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Heat Inactivation of *Mycobacterium avium-Mycobacterium intracellulare* Complex Organisms in Meat Products

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Wieners and sausages were prepared which contained the most heat-tolerant representative of the *Mycobacterium avium-Mycobacterium intracellulare* complex we were able to obtain. They also were prepared with infected tissues obtained from tuberculous swine. Processing conditions were as varied as possible. Neither incorporation of sodium nitrite in the emulsion nor presence of smoke during processing altered the heat susceptibility of the organisms. Substantial killing of the organisms occurred as wieners reached the upper processing temperatures, but hot oil or radiant heating of the "precooked" sausages allowed very short times within the killing range; hence, higher peak internal temperatures were necessary. The lethalities for these organisms of reaching and maintaining various processing temperatures are given.

The thermal inactivation of several serovars of the *Mycobacterium avium-Mycobacterium intracellulare* complex, which causes most tuberculous lesions of swine, has been described in a previous report (3). In that study, we investigated variables in aqueous suspension that could not be examined readily in meat products. This study was designed to provide information on the destruction of these organisms under actual meat processing conditions. Meat products containing the organisms were prepared and examined for survival of the organisms after various methods of processing.

MATERIALS AND METHODS

Meat. Freshly slaughtered beef and pork were hung at 5°C. The beef was aged 7 days; the pork was aged 2 days. The lean meat was separated from the fatty tissue. The lean beef, lean pork, and pork fat were ground through a ¼-inch (ca. 6.4-mm) plate, placed in plastic bags in approximately 1-kg quantities, and stored at −70°C. One day before use, bags of each ingredient were moved to a refrigerator at 5°C. Water analysis of each ingredient and of finished products was by drying samples in a microwave oven to constant weight. Fat analysis was by Soxhlet extraction of the dried samples with diethyl ether. All equipment used in the preparation and processing of products containing the organisms was operated in a series of class 1 hoods. Disposable gloves were always used, as well as face masks when appropriate. After use, all equipment was either autoclaved or soaked in 2% Amphyl (National Laboratories, Toledo, Ohio; contains 44% anhydrous soap, 15% *o*-phenylphenol, 6.3% *p*-tertamylphenol, 4.7% alcohol, and 30% inert ingredients). Between periods of use, ultraviolet germicidal lights were left on. After each use of the smokehouse, flowing steam was used to raise the temperature of the smokehouse to 80°C or above for at least 30 min.

Wiener emulsion. Wiener emulsions were prepared to contain 30% fat and no more than 10% added water after processing. A typical batch usually contained 1,200 g of lean beef, 970 g of lean pork, 1,217 g of pork fat, 600 g of ice, 1.6 g of sodium erythrobate, 56 g of sucrose, 72 g of sodium chloride, 14.8 g of spice mixture, and 4.6 ml of 10% sodium nitrite. Emulsions were prepared in a three-blade chopper, with the chilled emulsion chopped until its temperature reached 15°C. Suspensions of the previously determined (3) most heat-resistant isolant of *Mycobacterium intracellulare*, serovar 10, were added during the chopping process so that they would be thoroughly incorporated in the emulsion. These organisms had been grown on egg yolk medium as previously described (3). In vivo-grown organisms were incorporated in some batches of emulsion by use of infected liver or lymph node tissues to replace part of the lean pork. The tissues were provided by Federal Meat Inspectors who obtained them from slaughter pigs. Tissue specimens were cultured, and viable counts and serotypes were determined. Those specimens that contained about 10^4 *M. avium* serovar 2 were used to prepare wiener emulsion. The prepared emulsions were stuffed into size 84 (22-mm diameter) wiener casings. Links were formed by tying with cord. The stuffed links were stored at −17.8°C.

Sausage. Sausage was prepared with 64% lean pork, 34% pork fat, 1.8% sodium chloride, and a suspension of serovar 10 organisms. The meat, fat, and salt were reground through a ⅛-inch (ca. 3.2 mm) plate, then the organisms were added by hand mixing in a plastic bag. Sausage that was to be cooked in hot oil was stuffed into rib knit cotton tubing that had a diameter of ca. 16 mm when stuffed. Sausage that was to be cooked by radiant heating was stuffed into 5-cm diameter casings, frozen to −5°C, and sliced into sec-

tions 0.5 and 1 cm thick. The raw sausage contained 40% moisture and 39.3% fat. Both types of sausage were stored at −17.8°C until 1 day before use when they were moved to −5°C storage.

Heat processing. For each smoking-cooking cycle, four wieners were thawed at room temperature. One was processed for culturing without heat treatment. Recording thermocouples were inserted into each of the other three inside a smokehouse. Additional thermocouples recorded the wet-bulb (WB) and dry-bulb (DB) air temperatures near the wieners. The smoke was produced from hickory sawdust by an electrically heated smoke generator. Electric heating elements and a steam jet in the smokehouse were independently operated by a two-pen recorder controller. At the end of each heating cycle, the wieners were immersed in tap water at 20°C to cool them rapidly and to facilitate removal of the casings. Figure 1 illustrates the heating conditions of a typical smokehouse run.

Smoke was generated into the smokehouse until the wieners had reached and maintained a temperature of 50°C for 30 min. Runs to be conducted without smoking were maintained at the same temperatures without generating smoke. Operations of the heating elements and steam generator were varied to produce the desired relative humidities and internal temperatures. After the smoking cycle, some batches were removed to a preheated water bath to monitor death rate at more evenly controlled temperatures than could be maintained in the smokehouse. Figure 2 illustrates the internal temperatures of a batch of wieners tested at 70°C.

Oil-cooked sausage. Vegetable oil was preheated to 135°C or to 176.7°C. The sausages, each containing a centrally located thermocouple, were immersed in the hot oil to be cooked to various internal temperatures, then were transferred to 0.3% benzalkonium chloride (Zephiran-Winthrop Laboratories, Sterling Drug Inc., New York, N.Y.) solution to be rapidly cooled.

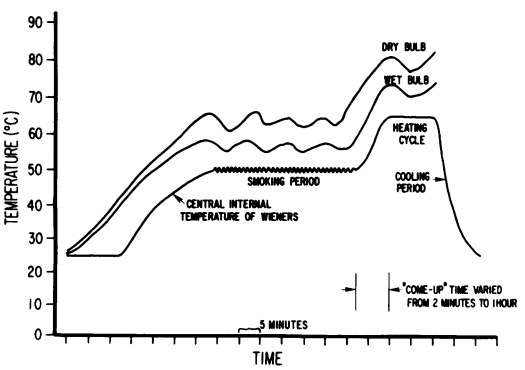

FIG. 1. *A typical smokehouse run. The smoking period was the same regardless of whether smoke was used. The rate of temperature increase ("come-up" time) from the end of the smoking period to the beginning of the heating cycle varied from 2 min to 1 h, and the time within the heating cycle varied from just reaching the temperature to 1 h. Cooling rates were uniform.*

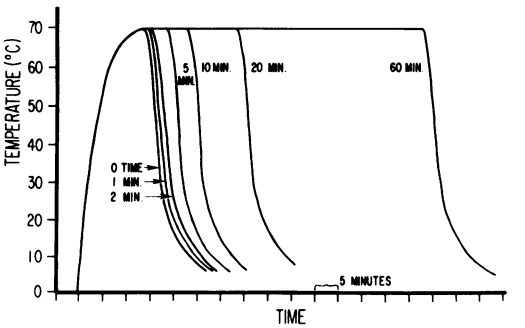

FIG. 2. *Internal temperatures of wieners immersed in a water bath at 70°C for various periods and then immersed in an ice bath. Numbers on curves represent immersion times at 70°C.*

Radiant-heat-cooked sausage. Dual 1,100-W coiled radiants, standing vertically 10 cm apart, were preheated. Sausage patties, each containing a thermocouple, were suspended vertically between the radiants to be cooked to various internal temperatures and then were transferred to 0.3% benzalkonium chloride solution to be cooled. Figure 3 illustrates the rise and fall of internal temperatures in oil- and radiant-heat-cooked sausages.

Culturing. Sections of wieners heated to 60°C were processed in Ten Broeck tissue grinders, in a high-speed blender (Lourdes) and in a bag-type blender (Stomacher) to determine which yielded the highest colony counts after culturing. No consistent difference was detected, but the bag-type blender required less preparation time than the others and allowed the maceration of an entire wiener.

After the heating cycle and cooling, the wieners or sausages were placed in blender bags and weighed. Four volumes of 0.3% benzalkonium chloride, used as decontaminating solution, was added, and the contents were blended for 1 min. The top of the bag was folded tightly to act as a coarse filter, and a portion of the filtrate was strained into a screw-capped tube. The filtrate was allowed to stand for 1 h at room temper-

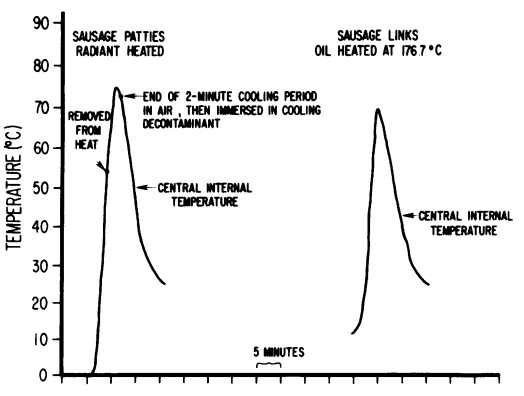

FIG. 3. *Internal temperatures of sausages cooked by radiant heat and by vegetable oil at 176.7°C.*

ature. Then the tube was shaken to assure an even distribution of fat droplets, and 0.9 ml of the filtrate was added to 0.9 ml of saline (0.85% NaCl) to provide a 10^{-1} dilution of the wiener or sausage. Subsequent serial 10-fold dilutions were prepared, usually to a final dilution of 10^{-8}.

The inocula consisted of 0.25 ml of the dilutions pipetted onto the surface of egg yolk medium in 25-cm^2 tissue culture flasks. The flasks were rotated to assure even distribution of the inocula on the surface. The cultures were incubated 30 days at 38°C before colonies were counted and recorded. Because of the dilution factor, counts of less than 40 viable units per gram of meat would not have been detected. This culturing procedure is highly selective for pathogenic mycobacteria, so the normal flora of wiener emulsion did not contaminate the cultures.

RESULTS

When the *M. avium-M. intracellulare* of tuberculous lymph nodes and livers of 286 swine were enumerated and serotyped, maximum viable cell counts of about 10^4/g of infected tissue were found in 17 specimens, and the predominant serovars were *M. avium* serotype 2.

Figure 4 shows the effect of time at 60, 63, 65.8, and 70°C for wieners heated in a water bath after 30 min of smoking at 50°C. This also illustrates that although the number of organisms killed during "come-up" time was negligible at the lower temperatures, a significant number of organisms was killed by the beginning of the higher temperature runs.

Figure 5 shows the effect of time in the smokehouse within the range of 60°C to 65°C central internal temperature. All wieners just reached 65°C, but the speed of heating and cooling varied. The counts were adjusted to 10^6 for unheated controls.

Because conditions within the smokehouse were varied as much as possible, humidity levels were from DB − WB = 0 to 54°C, and maximum house temperatures were from just above wiener temperature to 100°C. Figure 6 shows the $\log_{10}$ of counts versus peak internal temperature, regardless of smokehouse conditions. All spans of heating time and relative humidity are included, and the counts are adjusted to 10^6 for unheated controls. Under some conditions, more than 10^5 organisms were killed at peak internal temperatures of 66°C and under all conditions more than 10^5 were killed at peak internal temperatures of 70°C or above. Apparently, neither smoking nor presence or absence of nitrite in the emulsion altered the numbers of organisms killed.

The three important parameters that controlled the numbers of organisms killed were

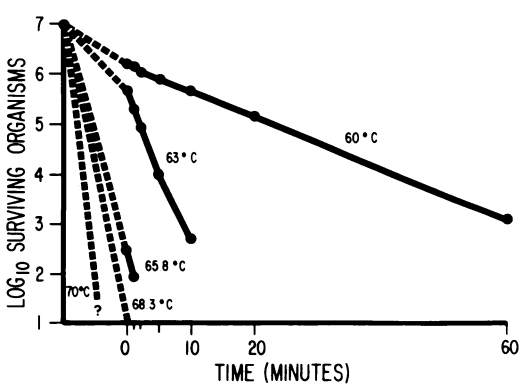

FIG. 4. *Survival of isolant 10C in wieners submerged in a water bath at temperatures shown.*

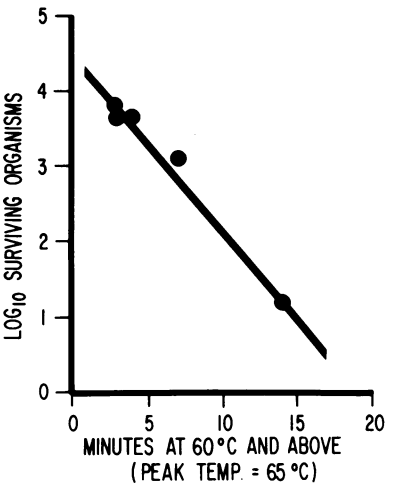

FIG. 5. *Survival of isolant 10C after wieners were heated in the smokehouse to 65°C, with the times shown above 60°C.*

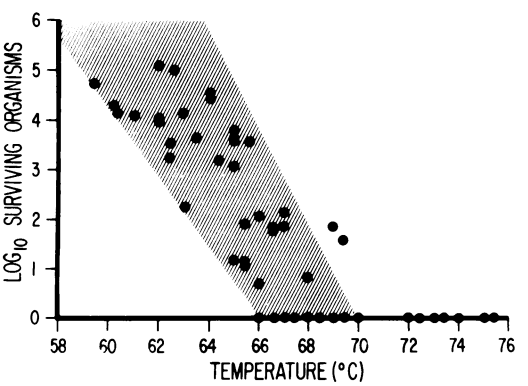

FIG. 6. *Survival of isolant 10C under variations of heating time, relative humidity, and smokehouse temperature. Temperatures shown are peak central internal temperatures of the wieners. The points with surviving organisms outside the shaded area were after extremely low humidity during heating.*

peak internal temperature, time within the killing range, and relative humidity. The number killed was reduced by low relative humidity only when humidity was extremely low. With ordinary humidity, at heating periods of 10 min or more above 65.5°C internal temperature of the wieners and a peak internal temperature of 68.3°C, more than 10^5 organisms were killed.

Heating to peak internal temperatures of at least 75°C with at least 5 min above 60°C was necessary to consistently kill 10^5 or more of the 10C organisms in the oil- or radiant-heat-cooked sausages.

DISCUSSION

Maximum counts of about 10^4 *M. avium* per g of lesion were found in a few of the lesions examined. Most of the tuberculous tissues contained serovar 2 organisms, a smaller number contained serovar 1, and only rarely did tissues contain any other serovar. The predominant serovars that occurred in these swine are the most heat sensitive, but the serovar 10 that was used in these studies was the most heat-resistant isolant we could obtain. Realistic kill rates should be based on enough organisms at the beginning of a heating cycle to allow countable numbers after a 5-log kill. Because naturally infected tissues did not contain that many organisms, kill rates could be based only on organisms grown in vitro.

The unheated wiener emulsions prepared with naturally infected tissues contained 10^3 to 10^4 *M. avium* serovar 2. When processed via various time-temperature protocols, organisms could not be recovered after any processing in which the internal temperature reached 65.5°C or above. With the method we used, any time the viable count was less than 40/g, organisms would not likely have been found.

In studies of the thermal destruction of microorganisms, investigators usually assume that all of the organisms in a particular test vessel are being heated to the same temperature for the same period of time. That assumption is not valid in the evaluation of processing of semisolid products such as wieners and sausages. In such processing, the temperature differential between the center and the outer parts of the product will vary with humidity, temperature difference between the smokehouse and the product, and distance between the center and outer surface. In radiant-heated products, the intensity of the radiation also will influence the differential.

Organisms of the *M. avium-M. intracellulare* complex are killed at conveniently measured rates in the range of 60 to 70°C. Very long exposure times are required to achieve even a 1-log decrease in viable units at 60°C, whereas extremely short exposure times are required at 70°C. In ordinary processing of meat products, temperatures of the outer part of a weiner or sausage would quite possibly be well above 70°C before they reached 60°C at the center. Therefore, the time required at a given internal temperature to kill a certain number of organisms in the whole product will vary with the processing conditions. These differences will produce alterations in the measured *D* values. For this reason, our processing conditions (relative humidities and temperature differentials) were as varied as possible.

Smoking and heating of the emulsion in wiener processing imparts a smoky flavor to the wiener, sets the emulsion, forms the skin of skinless wieners, accelerates the development of color, and kills trichinae and bacteria. The normal flora of commercial wiener emulsion may contain over 10^5 organisms/g, which is reduced to less than 10^3 during the normal heating cycle (4).

Dawson (1) and Palumbo et al. (4) recommend the processing of wieners to an internal temperature of 71.1°C (to allow a 2.8°C margin of safety) for the destruction of the most heat-resistant strains of *Salmonella*, which is commensurate with the 68 to 72°C used in commercial practice (2). According to Stumbo (5), a log cycle reduction of viable cells in the range of 12*D* to 15*D* is generally considered appropriate when pathogens are of chief concern. Because significant numbers of organisms in wieners are killed in reaching the upper temperatures, the total lethality of the process constitutes both those killed during come-up and those killed during holding at a specified temperature (Fig. 4). By extrapolation from survival curves, the additional killing of these organisms under various time-temperature conditions can be deter-

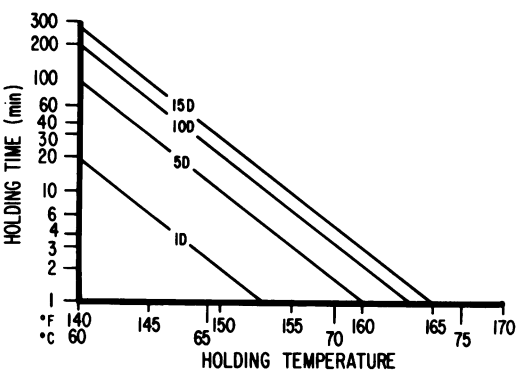

FIG. 7. *Relative holding times at various temperatures to accomplish different log cycle reductions in numbers of viable organisms. D values were calculated from survivor curves for water bath-heated wieners.*

mined (Fig. 7). For example, a 16*D* process can be calculated by adding the 6-log cycles of organisms that can be killed by the time wieners reach 68.3°C and an additional 10-log cycle reduction that could be achieved by holding them at 68.3°C for 7.5 min.

The commercial processing of wieners involves convection heating with moderately high relative humidity and gradually approached peak temperatures; this processing allows the product to be in the killing range for substantial periods of time. However, the processing of "precooked" sausages involves short, moisture-reducing heat periods either in hot oil or near radiant heating elements, followed by rapid chilling and freezing with refrigerated blast-air or CO_2; this preparation allows the product to be in the killing range for only a very brief period. Because relatively few of these organisms are killed during such brief come-up times, the lethality of the process must be derived primarily during the holding time at the elevated temperature unless higher peak temperatures are reached. For example, a minimum peak temperature of 75°C with heating periods of 5 min or more above 60°C would be required to kill 10^5 of these organisms within such sausages. As with wieners, maintaining the temperature at 71.1°C or above for at least 2 min would ensure a further destruction of 10^7 organisms for a total 12*D* kill capacity.

ACKNOWLEDGMENT

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