cheese at one time, about 2x10^7 \textit{Y. enterocolitica}/g are required to make one ill. Damietta cheese is often ripened in its salted whey for about 8 weeks before sale. This period of ripening presumed to be enough for destruction of potential disease agents which may be present. Therefore, the numbers of \textit{Y. enterocolitica} retained in the experimental Damietta cheese after two weeks of storage were lower than the level needed to cause illness.

On conclusion, contamination of Damietta cheese by \textit{Y. enterocolitica} emphasizes again the importance of stringent sanitation which must be followed during cheese making. Presence of even small numbers of virulent \textit{Y. enterocolitica} presents a public health hazard and should not be ignored.
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Table (1)
Changes in chemical composition of Damietta cheese during storage

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>Cheese prepared from milk with 5% added salt</th>
<th>Cheese prepared from milk with 10% added salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.S. %</td>
<td>Moisture %</td>
</tr>
<tr>
<td>0</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>57</td>
</tr>
</tbody>
</table>

Table (2)
Counts of *Y. enterocolitica* strain 0:8 and changes in pH during manufacture and storage of Damietta cheese.

<table>
<thead>
<tr>
<th>Storage period (weeks)</th>
<th>Cheese prepared from milk with 5% added salt</th>
<th>Cheese prepared from milk with 10% added salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yersinia/g</td>
<td>pH</td>
</tr>
<tr>
<td>Inoculum/ml.</td>
<td>$1 \times 10^6$</td>
<td>6.6</td>
</tr>
<tr>
<td>Curd</td>
<td>$2 \times 10^7$</td>
<td>6.3</td>
</tr>
<tr>
<td>0 time</td>
<td>$1 \times 10^6$</td>
<td>6.2</td>
</tr>
<tr>
<td>First week</td>
<td>$1 \times 10^6$</td>
<td>5.3</td>
</tr>
<tr>
<td>Second week</td>
<td>/ *100</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* : No colonies could be detected on the plate.

REFERENCES


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