FRI BRIEFINGS

Survival and Growth of *Clostridium perfringens* during the Cooling Step of Thermal Processing of Meat Products

A Review of the Scientific Literature

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Introduction

Inadequate thermal processing and post-processing contamination can result in the presence of foodborne pathogens in cooked ready-to-eat (RTE) meat products. While thermal processing of meat products should be sufficient to destroy vegetative bacterial pathogens, bacterial spores may survive cooking and multiply during cooling. Spores of foodborne bacterial pathogens, such as *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens*, are very heat-resistant and usually cannot be killed during processing without compromising the nutritional value and/or organoleptic properties of the food. Many spores are activated by heat and may germinate and grow if the process of cooling food to <5°C is prolonged. Post-cooking contamination with microbes such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. can also cause foodborne disease if the pathogens multiply or produce toxin.

Cooling processes must be designed to minimize or completely prevent growth of sporeforming foodborne pathogens. The USDA's draft compliance guidelines for RTE meat and poultry products state that during stabilization (cooling) there should be no growth of *Clostridium botulinum* and no more than 1 $\log_{10}$ growth of *Clostridium perfringens* throughout the meat (*40*).

Why should *C. perfringens* be the indicator organism for safety of cooling processes? Data from two studies on outgrowth of *B. cereus*, *C. perfringens*, and *C. botulinum*, during cooling of inoculated cooked ground beef, demonstrated that *C. perfringens* multiplied by 4–5 $\log_{10}$ if cooling took 18 hours while the other two organisms did not grow during this time (*72, 73*). Of the three pathogenic spore-formers, *C. perfringens* is most often associated with meat; improper holding temperatures and/or inadequate cooking are contributing factors in nearly all outbreaks investigated by the Centers for Disease Control (Table 1). Therefore, outgrowth of *C. perfringens* is a logical choice as the standard to assess the safety of cooling processes.

Since the optimal growth temperature for *C. perfringens* is in the range of 109–117°F (43–47°C) where the generation time may be as short as 8 minutes, it is important to rapidly cool foods through this temperature range. Therefore, the USDA guideline states that cooling of meat from 130 to 80°F (54.4 to 26.6°C) should take no longer than 1.5 hours and cooling from 80 to 40°F (26.6 to 4.4°C) take no more than 5 hours (*40*).

Recommendations from the Campden and Chorleywood Food Research Association (UK) are to cool uncured meats with low levels of contamination from 122 to 53.6°F (50 to 12°C) within six hours and from 53.6 to 41°F (12 to 5°C) within one hour. For cured meats containing salt and nitrites, the cooling time may be extended by approximately 25%. However, if the product contains high levels of spores, these recommended cooling times may be too long to ensure safety (*44*). (Further description of data from these experimental studies can be found in the section on Ham.)

Growth models for various foodborne pathogens have been developed by USDA scientists. These are the models currently available (*117*):

- Growth Models for *Aeromonas hydrophila, Bacillus cereus, Clostridium perfringens, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella, Shigella flexneri, Staphylococcus aureus*, and *Yersinia enterocolitica*
- Non-thermal inactivation/survival models for *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*
- Thermal inactivation models for *Clostridium botulinum*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*
- Gamma Irradiation models for *Salmonella typhimurium*, *Escherichia coli* O157:H7 and "Normal" flora in meats
- Cooling/Growth models for *Clostridium botulinum* and *Clostridium perfringens*
- Time-to-Toxigenesis model for *Clostridium botulinum* on fish
- Time-to-Turbidity models for *Clostridium botulinum*

The focus of this literature review is the presentation of published data on the potential survival of sporeformers during cooling. There are few journal articles that address this question directly. These papers as well as articles which describe compounds and conditions inhibitory to growth will be summarized. Some modeling programs, using data from growth studies, have been developed and these can aid in predicting growth under

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different conditions. In addition, some references on cooling rates of different meats will be included even where there is no mention of pathogen survival.

In order to address the issue of potential outgrowth in various meat products, information on the effects of water activity, pH, sodium chloride, curing salts, and other preservatives on growth and survival of *C. perfringens* will be included. These data may help food processors estimate the likelihood of spore outgrowth at different temperatures during cooling after normal processing conditions.

Clostridium perfringens and other foodborne spore-formers

Association with foodborne disease

Three species of spore-forming bacteria are associated with foodborne disease: *Clostridium botulinum, Clostridium perfringens,* and *Bacillus cereus*. Of these, *C. perfringens* causes the most outbreaks and cases but *C. botulinum* is most likely to cause death. Data reported to CDC on outbreaks caused by these pathogens during 1988–1997 are listed in Table 1 (*13, 14*). Most cases of botulism are recognized and reported, but illness due to the other sporeformers is generally mild and many cases are not reported. It has been estimated that there are 27,360 cases of foodborne *B. cereus* infections and 248,520 cases of foodborne *C. perfringens* occurring yearly in the U.S. (*90*).

Clostridium perfringens has been recognized as a cause of foodborne illness since the late 1940s. Typical outbreaks are associated with meat or poultry served in restaurants, homes for the elderly, or at large gatherings. Meats have often been cooked properly but invariably were held at room temperature for excessive lengths of time or cooled in large batches such that the cooling rate was insufficient to inhibit bacterial growth (*42*). The CDC reported on two such outbreaks related to corned beef served at large St. Patrick's Day dinners (*12*).

Early data (before 1980) on growth of *C. perfringens* in different meats has been summarized in a review. Cells grew at temperatures ranging from 15 to 51°C, with reported generation times as short as 8.5 min (*26*). At optimal temperatures, high concentrations of *C. perfringens* (10^6 to 10^8 cells per ml or gram) could accumulate and cause symptoms of diarrhea and abdominal pain. Further information on foodborne illness caused by *C. perfringens* has been reviewed (*36, 119*).

Bacillus cereus can also contaminate meat. *B. cereus* spores are commonly present in spices, and these may be the source of this bacterium in some

Table 1. Sporeformers in Foodborne Outbreaks, 1993–1997 (1988–1992)

	B. cereus		*C. botulinum*		*C. perfringens*	
Number of reported outbreaks	14	(21)	13	(40)	57	(40)
Number of reported cases	691	(433)	56	(133)	2772	(3801)
Number of reported deaths	0	(0)	1	(11)	0	(1)
Occurred in:						
Private home	3	(4)	10	(24)	3	(3)
Restaurant/cafeteria/deli	3	(9)	1	(0)	23	(12)
Church/school	2	(1)	0	(0)	7	(7)
Picnic	0	(0)	0	(0)	2	(1)
Vehicles:						
Beef/pork	1	(0)	0	(0)	12	(8)
Chicken/turkey/other meat	2	(2)	2	(10)	4	(9)
Fish/shellfish	0	(1)	1	(11)	1	(0)
Dairy products	0	(0)	1	(0)	1	(0)
Fruits/vegetables	0	(0)	6	(20)	0	(0)
Salads	1	(1)	0	(1)	2	(1)
Contributing factors:						
Improper holding temperature	11	(16)	6	(7)	46	(31)
Inadequate cooking	3	(4)	1	(13)	13	(13)
Contaminated equipment	2	(3)	0	(0)	5	(3)

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meats and gravies. *B. cereus* is also a problem in the dairy industry and is infamous for multiplying in cooked rice left at room temperature (*1*). A variety of foods have been implicated as vehicles for *B. cereus* food poisoning outbreaks (*47*).

Relatively few outbreaks of botulism in the U.S. are traced to meats, but this is not true in some other countries where home canning and preserving of meats is more prevalent. Proteolytic strains of *C. botulinum* produce spores resistant to heat pasteurization but vegetative cells can only grow and produce toxin at temperatures greater than 10°C. Theses strains may present a hazard if food is temperature abused. Non-proteolytic strains have spores that are less heat-resistant but cells can grow and produce toxin at refrigerator temperatures (3–4°C). Potential for growth of nonproteolytic *C. botulinum* in pasteurized restructured meat products has been reviewed (*69, 111*).

Effects of heat on vegetative cells in laboratory media

Strains of clostridia and bacilli vary somewhat in temperature requirements for growth and other environmental factors, e.g. acidity and water activity may affect heat sensitivity and spore germination. Table 2 lists the reported growth characteristics of the foodborne sporeformers (*32, 83*). All these bacteria are sensitive to pH <4.4. *C. perfringens* has a higher optimal growth temperature range, and *B. cereus* can tolerate lower water activities.

The potential for microbial growth in a particular meat product depends on a number of factors. Various nutritional and physical factors interact to determine whether, and how fast, an organism will grow. An assessment of the interactive effects of temperature, initial pH, sodium chloride, and sodium pyrophosphate on growth kinetics of *C. perfringens* in laboratory media demonstrated that the fastest growth (generation time of 12 min) occurred at 42°C, pH 6.25, 1.5% sodium chloride and 0.15% sodium pyrophosphate. Lower temperatures and pH depressed growth and higher salt concentrations were also inhibitory, particularly at colder temperatures and in more acidic solutions (*46, 70, 74*). Effects of variations in pH, salt and sodium pyrophosphate levels on the growth of proteolytic (*64*) and nonproteolytic *C. botulinum* have also been modeled (*69*). Other growth models for *C. perfringens*, *B. cereus*, and *C. botulinum* have been published by the USDA as part of its Pathogen Modeling Program (*117*).

Temperatures above 60°C will generally inactivate vegetative cells, although the presence of salts or fat in the surrounding medium can increase resistance of cells to heat. Previous growth conditions affect heat resistance with growth at a higher temperature and a heat shock of 55°C for 30 min increasing heat resistance (*32, 56*). In addition, strain differences exist: Some strains of *C. perfringens* associated with food poisoning have D (decimal reduction) values at 55°C which are twice that of strains not associated with food poisoning (*104, 105*).

Effects of heat on spores in laboratory media

Bacterial spores are very resistant to heat and survive many cooking and processing treatments. Transformation of these spores to actively growing, toxin-producing vegetative cells is a three stage process: activation, germination, and outgrowth. Heat often serves to activate the spores, and they will subsequently germinate and grow if conditions are favorable (*45*). High temperatures can injure spores, and depending on available nutrients, pH, temperature, and inhibitory substances, injured spores may or may not be able to

Table 2. Growth characteristics of sporeformers

	B. cereus	*C. perfringens*	*C. botulinum*[1]	*C.botulinum*[2]
Growth: temperature limits (°C)	10–50	12–50	10–48	3.3–45
Growth: optimal (°C)	28–35	43–47	30–40	25–37
Growth: pH range	4.35–9.3	5.5–9.0	4.6–8.0	5.0–8.0
Growth: a_w minimum	0.912	0.93	0.94	0.95

[1]Group I, proteolytic, toxin types A, B, F
[2]Group II, non-proteolytic, toxin types B, E, F

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recover and grow (*60*). A review on spore injury cites numerous research papers with data on resuscitation of injured spores (*39*). A model describing recovery of bacterial spores after heat stress has also been developed (*85*).

Significant variation in heat resistance has been observed for different strains of *C. perfringens*: Spores of some strains are completely destroyed after a few minutes' exposure to 100°C, while other isolates that were associated with foodborne outbreaks survived boiling for six hours (*119*). Average D (decimal reduction) values at 100°C of spores of five food poisoning isolates were reported to be 60-fold higher than the average for seven non-food poisoning isolates. For one extremely heat-resistant strain, 124 min at 100°C were required to achieve a one-log reduction in the number of spores (*104*).

Growth conditions of cells prior to sporulation affect the thermal resistance of spores. *C. perfringens* spores formed by cells grown at a higher pH (pH 9 compared to 7.5) and spores from heat-shocked vegetative cells are more heat tolerant (*27, 56*). Spores are also more heat resistant when tested in water compared to phosphate buffer and are even more resistant in meat (*32*).

Thermal destruction of *C. botulinum* spores does not follow first-order kinetics, indicating that some spores are more heat resistant than others. Resistance of *C. botulinum* spores to heat is inversely related to the water activity of the heating medium, with greatest survival at a_w of 0.2–0.4 (*93*), and is much higher in soybean oil as compared to phosphate buffer. This may be due to reduced water activity in the oil (*91*). Acidity reduces heat resistance and this is best modeled by a second degree equation (*84*). Other thermal inactivation models which reflect experimental data have been developed (*76, 98*). These models may be especially useful in estimating the safety of some marginal heat processes.

Acidity also decreases heat resistance of *B. cereus* spores and inhibits recovery of heat-damaged *B. cereus* (*11, 25*). Combined effects of temperature and pH on the heat resistance of *B. cereus* spores have been modeled (*32, 43, 82*).

Lower water activities (0.9 compared to 1.0) protect spores from destruction by heat (*86, 114*) but water activities of <0.98 in recovery media inhibited germination and growth of heated *B. cereus*. When spores were heated and recovered in media with the same water activity (as would occur during heating and cooling of a food), these protective and inhibitory effects nearly canceled each other and very little difference in D values for spores was observed in the water activity range of 0.92–1.0 (*24*). Unlike *C. botulinum* spores, *B. cereus* spores were more heat resistant in phosphate buffer than in soybean oil (*91*).

Most food sterilization procedures are based on data from studies involving isothermal heating: Small samples of media containing spores are placed in a hot liquid where they very quickly equilibrate with the high test temperature. However, heating and cooking of foods is a non-isothermal process: The temperature of the food gradually rises to some predetermined temperature. Experiments with *B. cereus* demonstrated that inactivation of spores was less and germination of spores was greater when media were heated non-isothermally as would occur during cooking or processing (*37*).

In summary, results from laboratory experiments and pathogen growth models can provide estimates of the times and temperatures required to inactivate vegetative cells and spores. But there are so many other factors that affect heat resistance of bacteria, that validation of a processing method should be undertaken under realistic conditions of use and with the meat or meat product involved.

Activation and outgrowth of spores in laboratory media

Spore germination is a complex process which requires numerous nutrients and is usually enhanced by heat treatment and (for clostridia) by anaerobic conditions. Generally, heat-resistant strains of *C. perfringens* can be activated by 10–20 min at 75–80°C although spores of some strains are activated at 100°C and others may be activated at 65°C. Only 0.13–3.6% of spores from *C. perfringens* strains associated with food poisoning germinate without heat activation (*32, 119*).

Exposure to heat during thermal processing may activate spores but can also injure them and germination will only occur if the damage is repaired. A nutrient-rich medium, such as meat, is required for repair. Lysozyme promotes repair of both *C. perfringens* and *C. botulinum* spores that have been damaged by heat. As with growth, germination depends upon the presence or absence of a variety of chemicals and environ-

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mental conditions. Germination of *C. perfringens* can occur at temperatures of 7–46°C and within a pH range of 5 to 9. Certain salts affect germination, and oxygen represses germination and outgrowth (*5, 32, 78*).

Germination kinetics of proteolytic *C. botulinum* spores were determined as a function of temperature (15, 22, 30°C), pH (5.5, 6.0, 6.5), and sodium chloride (0.5, 2.0, and 4.0%). Increasing sodium chloride concentrations inhibited germination, especially at low temperatures and/or pH values. Germination was also very slow or undetectable at pH 5.5. It is important to note that certain conditions, such as a low pH, retard germination but do not significantly affect growth of vegetative cells. Therefore, the cells arising from the few spores that germinate may grow and start producing toxin (*16*). Similar studies were done with a non-proteolytic strain of *C. botulinum* (*51*).

Non-proteolytic strains of *C. botulinum* can grow and produce toxin at refrigeration temperatures, and experiments with heat-shocked spores demonstrated that they could grow and produce toxin in media at 6–8°C within 28–40 days (*97*). Lysozyme aids germination and growth of non-proteolytic strains of *C. botulinum* that have been heat treated (*38, 96*).

Unlike the clostridia, *B. cereus* requires aerobic conditions for sporulation and not all strains require heat activation for spores to germinate. The optimal temperature for germination appears to be 30°C, although germination has been reported to occur at a wide range of temperatures (*32*).

Survival and Growth of *C. perfringens* in Uncured Meats

Pathogen modeling programs and in vitro experiments are useful in predicting ranges of temperatures, salt concentrations, etc. which may restrict pathogen growth in meat. But foods are complex systems which encompass important variables that may not be tested in carefully controlled laboratory experiments. For example, growth and toxigenesis of *C. botulinum* observed in 16 sous vide products was inconsistent with predictions from the Food MicroModel and the Pathogen Modeling Program. The inaccurate predictions were apparently the result of the limited number of factors considered in the models. This underlines the importance of studying pathogen survival in each type of food under realistic processing conditions (*59*).

Beef

Heat Resistance

Vegetative cells of *C. perfringens* are killed by heat but cells which have grown at higher temperatures (45–49°C) are more heat resistant than those grown at lower temperatures (*103*). Thus, *C. perfringens* may become more heat resistant during the long cooking of roasts at low temperatures. Some recommendations for cooking and cooling such roasts have been presented (*112*).

Other conditions also affect the sensitivity of vegetative cells to potentially lethal temperatures. Sodium pyrophosphate diminishes heat resistance of vegetative cells of *C. perfringens* in ground beef as indicated by D values for different time-temperature exposures (*66*). But a sublethal heat shock of 10 min at 48°C increases heat resistance of *C. perfringens* in beef gravy (*71*).

Cooling

Spores that survive cooking may be activated and start growing if cooling is slow enough. In experiments to determine the fate of *C. perfringens* during cooling, some researchers have added vegetative cells to hot beef (45–60°C) while others have used heat-activated spores.

Beef gravy inoculated with vegetative *C. perfringens* cooled slowly when packed into 33 lb cartons and refrigerated. Bacterial cells multiplied rapidly as the gravy cooled from 43.5°C (*116*). In fact, the generation time of *C. perfringens* in ground beef has been measured as only 7.1 min at 41°C (*80*).

Ground beef inoculated with heat-activated spores and cooled from 60 to 10°C at different linear rates of 5–25°C per hour was monitored for growth of *C. perfringens*. No growth occurred at the fastest cooling rates (15 and 25°C per hour) while rapid growth was observed at the slowest cooling rates (5 and 7.5°C per hour). Some growth occurred at the intermediate rate of 10°C per hr (*108*). Cooling of beef chunks (8 × 8 × 8 cm) from 55 to 10°C within 3 hours did not allow growth of *C. perfringens* but rapid growth occurred if cooling took 4 hours or longer (*77*).

In other experiments with heat-activated spores in cooked ground beef, cooling rate from 54.4 to 7.2°C was not linear but exponential, i.e. more rapid at the beginning and then slower. This type of cooling is more similar to what occurs in usual retail operations. No

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appreciable growth occurred if cooling took 15 hours or less; however, *C. perfringens* grew by 4–5 $\log_{10}$ if cooling took 18 hours (*72*). With the same meat and cooling regime, no growth was observed from spores of *B. cereus* or non-proteolytic *C. botulinum* (*73*).

Cooked meat that is in the process of cooling has a constantly changing temperature and this complicates calculations to determine potential growth of pathogens. Potential pathogen growth during cooling of animal carcasses has been modeled using data on the temperature history of the meat combined with data on growth rates of pathogens at different temperatures (*4, 48, 49*). At optimal temperatures, there may be a very short lag phase before growth occurs; at suboptimal temperatures, lag phase may be many hours or even days long.

Inhibitors

In the event that the cooling process is not rapid enough to prevent outgrowth of spores and multiplication of bacteria, some preservatives or other treatments may be used to ensure the safety of the meat. (Data on cured meats are presented in the next section.)

- Nisin added to precooked, vacuum-packaged beef prevented bacterial growth for up to 70 days at 10°C (*52*).
- Both 3% salt (sodium chloride) and a pH of 5.5 inhibited outgrowth of *C. perfringens* spores in cook-in-a-bag beef products at 15°C. Each factor was inhibitory while the combination completely suppressed growth for 20 days. Salt and acid alone were ineffective at 28°C and the combination delayed growth for only 36 hours. Neither factor was required to repress growth if the meat was kept at 4°C (*63*).
- Modified atmospheres can inhibit the growth of some bacteria in packages of meat. Because *C. perfringens* is an anaerobe, some oxygen should be present to inhibit its growth. An effective combination of gases for cooked beef included 10% oxygen (to inhibit *C. perfringens*), 75% carbon dioxide (to inhibit *Pseudomonas*, *Salmonella* and *Staphylococcus*), and 15% nitrogen (*57*). At 37 and 42°C, growth was only slightly inhibited in beef packaged aerobically as compared to vacuum-packaged beef. The inhibition by oxygen was more evident at 15°C but growth was completely prevented only by low temperatures (4–12°C) (68).
- Another strategy is post-packaging pasteurization (82°C for 16 min), which reduced populations of vegetative *C. perfringens* and of other microflora and significantly increased shelf life of packaged beef loin chunks stored at 4°C (*21*).

Pork

Clostridium perfringens has been detected in the liver and fluid from the body cavity of pork carcasses. In addition, it was reported from 100% of samples from scalding vat water (*7*).

Poultry

Heat Resistance

Sodium pyrophosphate diminishes heat resistance of vegetative cells as shown by the D values measured for different time-temperature exposures of vegetative *C. perfringens* in ground turkey (*66*).

Higher salt concentrations (2–3%) also reduced heat resistance of non-proteolytic *C. botulinum* spores in turkey slurry and D values at 75–90°C have been measured (*62*).

Cooling

Ready-to-eat turkey breast roasts, cooked in a steam oven to an internal temperature of 72°C, were then cooled at different rates to 12.8°C in walk-in coolers with forced air flow. Cooling of roasts for 6 and 8 hours in coolers set at –2 and 6°C, respectively, did not permit 1 log growth of *C. perfringens* (USDA standard). Cooling in a 10°C cooler for 10 hours was not fast enough to prevent significant multiplication of *C. perfringens*. Analysis of the data indicated that cooling from 48.9 to 12.8°C should take no longer than 8.9 hours in order to prevent possible *C. perfringens* growth with a 95% tolerance interval (*114*).

Commercial chicken croquettes in Spain are processed at 80°C for 30 min and then usually cooled in a cold room for 24 hours to reach a temperature of 5°C. Clostridia were detected in the raw chicken and onions used to make the croquettes and grew during the processing stages after heat treatment. A faster cooling process reduced the numbers of bacteria in

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the croquettes and is probably necessary to prevent *C. perfringens* poisoning (*22, 23*).

Cooking of chicken parts inoculated with *C. perfringens* in hot water destroyed all vegetative cells when the meat reached an internal temperature of 77°C but 40–60% of spores survived cooking to an internal temperature of 82°C. After cooking (at 82 or 93°C for 15–50 min), chicken pieces were immersed in tap water at room temperature for cooling. There was evidence that some spores germinated and grew during this cooling step. Freezing of the precooked chicken parts followed by reheating or frying killed more spores but some even survived these cooking methods (*28*).

Cooked meat that is in the process of cooling has a constantly changing temperature, complicating calculations to determine potential growth of pathogens. Potential pathogen growth during cooling of animal carcasses has been modeled using data on the temperature history of the meat combined with data on growth rates of pathogens at different temperatures (*4, 48, 49*). At optimal temperatures, there may be a very short lag phase before growth occurs; at suboptimal temperatures, lag phase may be many hours or even days long.

Inhibitors

Since cooking can activate spores, it is important to understand conditions that prevent outgrowth if cooling is not fast enough. Effects of storage temperature and atmosphere on growth of vegetative *C. perfringens* in cooked, ground turkey have been investigated. *C. perfringens* grew rapidly at 28°C in cooked ground turkey under both aerobic and anaerobic (vacuum-packed) conditions. At 15°C, growth was suppressed for about 4 days, and at 4°C bacterial populations remained stable or declined. If refrigerated samples were placed at 28°C for 5 hours and then returned to the refrigerator for 6 days (to simulate temperature abuse), no significant growth was observed. However, >10 hours of temperature abuse did permit multiplication of *C. perfringens* (*61*).

In similar experiments using ground turkey packaged under different modified atmospheres, bacterial growth was slowest in atmospheres containing 20% oxygen and 25–50% carbon dioxide at 15 and 28°C. Nevertheless, measurable growth occurred within 3 hours at 28°C and within 7 days at 15°C under all atmospheres. Only storage at 4°C prevented growth. No significant growth of *C. perfringens* occurred if temperature abuse (28°C) lasted for 8 hours, but cells did grow if this was extended to 12 hours (*67*).

Addition of 3% sodium chloride completely inhibited outgrowth of *C. perfringens* spores at 15°C and delayed growth for 12 hours at 28°C in cooked ground turkey containing 0.3% sodium pyrophosphate (*65*). Growth at 15°C was also slowed by 1–2% sodium chloride. Regardless of salt concentration, no growth occurred at 4°C.

Prevention of foodborne illness and growth of *C. perfringens* in turkey products in school lunch kitchens was reviewed with respect to time-temperature control of thawing, cooking, chilling and reheating (*9*).

Combination Foods

Chili

Growth of *C. perfringens* from activated spores was measured in commercial cooked chili held for 6 hours at several temperatures. No growth was observed at 26.7°C or below while growth was observed at 6 hours at 32.2°C and after 2 hours at 37.8–48.9°C. A model was developed to describe bacterial growth in chili during exponential cooling conditions (*8*).

Goulash (beef)

Effects of sodium and calcium lactate on growth from spores of *C. perfringens* and *B. cereus* in sous-vide beef goulash were measured at different temperatures. Calcium lactate (1.5 and 3.0%) was more inhibitory to both species at all temperatures during 28 days incubation. At 15°C, 3% sodium lactate inhibited growth, at 20°C it delayed growth for a week, and at 25°C it had little inhibitory effect (*3*).

Spaghetti with meat sauce

Salt concentrations of >1.5% and pH values of <5.25 prevented growth and toxigenesis of *C. botulinum* in a sous-vide spaghetti and meat sauce product maintained for 42 days under conditions of mild temperature abuse (15°C). At pH of 5.25 and 5.5, botulinum toxin was formed even though the food was not visibly spoiled. Because different batches of tomatoes vary in acidity, it is important to monitor pH (*110*).

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Survival and Growth of *C. perfringens* in Cured Meats

Clostridium perfringens has been detected in a high percentage of fresh pork sausage samples (*7, 53, 92*). Reviews of the production of cured meats with respect to the risk of botulism (*15*) and the risk for outgrowth of all foodborne sporeformers (*33, 34*) pointed out many factors that prevent growth and toxin production of these pathogens. No one factor in cured meats completely inhibits the growth of all pathogens. Rather, a combination of a mild heat treatment that sensitizes cells to curing agents, different salts and curing substances, a low pH, and refrigerated storage act to keep the foods safe.

Ham

Slow cooling of ham has been recognized as potentially hazardous. *C. perfringens* survived and grew in concentrations of curing salts greater than those usually used for ham. When inoculated into ham with curing brine, *C. perfringens* survived both curing and smoking (*50*). Even a secondary heat treatment of 121°C for 10 min only delayed but did not prevent outgrowth of *C. perfringens* spores (*29*).

A comprehensive series of experiments on hazards associated with slow cooling provides data on survival of *C. perfringens* in ham and other meat products (*44*). Good Practice recommendations for cooling cooked, lightly contaminated meat are a maximum of 8 hours from cooking temperature to 5°C for uncured meat and 10 hours for cured meats. If meat is expected to contain high levels of spores (for example, if it contains untreated herbs and spices distributed throughout the product) the safe cooling time may be as little as 3 hours. If a meat product is cooked and cooled and then reheated briefly (to give a roast flavor) and then cooled again, the total cooling time should not exceed these recommendations. Cooling requirements are not affected by the method of cooking or method of containment during cooking.

Frankfurters

Clostridium perfringens, inoculated into frankfurter emulsion containing sodium chloride, sodium nitrite and spices, survived processing at 69°C for 30–48 min. Under anaerobic conditions, cells multiplied rapidly at 23 and 37°C, slowly at 12–15°C, and not at all during 2 weeks' storage at 10°C or 4 weeks at 0–5°C (*113*).

Frankfurters formulated with several combinations of sodium nitrite, sodium isoascorbate, and potassium sorbate were inoculated with *C. perfringens* and processed to 67–70°C. During subsequent storage at both 20 and 7–9°C, *C. perfringens* counts slowly declined while total aerobic bacterial counts increased (*54*). Another comparison of the effects of sorbate vs. nitrite in frankfurters showed that both compounds caused a slow decline in numbers of *C. perfringens* during 9 days storage at 4 or 20°C (*118*).

Sausage

Turkey sausage processing — addition of *Pediococcus cerevisiae* and heating at 27–46°C for 12 hours followed by cooling and drying — was not sufficient to kill *C. perfringens* present in the meat (*6*).

Thuringer cervelat did not support the growth of *C. perfringens* during 23 days at 7–9°C or 96 hr at 20°C. This was apparently the result of the low pH (<5.0) produced by the lactic acid starter culture organisms (*54*).

Vacuum-packaged Vienna sausages, pasteurized after packaging, have been reported to have a longer shelf-life because of the destruction of spoilage bacteria. However, *C. perfringens* was more frequently detected in pasteurized samples, apparently as a result of decreased competition from the spoilage organisms (*41*).

Brine and nitrite concentrations were varied to determine combinations effective in preventing growth and toxigenesis of *C. botulinum* in liver sausage (*55*) and cured ground pork (*81*). Both sodium chloride and nitrite were inhibitory.

Inhibitors

Sodium nitrite

Sodium nitrite has long been used as a preservative in cured meats. Experiments in the late 1960s and early 1970s demonstrated that 0.02% and 0.01% sodium nitrite effectively inhibit germination of heat-sensitive and heat-resistant *C. perfringens* spores, respectively (*79, 106*). The combined effects of sodium chloride and sodium nitrite on growth of *C. botulinum* spores in canned, cured lunch meat have also been described

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(*99*). Spores which have been damaged by heat are more sensitive to the effects of nitrite (*17*). This inhibitory effect of nitrite was enhanced by acidic conditions and by prior heating of the medium containing nitrite. In experiments with several strains of *C. perfringens*, a five- to ten-fold higher concentration of nitrite was required to prevent outgrowth of some strains compared to others (*101*).

At a high concentration of 0.5 M, sodium nitrite interacts with the spore cortex, stimulating germination of *C. perfringens* spores (*2*). This could be detrimental to the spores if they started to outgrow prior to cooking and then became more vulnerable to heat during processing. But this stimulatory effect is not observed at the lower nitrite concentrations permitted for use in foods.

Sorbic Acid

In cured ground pork inoculated with *C. sporogenes* spores (a substitute for *C. botulinum*), a combination of 0.2% sorbic acid and 40 ppm sodium nitrite was more effective than 120 ppm sodium nitrite alone in delaying clostridial growth at 27°C (*102*) was cured and irradiated.

Sodium propionate

A shelf-stable beef product, inoculated with *C. botulinum* spores, was cured and irradiated. Although irradiation destroyed many spores, some survived even a high dose of 10 kGy. Addition of 2% sodium propionate inhibited spore outgrowth and toxigenesis even at a high dose of 10^5 spores and irradiation at 2.5 kGy (*115*).

Sodium hypophosphite plus ascorbate

A combination of sodium hypophosphite plus ascorbate exerted antibotulinal activity in meat and along with a colorant and an antioxidant may be an effective nitrite-free curing system (*120*).

SLEB (sucrose laurate, ethylaminediaminetetraacetate, butylated hydroxyanisole)

Several formulations of SLEB were found to effectively inhibit growth of *C. perfringens* in laboratory media. The ratios of the components in this preservative may be adjusted for use in different products (*109*).

Bacteriocins

Some lactic acid bacteria (LAB) used in the production of sausage can inhibit the growth of pathogenic bacteria. LAB compete with pathogens for nutrients and also produce antibacterial compounds called bacteriocins. Plantaricin, produced by a strain of *Lactobacillus plantarum* from dry sausage, inhibits growth of *B. cereus* and *C. perfringens* (*35*). In vitro tests of several bacteriocins revealed that enterocin L50 was the most effective inhibitor of *B. cereus, C. botulinum* and *C. perfringens* when compared to nisin A, lactocin S, and pediocin PA-1 (*20*). Both lacticin 3147 (from *Lactococcus lactis*) and nisin (commercially available) combined with sodium citrate or sodium lactate inhibited growth of *C. perfringens* in pork sausage during storage at 4°C (*107*).

Efficiency of Cooling Processes

Cooling rates of foods depend on the size and shape of the food or container holding the food and the methods used for cooling (*31, 94, 95*). Vacuum cooling reduces temperature most rapidly but may result in excessive moisture loss and toughening of meat (*30, 87, 89*). Immersion cooling in ice water is usually about twice as fast as forced cold air for large roasts (*10, 44*). Air and water spray cooling of sausages has also been compared (*100*). Other recent papers on chilling methodology include discussions of vacuum cooling (*88*), immersion chilling of trays (*75*), air blast chilling (*18, 58*), and sous vide technology (*19*).

Summary and Conclusions

Bacterial spores are not only very heat resistant but heat actually stimulates the spores to start growth. Generation times as short as 7 min have been recorded; therefore, it is important to cool cooked meats quickly to temperatures below the minimum that allows germination and growth of sporeformers. The critical temperature range for growth of *B. cereus, C. perfringens,* and some *C. botulinum* strains is approximately 10–50°C although some psychrotrophic *C. botulinum* strains can grow at refrigerator temperatures.

A variety of cooling methodologies exist, and the decision on which to use will depend on the type of meat product as well as economic costs. Another very important consideration is the avoidance of post-processing

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contamination during cooling and packaging. The actual cooling rate and safety of cooling processes should be investigated for each product because various factors such as sodium chloride concentration, fat percentage, and the presence of some inhibitory compounds may make a specific cooling regime safe for one product but not for another.

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