Inhibition of the germination and outgrowth of Clostridium perfringens by buffered sodium citrate (Ional) and buffered sodium citrate supplemented with sodium diacate (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 monopersulfate to yield final brine concentrations of 0.85, 0.25, and 0.2%, respectively. Products were ground and mixed with Ional or Ional Plus at 0.5, 1.0, 2.0%, and 2.5% (w/w). Each product was mixed with a three-strain C. perfringens spore cocktail to obtain final spore concentrations of ca. 2.5 log CFU 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 CFU/g for 18- and 24-h exponential chill rates, respectively, while chilling of injected pork resulted in increases of 3.70 and 4.41 log 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 CFU/g with 0.5, 1.0, and 2.0% Ional, respectively. For chilled, slowly-cooled beef only ≥1.0% Ional Plus was required to achieve similar reductions (reductions of 0.91 and 2.07 log 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 beef to 7.2°C or injected pork products after heat processing can be used to achieve ≥1.0% Ional or Ional Plus in 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, 10, 26, 28). 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 contaminates raw meat and poultry products. The U.S. Department of Agriculture—Food Safety and Inspection Service (USDA-FSIS) reported C. perfringens foodborne outbreaks of 1.0% for steers and heifers and 2.7% for cows and bulls (22). Furthermore, clostridia are present in raw meats or other ingredients, may be heat activated, germinate, and grow to hazardous levels in the final cooked products.

Clostridium perfringens as a potential pathogen is a concern to the food industry. The origin of the organism’s spores can survive. Meat-activated spores can germinate and grow in the meat-containing tissues of the organism. Theories that spores can be inactivated by heat activates spores in the meat product. The time-temperature guidelines for the cooling of cooked products confirm that the maximum internal temperature is not reached between 54.4 and 27.6°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 30 to 4°C (from 86 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 cooling medium. Junge et al. (16) reported minimal C. perfringens growth (<1.0 log CFU/g) from broiled beef strips in cooked beef during exponential cooling from 54.4 to 7.2°C at rates ranging from 6 to 15 h.

The USDA-FSIS approved the use of sodium or potassium salts of citric acid (up to 4.8% of product weight) and sodium diacate in meat products as antimicrobial ingredients for the control of L. monocytogenes and other pathogens (34). Up to 4.8% 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 shelf stability of perishable 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 a secondary inhibition method provides 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 diacate to control or inhibit C. perfringens vegetative cells in processed meat and poultry products.

MATERIALS AND METHODS

C. perfringens cultures and spore production. C. perfringens strains NCTC 8288 (Robbs strain), and NCTC 10430 (Robbs variant) were used in this study. The origins and sources of the strains and the production methods have been described elsewhere (11). Spore suspensions of C. perfringens vegetative cells in processed meat and poultry products. The time-temperature guidelines for the cooling of cooked products confirm that the maximum internal temperature is not reached between 54.4 and 27.6°C for >1.5 h or between 26.7 and 4.4°C for >5 h (30, 33).

In this study, the results of several experiments were analyzed to determine the effect of sodium citrate on C. perfringens spore growth. The results of several experiments were analyzed to determine the effect of sodium citrate on C. perfringens spore growth. The results of several experiments were analyzed to determine the effect of sodium citrate on C. perfringens spore growth. The results of several experiments were analyzed to determine the effect of sodium citrate on C. perfringens spore growth.
FIGURE 2. Mean pH values for roast beef and injected pork samples with no added antimicrobial ingredient (CON), with lonal (I) added at 0.5, 1.0, and 2.0%, and with lonal plus IP (IP) added at 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 (C) and after cooling from 54.4 to 7.2°C exponentially in 18 h (I). Lonal: IP; lonal plus; control. 1 and IP were added at concentrations of 0.5, 1.0, and 2.0%.

FIGURE 5. Mean levels (log CFU/g) of C. perfringens in roast beef immediately after heat shock at 75°C for 20 min (C) and after cooling from 54.4 to 7.2°C exponentially in 18 h (I). Lonal: IP; lonal plus; control. 1 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 (C) and after cooling from 54.4 to 7.2°C exponentially in 21 h (I). Lonal: IP; lonal plus; control. 1 and IP were added at concentrations of 0.5, 1.0, and 2.0%.

FIGURE 3. Mean levels (log CFU/g) of C. perfringens in roast beef immediately after heat shock at 75°C for 20 min (C) and after cooling from 54.4 to 7.2°C exponentially in 18 h (I). Lonal: IP; lonal plus; control. 1 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 (C) and after cooling from 54.4 to 7.2°C exponentially in 21 h (I). Lonal: IP; lonal plus; control. 1 and IP were added at concentrations of 0.5, 1.0, and 2.0%.

The programmed and observed product temperature profiles for 18- and 21-h exponential chill rates are shown in Figure 1. The 18- and 21-h temperature profiles represent extended chilling rates relative to the USDA-FSIS bag (Spiral Tech Corp., Bethesda, Md.). Steerle petrane water (0.1%, 20 ml) was added and stomached for 2 min (InterScience, St Nom, France). The samples were serially diluted in petrane water, plated on tryptone sulfite cycloserine (TSC, Difco Laboratories, Daitom, 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, St. Louis, Mo.), and typical C. perfringens colonies were enumerated.

Statistical analysis. 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 (SAS Institute, Cary, NC). The mean CFU/g for each treatment that did not differ significantly was used to separate means of the residual C. perfringens populations (log 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. The 18- and 21-h temperature profiles represent extended chilling rates relative to the USDA-FSIS 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 Jungea and Marks (14) and Jungea 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, 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 in the surface purge and "leakage" loss, and in injection needle channels in noninjected 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. Arun (1) reported that 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 15 or 30% prevented the outgrowth of C. perfringens even after 28 days of storage at 25°C. Arun (1) concluded that calcium lactate was more inhibitory to C. perfringens germination and outgrowth in sous vide beef goulash than sodium lactate. Arun attributed this improved antimicrobial activity partly to the ability of sodium lactate to lower the pH from an initial value of 6.5 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 (0.2%) on anaerobic, pathogenic, nonproteolytic, psychrotrophic, Clostridium species isolated from fresh, cooked chicken, and turkey meat. The incubated (pink discoloration and odor) cook-in-the-bag turkey meat did not contain refrigerated turkey breast meat product have been detected. 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 linalol at 0.5% and the subsequent chilling of ground roast beef and injected pork resulted in C. perfringens populations of reductions of 0.09 and -0.21 log CFU/g, respectively, for the 18-hour exponential chill rate. Extensive of the chill rate to 21 h resulted in C. perfringens population increases of 3.46 and 0.92 log CFU/g in roast beef and injected pork, respectively, with an 18-hour exponential chill rate. In contrast, 21 h of C. perfringens populations were larger (P < 0.05) for the 21-h chill rate (3.84 and 1.19 log CFU/g) for roast beef and injected pork, respectively.

The incorporation of linalol and linalool Plus into the meat formulation at ≥2.0% resulted in decreases (P < 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 ≥10 log CFU/g for both roast beef and injected pork, linalol Plus was incorporated into the formulation with the 18-h chill rate. C. perfringens grew by ≥10 log CFU/g when the chill rate was extended to 21 h. Thus, it is necessary to use concentrations of ≥2.0% for roast beef or injected pork with chill rates ≥18 h.

Miller et al. (24) found that citrate was more effective in delaying butylic toxin production than were propionic, lacticric, and lactic acids in uncured turkey breast. These authors reported that the inhibition of butyli
toxin production by monocarborylic (pyrlic, lactic, acetic, and propionic) acid esters in uncured turkey was proportional to the rate of 2-hydroxy acid follow this pattern, showing greater inhibitory activity on a molar basis. The antimicrobial 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 microbials 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 above-mentioned acidity.

Houtsm et al. (11) reported the inhibition of proteo
cytic C. botulinum growth and toxin production by sodium lactate in a peanut-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 delayed toxin production by proteolytic C. botulinum strains

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