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Thermal Inactivation and Injury of *Moraxella-Acinetobacter* Cells in Ground Beef

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The thermal inactivation and injury (sensitivity to 0.8% NaCl) of a radiation-resistant culture of *Moraxella-Acinetobacter* mixed in minced beef were determined. Survival curves for *Moraxella-Acinetobacter* cells in beef had an initial shoulder preceding a logarithmic decline when the cells were heated at 65, 70, and 75°C, but not at 80°C. In all cases, the experimental points not included in the shoulder were linearized by means of a least-squares straight line, and the latter was used to determine *D* values. Shoulder values of 12.2, 4.1, and 0.6 min at temperatures of 65, 70, and 75°C were added to the respective *D* values of 35.4, 6.6, and 1.4 min to determine the time required to destroy one log cycle. The *Z* value was 7.3°C. *Moraxella-Acinetobacter* cells in meat were more rapidly injured than inactivated, on initial exposure to heat. The number of cells injured by this initial exposure increased as the temperature was increased. At 65°C the percentage of injured cells increased more rapidly with exposure time than did the inactivated cells. As the temperature was increased, the rates of inactivation and injury became more and more similar.

Radiation has been proposed as a means of preserving a variety of highly perishable foods (e.g., pork, chicken, beef) for long periods of time under nonrefrigerated conditions (20). In this high-dose (>1 Mrad) process, the above foods are formulated to contain 0.5 to 0.8% (wt/wt) NaCl and 0.3 to 0.4% (wt/wt) sodium tripolyphosphate to improve the juiciness of the final product (21). The formulated food is placed in cellulose casing, heated to an internal temperature of 73 to 77°C to inactivate autolytic enzymes, chilled, vacuum packed in cans, frozen to about −40°C, and subjected to gamma irradiation. The dose used in the irradiation process is that which was determined by inoculated-pack studies to reduce the number of spores of the most radiation-resistant strain of *Clostridium botulinum* by 12 orders of magnitude, i.e., from 10^{12} to 10^{0} (120).

Cells are not only inactivated by irradiation, but some are injured, with resulting increased sensitivity to such factors as salts (12, 18, 19) and subsequent incubation temperature (4, 9).

A variety of microorganisms isolated from foods are more radiation resistant than spores of *C. botulinum*, which are used as the microbial index of safety. The radiation-resistant vegetative bacteria isolated from foods include *Micrococcus radiodurans* (6, 25) and other micrococci (5, 14) and *Moraxella-Acinetobacter* (M-A) (26). However, radiation-resistant viruses (24) and vegetative cells such as *Micrococcus radiodurans* (1) are heat sensitive (7, 23) and would be eliminated or reduced during the thermal process. Any vegetative cells surviving the heat treatment would probably be injured and sensitive to subsequent freezing or irradiation.

Welch and Maxcy (26) isolated a culture (isolate 7) of M-A from raw beef and showed that these asporogenous, gram-variable coccobacilli were able to grow on Trypticase soy agar (BBL Microbiology Systems) over a temperature range of 20 to 37°C and were extremely radiation resistant at −30°C. The purpose of this study was to determine the effect of heat treatment on the inactivation and injury (sensitivity to NaCl) of isolate 7.

MATERIALS AND METHODS

Test organism. M-A isolate 7 was kindly provided by R. B. Maxcy, University of Nebraska, Lincoln. The culture was maintained (at 5°C) on plate count agar (PCA; Difco Laboratories, Detroit, Mich.). Cultures for experiments were obtained by inoculating cells from a slant culture into plate count broth and incubating at 32°C on a rotary shaker (450 rpm) for 22 h. Preliminary experiments showed that a 22-h culture was the most resistant to heat treatment and that most of the coccobacilli were in pairs. The cultures were harvested by centrifugation (3,000 rpm) and suspended in 20 ml of sterile plate count broth to give a final concentration of about 7×10^{9} cells per ml.

Thermal treatment. Radiation-sterilized (4.1 Mrad) minced beef, containing (wt/wt) 0.75% NaCl and 0.375% sodium tripolyphosphate, was inoculated with a 22-h M-A culture to give a final concentration

of about 10^8 cells per g of meat. The inoculated meat was ground five times to assure the even distribution of M-A cells throughout the meat. The ground meat (20 g) was placed in a polyethylene pouch and pressed to a uniform thickness of 3 mm. Each pouch was heat sealed under vacuum and arranged on a metal frame to assure good contact with the water in the bath. The pouches were held for various time intervals in a water bath adjusted to different temperatures (e.g., 65, 70, 75, 80°C) and were subsequently cooled rapidly by dipping in an ice bath. Each experiment was repeated three times.

Recovery. After heat treatment and cooling, the surface of each plastic pouch was sterilized by dipping in 70% ethanol solution followed by washing in sterile water. The 20-g meat samples were aseptically introduced into a Waring blender jar with 180 ml of 0.1% peptone and blended at high speed for 2 min at ambient temperature.

The blended sample was appropriately diluted with 0.1% peptone and pour-plated with PCA, and the creamy-pink colonies were enumerated after incubation at 32°C for 5 to 7 days or as indicated in specific experiments.

Determination of heat-stressed cells. Uninjured cells were able to form colonies on both PCA and PCA plus 0.8% (wt/vol) NaCl. However, some of the heat-stressed cells produced colonies on PCA but not on PCA + 0.8% NaCl. The difference in colony counts with and without 0.8% NaCl was a measure of heat injury.

Come-up time. The come-up time, of meat packed in plastic pouches, to the different temperatures was measured using copper-constantan thermocouples. One thermocouple was placed in the center of the meat, and the other was attached to the metal frame holding the pouch. The latter showed the bath temperature. The results were recorded on a two-point recorder (Linear Instruments Corp., Irvine, Calif.; model 385) as millivolts and translated to temperature (°C). The times reported were the average of four experiments.

***D* value for logarithmic order of death.** The time (minutes) required at a given temperature to destroy 90% of the cells when the survivors were recovered on a salt-free medium was designated *D*.

***D'* value for logarithmic order of death.** The time (minutes) required at a specified temperature to destroy 90% of the cells when the survivors were recovered on a medium containing 0.8% NaCl was designated *D'*.

***Z* value.** The change in temperature (°C) which will alter the *D* value by a factor of 10 was called *Z*.

Calculation of thermal destruction. The time required for a 20-g meat sample (3 mm thick), in a vacuum-sealed plastic pouch, to reach an internal temperature of 65, 70, 75, or 80°C when placed in a water bath is shown in Fig. 1A. The data in these curves were used to take into account the come-up time when calculating the thermal inactivation of M-A cells.

To calculate *D* at any temperature, the destruction of bacteria, as a function of time, had to be found. Since time elapsed before the meat reached the bath temperature, the following calculations were made.

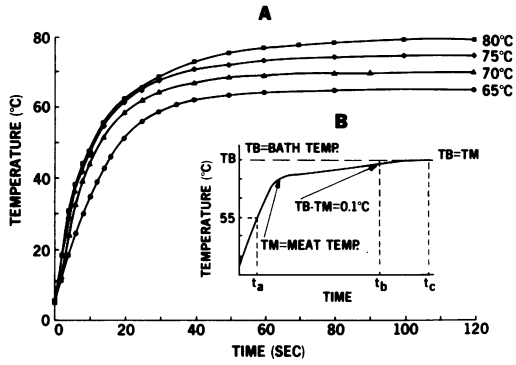

FIG. 1. *(A) Time required for a 20-g meat sample to reach an internal temperature of 65, 70, 75, and 80°C. (B) Schematic drawing showing terms (t_a, TB, TM, t_b, and t_c) described in the text.*

The total experimental treatment time (t_c) was divided into three parts (see Fig. 1B): (i) the time required for the internal temperature of the meat to reach 55°C (t_a) (during t_a, no bacteria were inactivated); (ii) the time required for the internal temperature of the meat to go from 55°C to within 0.1°C of the bath temperature ($t_b - t_a$); (iii) the time from t_b to t_c, in which the meat was held in a temperature varying no more than 0.1°C from the bath temperature.

Since no cells were inactivated during the time before the meat reached 55°C, t_a was deducted from the treatment time (t_c). For part (ii) the following calculations were made in order to find the equivalent time (t_{eq}) at bath temperature that will give the same destruction as come-up time. The relation between destruction of bacteria at different temperatures is (22):

$$D_{TM} = D_{TB} \cdot 10^{(TB-TM)\ Z} \quad (1)$$

where D_{TM} is the decimal reduction time of M-A at the meat temperature (TM), and D_{TB} is that time at the temperature of the bath (TB). The meaning of this relation is that the same destruction obtained by 1 min at bath temperature will be obtained by $10^{(TB-TM)/Z}$ min at any meat temperature (TM). Therefore, the time at the bath temperature that is equivalent to the come-up time is:

$$t_{eq} = \int_{t_a}^{t_b} 10^{-[(TB-TM)/Z]} \cdot dt \quad (2)$$

There is an exponential relation between time and temperature (Fig. 1), and therefore the following relation was found by linear regression:

$$\log (TB - TM) = A + B \cdot t \quad (3)$$

where *A* and *B* are constants.

Combining equations 2 and 3 and integrating gives us the equivalent time (t_{eq}). The total time of treatment is therefore:

$$t_{treatment} = t_c - t_b + t_{eq} \quad (4)$$

A computer program was written which assumed a *Z*

value and calculated the treatment time as explained. D and shoulder values were obtained by linear regression of the logarithm of survivors versus treatment time, and a Z value was obtained by linear regression of the logarithm of D values versus temperature. Using the new value of Z, a new calculation of t_{eq} and the treatment time was done. All this procedure was repeated until convergence to the real Z value was accomplished.

RESULTS

Tolerance of M-A isolate 7 cells to NaCl. The effect of various concentrations of NaCl on the recovery of M-A cells in PCA was studied to find the highest concentration that had no effect on unstressed cells but prevented colony formation by injured cells. The tolerance of unstressed M-A isolate 7 cells to NaCl is shown in Fig. 2. NaCl concentrations as high as 0.8%, added to PCA, had little if any effect on the number of colonies formed by unstressed cells. Increasing the concentration above 0.8% progressively decreased the percentage of survivors. No colonies were formed in PCA + 1.5% NaCl during an incubation period of 10 days at 32°C.

Thermal inactivation of M-A cells in meat. The survival curve had an initial shoulder of 12.2, 4.1, and 0.6 min in duration at 65, 70, and 75°C, respectively, followed by a logarithmic decline (Fig. 3). The decimal reduction (D) times at heating temperatures of 65, 70, 75, and 80°C were 35.4, 6.6, 1.4, and 0.32 min, respectively.

Injury due to thermal treatment. Figures 4, 5, and 6 show the kinetics of thermal inactivation and injury at 65, 70, and 80°C, respectively. At 65°C the percentage of injured cells increased with exposure time (Fig. 4). At 80°C the rates of inactivation and injury were almost the same; therefore the percentage of injured cells was essentially the same after exposure

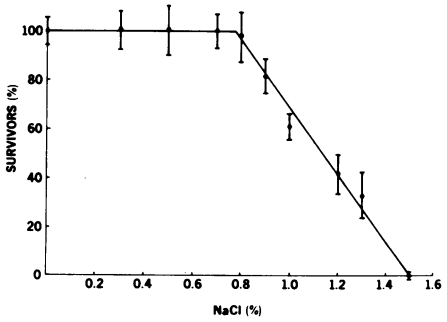

FIG. 2. *Effect of the concentration of NaCl in a plating medium (PCA) on the recovery of M-A isolate 7 at an incubation temperature of 32°C for 7 days. The points show the average result of the experiments at each concentration, and the bars give the confidence limits of 95%.*

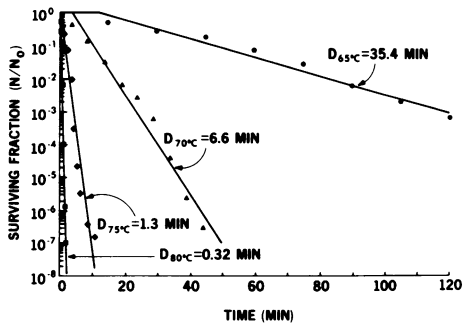

FIG. 3. *Thermal inactivation of M-A isolate 7 in ground beef. Twenty grams of radiation-sterilized ground beef, inoculated so as to contain about 10^8 cells per g, was vacuum sealed in a plastic pouch. Pouches were suspended in a water bath, adjusted to temperatures of 65 (●), 70 (▲), 75 (◆), and 80°C (■), for various time intervals as indicated. A straight line was fitted to the data by the method of least squares. The time shown on the abscissa is the treatment time as calculated by equation 4.*

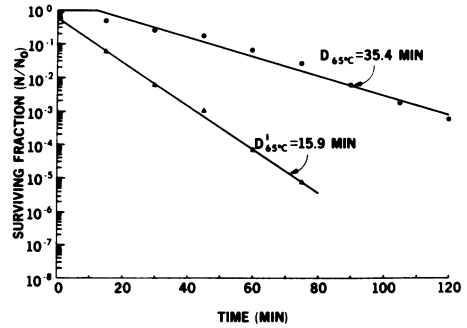

FIG. 4. *Thermal inactivation and injury (inability to form colonies in the presence of 0.8% NaCl) of M-A isolate 7 in vacuum-packed ground beef at 65°C. Symbols: (●) cells plated with PCA; (▲) cells plated with PCA + 0.8% NaCl. The times on the abscissa were obtained as described in Fig. 3.*

times of 0.08 to 1.23 min (Fig. 6). When the lines representing uninjured cells (i.e., capable of colony formation on PCA + 0.8% NaCl) were extrapolated, they did not intercept the ordinate at $N/N_0 = 1$. Rapid injury had resulted on initial exposure to heat. There was only a little of this injury at 65°C, but it increased with increasing temperature. The fraction of M-A cells that survived the heat treatment and were uninjured (capable of colony formation on media containing 0.8% NaCl) could be described by two straight lines (Fig. 5, 6): a sharp decline resulting from the initial thermal shock, and a more moderate decline after longer treatment times.

D' values at 65, 70, 75, and 80°C were 15.5, 4.6, 0.88, and 0.29 min, respectively.

Z values. Both D and D' values were plotted

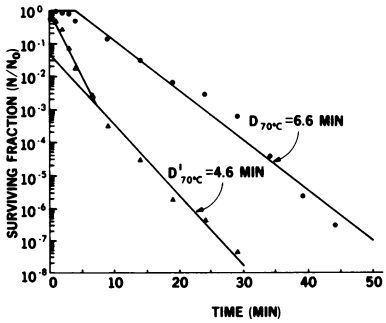

FIG. 5. *Thermal inactivation and injury (inability to form colonies in the presence of 0.8% NaCl) of M-A isolate 7 in vacuum-packed ground beef at 70°C. Symbols:* (●) *cells plated with PCA;* (▲) *cells plated with PCA + 0.8% NaCl. The times on the abscissa were obtained as described in Fig. 3.*

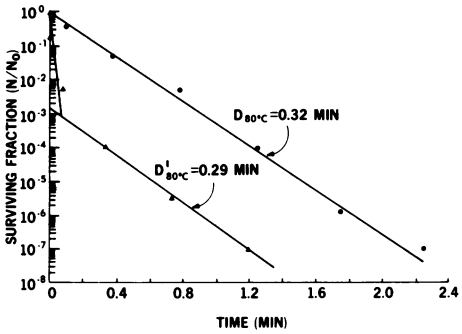

FIG. 6. *Thermal inactivation and injury (inability to form colonies in the presence of 0.8% NaCl) of M-A isolate 7 in vacuum-packed ground beef at 80°C. Symbols:* (●) *cells plated with PCA;* (▲) *cells plated with PCA + 0.8% NaCl. The times on the abscissa were obtained as described in Fig. 3.*

versus temperature (Fig. 7). The linear regression lines were used to obtain Z and Z' values of 7.3 and 8.1°C, respectively. As temperature increased, the rates of inactivation and injury became more and more similar. The shoulder shortening with increase of temperature was found to be proportional to changes in D value. At temperatures (e.g., 65, 70, 75°C) where the survival curve has an initial shoulder preceding a logarithmic decline, the time required to destroy 90% of the population at a certain temperature will be the D value plus the shoulder length; one tenth of that time will be required to destroy 90% of the population as the temperature is increased by 7.3°C.

DISCUSSION

Under our experimental conditions, isolate 7, mixed in minced beef containing 0.75%NaCl and 0.375% sodium tripolyphosphate, had a calcu-

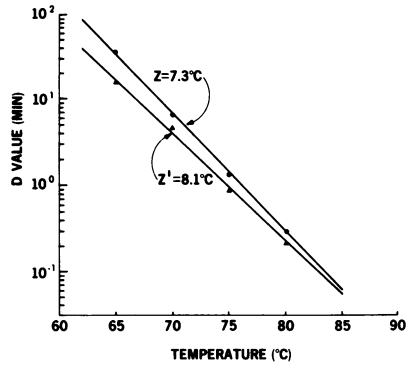

FIG. 7. *Thermal death time as a function of temperature. D values were based on the recovery of total survivors at 32°C on a salt-free agar medium* (●) *and uninjured survivors on agar medium containing 0.8% NaCl* (▲).

lated (by interpolation) $D_{68°C}$ of 13.7 min. M-A isolate 7 in beef with no added salt had a $D_{68°C}$ of 9.2 to 9.3 (17). The higher D value in the present study may be due to the salts having a protective effect similar to that found by Duncan and Foster (8) with spores of anaerobes.

Foodborne pathogens, such as *Salmonella* and *Staphylococcus aureus* mixed in meat, are not as heat resistant as M-A isolate 7. *Salmonella senftenberg* mixed in meat products had a $D_{60°C}$ of 9.2 to 13.3 and a Z value of 6.4 to 6.7°C (2). *Staphylococcus aureus* in meat products had $D_{60°C}$ and Z values of 4.9 to 8.2 min and 5.1 to 6.0°C, respectively (2). M-A isolate 7 was more heat resistant than the highly radiation-resistant cells of *Micrococcus radiodurans*. The latter, mixed in beef, had a $D_{60°C}$ = 0.75 min (7). Asporogenous cells having a greater heat resistance than M-A isolate 7 include certain thermoduric bacteria such as *Streptococcus thermophilus* ($D_{70°C}$ = 15 min) and *Lactobacillus bulgaricum* ($D_{70°C}$ = 30 min) (11).

During the thermal inactivation of enzymes, prior to irradiation, the formulated meat may be expected to be at an internal temperature of 70°C for 9 h. Since the $D_{70°C}$ for M-A isolate 7 was 6.6 min and the shoulder was evident for 4.1 min, the thermal treatment to inactivate enzymes should eliminate these radiation-resistant vegetative cells. However, in other products that may be preserved by irradiation, such as pork chops or beef steak, the internal temperature during heat treatment reaches 70°C for only a few minutes, and not all M-A cells may be destroyed by the heat process. The data on D and Z values, presented in the present study, will enable one to determine how many radiation-resistant M-A cells would be destroyed in beef given specific thermal treatments.

During a thermal treatment, cells may be inactivated while others remain viable, but injured (3, 13). Heat-injured bacteria may be more sensitive to subsequent treatments. Therefore, in a given process it is important to take into account the injured as well as the inactivated bacteria.

Unstressed cells of M-A were not sensitive to amounts (0.5 to 0.8%) of NaCl often present in meats formulated for the radiation process. However, thermally stressed cells were unable to form colonies in PCA with 0.8% (wt/vol) NaCl.

The results of this study showed two interesting features of the kinetics of thermal injury. (i) M-A cells were injured (sensitive to NaCl) very rapidly at the beginning of the thermal treatment. After longer treatment times, the increase in injured cells was more moderate. (ii) High rates of injury in comparison with inactivation were found at 65°C. However, as the temperature was increased the rate of injury approached that of inactivation (Fig. 4 to 6). Similar kinetic behavior of heat-injured bacteria was obtained with *Staphylococcus aureus* in milk (10). Thermally injured cells of M-A were more sensitive to subsequent irradiation at −30°C (16). The shoulder in the radiation survival curve was eliminated by a previous or subsequent thermal treatment which inactivated 90 to 99% of a given population.

The log survivor curves of M-A cells were characterized by a shoulder plus an accelerating death rate, which approached logarithmic death (Fig. 3). The initial shoulder observed on survival curves can be explained by the initial effect of heating, resulting in injury but no loss in viability. In this study the data analysis was based on the assumption of a shoulder in which no death occurred, followed by logarithmic inactivation. Another approach was recently published by King et al. (15). Regardless of the approach used, we found essentially no difference in the agreement between calculated values of log N/N_0 with experimental values. The method of King et al. (15) was not used in our studies because it would give results that would be difficult to compare with published D and Z values.

Since asporogenous M-A cells are more radiation resistant than *C. botulinum* spores, one initially wonders whether they might not survive and present a problem in radappertized meats. However, when one considers the total process (thermal + 12D irradiation dose for *C. botulinum* spores) it is evident that the radiation-resistant M-A cells at an estimated initial concentration of 10^2/g of beef (16) would not survive, due to thermal sensitivity, thermal injury, and sensitivity of these injured cells to NaCl and irradiation.

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