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# Control of *Clostridium perfringens* Germination and Outgrowth by Buffered Sodium Citrate during Chilling of Roast Beef and Injected Pork†

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## ABSTRACT

Inhibition of the germination and outgrowth of *Clostridium perfringens* by buffered sodium citrate (Ional) and buffered sodium citrate supplemented with sodium diacetate (Ional Plus) during the abusive chilling of roast beef and injected pork was evaluated. Beef top rounds or pork loins were injected with a brine containing NaCl, potato starch, and potassium tetrapyrophosphate to yield final in-product concentrations of 0.85, 0.25, and 0.20%, respectively. Products were ground and mixed with Ional or Ional Plus at 0, 0.5, 1.0, and 2.0%. Each product was mixed with a three-strain *C. perfringens* spore cocktail to obtain final spore concentrations of ca. 2.5 $\log_{10}$ spores per g. Chilling of roast beef from 54.4 to 7.2°C resulted in *C. perfringens* population increases of 1.51 and 5.27 $\log_{10}$ CFU/g for 18- and 21-h exponential chill rates, respectively, while chilling of injected pork resulted in increases of 3.70 and 4.41 $\log_{10}$ CFU/g. The incorporation of Ional into the roast beef formulation resulted in *C. perfringens* population reductions of 0.98, 1.87, and 2.47 $\log_{10}$ CFU/g with 0.5, 1.0, and 2.0% Ional, respectively, over 18 h of chilling, while ≥1.0% Ional Plus was required to achieve similar reductions (reductions of 0.91 and 2.07 $\log_{10}$ CFU/g were obtained with 1.0 and 2.0% Ional Plus, respectively). An Ional or Ional Plus concentration of ≥1.0% was required to reduce *C. perfringens* populations in roast beef or injected pork chilled from 54.4 to 7.2°C in 21 h. Cooling times for roast beef or injected pork products after heat processing can be extended to 21 h through the incorporation of ≥1.0% Ional or Ional Plus into the formulation to reduce the potential risk of *C. perfringens* germination and outgrowth.

*Clostridium perfringens* continues to be of concern to the food industry, particularly the retail food service industry, and has been implicated in several large outbreaks *(2, 3, 19, 28, 29)*. The U.S. Centers for Disease Control and Prevention estimate that more than 248,000 cases of foodborne illness due to *C. perfringens* occur annually in the United States *(21)*. *C. perfringens* and its spores are widely distributed in nature and often contaminate raw meat and poultry products. The U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) reported *C. perfringens* prevalence rates of 1.0% for steers and heifers and 2.7% for cows and bulls *(32)*. Furthermore, a wide variety of processed meat products, such as roast beef, turkey, and meat-containing Mexican foods, have been implicated in *C. perfringens* foodborne outbreaks *(6)*.

Although *C. perfringens* vegetative cells do not survive the normal heat-processing schedules employed in the meat industry, the organism's spores can survive. Heat-activated spores can germinate and grow rapidly on products that are improperly chilled. Juneja and Marmer *(15)* reported $D_{58°C}$-values of 1.15 to 1.60 min for 10 strains of *C. perfringens* (vegetative cells) in a model beef gravy system. These values are similar to the *D*-values (decimal reduction times) reported for vegetative foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* (7, 9, 17). Current USDA-FSIS *(32)* lethality standards require a 6.5-$\log_{10}$ reduction of *Salmonella* spp. during the processing of prepared meat products, and a 7.0-$\log_{10}$ reduction of *Salmonella* spp. is required for products containing poultry meat. Thus, thermal processing schedules designed to destroy *Salmonella* spp. should be adequate to control normal incidence levels of *C. perfringens* vegetative cells in processed meat and poultry products. However, if thermal processing deviations (heating or cooling) occur, spores of *C. perfringens,* if present in raw meats or other ingredients, may be heat activated, germinate, and grow to hazardous levels in the final cooked products.

The time-temperature guidelines for the cooling of cooked products specify that the maximum internal temperature should not remain between 54.4 and 26.7°C for >1.5 h or between 26.7 and 4.4°C for >5 h *(30, 33)*. The U.S. Food and Drug Administration (FDA) Division of Retail Food Protection recognized that inadequate cooling was a major food safety problem and established a recommendation that all food be cooled from 60 to 21°C (from 140 to 70°F) in 2 h and from 21 to 5°C (from 70 to 41°F) in 4 h *(35)*. Normally, the cooking or cooling of solid food products results in an exponential increase or decrease in temperature at the core of the product when that product is exposed to a cooking or a cooling medium. Juneja et al. *(16)* reported minimal *C. perfringens* growth (<1.0 $\log_{10}$ CFU/g) from heat-shocked spores in cooked beef during exponential cooling from 54.4 to 7.2°C at rates ranging from 6 to 15 h.

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The USDA-FSIS approved the use of sodium or potassium salts of lactic acid (at up to 4.8% of product weight) and sodium diacetate in meat products as antimicrobial ingredients for the control of *L. monocytogenes* and other pathogens *(34)*. Sodium and potassium salts of organic acids (such as propionic, lactic, pyruvic, acetic, and citric acids) are extensively used in meat and poultry products either as flavor enhancers or to extend the microbiological shelf stability of products. Sodium citrate and citric acid are generally recognized as safe ingredients and have been shown to inhibit the growth of pathogens in meat products. Sodium citrate is used primarily as a flavor enhancer and should be used according to current good manufacturing practices.

Cooling process (stabilization) deviations are common in the meat- and poultry-processing industry *(20)*. Large meat-processing establishments may have access to the technical expertise required to scientifically evaluate the safety of products that are subject to cooling deviations, but small and very small processors whose products are widely distributed throughout the United States do not possess such resources. The incorporation of antimicrobial agents such as buffered sodium citrate (BSC) as secondary inhibitors or barriers would provide an additional measure of safety for meat and poultry products when a cooling deviation occurs, thereby reducing the risk of product loss to the manufacturer and the risk of foodborne illness to the consumer.

The present study was undertaken to evaluate the use of BSC alone and in combination with sodium diacetate to control or inhibit the outgrowth of *C. perfringens* from spores in processed beef and pork products in extended-chill situations.

## MATERIALS AND METHODS

***C. perfringens* cultures and spore production.** *C. perfringens* strains NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13) were used in this study. The origins and sources of the strains and the spore production methods have been reported elsewhere *(13)*. Spore crops were prepared separately for each strain, washed twice, and resuspended in sterile distilled water. These stock spore suspensions were stored at 4°C. A three-strain *C. perfringens* spore cocktail was prepared immediately prior to experimentation by mixing ca. 1 ml of each suspension to obtain approximately equal numbers of spores. This spore mixture (0.75 ml) was then mixed with the beef and pork products (250 g of each product) for 1 min in a KitchenAid mixer (K5SSWH model; KitchenAid, Troy, Ohio), vacuum packaged in a Cryovac L340 bag (water vapor transmission rate: 10 g/m²/24 h at 37.8°C at 100% relative humidity; oxygen transmission rate: 3,000 cm³/m²/24 h at 23°C, 1 atm [101.29 kPa]; Model C-500, Multivac Inc., Kansas City, Mo.), and frozen until it was used.

**Preparation of meat and inoculation.** Beef top rounds and boneless pork loins were obtained from a retail store in Athens, Ga., and injected with minimal final levels of salt (0.85% NaCl), potato starch (0.25%), and potassium tetrapyrophosphate (0.2%) at a 12% pump rate (amount of marinate incorporated into the meat, wt/wt). The products were vacuum packaged separately and shipped overnight with ice packs to the USDA-ARS laboratories in Wyndmoor, Pa., and were stored refrigerated until they were used. The products were diced into ~14.2-mm³ pieces and ground through a 3.17-mm plate (Model 4822, Hobart, Troy, Ohio) to aid in the uniform distribution of the antimicrobial ingredients and the *C. perfringens* spores in the product during the subsequent inoculation and mixing steps. Separate packages containing 250 g of meat were prepared, vacuum packaged, and stored frozen. The products were thawed, mixed with BSC (Ional) or BSC supplemented with 8.0% sodium diacetate (Ional Plus, WTI Inc., Kingston, N.Y.) for 1 min in a mixer (KitchenAid), and subsequently mixed with the spore cocktail for 1 min to yield ca. 2.5 $\log_{10}$ spores per g. The product (10 g of roast beef or injected pork) was distributed into cook-in bags (5 by 7.6 cm; 0.8 mil nylon per 2.4 mil polyethylene; $O_2$ transmission rate: 52 cm³/m²/24 h at 23°C dry; water vapor transmission rate: 6.2 g/m²/24 h at 37.8°C at 90% relative humidity; Koch Supply Company, Kansas City, Mo.), and vacuum sealed at 12 mbar (1.2 kPa) vacuum with a Multivac Model A300/16 packaging machine.

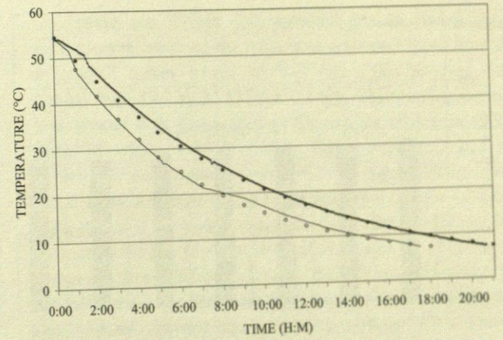


FIGURE 1. *Representative temperature profiles of meat products (roast beef and injected pork) chilled from 54.4 to 7.2°C in 18 h (○) and 21 h (●). Continuous lines indicate actual temperatures observed during chilling, and symbols represent the programmed temperature profile.*

**Treatments.** Seven treatments (BSC [Ional] at 0.5, 1.0, and 2.0%; BSC with sodium diacetate [Ional Plus] at 0.5, 1.0, and 2.0%; and no treatment [control]) were evaluated for each meat product (roast beef and injected pork).

**Heat shock and cooling procedures.** Prior to cooking, two sets of bags (beef and pork) were sandwiched in a stainless steel mesh (racks, 5 by 7.6 cm; Fisher Scientific, St. Louis, Mo.) to improve heat transfer and to uniformly heat and chill the product. The set of racks was submerged completely in a water bath set at 75.5°C (Exacal, Model RTE-221, NESLAB Instruments, Inc., Newington, N.H.), heat shocked for 20 min, removed, chilled immediately in an ice water bath, and plated as described below. A second set of racks containing the product for each treatment was heat shocked as described, transferred to a water bath set at 54.5°C, allowed to equilibrate at this temperature for 10 min, and chilled from 54.5 to 7.2°C at an 18- or 21-h exponential rate (Fig. 1).

**Enumeration procedure.** After chilling to 7.2°C, each packaged meat sample was transferred aseptically to a filter stomacher

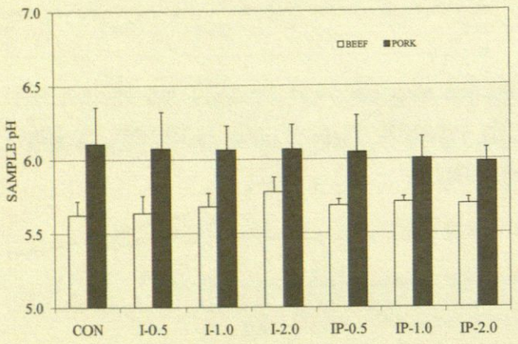


FIGURE 2. *Mean pH values for roast beef and injected pork samples with no added antimicrobial ingredient (CON), with Ional (I) added at 0.5, 1.0, and 2.0%, and with Ional Plus (IP) added at 0.5, 1.0, and 2.0%.*

bag (Spiral Biotech, Bethesda, Md.). Sterile peptone water (0.1%, 20 ml) was added and stomached for 2 min (Interscience, St. Nom, France). The samples were serially diluted in peptone water, plated on tryptose sulfite cycloserine (TSC; Difco Laboratories, Detroit, Mich.) agar by pour or spiral plating, and overlaid with an additional 10 ml of TSC. The TSC plates were then incubated at 37°C for 18 to 24 h in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, Oreg.), and typical *C. perfringens* colonies were enumerated.

**Statistical analyses.** Three independent trials were performed for each of the exponential chilling rates (18 and 21 h). Data were analyzed by analysis of variance with the use of the general linear model procedure of the Statistical Analysis System (Release 8.01, SAS Institute, Inc., Cary, N.C.). Fisher's least significant difference was used to separate means of the residual *C. perfringens* populations ($\log_{10}$ CFU/g) for the samples.

## RESULTS AND DISCUSSION

The programmed and observed product temperature profiles for 18- and 21-h exponential chill rates are shown in Figure 1. Both the 18- and the 21-h temperature profiles represent extended chilling rates relative to the USDA-FSIS

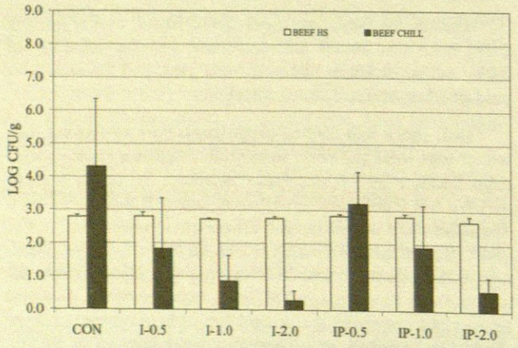


FIGURE 3. *Mean levels (log CFU/g) of* C. perfringens *in roast beef immediately after heat shock at 75°C for 20 min (□) and after cooling from 54.4 to 7.2°C exponentially in 18 h (■). I, Ional; IP, Ional Plus; Con, control. I and IP were added at concentrations of 0.5, 1.0, and 2.0%.*

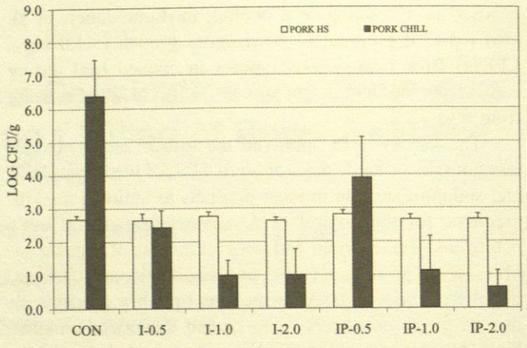


FIGURE 4. *Mean levels (log CFU/g) of* C. perfringens *in injected pork immediately after heat shock at 75°C for 20 min (□) and after cooling from 54.4 to 7.2°C exponentially in 18 h (■). I, Ional; IP, Ional Plus; Con, control. I and IP were added at concentrations of 0.5, 1.0, and 2.0%.*

or the FDA stabilization requirements for the chilling of cooked meat and poultry products. The pH values for roast beef and injected pork, shown in Figure 2, correspond to normal pH values reported in the literature for these products *(8)*.

Chilling of control roast beef samples from 54.4 to 7.2°C resulted in *C. perfringens* population increases of 1.51 and 5.27 $\log_{10}$ CFU/g for the 18- and 21-h exponential chill rates, respectively (Figs. 3 and 5). Chilling of control samples of injected pork at similar chill rates resulted in *C. perfringens* population increases of 3.70 and 4.41 $\log_{10}$ CFU/g (Figs. 4 and 6). Higher *C. perfringens* levels were observed for injected pork than for roast beef with the 18-h chill rate. These differences in the germination and outgrowth of *C. perfringens* could be due to the higher pH of the injected pork ($P \leq 0.05$) (Fig. 2) or the inherent differences in muscle food species (beef versus pork).

The pH values for roast beef for each of the treatments (5.62 to 5.78) were lower ($P \leq 0.05$) than those for injected pork (5.98 to 6.11). The addition of Ional tended to slightly

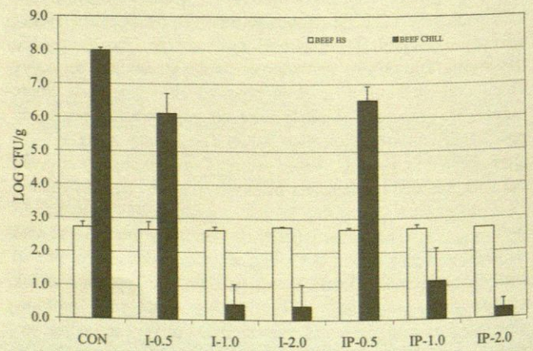


FIGURE 5. *Mean levels (log CFU/g) of* C. perfringens *in roast beef immediately after heat shock at 75°C for 20 min (□) and after cooling from 54.4 to 7.2°C exponentially in 21 h (■). I, Ional; IP, Ional Plus; Con, control. I and IP were added at concentrations of 0.5, 1.0, and 2.0%.*



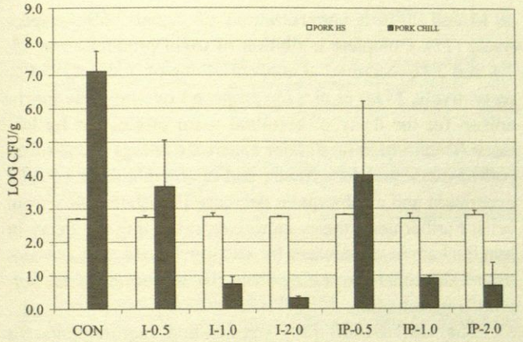


FIGURE 6. *Mean levels (log CFU/g) of* C. perfringens *in injected pork immediately after heat shock at 75°C for 20 min (□) and after cooling from 54.4 to 7.2°C exponentially in 21 h (■). I, Ional; IP, Ional Plus; Con, control. I and IP were added at concentrations of 0.5, 1.0, and 2.0%.*

increase the pH of roast beef from 5.62 to 5.78, while it tended to slightly reduce the pH of injected pork from 6.11 to 6.07. Although such differences appear minor when pH units are considered (with greater differences in $H^+$ concentrations), they may be biologically significant with regard to the control of *C. perfringens* germination and growth. The addition of Ional Plus did not result in pH changes ($P > 0.05$) for either of the products.

Juneja et al. *(16)* reported germination and outgrowth of *C. perfringens* from 1.5 to 6.0 $\log_{10}$ CFU/g during exponential cooling of autoclaved ground beef from 54.4 to 7.2°C in 18 h. However, in the present study, the growth observed was slower, resulting in increases of 1.51 log units for roast beef with the 18-h exponential cooling rate. This minimal growth of *C. perfringens* in the roast beef system was probably due to differences in the meat substrate. While Juneja et al. *(16)* employed autoclaved ground beef without added ingredients such as NaCl and phosphates, which are normally used in processed meat products including roast beef, we used a product containing minimal levels of these ingredients to establish conservative estimates of the growth of *C. perfringens* from spores. Furthermore, in the present study, we inoculated non–heat-shocked spores and heat treated the product at 75°C for 20 min with the dual purpose of heat activating the spores in the meat substrate and simulating the cooking of meat products. Traditionally, many processed meat products are not cooked to this target temperature. We used 75°C for 20 min as a worst-case scenario that would result in heat activation of the spores and subsequent growth during abusive cooling from 54.4 to 7.2°C.

Heat-activated spores in the injected beef system resulted in larger *C. perfringens* populations, with initial populations of ca. 2.75 $\log_{10}$ CFU/g increasing by 5.27 $\log_{10}$ CFU/g during 21 h exponential chilling. Thus, caution should be exercised in extending the results of this and other studies to different meat systems, such as poultry, or to situations in which the pH of the meat in question differs considerably from the meat systems evaluated herein.

Steele and Wright *(27)* reported growth (>1.0 $\log_{10}$ CFU/g) of *C. perfringens* from heat-activated spore inocula in cooked turkey breast product when this product was cooled from 48.9 to 12.8°C for >8.9 h. This cooling period was shorter than those used in the studies of Juneja et al. *(16)*, in which longer cooling times were required to achieve *C. perfringens* growth of >1.0 $\log_{10}$ CFU/g from spore inocula. These authors attributed the shorter cooling rates required for turkey breast to differences in the model systems used by Snyder *(26)* and Juneja et al. *(16)*. Nevertheless, these differences could be due to differences in the pH values for the meat systems evaluated: autoclaved ground beef *(16, 26)* and turkey breast roasts *(27)*. Steele and Wright *(27)* stated that cooling periods determined from model systems may not be appropriate for other food systems that may behave differently with regard to heat transfer because of variations in the product. In our studies, we evaluated the germination and outgrowth of *C. perfringens* spores that had been heat activated in roast beef or injected pork during simulated cooking processes and controlled chilling at exponential rates as described by Juneja and Marks *(14)* and Juneja et al. *(16)*. Furthermore, results from our studies indicate that the chilling rates obtained with model systems such as autoclaved roast beef may not be applicable to other products with different compositions (moisture, NaCl, phosphates, starch, etc.) and different intrinsic characteristics such as pH. Moreover, roast beef and injected pork were ground in the present study, resulting in the uniform distribution of the antimicrobial agents. Results obtained for ground meat systems will be conservative estimates of the antimicrobial activity of the compounds, since these compounds are generally concentrated on the surface (purge and "leak-out" of injected marinade) and in injection needle channels in nonintact injected whole-muscle meat products where microbial contamination is expected.

A review of the literature provided no data on the effect of chemical antimicrobial agents on *C. perfringens* outgrowth during the chilling of heat-processed meat and poultry products. Aran *(1)* reported on the use of sodium and potassium lactates for the inhibition of *C. perfringens* growth in processed beef goulash under isothermal storage conditions. *C. perfringens* growth was observed in goulash with 1.5% sodium lactate at all three temperatures evaluated (15, 20, and 25°C). The use of calcium lactate at either 1.5 or 3.0% prevented the outgrowth of *C. perfringens* even after 28 days of storage at 25°C. Aran *(1)* concluded that calcium lactate was more inhibitory to *C. perfringens* germination and outgrowth in sous vide beef goulash than was sodium lactate. Aran attributed this improved antimicrobial activity partly to the ability of calcium lactate to lower the pH from an initial value of 6.0 to values of 5.0 and 5.5 at 1.5 and 3.0% concentrations, respectively.

Similar antimicrobial effects of sodium lactate (1.5%) and sodium diacetate (0.25%) on nonpathogenic, nonproteolytic, psychrotrophic *Clostridium* species isolated from a spoiled (pink discoloration and off-odor) cook-in-the-bag refrigerated turkey breast meat product have been reported *(23)*. Synergistic antimicrobial activity was observed when

the two antimicrobial agents were used in combination for a cook-in-bag refrigerated turkey breast product, with an extension of shelf life and the inhibition of off-odor development beyond 22 weeks of refrigerated storage, while the control product spoiled within 7 weeks.

The addition of Ional at 0.5% and the subsequent chilling of ground roast beef and injected pork resulted in *C. perfringens* population reductions of 0.98 and 0.21 $\log_{10}$ CFU/g, respectively, for the 18-h exponential chill rate. Extension of the chill rate to 21 h resulted in *C. perfringens* population increases of 3.46 and 0.92 $\log_{10}$ CFU/g in roast beef and injected pork, respectively. The addition of Ional Plus at 0.5% resulted in *C. perfringens* population increases of 0.37 and 1.10 $\log_{10}$ CFU/g in roast beef and injected pork, respectively, with an 18-h exponential chill rate. Increases in *C. perfringens* populations were larger ($P \leq 0.05$) for the 21-h chill rate (3.84 and 1.19 $\log_{10}$ CFU/g for roast beef and injected pork, respectively).

The incorporation of Ional and Ional Plus into the meat formulation at ≥1.0% resulted in decreases ($P \leq 0.05$) in *C. perfringens* populations in both ground roast beef and injected pork products. These reductions were larger when a 21-h chill rate was used, indicating that the antimicrobial activity of the sodium citrate was dependent on temperature, with larger reductions being observed with longer exposures to higher temperatures. Although *C. perfringens* populations increased by <1.0 $\log_{10}$ CFU/g for both roast beef and injected pork when Ional Plus was incorporated into the formulation with the 18-h chill rate, *C. perfringens* grew by >1.0 $\log_{10}$ CFU/g when the chill rate was extended to 21 h. Thus, it is necessary to use concentrations of ≥1.0% for roast beef or injected pork with chilling rates of >18 h.

Miller et al. *(24)* found that citrate was more effective in delaying botulinal toxin production than were propionate, acetate, and lactate on a molar basis in uncured turkey breast. These authors reported that the inhibition of botulinal toxin production by monocarboxylic (pyruvic, lactic, acetic, and propionic) acid esters in uncured turkey was proportional to $pK_a$. However, citrate did not follow this pattern, showing greater inhibitory activity on a molar basis. The antibotulinal mechanism of citrate was attributed to the chelation of metals and the subsequent deprivation of minerals needed for germination and growth *(10–12, 25)*. The inhibitory action of organic acid esters has been attributed to the lowering of the intracellular pH within the microbial cells and to alterations in cell membrane permeability that affect substrate transport and the inhibition of electron transport systems necessary for energy regeneration *(4)*. Similar mechanisms may be responsible for the inhibition of the outgrowth of *C. perfringens* on meat products with the abusive chilling rates used in this study.

Houtsma et al. *(11)* reported the inhibition of proteolytic *C. botulinum* growth and toxin production by sodium lactate in a peptone–yeast extract medium (pH 6.1). However, this inhibitory effect gradually decreased as incubation temperatures were increased *(11, 22)*. Furthermore, sodium lactate alone or in combination with NaCl was shown to delay toxin production by proteolytic *C. botulinum* strains at 15 and 20°C at concentrations of 2 and 2.5%, respectively *(11)*. Complete inhibition of toxin production at 15, 20, and 30°C occurred at concentrations of 3, 4, and >4%, respectively. Maas et al. *(18)* proposed two possible mechanisms for the delay of botulinal toxin production by lactates: (i) inhibition of a major anaerobic energy metabolism pathway essential for growth, and/or (ii) inhibition of ATP generation and a subsequent decrease in lactate efflux from cells. Furthermore, these authors reported that the delay in botulinal toxin production by sodium lactate was concentration dependent, resulting from the inhibition of *C. botulinum* germination.

The USDA-FSIS *(33)* stabilization requirements for processed meat and poultry products were established for the prevention of the germination and outgrowth of spore-forming bacteria that survive the normal heating regimes employed in the meat-processing industry. Furthermore, the USDA-FSIS has stated that *C. perfringens* can be used alone in an inoculated pack (challenge) study to demonstrate that the cooling performance standard is met for both *C. perfringens* and *Clostridium botulinum,* since the time-temperature conditions that would limit the growth of *C. perfringens* to ≤1 $\log_{10}$ would also prevent the multiplication of *C. botulinum,* which is much slower. It is evident from the literature that organic acid esters such as sodium or potassium salts of lactic, acetic, and citric acids inhibit the germination and/or outgrowth and botulinum toxin production of *C. perfringens* as well as *C. botulinum.*

The USDA-FSIS stabilization guidelines for the cooling of cooked meat products (6.5 h) can be extended by 14.5 h for roast beef and injected pork through the incorporation of either BSC (Ional) or BSC in combination with sodium diacetate (Ional Plus at >1.0%) into the formulation. Presently, a maximum BSC (Ional) level of 1.3% is approved for meat and poultry products for flavor retention and microbiological control *(31)*. The incorporation of these antimicrobial agents into product formulations can be advantageous to meat processors in situations in which a product may not be cooled within the USDA-FSIS guidelines and can provide additional safety for meat products through the prevention of the potential germination and outgrowth of *C. perfringens* when cooling process deviations occur. Caution should be exercised in extrapolating data obtained with model systems to other food systems, since *C. perfringens* germination and outgrowth may reach potentially hazardous levels when spores are present in raw meat ingredients.

## ACKNOWLEDGMENTS

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