Survival of *Clostridium perfringens* and Aerobic Bacteria in Ground Beef Patties during Microwave and Conventional Cookery

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**ABSTRACT**

Conventional oven cookery was more effective than microwave cookery for reducing numbers of aerobic microorganisms and *Clostridium perfringens* in ground beef patties when the meat was heated to approximately the same internal temperatures of 65-71°C for rare or 77-93°C for well done. Reductions in numbers of *C. perfringens* during microwave cookery of patties inoculated with 10^5 vegetative cells/g ranged from 0.75 to 1.48/g (log values); for conventional cookery, these reduction values ranged from 3.51 to 8.06/g (log values). Recovery of heat-stressed cells of *C. perfringens* was equally efficient in Trypton-Sulfite-Cycloserine agar and Sulfite-Polymyxin-Sulfadiazine agar.

Survival of *Clostridium perfringens* in chicken pieces, precooked chicken and cooked whole turkeys during heating and cooking in the microwave oven has been described in several publications (1,5,8). Little information is available on survival of this bacterium in ground beef although considerable information is available on other bacteria in ground meat and other foods (9,10,15,17). Ground beef in one form or another accounts for nearly one-half of all beef consumed in the United States (14). Therefore, studies were undertaken to observe survival of *C. perfringens* and aerobic bacteria in ground beef patties after cooking in a microwave oven and a conventional oven and to determine the effect of incubation at 5, 15 and 27°C on growth of *C. perfringens* and other surviving organisms in ground beef patties after microwave cooking. Also, a comparison of Sulfite-Polymyxin-Sulfadiazine agar (SPS) and Tryptose-Sulfite-Cycloserine agar (TSC) was made to determine if one medium was superior to the other for recovery of heat-stressed cells.

**MATERIALS AND METHODS**

Vegetative cells of *C. perfringens* strains HR2 and FD1, originally obtained from H. E. Hall, R. A. Taft Engineering Center, Cincinnati, Ohio, were used as inocula. Vegetative cells were prepared by inoculating 1 ml of stock culture into Tryptic Soy Broth (TSB) and incubating for 16 h at 37°C; then consecutive transfers at 4-, 4- and 2-h intervals were made into 10 ml of TSB and incubated at 37°C. The broth in the final tubes contained approximately 10^5 cells/ml and was used for inoculating samples of ground beef.

Media used for enumeration of *C. perfringens* were Sulfite-Polymyxin-Sulfadiazine agar (SPS) (2) and Tryptose-Sulfite-Cycloserine agar (TSC) (13). These media were made in the laboratory from the basic ingredients. The pouch method described by Bladel and Greenberg (4) was used. Incubation was for 24 h at 37°C. Confirmatory testing for *C. perfringens* consisted of the gram stain, nitrate-motility test, gelatin liquefaction and acid-gas production in lactose-gelatin medium (11). Aerobic counts were done using Plate Count agar (PCA) (Difco) and incubating at 32°C for 40 h (11).

Ground beef was divided aseptically into 120-g patties. Disposable vinyl medical gloves were worn when the meat was handled. A mold was used to form the patties (3 1/2 in. × 3 3/4 in. × 1/2 in.). The patties were separated into three groups: the uninoculated controls, those inoculated with *C. perfringens* strain FD1 and with strain HR2. Patties were inoculated with vegetative cells by adding 1.0 ml of culture to the surface of the patty. Each patty was kneaded 10 to 15 times to distribute the cells as well as possible throughout the meat and then reshaped in the patty mold. At this time a small indentation was made by pressing the thumb into the center of the patty. After storage for 1 d at 5°C, counts of *C. perfringens* and aerobes were made. After microwave cooking, samples were stored at 5, 15 and 27°C for 24 h when aerobic plate counts and counts of *C. perfringens* were made. Analysis of variance was used to analyze the data (19).

Procedures recommended by the National Livestock and Meat Board (Chicago) were followed for uniform microwave cooking of the patties (20). These recommendations were: (a) make a small indentation or hollow in the center of the patty, (b) place a piece of waxed paper over the patties' container before cooking, (c) rotate the container 1/2 turn at some point in the cooking time, and (d) let the container of patties stand for 1 to
2 min after removal from the microwave oven. Sawyer et al. (16) found that use of a plastic film wrap for single servings of chicken drumsticks, ham slices and pork slices produced a decrease in end temperature range but not in end temperature.

Two patties of each type, treatment and heat exposure were placed in plastic containers and covered with waxed paper and set into a Hobart microwave oven (Model M-321). The oven operated at a frequency of 60 Hz, 208-240 volts and 2860 watts. The container was rotated 1/2 turn after 45 s of exposure. The final internal temperature was measured by inserting a thermometer half-way between the center and edge of the patties after removal from the oven and standing 1 min. The times required to reach very rare, rare, medium and well done were determined in preliminary studies. As a result of these observations, the following guidelines were used during microwave cookery: for each an initial time of 45 s was used and then after turning the container an additional 10 to 20 s to obtain a final internal temperature of 60 to 65°C (very rare); an additional 20 to 30 s to obtain a final internal temperature of 65 to 71°C (rare); an additional 30 to 35 s to an internal temperature of 71 to 77°C (medium); and an additional 45 s to a temperature of 77 to 93°C (well done).

For conventional-oven cooking, patties were placed in glass containers and set into a General Electric oven preheated to 177°C. The time to reach a final temperature of 60 to 65°C (rare) was 25 to 30 min and 40 to 45 min to reach 77 to 93°C (well done).

RESULTS AND DISCUSSION

Storage of raw uninoculated and inoculated ground beef patties at 5°C for 24 h before cooking caused a decrease in numbers of *C. perfringens* in patties to which this organism had been added; no change occurred in the aerobic count (Table 1). Reduction in numbers of *C. perfringens* in the inoculated patties can be attributed to refrigeration at 5°C; this organism is sensitive to temperatures below the minimum for its growth and tends to die under such conditions (5,12). Storage at 5°C for 24 h had no apparent effect on numbers of *C. perfringens* in uninoculated meat. The resident flora apparently had adapted or was inherently stable to temperatures at these levels.

Cooking times for the patties can be affected by a number of variables such as the initial temperature of the meat patties to an internal temperature of 65 to 71°C.

**TABLE 1. Log of aerobic plate counts and of numbers of Clostridium perfringens in raw uninoculated and inoculated ground beef patties before and after storage at 5°C for 24 h. Patties were inoculated with *C. perfringens* strains FD1 or HR2.**

<table>
<thead>
<tr>
<th>Patty treatment</th>
<th>Counta</th>
<th>No. <em>C. perfringens</em>b</th>
<th>(CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time(h)</td>
<td>TSC</td>
<td>SPS</td>
<td></td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>0</td>
<td>7.05</td>
<td>2.40</td>
</tr>
<tr>
<td>24</td>
<td>7.65</td>
<td>2.40</td>
<td>2.40</td>
</tr>
<tr>
<td>Inoculated: strain FD1</td>
<td>0</td>
<td>6.70</td>
<td>5.29</td>
</tr>
<tr>
<td>24</td>
<td>6.74</td>
<td>4.63</td>
<td>4.86</td>
</tr>
<tr>
<td>Inoculated: strain HR2</td>
<td>0</td>
<td>7.02</td>
<td>5.11</td>
</tr>
<tr>
<td>24</td>
<td>7.68</td>
<td>4.41</td>
<td>4.45</td>
</tr>
</tbody>
</table>

**TABLE 2. Logs of aerobic plate counts and of numbers of Clostridium perfringens in ground beef patties before and after microwave and conventional cookery. Patties were inoculated with *C. perfringens* strains FD1 and HR2. (Average of 4 replicates).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Raw count (CFU/g)</th>
<th>Aerobic count (CFU/g)</th>
<th>C. perfringens (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave: rare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.05</td>
<td>4.26</td>
<td>2.40</td>
</tr>
<tr>
<td>FD1</td>
<td>6.70</td>
<td>4.18</td>
<td>5.29</td>
</tr>
<tr>
<td>HR2</td>
<td>7.02</td>
<td>3.86</td>
<td>5.11</td>
</tr>
<tr>
<td>Microwave: well done</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.05</td>
<td>3.63</td>
<td>2.40</td>
</tr>
<tr>
<td>FD1</td>
<td>6.70</td>
<td>4.25</td>
<td>5.29</td>
</tr>
<tr>
<td>HR2</td>
<td>7.02</td>
<td>3.74</td>
<td>5.11</td>
</tr>
<tr>
<td>Conventional: rare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.05</td>
<td>2.26</td>
<td>2.40</td>
</tr>
<tr>
<td>FD1</td>
<td>6.70</td>
<td>2.08</td>
<td>5.29</td>
</tr>
<tr>
<td>HR2</td>
<td>7.02</td>
<td>2.15</td>
<td>5.11</td>
</tr>
<tr>
<td>Conventional: well done</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.05</td>
<td>2.20</td>
<td>2.40</td>
</tr>
<tr>
<td>FD1</td>
<td>6.70</td>
<td>2.08</td>
<td>5.29</td>
</tr>
<tr>
<td>HR2</td>
<td>7.02</td>
<td>1.79</td>
<td>5.11</td>
</tr>
</tbody>
</table>

*Average of 4 samples, 1 sample from each of 4 replicates.
Comparison of counts on TSC (Tryptose-Sulfite-Cycloserine agar) and SPS (Sulfite-Polymyxin-Sulfadiazine agar).
pared to 10^2/g for the same "doneness" when cooked with microwave cookery or greater for conventional cookery. Also, total counts were reduced by a log factor of 3 from approximately 10^7 CFU/g to 10^4/g for microwave-cooked product for both rare and well-done as compared to 10^2/g for the same "doneness" when cooked in a conventional oven. The longer times required for cooking by conventional means than by microwave probably was the main factor contributing to the differences in numbers of survivors in the meat because destruction of bacteria is time-temperature dependent.

Reductions of comparable magnitudes in total numbers of organisms have been reported by Mueller (15). Fung and Cunningham (10), in an extensive review on the effect of microwaves on microorganisms in foods, cited publications in which the differences in numbers of survivors between microwave cookery and conventional cookery were observed to be insignificant or greater for microwave cookery or greater for conventional cookery. Difference in these instances may be attributed to differences in heat resistance and numbers of the microbial flora, differences in heating dependent on composition of the food and inability to heat to the same final temperature throughout the food. Sawyer et al. (17), for example, observed that aerobic plate counts of individual pre-cooked samples of beef loaf, peas and potatoes reheated by a conduction system were less than but not significantly different than counts for food reheated in convection or microwave systems. The similarity in counts in this instance could be attributed to survival of thermoduric organisms during the initial cooking and their ability to withstand reheating temperatures. Dahl et al. (9) evaluated the microbiological quality and end temperature of portioned food after heating in a microwave oven as used in a hospital cook/chill foodservice system and observed wide ranges of end temperatures and of aerobic plate counts. They concluded that there was a need for better control of microwave-heating to produce food with constant microbiological quality.

C. perfringens also survived to a greater extent during microwave heating than during conventional cookery. Log values of reductions of numbers of C. perfringens for microwave cookery of inoculated patties ranged from 0.75 to 1.48; for conventional cookery, these values ranged from 3.06 to 3.51 (Table 2). These values were obtained by subtracting logs of counts for cooked product from those for raw product. Reduction in numbers of C. perfringens in uninoculated meat was usually one log cycle or less. Perhaps the indigenous strains were more resistant than the laboratory strains, or it may be a reflection of the low numbers of C. perfringens (usually < 100/ g) in the raw product. These results agree with work completed by several investigators working with C. perfringens in other foods. Blanco and Dawson (5) reported a two-log cycle reduction in resident vegetative and inoculated C. perfringens cells on chicken pieces heated to 98°C. Craven and Lillard (8) concluded that heating pre-cooked chicken by microwaves to internal temperatures up to 84°C is not adequate to eliminate the possibility of C. perfringens food poisoning. Aleixo et al. (1) cooked whole turkeys inoculated with C. perfringens in microwave ovens to an end point of 76.6°C and found that the extent of survival of C. perfringens on the cooked, unstuffed turkeys was proportional to the number of spores in the initial inoculum.

The numbers of aerobic organisms as well as numbers of C. perfringens did not increase during storage of the microwave-cooked patties for 24 h at 5 or 15°C. No increase in numbers of C. perfringens was expected at 5 or 15°C because these temperatures are below its minimal growth temperature (3). At 27°C (Table 3), however, significant increases in numbers of aerobes and of C. perfringens were observed. Numbers of C. perfringens increased as much as two log cycles or more in some instances and the aerobic counts as much as three log cycles. Numbers of C. perfringens in patties cooked to the very rare or rare stages after abusive storage for 24 h were in the range that can produce food poisoning (3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aerobic count (CFU/g)</th>
<th>C. perfringens (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Very rare - C</td>
<td>4.44</td>
<td>6.29</td>
</tr>
<tr>
<td>Rare - C</td>
<td>4.55</td>
<td>6.84</td>
</tr>
<tr>
<td>Medium - C</td>
<td>3.30</td>
<td>4.71</td>
</tr>
<tr>
<td>Very rare - F</td>
<td>5.04</td>
<td>7.98</td>
</tr>
<tr>
<td>Rare - F</td>
<td>5.39</td>
<td>7.52</td>
</tr>
<tr>
<td>Medium - F</td>
<td>3.20</td>
<td>4.52</td>
</tr>
<tr>
<td>Very rare - H</td>
<td>5.00</td>
<td>8.69</td>
</tr>
<tr>
<td>Rare - H</td>
<td>3.99</td>
<td>8.07</td>
</tr>
<tr>
<td>Medium - H</td>
<td>3.67</td>
<td>4.94</td>
</tr>
</tbody>
</table>

*C = Control, uninoculated; F = inoculated with strain FD1; H = inoculated with strain HR2.

An evaluation of TSC and SPS agars for recovery of C. perfringens stressed by heat showed no differences between the two media. TSC medium, however, was more effective than SPS medium for eliminating interfering facultative organisms. Several workers have reported previously on the inhibition of fecal streptococci in TSC agar (6,7,18,21).

This work shows that conventional cookery reduces total numbers of aerobes and vegetative cells of C. perfringens in ground beef patties to a greater extent than microwave cookery when heated to approximately the same internal temperature. Storage of cooked patties 24 h at 5 and 15°C inhibited growth of both aerobes and C. perfringens but an abusive temperature of 27°C resulted in a one- to three-log increase in numbers depending.
upon degree of doneness of the patties. During both types of cookery, thermodynamically organisms survived, which reemphasizes the need for proper cooling and storage of cooked patties as well as the need for a sufficiently high end temperature upon reheating.

REFERENCES