Influence of Heating and Cooling Rates on Spore Germination and Growth of Clostridium perfringens in Media and in Roast Beef

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ABSTRACT. Spore germination, growth and survival of Clostridium perfringens during heating and/or cooling were examined. The spores in media (FTM or ground beef) were exposed to rising temperature from 20 to 60°C at increment rates of 7–25°C/h or to falling temperature from 60 to 15 or 10°C at decrement rates of 5 to 25°C/h. Roast beef was prepared with various muscles inoculated with the spores. Although germination and growth occurred while heating, the vegetative cells were killed at 60°C in media and in roast beef. During exposure to falling-temperature rates of 25 to 15°C, no change in the population was observed; multiplication of not more than 10-fold at 10°C/h and exponential multiplication at 7.5 and 5°C/h were observed. Roast beef prepared with gluteus, semitendinosus, or halved semitendinosus muscle did not support any growth of C. perfringens during cooling/chilling. That prepared with the whole muscles of semimembranosus supported some growth, but it did not surpass the inoculum size.—KEY WORDS: Clostridium perfringens, roast beef, spore.

A provision of the Japanese Specifications and Standards of Food and Additives [9] provides that all the meat products but dried ones and unpasteurized cured ones shall be pasteurized by a method of heating at an internal temperature of 63°C for 30 min or by an equivalent or superior method thereto. The provision does not seem to fulfil the required quality of such meat products that the external parts are fully cooked but the internal parts are rather rare; i.e., roast beef, beef of "Tataki" (a Japanese way of cookery, applied to "Sashimi" or raw fish, by which only the surface of meat is browned), and similar products. Consequently, these precooked meat products are not authorized to be supplied by manufacturers of meat products in this country; nevertheless, dishes of roast beef or "Tataki" are served at restaurants or food service establishments.

The desirable quality of roast beef depends upon the degree of doneness characterized by interior bright red color, juiciness, and tenderness. Some investigators reported the relationship between the rare quality of beef roasts and the final roast temperatures. Visser et al. [16] defined the rare roast as the one cooked to a final temperature of 55°C. Bernofsky et al. [2] regarded the beef cooked to 60°C or lower temperature to be rare. The rare roast according to the definition by Goodfellow and Brown [7] is the one cooked to 57°C or bellow. According to Blankenship et al. [3], about 2% reduction in the rare area may be expected for each degree increasing in the maximum internal temperature between 54.4 and 64.1°C.

Requirements for production of precooked roast beef have been established in the United States [1]. The current requirements have been effective with some modifications since July, 1983 [6]. The regulations require that cooked beef shall be heated to an internal temperature of 145°F (62.8°C) or by one of the fifteen alternative cooking procedures in regard to both time and temperature. These requirements were designed to assure a 7D reduction of salmonellae and to retain rare appearance [4, 8]. Our previous study [13] also showed that about 10 million colony-
forming units (CFU)/g of *Escherichia coli*, salmonellae or *Staphylococcus aureus* inoculated on the surface or into the geometric center of 2 kg blocks of beef were inactivated by heating to 57°C in a convection oven at a temperature higher than 120°C. The products were judged to be organoleptically satisfactory by a panel.

The present study dealt with the fate of *Clostridium perfringens* spores exposed to rising and/or falling temperature environments for the following reasons; (1) *C. perfringens* is a potential contaminant of raw meat, (2) many outbreaks of food-borne *C. perfringens* infections have been traced to the consumption of roast beef in foreign countries [4, 5, 17], (3) its spore may germinate and grow inside the roasts where the conditions are anaerobic and where heat penetration and heat loss are poor, (4) internal parts of roasts may be contaminated with its spores by mechanical tenderization, pickle injection and/or re-structuring meat, and (5) few reports have been published on germination and growth of *C. perfringens* spores during the entire processes of production of roast beef.

**MATERIALS AND METHODS**

**Test Organism:** Spores of *C. perfringens* H-3 were prepared as reported by Uemura [15].

**Growth Media:** Either fluid thioglycollate medium II (FTM, Nissui) or sterilized ground beef was used. Sterilized ground beef was prepared by a modification of the method of Willardsen et al. [18]. A 15 g portion of twice-ground (a 3 mm plate) lean beef (pH 6.0±0.2) was stuffed into a #24 NoJax casing (cellulose tubing), that had been tied off at one end and placed in a test tube (21 mm in diameter), through a pneumatic stuffer (Nantsune, Osaka) and sterilized at 120°C for 15 min to eliminate naturally occurring *C. perfringens* and competing organisms.

**Inoculation:** A 0.2 ml portion of an appropriately diluted spore suspension was either pipetted into a 15 ml FTM or injected (a one-ml disposable syringe with a 9.5 cm needle) into the geometric center of 15-g sterilized ground beef to achieve a population of about 10⁴ spores per ml or g. The other end of the casing was then tied off. All the inoculated growth media were heat-activated at 80°C for 15 min immediately before the following tests.

**Heating at Constantly-Rising Temperature:** Heat-activated tubes were submerged in a 50 liter water bath (Model TS-2, Tabai, Kyoto) adjusted to 20°C. After the growth media were tempered to 20°C, heaters of the bath were turned on to attain a rising-temperature rate of 7, 13, 20, or 25°C/hr. Preliminary test proved that various rates of constantly-rising temperature were obtained with combinations of the heaters installed. Tubes were withdrawn periodically and immediately transferred to an ice bath. Sampling was repeated till the temperature reached 60°C. All temperatures were monitored with a recorder equipped with thermocouples.

**Cooling at Constantly-Falling Temperature:** Heat-activated tubes were submerged in a 50 liter water bath adjusted to 60°C. After the growth media were tempered to 60°C, the water bath was adjusted manually so as to attain a constantly-falling-temperature rate of 25, 20, 15, 10, 7.5 or 5°C/hr.

The differences between the desired and the actual temperature were not larger than 2°C at any intervals of 10 min. Tubes were withdrawn periodically and immediately transferred to an ice bath. Sampling was repeated until the temperature reached 15 or 10°C. All temperatures were monitored.

**Preparation of Roast Beef:** Frozen boneless beef muscles of biceps femoris, semimembranosus, gluteus, and semitendinosus were used.

After thawed in a cold room (2–4°C), each beef mass was artificially contaminated with *C. perfringens* spores; the approximate center
of each mass was cut open, and two tubings prepared as described above were placed therein. The inoculated beef masses were roughly sutured, and suspended into cotton stockinetos or stringed.

They were roasted in a convection oven (K-20, Electrolux, Sweden) at 130°C. During the operations, the pans beneath the racks were filled with water, and the doors of the oven were kept closed. The oven and the roast temperatures were monitored with thermocouples. The dry and the wet temperatures inside the oven ranged from 107 to 139°C (mean; 131°C), and from 54 to 58°C (mean; 56°C) in a cycle of about 5 min, respectively. The roasts were removed from the oven when their internal temperature reached 55°C. During cooling at room temperature for an hour, the internal temperature of the roasts reached a maximum of 63–64°C. The roasts were transferred to a cold room and refrigerated overnight.

Two samples were taken from each roast for bacterial examinations. Immediately after the internal temperature reached 60°C, one tubing was withdrawn, vacuum-packaged, and chilled in an ice bath (60°C samples). The other one was sampled from the chilled roast after overnight refrigeration of the roast (overnight samples).

Enumeration of Viable Cells: A suspension of treated ground beef (1:10) was prepared by maceration in a Stomacher 400 (Colworth). The total C. perfringens population in each growth medium was determined. Heat-resistant spore population thereof was determined after heat treatment at 80°C for 10 min. Each 1 ml portion of an appropriately diluted specimen was transferred to an anaerobic pouch (PT pouch; Sakami Rika, Osaka). A 15 ml amount of half-strength Handford agar (Eiken, Tokyo) was poured into each pouch and the contents were mixed well. After the agar was allowed to solidify, the pouches were sealed by an impulse-heat-sealer (Poly-sealer 210, Fuji MFG) and incubated overnight at 40°C. Typical black colonies were regarded as those of C. perfringens.

RESULTS

Internal Temperature Profile: A typical internal temperature profile of roast beef from biceps femoris during heating, cooling, and chilling versus time is illustrated in Fig. 1. For about an hour after start of roasting, the
Table 1. Rising-temperature rates and falling-temperature rates of roast beef from various muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Green Weight (kg)</th>
<th>Cooking Time (h)</th>
<th>Temperature Rates (°C/hr) during</th>
<th>Time to Chill (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>60–40°C</td>
<td>40–20°C</td>
</tr>
<tr>
<td>biceps femoris</td>
<td>10.2</td>
<td>4.8</td>
<td>15.0a</td>
<td>10.0</td>
</tr>
<tr>
<td>semimembranosus</td>
<td>7.2</td>
<td>3.9</td>
<td>20.0</td>
<td>13.3</td>
</tr>
<tr>
<td>gluteus</td>
<td>4.3</td>
<td>2.8</td>
<td>22.0</td>
<td>13.6</td>
</tr>
<tr>
<td>semitendinosus</td>
<td>3.9</td>
<td>3.0</td>
<td>21.5</td>
<td>13.3</td>
</tr>
<tr>
<td>half-sized</td>
<td>2.0</td>
<td>2.8</td>
<td>25.0</td>
<td>20.0</td>
</tr>
<tr>
<td>semitendinosus</td>
<td></td>
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</table>

a) Mean of duplicate or triplicate samples.

Fig. 2. Total (open symbols) and heat-resistant spore (closed symbols) populations of C. perfringens in FTM heated at various rates of rising temperature. Heat-activated C. perfringens spores inoculated into FTM were exposed to rising temperature from 20 to 60°C at increment rates of (a) 25°C/h; top left, (b) 20°C/h; top right, (c) 13°C/h; bottom left, and (c) 7°C/h; bottom right.

Internal temperature remained almost constant, but it continued to rise rather linearly at a rising-temperature rate of about 15°C/hr. During cooling at room temperature, the internal temperature exceeded 55°C, at which temperature the roast was removed from the oven, and reached a maximum temperature of 64°C at a lower rate of rising temperature. The internal temperature then began to fall gradually; it decreased from 60 to 40°C at a rate of 10°C/hr, from 40 to 20°C at about 6.7°C/hr, and from 20 to 10°C at a lower rate of 3.3°C/hr. It took about 6.5 hr for the product to be chilled from 49°C to 12°C. The rising-temperature rates and falling-temperature rates obtained with other beef muscles are summarized in Table 1.

Changes in Population at Constantly Ris-
ing Temperature: Total and spore populations of *C. perfringens* in FTM heated at various rising-temperature rates are presented in Fig. 2. Colony-forming units (CFU) are plotted against temperature.

When the growth media were heated at a rising-temperature rate of 25°C/hr (Fig. 2; a), the count did not change greatly in a temperature range between 20 and 30°C. The total population then somewhat increased, whereas the heat-resistant spore population decreased gradually. The maximum differences were noticed at a temperature range between 48 and 53°C or at a time interval between 75 and 90 min after start of heating. The total population continued to decrease to almost the same number as that of heat-resistant spores at 60°C. The terminal counts were lower than the initial counts by approximately one log cycle.

The behavior patterns of *C. perfringens* spores heated at rising-temperatures of 20 and 13°C/hr were generally identical to that obtained at 25°C/hr (Fig. 2; b and c).

When FTM was heated at a rising-temperature rate of 7°C/hr (Fig. 2; d), the heat resistant spore population of *C. perfringens* decreased continuously from 30 to 50°C and reached about one-hundredth level of the initial number. The total population increased exponentially in a temperature range between 40 and 50°C or during the period between 160 and 260 min after the start of heating. The total population then decreased markedly and reached the same level as that of the heat-resistant spore population at 60°C.

Changes in Population at Constantly Falling Temperatures: Growth characteristics of *C. perfringens* spores at different falling-temperature conditions are shown in Fig. 3.

At falling-temperature rates of 25, 20 and 15°C/hr (Fig. 3; a, b and c), no differences between total and heat-resistant spore population, nor between the initial and the terminal CFU were found during the entire range of 60–10°C. In these cases, the period of time
required to traverse a temperature range of 50–20°C, the zone in which *C. perfringens* can grow [14], were only 1.2, 1.5 and 2.0 hr, respectively.

When the growth media were cooled at a falling-temperature rate of 10°C/hr from 60 to 10°C (Fig. 3; d), the heat resistant spore population of *C. perfringens* remained almost constant throughout the period. The total population showed no change in a range between 60 and 35°C or for first 150 min, whereas it increased by no more than one log cycle thereafter.

At a falling-temperature rate of 7.5°C/hr (Fig. 3; e), the heat resistant spore population of *C. perfringens* began to decrease after the temperature had reached about 40°C. The ultimate spore population was, however, not less than half the initial one. The total population changed noticeably, followed by initiation of spore germination. It did not increase until the temperature reached 30°C or within 260 min.

A similar tendency was shown at 5°C/hr, although the temperatures for germination and multiplication were somewhat higher than those observed at 7.5°C/hr; i.e., 42.5°C for germination and 37.5°C for multiplication.
Growth dynamics of *C. perfringens* at falling temperature conditions were also determined in ground beef (Fig. 4). The results were almost the same as those in FTM. Nevertheless, the heat-resistant population was smaller than the total, even though determinations were made immediately after inoculation of the spores.

**Changes in Population in Roast Beef:** Roast beef was prepared from various muscles, internal parts of which had been contaminated with *C. perfringens* spores (Table 2).

All the total populations of 60°C samples were significantly (p<0.05) smaller (about 1/10) than those originally inoculated, but not significantly different among muscles. All the spore populations except for that in semimembranous muscle were not significantly different from the respective total populations, although all the former figures were smaller than the latter ones. Heat-resistant populations of 60°C samples were not necessarily different one another.

The clostridial populations in overnight samples with gluteus, semitendinosus and halved semitendinosus muscle were not significantly different from those of the 60°C samples. Overnight samples from biceps femoris and semimembranosus muscles significantly differed in both the counts from 60°C samples. Namely, the spore populations of the overnight samples were smaller than those of 60°C samples. Simultaneously the total populations of the overnight samples were larger than those of the 60°C samples. In the case of semimembranosus, however, the total populations of the overnight samples were not larger than those of the inoculum or before heating.

**DISCUSSION**

Although roast beef is popular in Japan for banquet meals, for entrees at restaurants, and in sandwiches at fast-food restaurants, no regulation for its commercial production has been established. To establish such regulations, considerations should be taken from two distinct viewpoints; keeping satisfactory quality and preventing public health hazards. In this sense, a series of studies were undertaken by the authors.

Willardsen et al. [19] reported growth and survival characteristics of *C. perfringens* vegetative cells in constantly rising temperature environments ranging from 4.1 to 12.5°C/hr. They stated that the internal temperature of 3 kg inside-round roast was raised at a rising rate of 7.3°C/hr in a conventional home oven at 85°C, and 10.0°C/hr at 107°C. The present study, 2 to 10 kg beef roasts were cooked at rising temperature rates of about 15–25°C/hr in a convection oven at 130°C (Table 1). The differences might depend on the ovens (conventional vs. convection), the cooking temperatures and others.

During heating *C. perfringens* spores at rising-temperature from 20 to 60°C at a rate of 13–25°C/hr, germination characterized by loss of heat resistance (80°C for 10 min) and growth were observed at temperatures higher than approximately 35°C and lower than 60°C (Fig. 2). All vegetative cells were killed at 60°C, for no numerical differences between the total and the spore populations were observed. The terminal populations obtained at rising-temperature rates of 13–25°C/hr were smaller than the initial population and hardly different among test variables. During heating at 7°C/hr, *C. perfringens* spores germinated at nearly 30°C, somewhat lower than the previous variables. Although the total population increased exponentially and reached a maximum in a temperature range of 46–50°C, all vegetative cells were inactivated also at 60°C. The population at 60°C reduced to approximately onehundredth of the initial.

Roast beef was prepared in a convection oven with various muscles, internal parts of which had been contaminated with *C. perfringens* spores. The internal temperature was
raised rather linearly at a rising-temperature rate of 15–25°C/hr (Table 1). Viable cells were determined no sooner than the internal temperature reached 60°C. The results with 60°C samples show (Table 2) that the population was smaller than the initial one and there were no significant differences among muscles. This might correspond with the facts shown in Fig. 2, where the terminal population was smaller than the initial but not different among rising-temperature rates of 13–25°C/hr.

With ground beef as growth medium, the heat treatment (80°C for 10 min) must have reduced the count. Despite immediate enumeration after spore inoculation, the heat-resistant population of the inoculum was about half the total. Such a tendency in ground beef was reproducible (Fig. 4) but not the case in FTM (Figs. 2 and 3). This may have been caused by some other factors than the heat treatment, for the spores used in this study were heat resistant (data not shown) categorized by Oka et al. [10]. At any rate, it seems justifiable to presume the growth pattern of C. perfringens spores.

Toumy and Lechnir [14] found that the color of cooked beef depended on the temperature rather than the period of time of exposure and that beef samples still appeared rare in color after 7 hr at 60°C. Smith et al. [13] found cooking beef in a water bath to an internal temperature of 60°C and holding for at least 12 min reduced a population of C. perfringens by approximately 3 log cycles. The present data further indicate that cooking roast beef to 60°C may be a promising method to reduce the spores loaded inside the meat. In this study, beef roasts were removed from the oven at an internal temperature of 55°C; it exceeded 60°C and reached 63–64°C during cooling. Since Blankenship et al. [3] reported that the center temperatures of roast at the time of removal from the oven correlated significantly with the maximum temperature of the center, it may be easier to settle the temperature for removal.

During cooling and chilling processes, thermal curves of the center of roast beef were not linear but rather parabolic (Fig. 1), but germination and growth patterns of C. perfringens spores were examined at constantly falling temperatures. At such rapid rates as 25 to 15°C/hr, no germination nor growth was observed. At 10°C/hr, germination and appreciable growth were not necessarily clear. At falling-temperature rates of 7.5 and 5°C/hr, exponential growth was observed, followed by spore germination. It is emphasized that meat should be refrigerated not later than 90 min after cooking [6, 17]; during the cooling period, it should be placed in such a position that cool air can circulate underneath and around it [17].

At smaller falling-temperature rates, temperatures for germination and growth tended to be higher. The fact may be reconcilable, although the ways of changing temperatures were reverse, with the findings by Willardson et al. [19] and Roy et al. [11] that the higher the rate of rising temperature or the higher the temperature for subculture, the higher the temperature to kill C. perfringens vegetative cells.

The total and the heat-resistant spore populations in roast beef were determined after fully refrigerated (Table 2). No germination nor growth was observed at all in roast beef of gluteus muscle, whole and half sized muscles of semitendinosus, but germination and growth were observed in roast beef of biceps femoris and semimembranosus muscles. In the cases of the latter two, more than 5 hr were consumed to traverse a temperature interval of 49–12°C, because the whole muscles were employed. In the United States, it is mandatory for all products to be chilled from 120°F (48.8°C) to 55°F (12.7°C) in no more than 6 hr [6].

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REFERENCES


要 約

加熱と冷却速度が培地とローストビーフ中のウエルシュ菌芽胞の挙動に及ぼす影響: 久保・中上亘幸・大沼司郎（日本ハム株式会社中央研究所）——ウエルシュ菌芽胞を接種した培地（FTM または牛挽肉）を 20°C から 60°C に加熱し、ついで 60°C から 15°C または 10°C まで冷却した。7-25°C/h の加熱速度で加熱中に芽胞の発芽芽胞がみられれたが、培地が 60°C に達したときには栄養型細胞の死滅がみられた。15-25°C/h の冷却速度では菌数の変化はみられなかったが、5-10°C/h では菌数の増加がみられた。浅焦遅、半浅味状態で半分に切断した半璧麻筋では冷却工程中にウエルシュ菌の増殖はみられなかった。半璧動筋では冷却工程中にウエルシュ菌の発芽芽胞がみられれたが、接種菌数以上になることはなかった。