Effect of Phosphate and Meat (Pork) Types on the Germination and Outgrowth of *Clostridium perfringens* Spores during Abusive Chilling†

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**ABSTRACT**

The effect of phosphate blends and meat type (pale, soft, and exudative [PSE]; normal; and dark, firm, and dry [DFD]) on the germination and outgrowth of *Clostridium perfringens* during abusive exponential chilling times was evaluated. Two phosphates were used: tetrathionate pyrophosphate (TSPP) and sodium acid pyrophosphate (SAPP, from two different sources, SAPP1 and SAPP2). The pork loins representing each meat type were ground (1/8-in. [0.3-cm] plate), and one of the three phosphate blends (SAPP1 + SAPP2, TSPP + SAPP1, or TSPP + SAPP2) was added (0.3% total, equal proportions of 0.15% each type) with salt (1.0%). The pork was then mixed with a three-strain *C. perfringens* spore cocktail to obtain a final concentration of 2.0 to 2.5 log spores per g. The inoculated product was heat shocked for 20 min at 75°C and chilled exponentially from 54.4 to 4°C in a period of 6.5, 9, 12, 15, 18, or 21 h. In control samples (PSE, normal, and DFD), the increase in *C. perfringens* population was <1 log CFU/g within the 6.5-h chilling period, and longer chilling times resulted in greater increases. *C. perfringens* population increases of 5.95, 4.73, and 5.95 log CFU/g of meat were observed in normal, PSE, and DFD pork, respectively, during the 21-h abusive chilling period. The combination of SAPP1 + SAPP2 was more effective than the other treatments for inhibiting *C. perfringens*. The types of phosphate and their blends and the type of meat affected the germination and outgrowth of *C. perfringens* spores in cooked pork during abusive chilling periods.

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*Clostridium perfringens* is a nonmotile, endospore-forming, rod-shaped, gram-positive bacterium (20). Typically, *C. perfringens*-associated foodborne illness is caused by consumption of foods that are temperature abused during either processing or subsequent handling, resulting in *C. perfringens* populations greater than 6.0 log CFU/g (19). Although the initial *C. perfringens* spore population in meat processing ingredients or raw product could be low, the organism can germinate and grow to reach hazardous levels in food matrices because of its short generation time (typically <10 min under optimal conditions) (20). The majority (>75%) of the foodborne illness outbreaks caused by *C. perfringens* have been linked to consumption of meat and meat products (6). The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) (36) nationwide microbiological baseline data indicate a *C. perfringens* prevalence of 10.4% on market hog carcasses. Use of meat from these animals can result in the presence of *C. perfringens* spores in the raw meat and their potential germination and their outgrowth during chilling.

Typical thermal treatments applied during manufacture of meat products can destroy the vegetative cells of *C. perfringens* but activate its spores. Abusive chilling of meat or food products containing meat in food service settings has been reported as the primary factor in *C. perfringens*-related foodborne illness outbreaks (13). The activated spores in meat can germinate, grow, and multiply during thermal process deviations or abusive holding temperatures at food service operations (13). Fresh meat quality (e.g., color and purge) is a characteristic of major significance to pork processors and influences the buying decisions of the consumer (4, 24). The muscle postmortem condition (rate of pH decline) and the ultimate pH of the muscle determine the characteristics of the muscle and have been characterized as pale, soft, and exudative (PSE), normal, or dark, firm, and dry (DFD). Approximately 10 and 4% of total pork meat in the U.S. market is PSE pork and DFD pork, respectively (24), resulting in an economic loss to the producer because these conditions are not desirable in good quality meat.

Use of PSE pork in the manufacture of processed meat products can result in problems such as pale color, low water holding capacity (resulting in low cook yields), poor fat emulsifying capacity, granular and crumbly texture, and a poor consistency (34, 42). Some of the loss in functionality due to use of PSE pork for manufacturing processed meat products can be overcome by manipulating the processing conditions such as ionic strength through the use of ingredients (NaCl and phosphates) and optimizing cooking temperatures.
Recent work in our laboratory has revealed that minor differences in pH of the meat can have a significant effect on C. perfringens spore germination and outgrowth. Two factors that can determine the final pH of the meat product are the muscle type (PSE, normal, or DFD) and the type and concentration of phosphate, which is an ingredient commonly used in meat processing.

Several phosphate types are used in the food industry. Inorganic phosphates have a number of functional effects on meat, including improving water binding properties, curing color development, and retarding oxidation and discoloration. The phosphates differ in chain length; ortho, pyro, and poly forms have one, two, and multiple phosphate groups, respectively. The remaining sites (two or three) of each of the phosphate groups (PO$_4^{3-}$) are commonly occupied by sodium or potassium ions. Of all the phosphates, alkaline phosphates (Na or K salts of pyro- or polyphosphates) are most commonly used in the meat industry for their effects on the final pH of the meat products and other functional properties. Although acid phosphates are not commonly used in meat processing, they may be used in combination with other phosphates to deliver a product with a particular pH.

The role of pH of the meat and the effect of phosphate (type and blends) on C. perfringens spore germination and outgrowth during chilling of processed meat products have not been systematically evaluated. For fair comparison between the treatments in this study, we used three pork types (PSE, normal, and DFD) from the same muscle and two pyrophosphates types, sodium acid pyrophosphate (SAPP) and tetrasodium pyrophosphate (TSPP), each containing two phosphate groups. The goal of the current research was to evaluate the effect of meat type (pork and its effect on pH) and pyrophosphate blends (types and their effect on pH) on C. perfringens spore germination and outgrowth during exponential chilling of pork.

MATERIALS AND METHODS

Cultures. Three different enterotoxin-producing strains of C. perfringens were used in the study: NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13). Additional information about these cultures and their maintenance has been reported elsewhere (12).

Phosphates. SAPP obtained from two different sources, SAPP$^+$ (sodium pyrophosphate dibasic, Sigma-Aldrich, Co., St. Louis, MO) and SAPP$^-$ (BK Giulini Corporation, Simi Valley, CA), and TSPP (sodium pyrophosphate tetrabasic, dechydrate, Sigma-Aldrich) were used.

Preparation of C. perfringens spore cocktail. Spores of each strain of C. perfringens were prepared following the procedure outlined by Juneja et al. (12). An aliquot (0.1 ml) from the stock culture was inoculated into 10 ml of freshly prepared fluid thioglycollate medium (FTM; Becton Dickinson, Sparks, MD). The C. perfringens spore cocktail in the inoculated FTM was heat shocked at 75 °C for 20 min in a circulated water bath (Isotemp 3013H, Fisher Scientific, Fair Lawn, NJ), cooled in ice-chilled water, and then incubated at 37 °C for 18 h. A 1.0-ml portion of this culture was transferred to 10 ml of freshly steamed FTM and incubated for 4 h at 37 °C. The fresh culture (1%) was transferred to modified Duncan Strong (DS) (8) medium and incubated aerobically at 37 °C for 24 h. The original DS formulation was modified by replacing starch with 0.4% raffinose (Sigma-Aldrich) and supplementing with 100 mg/ml caffeine (Sigma-Aldrich) to support sporation. Spores of each strain were harvested by centrifugation (CS-15R, Beckman, Palo Alto, CA) at 7,012 × g for 20 min at 4 °C and washed twice with 50 ml of sterile distilled water. The spore crop of each strain was resuspended in 10 ml of sterile deionized water and stored separately at 4 °C until use. A spore cocktail containing all three strains of C. perfringens spores was prepared immediately before experiments by mixing approximately equivalent numbers of spores from each suspension.

Meat preparation. Pork loins to represent PSE, normal, and DFD pork were obtained from a local meat processor under refrigeration and ground (1/4-in. [0.3-cm] plate) using a meat grinder (model 4732, Hobart, Troy, OH). The ground pork was vacuum packaged (A300/H, Multivac, Woffelschwend, Germany) and stored frozen at −20 °C until use (a maximum of 3 months). The ground pork representing PSE, normal, and DFD conditions was thawed overnight under refrigeration. Salt (NaCl; 1.0%, wt/wt) and phosphate combinations (0.3%, wt/wt) were dissolved in water and added to the ground pork at 20% pump rate, and the meat was mixed for 3 min in a kitchen mixer (model K55SWH, Kitchen Aid, Troy, OH). Five-gram portions of the meat mixture representing each treatment were weighed into vacuum pouches (3-mil standard barrier polyethylene-nylon bag with a water vapor transmission rate of 10 g/liter/m$^2$/24 h at 37.8 °C and 100% relative humidity and an oxygen transmission rate of 3,000 cm$^3$/liter/m$^2$/24 h at 23 °C and 1 atm) that were 2.5 by 5.0 in. (6.35 by 12.7 cm) (Prime Source, Kansas City, MO), and the pouches were vacuum sealed at 12 mbar (1.2 kPa) with a Multivac vacuum packaging machine and stored frozen at −20 °C until used.

Treatments. A 3 (meat type: PSE, normal, and DFD pork) × 4 (treatments: control and three phosphates) experimental structure was used. The phosphate combinations were 0.15% each of (i) SAPP$^+$ + SAPP$^-$, (ii) SAPP$^+$ + TSPP, or (iii) SAPP$^-$ + TSPP. The total phosphate concentration was 0.3% by weight of the meat.

Spore inoculation, heat shock, and cooling procedure. Samples were thawed overnight at 5 °C in a refrigerator before the experiment, and each pouch containing the meat was aseptically inoculated with 100 μl of the three-strain spore cocktail of C. perfringens to attain a final population of ca. 2.0 to 2.5 log spores per g of meat. The pouches containing the inoculated meat were vacuum sealed again as described above, massaged manually for 30 s for even distribution of spores, and flattened to a uniform thickness of ca. 2 mm before heat shock. Two pouches from each treatment were submerged in a water bath (Isotemp 3013H) set at 75 °C for 20 min to activate the C. perfringens spores. After heat activation, one of the two pouches was chilled immediately in ice water and used for the enumeration of C. perfringens. The second pouch was transferred to a refrigerated bath with water circulation capabilities (RTE 740, Thermo Neslab, Portsmouth, NH) that was set at 54.4 °C. The pouch was allowed to equilibrate at this temperature for 10 min and then chilled exponentially from 54.4 to 4.0 °C within 6.5, 9, 12, 15, 18, and 21 h.

C. perfringens populations in the meat samples were enumerated for each sample following the protocol described by Velugoti et al. (39) and expressed as log CFU per gram of meat.

Measurement of pH and water activity. The pH and water activity ($a_w$) of the meat was measured in noninoculated samples. Five-gram portions of the sample were homogenized with 20 ml of...
Two independent replications were performed, and the pH and water activity (a_w) of PSE, normal, and DFD pork containing different combinations of sodium acid pyrophosphate (SAPP) and tetrasodium pyrophosphate (TSPP) were measured. The pH and a_w values for PSE, normal, and DFD pork were 5.31, 5.58, and 5.78, respectively. The addition of SAPP did not affect the a_w of normal or PSE meat (P > 0.05). Tompkin (22) reported that phosphates have little or no effect on a_w of foods at concentrations generally used in foods.

The pH of normal muscle declines from 7.2 (physiological) to approximately 5.5 to 5.8 during the 24 h subsequent to slaughter. Depletion of muscle glycogen before slaughter can result in a decline of muscle pH to ca. 6.1 to 6.2 and produce the DFD muscle condition. The high ultimate pH of the muscle traps the light, resulting in a darker appearance. Alternatively, a rapid decline in postmortem muscle pH from 7.2 to 5.8 within 45 min and low ultimate (24-h) pH may have a variety of causes, including breed, genotype, feeding, pre-slaughter handling, stunning, slaughter method, and chilling and storage conditions (27). These conditions result in a muscle that looks pale, soft, and exudative (PSE type). The muscle type (PSE, normal, or DFD) affects microbial growth, with low growth observed in PSE pork and the faster growth observed in DFD pork (10). Although other meat processing ingredients such as salt and phosphates can affect microbial growth, differences in microbial growth, differences can be evident in the finished product (25, 33).

Lower ultimate pH values in the range of 5.20 to 5.47 and 5.58 to 5.69 were reported for PSE and normal pork loins, respectively (10). In processed meat products, use of PSE pork can result in problems such as pale color, low water holding capacity (thus low cook yields), poor fat emulsifying capacity, granular and crumbly texture, and a poor consistency (25, 34, 42). Some of the loss in functionality due to use of PSE pork for manufacturing processed products can be overcome by manipulating the processing parameters such as ionic strength, pyrophosphate addition, and cooking temperature.

The pH and a_w of a food product can affect microbial growth significantly. The addition of SAPP + SAPP (0.3%) resulted in a lower pH (P < 0.05) of 5.69, 5.29, and 5.78 in normal, PSE, and DFD pork, respectively. The addition of phosphate combinations containing SAPP or SAPP + TSPP resulted in a slight increase in pH (P > 0.05) with all types of meat compared to control samples. Molins et al. (21) reported that addition of sodium acid pyrophosphate at 0.5 and 1.0% to ground pork reduced pH by 0.05 to 0.16 units from an initial pH of 5.49.

Although the phosphates can be used in processed meats at up to 0.5% concentration of the final meat product, concentrations of ≤0.3% are traditionally used because of soapy or bitter flavor defects in meat associated with use of higher concentration of phosphates. Use of phosphates in the meat formulation allows meat processors to reduce the salt level from 3 to 1.5% because the phosphates provide the desired ionic strength and hence the functional properties (7, 33). Combinations or blends of phosphates are used in the processed meat industry, and the criteria for selecting phosphate blends are mostly based on desired functional properties in foods rather than microbial control (32).

Phosphates exhibit antimicrobial properties in food products. Akhtar et al. (1) reported inhibition of C. \( \text{perfringens} \) in pork.
perfringens spore germination and outgrowth by polyphosphates in laboratory medium and cooked chicken. Similarly, Molins et al. (23) reported SAPP inhibition of Clostridium sporogenes PA3679 in cooked vacuum-packaged bratwurst held at 24 to 25°C for 24 and 48 h. The phosphates are effective for controlling growth of Salmonella, Staphylococcus, and Pseudomonas in laboratory media (9, 26), with greater efficacy against gram-positive bacteria (22). In our research, we wanted to minimize the effect of phosphate type (and number of phosphates in the chain) on the C. perfringens spore germination and outgrowth. Thus, we selected as ingredients pyrophosphates that contain only two phosphate groups.

The growth of C. perfringens in different types of pork meat containing different phosphate blends during exponential chilling times of 6.5, 9, 12, 15, 18, and 21 h are shown in Figures 1 through 6, respectively. In all three types of control pork meat, C. perfringens populations increased, but the increase was <1 log CFU/g of meat during the 6.5-h chilling period (Fig. 1), meeting the USDA-FSIS (35) compliance guidelines for cooling of cooked meat and poultry products. The C. perfringens population increase was lower (P ≤ 0.05) in PSE pork (0.09 log CFU/g) than in normal (0.89 log CFU/g) and DFD (0.79 log CFU/g) pork. The C. perfringens population increased by >1 log CFU/g in all three pork meat types during the extended 9-h chilling period, but the increase was smaller in PSE pork (1.15 log CFU/g) than in normal (2.27 log CFU/g) and DFD (2.62 log CFU/g) pork. The same trend of growth continued for all the chilling periods.

Thippareddi et al. (31) reported that chilling of roast beef and injected pork from 54.4 to 7.2°C resulted in C. perfringens population increases of 1.51 and 3.70 log CFU/g, respectively, for the 18-h chill time. The authors

FIGURE 1. Mean Clostridium perfringens populations (log CFU per gram) in pork immediately after heat shock at 75°C for 20 min and before chilling (BEF) and after chilling from 54.4 to 4°C exponentially in 6.5 h (AFT). Normal, normal pork; PSE, pale, soft, and exudative pork; DFD, dark, firm, and dry pork; SAPP¹, sodium acid pyrophosphate from source 1; SAPP², sodium acid pyrophosphate from source 2; TSPP, tetrastomide pyrophosphate. All phosphate blends were added at 0.15% final concentration. Bars with the same letter are not significantly different (P > 0.05).

FIGURE 2. Mean Clostridium perfringens populations (log CFU per gram) in pork immediately after heat shock at 75°C for 20 min and before chilling (BEF) and after chilling from 54.4 to 4°C exponentially in 9 h (AFT). Normal, normal pork; PSE, pale, soft, and exudative pork; DFD, dark, firm, and dry pork; SAPP¹, sodium acid pyrophosphate from source 1; SAPP², sodium acid pyrophosphate from source 2; TSPP, tetrastomide pyrophosphate. All phosphate blends were added at 0.15% final concentration. Bars with the same letter are not significantly different (P > 0.05).
suggested that these differences in the germination and outgrowth of *C. perfringens* could be due to the higher pH of the injected pork (5.62 for beef and 6.11 for pork) or the inherent differences in muscle food species (beef versus pork) (31). However, chilling a turkey roast at a pH of 5.94 for the same period (18 h) resulted in a *C. perfringens* population increase of 4.66 log CFU/g (14). There seem to be inherent differences in the muscle, other than pH, that affect the germination and outgrowth of *C. perfringens* spores in meat systems.

The addition of the SAPP combination (SAPP<sub>1</sub> or SAPP<sub>2</sub>) resulted in a reduction in the *C. perfringens* population in PSE pork by 0.71 log CFU/g but an increase of 0.40 and 0.70 log CFU/g in normal and DFD pork meats, respectively, during the 6.5-h chilling period. The *C. perfringens* population in DFD pork increased by 1.76 log CFU/g within the 9-h chilling period, whereas in normal meat, a similar increase (1.57 log CFU/g) was observed for longer chilling times (15 h; Fig. 4).

The combination of TSPP and SAPP (SAPP<sub>1</sub> or SAPP<sub>2</sub>) failed to control *C. perfringens* growth to <1 log CFU/g in normal and DFD pork meats beyond the 6.5-h chilling period. The *C. perfringens* population increase in the presence of pork meat containing TSPP + SAPP<sub>1</sub> was greater in DFD pork than in normal pork for all the chilling times, and the increase was 5.57 and 6.08 log CFU/g, respectively, for the 21-h chilling period (Fig. 6). The source of SAPP did not affect *C. perfringens* germination and outgrowth, and an increase in population by 5.79 and 6.11 log CFU/g in normal and DFD pork, respectively, was observed in the pork meat containing TSPP + SAPP<sub>2</sub> during the 21-h chilling period. Although *C. perfringens* population increased by >1 log CFU/g during the 9-h chilling period in DFD and normal pork, the presence of

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**FIGURE 3.** Mean *Clostridium perfringens* populations (log CFU per gram) in pork immediately after heat shock at 75°C for 20 min and before chilling (BEF) and after chilling from 54.4 to 4°C exponentially in 12 h (AFT). Normal, normal pork; PSE, pale, soft, and exudative pork; DFD, dark, firm, and dry pork; SAPP<sub>1</sub>, sodium acid pyrophosphate from source 1; SAPP<sub>2</sub>, sodium acid pyrophosphate from source 2; TSPP, tetrasodium pyrophosphate. All phosphate blends were added at 0.15% final concentration. Bars with the same letter are not significantly different (*P* > 0.05).

**FIGURE 4.** Mean *Clostridium perfringens* populations (log CFU per gram) in pork immediately after heat shock at 75°C for 20 min and before chilling (BEF) and after chilling from 54.4 to 4°C exponentially in 15 h (AFT). Normal, normal pork; PSE, pale, soft, and exudative pork; DFD, dark, firm, and dry pork; SAPP<sub>1</sub>, sodium acid pyrophosphate from source 1; SAPP<sub>2</sub>, sodium acid pyrophosphate from source 2; TSPP, tetrasodium pyrophosphate. All phosphate blends were added at 0.15% final concentration. Bars with the same letter are not significantly different (*P* > 0.05).
TSPP+SAPP inhibited *C. perfringens* growth in PSE pork (Fig. 2).

Apart from providing functionality, the added phosphates also may enhance the safety and shelf life of meat and meat products. The incorporation of SAPPs (SAPP₁ + SAPP₂; 0.3%) was more inhibitory to the germination and outgrowth of *C. perfringens* spores in all three pork meat types compared with other phosphate blends containing TSPP. As in control products (PSE, normal, DFD), *C. perfringens* growth in PSE meat was lower than that in other meat types for each of the respective phosphate blends.

Incorporation of the SAPP blend (0.3%) reduced the pH of PSE pork (5.29) by 0.02 units, but the *C. perfringens* population increased by 0.38 log CFU/g for the extremely abusive chilling time of 21 h. In contrast, *C. perfringens* population increased by 4.73 log CFU/g during the same chilling time in PSE pork (pH 5.31) without addition of the phosphate blend. This finding indicates that the inhibitory activity of the phosphates does not rely solely on pH reduction of the medium, and probably a mechanism other than pH reduction also contributes to the antimicrobial activity. Molins et al. (21) reported an increase in the shelf life of fresh ground pork with the addition of SAPP, with minimal reduction in the pH (by 0.05 units). Madril and Sofos (18) reported that the addition of 0.5% SAPP improved the shelf life of comminuted meat products to an extent that exceeded the inhibitory effect of reduced pH on microorganisms, and SAPP was more inhibitory at pH 6.0 than at pH 5.7. The inhibition of *C. perfringens* by SAPP with a minimal reduction in pH of the meat could be attributed to the ability of SAPP to chelate metal ions. The phosphates act as chelators of cations essential for microbial growth (28). Akhtar et al. (1) reported that among all the...
tested polyphosphates, SAPP had the highest inhibitory activity against the food poisoning isolate C. perfringens SM101. The reduced efficacy of TSPP + SAPP and TSPP + SAPP against the germination and outgrowth of C. perfringens could have been the result of increased pH, which would have decreased the chelating efficiency of the added phosphates. Irani and Morgenenthaler (11) found a decrease in the sequestration of metal ions (ferrous and ferric) by polyphosphates with an increase in pH or polyphosphate chain length.

Inhibition of Clostridium botulinum toxin formation by SAPP (0.4%) in frankfurter emulsions was reported, with minimal inhibition of C. botulinum cell growth and total microbial populations (40). Wagner and Busta (41) reported that SAPP inhibited or delayed production of toxin by C. botulinum 52A by binding to the toxin molecule or inactivating the C. botulinum proteases responsible for activation of the proteotoxin molecule by binding to the enzyme (protease), substrate, or cations needed for proteotoxin activation. The latter mechanism was proposed for the phosphate chelation of the cations (Mg$^{2+}$) needed by the enzyme systems such as lipoygenase in sponge cake and proteolytic activity in eggs (29). In the current study, addition of SAPP (0.3%) to the meat resulted in pH decreases of 0.02, 0.11, and 0.14 units for PSE, normal, and DFD pork meat types. Although the pH reduction was minimal (0.02) for PSE meat, addition of SAPP (0.3%) resulted in significant differences in C. perfringens germination and outgrowth for all chill periods, with increasing differences with longer chill times. Below a certain pH minimum, exposure of the heat-activated spores that have progressed beyond germination and/or outgrowth in response to low pH for prolonged periods may result in lysis of the cells.

Although the pH of the PSE meat containing blends of SAPP and TSPP was higher than that of the PSE meat alone, the germination and outgrowth of C. perfringens spores was minimal, with reductions in total populations observed up to chill times of 15 h. A similar trend, with higher C. perfringens populations in untreated PSE meat compared with PSE meat containing SAPP and TSPP mixtures, was observed at 18- and 21-h chill times. This finding indicates that the phosphates rely on mechanisms other than that of reducing pH (probably chelation of metal ions) to inhibit C. perfringens spore germination and outgrowth. However, a similar trend of inhibition of germination and growth of C. perfringens spores was not observed in high pH meat (DFD) with the addition of SAPP and TSPP mixtures at the same concentrations (0.3%). Thus, the mechanism of metal ion chelation might be more prominent in meat with low pH values compared with meat at higher pH values.

Iron is an essential metal for all living systems, and all microorganisms except lactic acid bacteria require iron for growth and proper functioning (5). Iron is required for the vegetative growth of C. perfringens (2 ppm), and sporulation requires even greater concentrations (7 ppm) (16). Iron exists in fresh meat within the porphyrin ring in myoglobin (mostly as heme-iron) or hemoglobin (a minor component) or as free iron (nonheme iron) (17). The concentrations of the heme and nonheme iron in pork meat are 0.26 and 0.17 mg/100 g of meat, respectively, and the nonheme iron concentration increases during cooking (17). The use of ingredients such as citrates and phosphates in meat can effectively bind this iron in the meat (2). This binding or chelation can make iron unavailable for microbial growth.

Allen and Cornforth (2) evaluated several type II (metal chelating) antioxidants, including milk mineral (complex containing calcium phosphate), sodium tripolyphosphate (STPP), calcium phosphate (monobasic), and calcium pyrophosphate and reported that milk mineral had the highest iron-binding capacity followed by STPP, calcium pyrophosphate, and calcium phosphate (monobasic). Allen and Cornforth (3) also reported that STPP at 0.1 and 0.5% concentrations chelated 39.5 and 81.1% of the iron, respectively, in a myoglobin model system with iron supplemented as ferrous chloride.

Assessment of the specific role of a mechanism (pH reduction or chelation of metal ions) is difficult in a complex system such as meat. Research on inhibition of C. perfringens spore germination and outgrowth by different phosphates in a model system at different pH values is in progress in our laboratory to elucidate the role and contribution of each of these antimicrobial mechanisms.

The USDA-FSIS (36) nationwide microbiological baseline data indicate C. perfringens prevalences of 10.4, 8.4, and 2.6% in market hogs, cows and bulls, and steers and heifers, respectively. However, the report does not distinguish between the vegetative cells and the spores, an important consideration when these products are used for preparation of processed meat products. Vegetative cells are destroyed during thermal processing, but spores can survive and may germinate, with subsequent bacterial growth and multiplication, if the products are not chilled properly or if subsequent temperature abuse occurs in food service settings such as large gatherings or restaurants where foods are prepared in bulk and held for long periods.

Taormina et al. (30) reported a higher prevalence of C. perfringens mean total and spore populations of 2.01 and 1.62 log CFU/g, respectively, in raw meat product mixtures. In particular, the prevalence of C. perfringens spores was greater in ground or emulsified products (cured and uncured), at 5.3 and 16.7%, respectively. The mean spore populations in these products were 1.56 and 1.75 log CFU/g, respectively. Although the mean prevalence of C. perfringens (total) was lower in cured or uncured whole muscle products (1.6 and 14.8%, respectively) than in ground products, these lower levels may indicate the sampling constraints. For the whole muscle products, only 25-g samples were taken, highlighting sampling limitations that are especially important because of the nonhomogeneous nature of the distribution of microorganisms in meat and poultry products. However, for the ground or emulsified products, larger portions of the muscle or meat chunks would have been used in preparation of these products in the manufacturing facility, increasing the probability of finding the organism in the meat. Kalinowski et al. (15) reported C. perfringens spore prevalence of 1% (2 of 197 samples) in raw meat blends from commercial USDA-FSIS–inspected
facilities, with spore populations between 0.5 and 2 log CFU/g. None of the samples contained C. perfringens spore populations >2.0 log CFU/g. These differences in the prevalence of C. perfringens total and spore populations could be due to the differences in sources of the products, types of product, or slaughter and processing conditions within these meat processing operations.

Considering the mean C. perfringens spore population of 1.62 log CFU/g (30) and the mass of the total meat used in preparation of the ground and comminuted products, the levels of these spores could have been very high in some of the components used in preparation of these products, and these spores may have come from either the meat or other ingredients. Thus, the risk of C. perfringens spore germination and outgrowth should be considered when preparing RTE meat and poultry products, and the effects of the ingredients (salt, phosphates, and spices), the meat species (beef, pork, or poultry) and the types (PSE, normal, and DFD) can be critical, especially when evaluating the microbial safety of these products that have been subjected to deviations in the cooling process.

Care should be exercised in the manufacture of prepared RTE meat and poultry products, especially during chilling of the products after thermal processing. A prudent approach would be to follow the USDA-FSIS Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization) (37), especially the first option of cooling the cooked product from 54.4 to 26.7°C within 1.5 h and subsequently from 26.7 to 4.4°C in 5 h. This particular option of the cooling guideline was shown to be safe (<1.0 log CFU/g growth of C. perfringens) for all product types, regardless of the product ingredients, meat species and type, and other factors (12, 31, 39). Although the propriety and scientific validity of application of the performance standard of <1.0 log CFU/g growth of C. perfringens during cooling of RTE meat and poultry products is arguable, its application regardless of the other factors seems to provide safe cooked RTE meat and poultry products, as evidenced by the minimal number of C. perfringens–related outbreaks (38) from such products commercially produced in federally inspected meat processing operations. One alternative to such an approach was specified in the compliance guidelines: validating the safety of alternative chilling processes through microbiological challenge studies. Regardless of the approach taken, an understanding of the effects of the ingredients and their concentrations used in the manufacture of RTE meat and poultry products will enable processors to design their products and/or processes to assure microbiological safety of their products.

Germination and outgrowth of C. perfringens spores in pork meat was affected by both the meat type (PSE, normal, and DFD) and phosphate type (SAPP and TSPP) and source. The growth was slowest in PSE pork followed by normal and DFD pork. Among the phosphates, SAPP combinations were more effective in preventing the germination and outgrowth of C. perfringens spores than were other combinations. The phosphates reduce the NaCl content needed, impart the desired functional properties, and contribute to inhibition of C. perfringens spore germination and outgrowth to <1 log CFU/g during extended chilling times of 6.5 to 21 h (if appropriate combination of phosphates are used). Proper care must be taken for the selection of appropriate pork meat for experimental and validation studies because the growth of C. perfringens is largely influenced by the type of pork meat.

REFERENCES


