Available evidence indicates that enterococci\(^1\) may, on occasion, be associated with food poisoning; however, their role as etiological agents of gastroenteritis is not universally accepted. In those instances in which they have been incriminated, they have usually represented the predominant flora, and other types of food-poisoning organisms were present in small numbers or undetected (1-3). In view of the inconclusive results of feeding experiments conducted by various investigators (3-9), their role is unknown. Nevertheless, until additional information is provided, which unequivocally demonstrates that this group is or is not pathogenic, the entry or development of large numbers of enterococci in foods should be avoided.

Of the various procedures by which microbial development may be prevented in food, the control of time and temperature is achieved most easily and is, therefore, most widely used. In practice, temperature control is often based on operational experience rather than the results of experimental time-temperature data on the behavior of microbial pathogens in perishable foods. Due to the fairly recent and rapid technological development of the food industry, many products and processes are available today that did not exist a few years ago. Precooked ready-to-serve foods, perishable meals vend-ed from machines, pressure- or vacuum-packed items packaged in unique and newly developed forms and containers, and dehydro-frozen foods are a few samples of modern food processing. Knowledge relative to the precautions necessary to safeguard the public health has not kept pace with rapidly advancing technological developments, and the problem of insuring adequate time-temperature control over perishable foods has become vastly complicated. For this reason, our laboratories have undertaken to develop an organized body of information on the effects of time and temperature upon the response of various food-poisoning bacteria in potentially hazardous foods. This information is intended to serve as a guide in the formulation of safe food-handling practices.

Our previous reports (10-12) dealt with the response of salmonellae and staphylococci in potentially hazardous foods held in the temperature range of 40 F through 150 F and revealed that these organisms do not multiply at temperatures of 42 F and below or at 116 F and above. The data also revealed that heating perishable foods to 150 F and holding every particle of the food at this temperature for at least 12 min reduced 10,000,000 salmonellae or staphylococci per g to non-detectable levels. Comparable effects were achieved in similarly contaminated foods when held at 140 F for 78 to 83 min.

The present report is an extension of our previous work, and is specifically directed toward determining the temperature limits of growth for fecal streptococci and toward defining the "incubation danger zone" for these organisms in ham salad, chicken à la king, and custard - foods frequently implicated in disease outbreaks.

**METHODS**

**Test Cultures**

The selection of test cultures was based on previous investigations conducted in our laboratory in which the response of 17 strains of various enterococci was determined in brain heart infusion broth at temperatures in the range of 40 F through 158 F. The enterococci were isolated from the following sources: river water, pig feces, raw municipal sewage, sewerage lagoon water, and foods in which enterococci were implicated as the etiological agents of gastroenteritis. Twelve of the cultures were identified as enterococci according to the classification of Sherman (13). The remaining five cultures, in addition to meeting Sherman’s criteria as enterococci, did not reduce tetrazolium, failed to grow in the presence of 0.04% potassium tellurite, were not beta hemolytic but did ferment mannitol and were, therefore, identified as *Streptococcus faecium*. No appreciable difference between the 17 strains was noted in their ability to survive or multiply in the cold

---

\(^1\)For purposes of brevity, the term enterococci as applied in this report includes the species *S. faecalis*, *S. faecalis* var. *zymogenes*, *S. faecalis* var. *lilquofaciens*, *S. durans*, and *S. faecium*, though it is recognized that the latter species is not universally accepted as an enterococcus.
(40 to 50 F). Their individual responses to higher temperatures, however, were related primarily to strain differences rather than species. For example, of three strains of S. faecalis var. liquefaciens, two died off slowly during 24 hours of incubation at 122 F. The third strain displayed a three-fold increase in numbers under the same test conditions. Though variation in response to temperature was observed among the 12 strains of enterococci, as a group they were not as heat resistant as the remaining five strains of S. faecium.

To obtain data representative of the general temperature response of enterococci in foods, we employed mixtures of the enterococci. On the basis of their behavior in brain heart infusion broth incubated at temperatures in the range of 113 F through 158 F, the following five strains were selected for study in food: S. faecalis (low heat resistance); S. faecalis var. liquefaciens, S. faecalis var. zymogenes, and S. durans (intermediate heat resistance); and S. faecium (high heat resistance).

The enterococci selected were lyophilized and prepared for inocula in a manner similar to that described previously for salmonellae and staphylococci (12). One-ml volumes of each of the appropriately diluted cultures were pooled to obtain a mixed culture containing approximately equal numbers of each of the five organisms. The mixed-culture inoculum so obtained for each experiment consistently yielded approximately 5,000,000,000 viable cells per ml, as determined by plate count.

Preparation of Food

Custard, ham salad, and chicken à la king were prepared according to the recipes of Angelotti, et al (12). They were weighed out in 100-g aliquots, placed in 6-oz, screw-capped jars, sterilized, and tested for sterility, as previously described (12). Each jar of food to be inoculated was allowed to equilibrate to the desired test temperature, as follows. Upon removal from refrigerated storage, duplicate jars were placed in 4-in. x 9-in. plastic bags. The air was evacuated from the plastic bags, which were twisted and closed with a rubber band, then suspended in a water bath adjusted to the desired temperature. The bags were so suspended that the hand-closed end of the bag was above the water surface, but the jars of food were completely submerged. This prevented seepage of water into the jars. Depending upon the temperature, the jars of food usually equilibrated within 1 to 2 hours.

Inoculation, Test Temperatures, and Bacteriological Examination

Aliquots of 0.2 ml of the mixed suspension of enterococcal culture were inoculated into duplicate jars of each of the test foods. This volume of suspension added to the 100 g of food in each jar resulted in a final inoculum of approximately 10,000,000 cells per g. (See last paragraph of "Test Cultures" above.) After the test foods were inoculated, the screw caps were replaced and the jars returned to their plastic bags and closed as described. The jars were vigorously shaken for 50 return strokes to distribute the inoculum and were then re-suspended in their respective water baths. Temperatures in the baths (plus or minus 0.18 F) were determined with dual-scale Centigrade- Fahrenheit thermometers, checked for accuracy against a Bureau of Standards thermometer, throughout the experiments.

Growth responses of the mixed enterococcal culture in the foods were determined as follows: (a) 40 F through 50 F at 2 F intervals - bacteriological analyses were performed every 24 hr for five days; (b) 60 F - bacteriological analyses were performed every 24 hr for five days; (c) 70 F - bacteriological analyses were performed every 12 hr for three days; (d) 80, 95, 105, and 115 F - bacteriological analyses were performed at 6-hr intervals for 36 hr; and (e) 118 F through 128 F at 2 F intervals - bacteriological analyses were performed at 6-hr intervals for 24 hr. At each sampling interval, 10 g of food was removed aseptically from each of the duplicate test jars and placed in a sterile mechanical-blender cup containing 90 ml of phosphate buffered dilution water (14). The resulting 1:10 food blend was homogenized for 2 min at approximately 8,000 rpm, and further serial 10-fold dilutions were prepared. Duplicate plates of each dilution were poured in Bacto plate count agar and incubated at 95 F for 24 hrs. To determine the number of organisms added per g, duplicate jars of inoculated food were similarly tested immediately after distribution of the inoculum. A single jar of sterile food was incubated in each of the water baths to serve as a "leak" control and was bacteriologically examined as above at the end of the incubation period.

Results

The growth response of the enterococci in ham salad, chicken à la king, and custard incubated at 40 F through 70 F are shown in Figures 1, 2, and 3. Curves depicting the growth response at 80 F through 128 F are shown in Figures 4, 5, and 6. Each point on these growth curves represents the mean of duplicate plate counts obtained from each of the paired jars of food.

In ham salad (Figure 1) the number of enterococci slowly declined throughout the 5-day incubation period at temperatures of 40 F through 46 F. The population density remained unchanged at 48 F but increased after four days' incubation at 50 F. An additional slight increase in numbers occurred after
Fecal streptococci in foods

Data collected to determine the highest incubation temperature at which the enterococci were capable of multiplying in these foods revealed that in all cases some multiplication occurred in the first few hours of incubation at 128 F, followed by a gradual decline in numbers. At 128 F, however, a fairly rapid rate of death was observed in all three foods. (See Figures 4, 5, and 6.) In chicken à la king (Figure 5) and custard (Figure 6) the organisms displayed a progressively increasing rate of multiplication at 80 F, 95 F, and 105 F, and the final concentration of organisms per g was quite similar in both foods at all three temperatures. Rapid multiplication occurred in ham salad (Figure 4) during the first 18 hr of incubation at 105 F; however, the final cellular concentration was considerably below that achieved at 95 F and 80 F.

Reproduction was fairly rapid in all three foods held at 115 F and the maximum cellular concentration was achieved in approximately 12 hr. Though the stationary phase of growth was attained early, the numbers of organisms developed per g were considerably less than those recorded at 80 F through 105 F.

Figure 1. Growth of fecal streptococci in ham salad temperature 40-70 F.

five days at 50 F. Good growth occurred at 60 F, and a significant increase in numbers was noted after 48 hr of incubation. At 70 F the maximum stationary phase of growth was achieved (4.3 x 10⁹ organisms per gram) after 48 hr of incubation.

In chicken à la king (Figure 2), the growth of the enterococci declined at 40 F, whereas no change was noted at 42 F. Temperatures of 44 F through 50 F produced growth, but several days of incubation were required before significant increases occurred. Rapid multiplication occurred at 60 F and 70 F; significant increases were noted after 48 hr at 60 F and in less than 24 hr at 70 F.

Custard (Figure 3) permitted more rapid multiplication of the enterococci than did either ham salad or chicken à la king. No detectable change occurred at 40 F, and a decrease in numbers, followed by a slight increase after 3 days of incubation, occurred at 42 and 44 F. Growth was poor at 46 F and good at 48 through 70 F. Though the maximum concentration of cells attained in both chicken a la king and custard incubated at 70 F were approximately equal (2.4 x 10⁹ and 2.0 x 10⁹ per g, respectively), the former concentration was achieved in less than 48 hr, whereas the latter required 72 hr.

Figure 2. Growth of fecal streptococci in chicken a la king temperature 40-70 F.
Fecal Streptococci in Foods

In comparing the data presented above to the data on salmonellae and staphylococci (10, 11) it is readily apparent that the enterococcal species are capable of growing in chicken à la king and custard at both lower and higher temperatures than are salmonellae and staphylococci. In no instance did the salmonellae or staphylococci multiply during a five-day period at 42 F or below (10, 11). By contrast, the enterococci grew slowly in custard incubated at 42 F; an approximately two-fold increase developed during the five-day period. The rate of multiplication and the final concentrations of the enterococci were significantly greater in custard and chicken à la king at temperatures of 44 F through 50 F than those of salmonellae and staphylococci.

In view of the most recent recommendation of the Public Health Service as set forth in the "Food Service Sanitation Manual, 1962 Recommendations of the Public Health Service" (15) it may be well to review these data in light of this Public Health Service recommendation. The Food Service Manual states that "All potentially hazardous food shall be maintained at safe temperatures (45 F or below, or 140 F or above), except during necessary periods of preparation and service." Potentially hazardous food is defined as "any perishable food which consists in whole or in part of milk or milk products, eggs, meat, poultry, fish, shellfish, or other ingredients capable of supporting rapid and progressive growth of infectious or toxigenic microorganisms."

The data presented above for enterococci and that reported earlier for salmonellae and staphylococci (10) reveal that growth of food-poisoning organisms occurs very slowly or not at all in potentially hazardous foods stored at 46 F and below. Thus foods with an internal temperature of 45 F may be stored safely for short periods. In order to chill foods rapidly to this temperature, it is desirable to operate the refrigerator at a lower temperature. In view of the cold-temperature tolerance of the enterococci, the time-temperature relationships shown in Table 1 should be observed to ensure that no significant increases of the enterococci occur. In comparing the data presented in Table 1 for enterococci to that for salmonellae and staphylococci similarly presented in a former report (10), it should be noted that the enterococci are capable of slightly better growth at the lower temperatures than either salmonellae or staphylococci.

The response of the enterococci to intermediate and warm holding temperatures indicates that some
growth of these organisms is possible at 126°F. A significant observation relative to the enterococci is that they typically displayed a very rapid rate of multiplication within the mid-portion of the temperature range investigated (70°F through 115°F) and may be expected to multiply rapidly in foods passing through this range during "heat-up" and "cool-down" intervals in food preparation and service operations.

The very rapid rate of multiplication displayed by the enterococci in the range of 95 to 115°F points up the fact that cooling foods slowly at room temperature is an unsatisfactory practice. The introduction of inadvertent contamination to heated foods passing slowly through this range in the cooling cycle may permit rapid proliferation and marked deterioration of an otherwise excellent product. Because of the increased rate of multiplication at the upper temperature limits that permit growth, it is essential that potentially hazardous foods be refrigerated immediately after cooking, or at least before they cool below 140°F.

Summary

Mixtures of enterococcal species were cultured in ham salad, chicken a la king, and custard at temperatures ranging from 40°F through 128°F. In ham salad, no growth occurred at temperatures of 40°F through 48°F, whereas slight growth occurred after 4 to 5 days at 50°F and good growth occurred at 60°F through 115°F. In chicken a la king, no growth occurred at 42°F or below, and good growth developed at 48°F through 115°F. In custard, poor growth was observed at 42°F and rapid growth was noted at temperatures of 48°F through 115°F. In all three foods, some

Table 1—Time-Temperature Relationship Necessary to Prevent Growth of Enterococci in Potentially Hazardous Foods

<table>
<thead>
<tr>
<th>Storage temperature in °F</th>
<th>Longest storage interval of food without growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>&lt; 1 day</td>
</tr>
<tr>
<td>48</td>
<td>1 day</td>
</tr>
<tr>
<td>46</td>
<td>2 days</td>
</tr>
<tr>
<td>44</td>
<td>3 days</td>
</tr>
<tr>
<td>42</td>
<td>4 days</td>
</tr>
<tr>
<td>40</td>
<td>5 days +</td>
</tr>
</tbody>
</table>
Fecal Streptococci in Foods

Multiplication occurred in the first few hr of incubation at 126 F, followed by a gradual decrease in numbers. A rapid rate of decline in numbers was observed in all three foods at 128 F.

REFERENCES


Plan To Attend The Fiftieth
ANNUAL MEETING
OF
The International Association of Milk, Food and Environmental Sanitarians

Find enclosed inserted in the loose-leaf program,
a reservation card for the Royal York Hotel, Toronto. If you have not already done so, please complete the card and mail it in immediately to assure your reservations for the 50th Annual Meeting.

See you there!

October 22-25, 1963
Royal York Hotel
Toronto, Ontario

Guest Banquet Speaker
Dr. Carl C. Byers
General Motors Corp.