Behavior of Enterobacter aerogenes and Hafnia Species During the Manufacture and Ripening of Camembert Cheese

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ABSTRACT

Camembert cheese was made from pasteurized milk contaminated with about $10^7 - 10^8$ cells of Enterobacter aerogenes or Hafnia sp. The coliform bacteria were enumerated with a Most Probable Number method and with violet red bile agar. Numbers of viable E. aerogenes decreased rapidly during ripening at 15.5 or 10 C when cheese was made with the commercial lactic starter cultures OD or C-S. No viable E. aerogenes was detected in cheese ripened at 10 C for 3 weeks. Ripening of cheese made with starter culture OD or C-S, for 1 week at 15.5 C was accompanied by a decrease in numbers of viable Hafnia sp. to 10^7/g. The number of Hafnia sp. increased markedly during 7 weeks of additional ripening at 10 C to yield cheese which contained numbers in excess of $10^7/g$ when ripening was almost completed. Growth of Hafnia sp. during the storage period coincided with an increase in pH of the cheese.

The possible presence of coliform bacteria in cheese has been recognized since 1895 when Russell (15) studied the defects caused by these organisms. These defects include off-odors and -flavors and excessively open texture resulting from gas produced by the bacteria. Coliform bacteria commonly are in raw milk, and, although killed by pasteurization, sometimes appear as contaminants in pasteurized milk. This, together with such conditions as improperly cleaned equipment, inadequate personal hygiene of workers in the cheese factory or a contaminated lactic starter culture, can lead to the presence of coliform bacteria in cheese. The last source should be extremely rare.

Survival of coliforms and other bacteria in cheese is related to pH (13,15,16). Coliforms prefer mildly acid to mildly alkaline conditions for maximum growth and survival in cheese. Cheese manufacturers rely on the ability of the lactic starter culture to successfully compete with coliforms, if present in milk, and produce sufficient acid to inhibit or inactivate them. However, a low pH, within limits, does not insure that all coliforms will be eliminated since some are more resistant to acidic conditions than are others. As cheese ripens, various areas of cheese may have different pH values. Mold-ripened cheese has a tendency to develop regions that are neutral or alkaline in pH and thus suitable conditions for growth and/or survival of coliforms may exist (9).

Enteropathogenic strains of Escherichia coli and other coliforms have been found in market milk and market cheese, especially in Camembert cheese from France, Sweden and Denmark (2,8) and also in cheese from the U.S. (3). Late in 1971, several outbreaks of acute gastroenteritis were reported in the U.S. These were associated with consumption of soft, ripened cheese of the Camembert-type that was contaminated with enteropathogenic E. coli (EEC). This was the first well-documented cheese-related outbreak of EEC foodborne illness in the U.S. (11). This incident prompted a renewed interest in the problem of coliforms and lead to studies by Frank et al. (4) and Park et al. (14) on the behavior of EEC in Camembert cheese and by Frank et al. (5) on the behavior of EEC in brick cheese.

Information is lacking on the fate of Enterobacter aerogenes and Hafnia sp. during the manufacture and ripening of Camembert cheese. This study was undertaken to determine growth or inactivation of these coliforms in Camembert cheese made with different commercial lactic starter cultures.

MATERIALS AND METHODS

Cultures

Cultures of E. aerogenes (MF-1) and Hafnia sp. (14-1) were obtained from the Food and Drug Administration. Each culture was transferred to nutrient broth daily for 3 days before it was used for an experiment. Incubation was at 35 C for 24 h. A sufficient amount of a 24-h-old nutrient broth culture (35-C incubation) was added to pasteurized milk to provide approximately 100 cells/ml of milk. Numbers of bacteria in broth were determined using a standard curve derived from absorbance measurements of nutrient broth containing E. aerogenes or Hafnia sp. and from plate counts of organisms. A spectrophotometer (Model 20, Bausch and Lomb, Rochester, New York) was used to make absorbance measurements. All necessary dilutions were made using sterile nutrient broth.

Lactic starter cultures used to make cheese included two commercial mixed strain lactic cultures obtained from the Marschall Division of Miles Laboratories, Inc., Madison, Wisconsin. These cultures were grown in sterile skim milk at 21 C for 24 h, frozen at -57 C in 10-ml quantities and stored frozen until they were to be used. Each 10-ml quantity was subcultured once in 35 ml of sterile skim milk and incubated at 21 C for 24 h before use in cheesemaking. The cultured skim milk served as inoculum for 18 kg of pasteurized milk.

Penicillium camembertii. obtained from K. B. Raper, Department of Bacteriology, University of Wisconsin, Madison, was grown on slants of Czapek agar at 21 C for 1 week. Mold growth on one agar slant was blended (Waring blender) with 50 ml of sterile citrate-buffered distilled water and the mixture was added to 18 kg of milk at the start of cheesemaking.

Manufacture and sampling of cheese

Camembert cheese was made according to procedures described by Park et al. (14). Cheese was made from 18-kg lots of pasteurized whole milk adjusted to 33 C; this temperature was maintained from the time
of inoculation until the cut curd was dipped into hoops. Before addition of 4.8 ml of commercial rennet extract to the milk, 363 ml of starter culture, blended mold from one agar slant and the coliform culture were added. The mixture was stirred, covered and allowed to stand undisturbed. Fifty minutes later the curd was cut and 20 min after cutting, the curd was dipped into eight hoops which when filled were held at room temperature. Six hours after addition of rennet extract to milk, the hoops of cheese were turned and holding at room temperature was continued. After 24 h, 69 g of salt were distributed over the surface of the cheese. The cheese was ripened for 1 week at 15.5 C with high humidity (80-90%) and turned daily to allow for mold development. Each cheese (approx. 275 g) was then wrapped in aluminum foil and ripened at 10 C for up to 8 additional weeks.

Each test coliform was used to inoculate two vats of milk which was made into cheese with one of the lactic starter cultures. This was repeated with the other lactic culture.

The schedule for sampling the cheese and for microbiological testing is indicated in Fig. 1 and 2. The following samples were taken: (a) milk after addition of rennet extract, (b) curd during dipping, (c) cheese before salting, (d) cheese 1 day after salting, (e) cheese after 7 days at 15.5 C and (f) cheese after 1, 3, 5, 7 and 8 weeks at 10 C.

Enumeration of coliform bacteria

A Most Probable Number (MPN) technique and plating on a selective medium were used to enumerate *E. aerogenes* or *Hafnia* sp. in samples of milk, curd or cheese. Each value reported in Fig. 1 and 2 represents the average count obtained with the MPN technique or violet red bile agar (VRBA) in two trials. The MPN method involved (a) blending 20 g of cheese curd (Waring blender) for 3 min with 180 ml of sterile 2% sodium citrate solution, (b) making 1:10 dilutions in the sodium citrate solution, (c) adding 1-ml quantities of the appropriate dilutions to tubes containing 9 ml of sterile nutrient broth, (d) incubating tubes at 35 C for 24 h, (e) streaking material from each tube onto eosin-methylene blue (EMB) agar (Difco), and (f) incubating the plates at 35 C for 24 h.

Use of VRBA (Difco) and incubation of plates at 35 C for 24 h also served to enumerate *E. aerogenes* and *Hafnia* sp. VRBA in plates was allowed to solidify before being overlaid with an additional 5 ml of medium. Values in Fig. 1 and 2 represent the averages of duplicate platings and duplicate trials of cheesemaking.

Measurements of moisture, NaCl and fat

Approximately 3 g of cheese placed in a previously dried moisture dish made of aluminum foil were dried for 16 h in a forced-draft air oven at 110 C. Samples were then removed and placed in a desiccator to cool. The amount of weight lost was considered to be the amount of moisture in the cheese. Values reported are averages of duplicate trials. The Volhard method (8) was used to determine the amount of NaCl in cheese. Samples were done in triplicate and results reported are average
values. The amount of fat in the cheese was determined by the 
Mojonnier method (12). Experiments were done in duplicate and 
results reported are average values.

Measurement of pH

The pH values of the milk and curd were determined with a pH 
meter (Model 10, Corning, Corning, New York) equipped with a 
miniature combination glass electrode. The pH values of cheese 
were determined with a saturated calomel half-cell, gold electrode and a 
portable potentiometer (Leeds and Northrup, North Wales, Pennsyl-

RESULTS

Eight vats of Camembert cheese were made; two for 
each combination of lactic starter culture and coliform 
being studied. E. aerogenes (MF-1) was selected for these 
experiments because of its inability to survive in 
skimmilk during fermentation by the above mentioned 
lactic starter cultures (unpublished data). Ḥafnia sp. 
(14-1) was chosen because it was generally the hardest of 
the coliform organisms tested in skimmilk during 
fermentations by lactic starter cultures (unpublished 
data).

Behavior of E. aerogenes during cheese manufacture

Relatively little growth of E. aerogenes occurred 
during the first 45-50 min of the cheesemaking operation 
(Fig. 1). Numbers of E. aerogenes increased during the 
interval between cutting the curd and turning the hooped 
cheese. Slightly larger numbers were attained when 
cheese was made with the lactic starter culture OD rather 
than with C-5. The increase during this time can be 
attributed both to bacterial growth and concentration of 
cells through entrapment in the curd (14). A 10- 
100-fold increase in numbers was detected when either 
lactic starter culture was used.

Numbers of E. aerogenes in cheese decreased slowly 
during the next 18 h until salting took place. The pH of 
the cheese dropped to 5.0 during the first 6 h and 
decreased further to 4.6-4.8 in the next 24-48 h. The 
resultant increase in acidity probably served to inactivate 
a portion of the coliform population. Other investigators 
also have noted that coliform bacteria are either 
inhibited or inactivated by pH values of 5.0 or below when 
they occur during production of Cheddar or cottage 
cheese or in other cultured products (6, 7, 14, 16, 17).

Behavior of E. aerogenes during ripening of cheese

After storage for 24 h, hoops were removed, salt 
(approximately 2%, wt/wt) was rubbed on the outside of 
the cheese, and cheese was ripened at 15.5 C and 80-90% 
relative humidity. During the first day of such ripening 
there was a further decrease in number of E. aerogenes in 
cheese made with either of the two starters (Fig. 1). 
Cheese made with culture OD showed a decrease of two 
logs in number of E. aerogenes during this 24-h period. A 
decrease of 1.5 logs in number was noted when C-5 
was used as the starter culture. The pH of the cheese did 
not change appreciably during this period, but remained 
at 4.6-4.8.

The number of viable E. aerogenes continued to 
decrease at 15.5 C; however, it was more rapid than 
during the initial storage (Fig. 1). Cheese made with 
lactic culture OD was free of viable E. aerogenes at the 
end of 1 week at 15.5 C. In contrast, the number of E. 
aerogenes in cheese made with lactic culture C-5 
decreased only slightly during the same time. Continued 
ripening at 10 C was marked by a further decrease in 
number of organisms until the cheese was free of viable 
E. aerogenes after 3 weeks. The pH of the cheese 
increased to 5.6-6.1 after 3 weeks at 10 C.

Behavior of Ḥafnia sp. during cheese manufacture

Behavior of Ḥafnia sp. was very similar to that of E. 
aerogenes during the first 6 h of cheese manufacture 
(Fig. 2). After turning of hooped cheese, numbers of 
Ḥafnia sp. in the cheese made with lactic culture OD 
decreased slightly for the next 18 h as did numbers of E. 
aerogenes. However, during this period there was a 
distinct increase in number of Ḥafnia sp. (.5 log/g) in 
cheese made with lactic culture C-5. The pH of the 
cheese decreased to 5.25 during the first 6 h and 
decreased further to 4.6-4.8 during the next 24-48 h, as 
did the pH of cheese containing E. aerogenes. Lactic 
culture OD appeared to inhibit Ḥafnia sp. more than did 
culture C-5 during the 24 h of cheese manufacture.

Behavior of Ḥafnia sp. during ripening of cheese

The number of Ḥafnia sp. in cheese made with lactic 
culture C-5 decreased by one log during the first day of 
ripening at 15.5 C (Fig. 2). This decrease in numbers was 
not as evident in cheese made with lactic culture OD. 
Further ripening at 15.5 C for 1 week resulted in a 
100-fold decrease in the population of Ḥafnia sp. in 
cheese made with either lactic starter culture. The number of viable Ḥafnia sp. was approximately 10/g at 
this time.

Ripening at 10 C failed to eliminate all viable Ḥafnia 
sp. from cheese made with either lactic culture OD or 
C-5. Rapid growth of Ḥafnia sp. was clearly evident 
during the first 5 weeks of ripening at 10 C (Fig. 2). 
Increased growth coincided with a marked increase in 
the pH of cheese. The population of Ḥafnia sp. in cheese 
made with lactic culture OD increased from 10^2 to 10^7/g 
during the 5-week period. Further holding at 10 C 
resulted in another increase in number of Ḥafnia sp. 
until more than 10^8/g were detected at the end of cheese 
ripening.

Cheese made with lactic culture C-5 contained 
numbers of Ḥafnia sp. in excess of 10^7/g after 5 weeks 
of ripening at 10 C. This was followed by a slight decrease 
in numbers during the final 3 weeks of ripening; however, 
the population of Ḥafnia sp. at the conclusion of 
ripening still exceeded 10^7/g. Ripening of cheese at 
10 C for 8 weeks was accompanied by a steady increase 
in pH of cheese from 4.6 to 7.2

DISCUSSION

Numbers of coliforms detected in samples of milk, 
curd and cheese generally were larger when the MPN 
procedure rather than VRBA plates was used. VRBA
observations were reported by Frank et al. They studied the fate of EEC in Camembert and brick cheese when only a selective medium (VRBA) was used. Similar observations were reported by Frank et al. (4,5) when they studied the fate of EEC in Camembert and brick cheese.

Information on the ripening process required for production of soft cheese and on the physical properties of the final product is useful to explain how large numbers of coliforms can occur in the cheese. Growth of certain coliforms during ripening and subsequent extended survival of these organisms may, in part, be attributable to the high moisture and high pH of the cheese. Soft-ripened cheese has a moisture content generally greater than 50% (Table 1). After formation of the curd, ripening of the cheese entails degradation of protein through activity of the mold. Knoop and Peters (II) studied microscopic structures that exist in Camembert cheese during its ripening. They reported that hyphae of P. camembertii were seen only in the ripened areas of the cheese. Inward progression of ripening results from diffusion of extracellular enzymes originating at the surface of cheese where they are generated by the mold mycelium. This activity reduces the acidity of the curd and the pH of cheese increases from about 4.9 to 6.0 or more, depending on the lactic culture that was used. Thus, the acid produced originally by the lactic bacteria is neutralized and an improved environment is created for growth of coliforms. Those cheeses found to contain large numbers of coliform bacteria had elevated pH values in the range of 7.0 to 7.3 (Fig. 2) at the end of the ripening process.

These data again emphasize the importance of active lactic cultures and of attention to sanitary practices in the manufacture of Camembert cheese.

**ACKNOWLEDGMENT**

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**REFERENCES**


Behavior of *E. aerogenes* in the manufacture of Camembert cheese, as described in this paper, generally was similar to that of EEC in Camembert cheese, as reported by Park et al. (14) and Frank et al. (4). However, of all the coliforms studied in this laboratory (4,5,14), the *Hafnia* spp. used in this study proved to be the only ones that were able to initiate growth in cheese and then attain rather substantial populations.

**TABLE 1.** Percent of moisture, NaCl, and fat in Camembert cheese made with the commercial lactic starter OD or C-5.

<table>
<thead>
<tr>
<th>Coliform</th>
<th>Commercial lactic starter</th>
<th>Moisture (%)</th>
<th>NaCl (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. aerogenes</em></td>
<td>OD</td>
<td>60.0</td>
<td>1.10</td>
<td>24.5</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>C-5</td>
<td>56.5</td>
<td>1.59</td>
<td>23.8</td>
</tr>
<tr>
<td><em>Hafnia</em></td>
<td>OD</td>
<td>52.5</td>
<td>1.30</td>
<td>27.6</td>
</tr>
<tr>
<td><em>Hafnia</em></td>
<td>C-5</td>
<td>60.5</td>
<td>1.27</td>
<td>23.2</td>
</tr>
</tbody>
</table>