Assessment of the Sanitary Effectiveness of Holding Temperatures on Beef Cooked at Low Temperature

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Beef cubes cooked at low temperature were surface inoculated and incubated at 43.3 through 53.3 C to establish temperature limits for growth of staphylococci, salmonellae, and Clostridium perfringens. A greater than 99% reduction in staphylococci was achieved after 6 hr at 48.8 C, of salmonellae at 51.1 C, and of C. perfringens at 53.3 C. There were no survivors of a mixed inoculum of Staphylococcus aureus, Salmonella enteritidis, and C. perfringens after 12 hr at 51.1 C.

The prevention of microbial development in prepared food has most often depended upon control of time and temperature. Among recent innovations in the food industry is the precooked product served to order by carry-out vendors. Temperature control in these operations is frequently based upon quality considerations rather than public health. One such precooked product is the sliced beef sandwich, prepared at the customer's request from beef cooked at low temperature (60 C). The cooked beef is maintained at approximately 48.8 C by commercial heat lamps for as long as 12 hr. A similar situation exists in the case of beef roasts held on conventional cafeteria serving lines.

The practice of holding cooked beef for extended periods in the operations mentioned poses the question of whether enteropathogens would survive and multiply at the prevailing temperatures. Angelotti et al. (1) studied the growth of strains of salmonellae and staphylococci in custard, ham salad, and chicken a la king. They found that both groups of organisms decreased at temperatures of 46.6 C and above. Bryan and Kilpatrick (2) made a bacteriological survey of raw and cooked meat and the kitchen environment in a fast-food service operation. They isolated Clostridium perfringens from 11 of 36 samples of sliced roast beef, cooked to an internal temperature of 68.4 C or higher and held under heat lamps. They demonstrated that beef roasts are held at optimum temperatures for the multiplication of C. perfringens for many hours in such an operation.

The purpose of this study was to obtain data on the growth of staphylococci, salmonellae, and C. perfringens on roast beef when held at various temperatures.

MATERIALS AND METHODS

Test organisms. Test cultures, selected on the basis of incrimination in food poisoning outbreaks, included Salmonella typhimurium; S. anatum; S. enteritidis; Staphylococcus aureus MS149, 196E, and 161; and C. perfringens E-3, A86, and A91. The salmonellae and staphylococci were maintained on Trypticase soy agar (BBL), and the clostridia were maintained in cooked meat medium (Difco). Inocula for a given experiment were prepared by transferring the stock cultures of staphylococci and salmonellae to Trypticase soy broth (BBL) and the clostridia to fluid thioglycolate medium (Difco). All cultures were incubated 24 hr at 35 C.

Preparation of beef. Commercial grade top-round beef roasts, approximately 15 lb each, were purchased locally over-the-counter. The meat, freshly cut at the time of purchase, was brought to the laboratory, where all fat and sinew were removed.

The roasts were cooked to an internal temperature of 60 C in a standard electric oven, modified to provide a maximum cooking temperature of 98.8 C. The accuracy of the oven temperature was determined by using thermocouples distributed at the bottom, top, sides, and center of an empty oven and inserted similarly into the beef roast during control and alternate experimental runs.

Two "thermo-pins" (5) were placed in parallel positions in the roast in such a manner that during cooking they would direct the heat from the bottom
upward into the center of the roast. Cooking oil (Mazola) was poured over the surface of heavy parchment paper that lined the bottom of the cooking pan. The roast was placed on the paper, covered with an oil-soaked cloth towel, and an ordinary meat thermometer was inserted into the center of the roast.

The pan containing the roast was put into the oven, which had been preheated to 98.8 C, and the roast was cooked for 3.5 hr, after which the thermometer in the center of the roast registered the desired internal temperature of 60 C. This procedure duplicates commercial practice (9), and the resulting roasts are well done externally and rare internally.

Temperature studies. Each roast was removed from the oven, cooled at room temperature for 15 min, and the thermo-pins were removed. The center, rare portion of beef was cubed into 50-g samples (2-inch cubes), and each cube was placed in a 9-oz wide-mouth screw-capped jar that had been equilibrated to the appropriate incubation temperature. Four jars and a control were prepared for each temperature and each organism. The upper surface of each experimental beef cube was covered with 0.1 ml of 24-hr broth inoculum of the organism to be tested. This volume yielded an initial population of approximately 4 x 10^4 to 4 x 10^6 clostridia, 3 x 10^4 salmonellae, or 5 x 10^4 staphylococci, determined by viable plate counts of broth cultures as well as of the 0-hr blended sample.

Each organism was tested three times. Each jar, tightly capped, was heat-sealed inside a plastic bag and was submerged in one of a series of carefully controlled water baths set at selected temperatures between 43.3 and 53.3 C. Each strain was incubated at all temperatures in a given experiment.

At 6-hr intervals for 24 hr, an inoculated jar was removed from each water bath, and the contents were transferred to a sterile Waring blender cup to which 400 ml of sterile, cold (7.2 C) 0.1% peptone water had been added. Each jar was rinsed with an additional 50 ml of peptone water, which was then added to the blender cup. The mixture was blended for 2 min at low speed, and appropriate tenfold dilutions were prepared from the blend in sterile 0.1% peptone water. Duplicate plates were poured from these dilutions using plate count agar (Difco) for the total aerobic counts and sodium sulfate polymyxin sulfadiazine (SPS, BBL) for the anaerobic counts.

Egg-yolk medium 110 was used as a selective medium for staphylococci, and Brilliant Green sulfadiazine was used as the selective medium for salmonellae. Preliminary studies showed that plate count agar was the most effective recovery medium for staphylococci and salmonellae, and anaerobic SPS was the most effective medium for recovery of C. perfringens.

Verification. Three typical colonies from each medium were confirmed biochemically. The control, or uninoculated, meat sample was also tested for any growth or contamination, in the same manner as the inoculated samples. None of the uninoculated meat samples yielded any significant bacterial growth.

In one experiment, 0.1 ml of inoculum of a representative strain from each bacterial group (S. aureus 161, S. enteritidis, and C. perfringens E3) was added to a 50-g cube of beef to approximate natural mixed contamination. Only temperatures between 51.1 and 53.3 C were used, because the individual strains of salmonellae and C. perfringens had survived the 48.8 and 50-C exposure temperatures in earlier experiments.

RESULTS

The results of this study are compiled in Table 1. Each value tabulated represents the mean of three separate analyses. The resistance of individual strains of each of the three groups to the temperatures tested was quite similar.

The growth of S. aureus MS149, the most resistant of the staphylococci, is shown in Fig. 1. The organisms grew well at 43.3, 44.4, and 45.5 C. At 46.6 C, a slow decrease in numbers took place during 24 hr. A greater than 99% reduction in staphylococci occurred after 6 hr at 48.8 C. Some survivors were detected after 12 hr, but none after 18 hr.

Salmonellae grew well at all temperatures below 48.8 C, requiring that this species be tested at higher temperatures. The growth of S. typhimurium at temperatures between 48.8 and 53.3 C is shown in Fig. 2. At 48.8 C, a slight increase occurred after 6 hr, a decrease of greater than 90% after 18 hr, and greater than 99% after 24 hr. A greater than 99% reduction in viable organisms occurred in 18 hr at 50 C, in approximately 6 hr at 51.1 C, and in less than 6 hr at 53.3 C.

The growth of C. perfringens A86 at the higher temperatures is shown in Fig. 3. The organisms grew extremely well during the first 12 hr at 48.8 C, and declined sharply after 18 and 24 hr. The number of viable organisms increased 90% or more by 6 hr at 50 C and by 12 hr at 51.1 C. A subsequent reduction of approximately 90% or more was exhibited by 18 hr at both temperatures. A greater than 99% reduction was achieved in less than 6 hr at 53.3 C.

The growth responses of a representative strain from each of the genera inoculated together onto beef cubes and held at 51.1 C are shown in Fig. 4. No S. enteritidis or S. aureus 161 survived after 6 hr at 51.1 C; a few C. perfringens E-3 cells survived 6 hr at this temperature, but none remained after 12 hr. No survivors of the three genera were found after 6 hr at 52.2 and 53.3 C.
| Strain            | Temp (C) | 0 hr  | 6 hr  | 12 hr | 18 hr | 24 hr |
|------------------|----------|-------|-------|-------|-------|-------|
|                  |          | 510,000 | 8,500,000 | 9,200,000 | 3,800,000 | 4,000,000 |
|                  |          | 4,800,000 | 4,000,000 | 4,000,000 | 330,000 |
|                  |          | 590,000 | 400,000 | 320,000 | 4,000 |
|                  |          | 1,400 | <300 | <300 | <300 | <300 |
|                  |          | <300 | <300 | <300 | <300 |
| *S. aureus* MS149 | 43.3     | 580,000 | 140,000,000 | 740,000,000 | 170,000,000 | 490,000,000 |
|                  | 44.4     | 87,000,000 | 160,000,000 | 110,000,000 | 140,000,000 |
|                  | 45.5     | 98,000,000 | 170,000,000 | 40,000,000,000 | 110,000,000 |
|                  | 46.6     | 59,000 | 810,000 | 1,300 | 5,000 |
|                  | 48.8     | 650 | 800 | <300 | <300 | <300 |
| *S. aureus* 161  | 43.3     | 940,000 | 44,000,000 | 12,000,000 | 69,000,000 | 4,600,000 |
|                  | 44.4     | 8,300,000 | 5,200,000 | 71,000,000 | 430,000 |
|                  | 45.5     | 58,000,000 | 120,000,000 | 120,000,000 | 74,000,000 |
|                  | 46.6     | 9,500 | 1,000 | <300 | <300 | <300 |
|                  | 48.8     | <300 | <300 | <300 | <300 |
| *Salmonella typhimurium* | 48.8 | 3,200,000 | 10,000,000 | 1,200,000 | 50,000 | 5,000 |
|                  | 50.0     | 97,000,000 | 83,000 | 4,100 | <300 |
|                  | 51.1     | 53,000 | 300 | <300 | <300 |
|                  | 53.3     | <300 | <300 | <300 | <300 |
| *S. anatum*      | 48.8     | 3,300,000 | 300,000 | 32,000 | 2,900 | 500 |
|                  | 50.0     | 6,000 | 600 | <300 | <300 |
|                  | 51.1     | 1,200 | <300 | <300 | <300 |
|                  | 53.3     | <300 | <300 | <300 | <300 |
| *S. enteritidis* | 48.8     | 1,200,000 | 1,500,000 | 200,000 | 36,000 | 1,200 |
|                  | 50.0     | 94,000 | 7,600 | 2,200 | 360 |
|                  | 51.1     | 34,000 | 300 | <300 | <300 |
|                  | 53.3     | <300 | <300 | <300 | <300 |
| *Clostridium perfringens* E-3 | 48.8 | 350,000 | 3,600,000 | 1,200,000 | 670,000 | 1,100,000 |
|                  | 50.0     | 870,000 | 490,000 | 540,000 | 520,000 |
|                  | 51.1     | 13,000 | 35,000 | 6,400 | 3,000 |
|                  | 53.3     | <300 | <300 | <300 | <300 |
| *C. perfringens* A-86 | 48.8 | 40,000 | 5,700,000 | 170,000,000 | 890,000 | 100,000 |
|                  | 50.0     | 800,000 | 900,000 | 7,000 | <300 |
|                  | 51.1     | 120,000 | 350,000 | 3,800 | <300 |
|                  | 53.3     | <300 | <300 | <300 | <300 |
| *C. perfringens* A-91 | 48.8 | 66,000 | 35,000,000 | 40,000,000 | 11,000,000 | 13,000,000 |
|                  | 50.0     | 8,600,000 | 7,800,000 | 7,000,000 | 450,000 |
|                  | 51.1     | 590 | <300 | <300 | <300 |
|                  | 53.3     | <300 | <300 | <300 | <300 |

*Total counts for staphylococci and salmonellae in plate count agar, and *Clostridium* in sulfadiazine polymyxin sulfite agar.

**DISCUSSION**

The results of this study show that the strains of staphylococci, salmonellae, and *C. perfringens* used in this study are capable of growing on the surface of roast beef at temperatures as high as 43.3 to 45.5 C. Death of staphylococci took place in 18 hr at 48.8 C. The population of salmonellae decreased rapidly above 48.8 C. Destruction of *C. perfringens* occurred above 51.1 C. Strains representing all three groups in a mixed inoculum were destroyed in less than 12 hr at 51.1 C.

It is apparent that the growth curves which...
Fig. 1. Growth of Staphylococcus aureus MS 149 on roast beef incubated at various temperatures.

Fig. 2. Growth of Salmonella typhimurium on roast beef incubated at various temperatures.

Fig. 3. Growth of Clostridium perfringens A86 on roast beef incubated at various temperatures.

Fig. 4. Growth of Clostridium perfringens E-3, Salmonella enteritidis, and Staphylococcus aureus 161 inoculated together on roast beef and incubated at 51.1 C.
resulted when all three genera were added to beef cubes simultaneously (Fig. 4) represent a more dramatic decline than when the organisms were tested alone. No Salmonella or Staphylococcus, and only a few Clostridium, survived 6 hr at 51.1°C in a mixed inoculum. When tested individually, the genera showed sharp decreases at 6 hr, but significant numbers of survivors were still present at this temperature. One explanation that could be offered for this difference in resistance is competitive inhibition. The metabolic activities of a mixed flora inhibit the growth of individual species in the population. Recently, Speck (6) offered another illustration of inhibitory competition when he studied the interactions among commercial lactic streptococci and different foodborne pathogens. When inoculated alone into sterile, reconstituted, nonfat milk solids, Salmonella gallinarum grew rapidly. However, when a mixed-strain starter culture was present, growth was depressed remarkably.

The cooking process eliminated all significant contamination on the surface of the roast beef. The general distribution of the three groups of organisms in the environment, however, would facilitate recontamination. Furthermore, the continued handling of a vended product such as roast beef would transfer enteropathogens from human sources to the food. When conditions of incubation, time, and temperature are proper, these cells multiply and produce toxins.

The recommended temperature for holding potentially hazardous foods in food-service operations, in vending machines, and aboard aircraft is 60°C (3, 4). Our data on C. perfringens, staphylococci, and salmonellae substantiate the earlier studies of Angelotti et al. (1) and Bryan and Kilpatrick (2) and suggest that reducing the holding temperature of 60°C for beef served to order is possible without endangering the public health from growth of staphylococci, salmonellae, or C. perfringens.

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LITERATURE CITED

1. Angelotti, R., M. J. Foter, and K. H. Lewis. 1960. Time-temperature effects on salmonellae and staphylococci in foods. II. Behavior at warm holding temperatures. Thermal-death-time studies. U.S. Pub. Health Serv. Tech. Rep. no. F60-5.
2. Bryan, F. L., and E. G. Kilpatrick. 1971. Clostridium perfringens related to roast beef cooking, storage, and contamination in a fast food service restaurant. Amer. J. Pub. Health 61:1869-1885.
3. Public Health Service. 1962. Food Service Sanitation Manual. Pub. Health Serv. Publ. No. 934.
4. Public Health Service. 1965. The vending of foods and beverages. A sanitation ordinance and code. Publ. Health Serv. Publ. No. 546.
5. Sam's Roast Beef Sandwich Division Manual. 1969. Denney's Restaurants, Inc., Le Marada, Calif.
6. Speck, M. L. 1972. Control of foodborne pathogens by starter cultures. J. Dairy Sci. 55:1019-1021.