Inhibition of Germination and Outgrowth of *Clostridium perfringens* Spores by Lactic Acid Salts during Cooling of Injected Turkey†

PADMANABHA REDDY VELUGOTI,† LALIT K. BOHRA,† VIJAY K. JUNEJA,‡ AND HARSHAVARDHAN THIPPAREDDI††

1Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68583; and 2Food Safety Intervention Technologies Research Unit, Eastern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 06-415: Received 1 August 2006/Accepted 31 October 2006

ABSTRACT

Inhibition of *Clostridium perfringens* spore germination and outgrowth by lactic acid salts (calcium, potassium, and sodium) during exponential cooling of injected turkey product was evaluated. Injected turkey samples containing calcium lactate, potassium lactate, or sodium lactate (1.0, 2.0, 3.0, or 4.8% [w/w]), along with a control (product without lactate), were inoculated with a three-strain cocktail of *C. perfringens* spores to achieve a final spore population of 2.5 to 3.0 log CFU/g. The inoculated product was heat treated and exponentially cooled from 54.5 to 72°C within 21, 15, 12, 9, or 6.5 h. Cooling of injected turkey (containing no antimicrobials) resulted in *C. perfringens* germination and an outgrowth of 0.5, 2.4, 3.4, 5.1, 5.8, and 5.8 log CFU/g when exponentially cooled from 54.4 to 7.2°C in 6.5, 12, 15, 18, and 21 h, respectively. The incorporation of antimicrobials (lactates), regardless of the type (Ca, Na, or K salts), inhibited the germination and outgrowth of *C. perfringens* spores at all the concentrations evaluated (1.0, 2.0, 3.0, and 4.8%) compared to the injected turkey without acetate (control). Increasing the concentrations of the antimicrobials resulted in a greater inhibition of the spore germination and outgrowth in the products. In general, calcium lactate was more effective in inhibiting the germination and outgrowth of *C. perfringens* spores at ≥1.0% concentration than were sodium and potassium lactates. Incorporation of these antimicrobials in cooked, ready-to-eat turkey products can provide additional protection in controlling the germination and outgrowth of *C. perfringens* spores during cooling (stabilization).

Proper cooling of meat and poultry products after cooking is an important process parameter for the ensuring the safety of meat and poultry products, as it can allow the germination and outgrowth of spore-forming pathogens such as *Clostridium perfringens*. The genus *Clostridium* is composed of gram-positive bacteria that are ubiquitous in the environment and in the intestines of humans and animals (19). These organisms typically persist in food-processing environments as heat-resistant spores. When products containing meat and poultry as ingredients are cooked, the thermal process can activate spores of this organism, and if the products are not cooled rapidly, it can lead to the outgrowth and multiplication of the germinated spores (2).

Food poisoning due to *C. perfringens* is one of the more commonly reported foodborne illnesses that is caused by the ingestion of high levels of cells (ca. 8 log CFU) (18). Symptoms of *Clostridium* poisoning include diarrhea and severe abdominal pain, while nausea is less common. Symptoms typically occur within 8 to 22 h of consumption of toxin-producing strains, and illness usually lasts 24 h after the onset of symptoms. The U.S. Centers for Disease Control and Prevention estimates that 248,000 cases occur in the United States annually due to *C. perfringens* (8), and the majority of them are foodborne.

Because of the potentially high contamination levels (ca. 4 log CFU), the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has established a performance standard specifying that the cooling process should not allow more than a 1-log increase of *C. perfringens* in cooked meat and poultry products (16). Compliance guidelines for stabilization indicate that cooked, ready-to-eat (RTE) meat and poultry products should preferably be cooled from 54.4 to 26.7°C in less than 1.5 h and from 26.7 to 4.4°C in less than 5 h.

The USDA-FSIS has approved the use sodium and potassium lactates as part of the formulation (up to 4.8% of product weight) (17) to control the growth of *Listeria monocytogenes* in RTE meat and poultry products. The majority of the RTE meat and poultry processors in the United States incorporate these antimicrobials in combination with sodium diacetate to improve the efficacy of the lactates. The antimicrobial effects of organic acids are primarily because of the lowering of the internal pH of the cell and the inhibition of metabolic activity by the undissociated molecules of these acids (11). These antimicrobials can act as a secondary layer of safety for products when there is a cool-

† Mention of trade names or commercial products in this publication is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

‡ Author for correspondence. Tel: 402-472-3403; Fax: 402-472-1693; E-mail: hthippared2@unl.edu.
ing process deviation (15). They are generally recognized as safe and have been used as flavor enhancers, antimicrobials, or both in the meat industry.

The objective of this study was to evaluate the control of C. perfringens spor germination and outgrowth by sodium, potassium, and calcium lactates at different concentrations (1, 2, 3, and 4.8%) in injected turkey during exponential cooling of the product from 54.5 to 7.2°C in 6.5, 9, 12, 15, 18, and 21 h.

MATERIALS AND METHODS

Bacterial cultures. A three-strain cocktail of enterotoxin-producing C. perfringens strains (NCTC 8238; Hobbs serotype 2, NCTC 8239; Hobbs serotype 3, and ATCC 10388; Hobbs serotype 13) was used in the study. Cultures were maintained at 4°C in tubes (10 ml) containing cooked meat medium until ready for use in the study. The origin and sources of these cultures have been described elsewhere (7). C. perfringens inocula were activated by transferring a loopful of the culture into freshly prepared fluid thioglycolate medium and incubated at 35°C for 24 h aerobically under stationary conditions. All media were obtained from Difco, Becton Dickinson (Sparks, Md.) unless specified otherwise.

Preparation of the spore cocktail. A 0.1-ml aliquot of stock culture was inoculated into 10 ml of freshly prepared fluid thioglycolate medium. Inoculated media were heat shocked for 20 min at 75°C in a water bath (Isotemp 3013H, Fisher Scientific, Pittsburgh, Pa.), cooled in chilled water, and incubated for 18 h at 37°C. A 1.0-ml portion of this culture was transferred to 10 ml of freshly steamed fluid thioglycolate medium and then incubated for 4 h at 37°C. The activation procedure was repeated two times. Contents of fluid thioglycolate medium were transferred to 100 ml of modified Duncan-Strong medium and incubated for 24 h at 37°C aerobically under stationary conditions. The original Duncan-Strong formulation was modified by replacing starch with 0.4% raffinose (Sigma, St. Louis, Mo.) and supplemented with caffeine (Sigma) at 100 mg/liter to enhance sporulation. The cultures of each strain were harvested by centrifugation at 7,012 × g for 20 min at 4°C (J2-HS, Beckman Instruments, Fullerton, Calif.) and washed twice with 50 ml of sterile distilled water. The spore crop of each strain was stored separately at 4°C for up to 6 months until used. A spore cocktail containing all three strains of C. perfringens was prepared immediately before experiments by mixing equivalent numbers of spores from each suspension.

Product. Injected turkey breast meat containing 0.85% salt, 0.25% potato starch, and 0.2% potassium tetrapyrophosphate was obtained from a local processor. The average moisture, protein, fat, carbohydrate, and ash composition of the product was 76.2, 20.7, 0.7, 0.09, and 2.30%, respectively, for the product without antimicrobials. Three separate samples from each replication were obtained and analyzed in triplicate for proximate composition. Injected turkey with antimicrobial agents was prepared by mixing injected turkey breast meat with the appropriate type and level of antimicrobial to achieve products containing 1.0, 2.0, 3.0, and 4.8% calcium lactate, potassium lactate, or sodium lactate on a weight/weight basis, considering the moisture content in each of the antimicrobials. Calcium, potassium, and sodium lactates were obtained from Purac (Lincolnshire, Ill.).

For the preparation of equinormal concentrations of the three lactate types in the meat, quantities of each lactate were added to attain a 0.1 N final concentration (1.87, 2.13, and 1.09 g of sodium lactate, potassium lactate, and calcium lactate, respectively, weight/weight basis) of each lactate, considering the moisture con-
tent of various lactates as described above. We did not consider the concentration of the lactates in the water phase of the meat product (76.2% moisture), as the objective of the experiment was to attain the same concentrations of the lactate ion in each of the three types. The meat product was mixed, prepared, and packaged as described earlier.

Sample preparation. Five-gram portions of treated meat samples were weighed in a vacuum pouch measuring 6.35 by 12.7 cm (3-mil standard barrier nylon vacuum pouch with a water vapor transmission rate of 10 g/m²/24 h at 37.8°C and 100% relative humidity and an oxygen transmission rate of 3,000 cm³/m²/24 h at 23°C and 1 atm [101.29 kPa]; Prime Source, Kansas City, Mo.). Each subsample was inoculated with 0.1 ml of the three-strain C. perfringens spore cocktail to yield a final spore population of ca. 2.5 to 3.0 log CFU/g of meat. Inoculated samples were vacuum sealed (A300/H, Multivac, Wolfskrecht, Germany), massaged manually for 30 s to distribute the inoculum homogeneously, and flattened to a uniform thickness of approximately 0.2 mm prior to use in the study.

Spore activation and cooling profiles. Spores were activated by submerged inoculated bags for 20 min in a water bath (Isotemp 3013H) set at 75°C. Heat-shocked samples were then transferred to a refrigerated bath (submerged coil apparatus), with water circulation capabilities (RTE 740, Thermo Neslab, Portsmouth, N.H.), that was programmed to cool from 60 to 54.5°C in 5 min and subsequently to 7.2°C within 6.5, 9, 12, 15, 18, or 21 h. The product was cooled further to 4.4°C within 30 min and maintained in the water bath at this temperature to simulate industry practices of chilling the product. To develop the cooling schedules, core temperature profiles of products were obtained from an RTE meat manufacturer. Commercial profiles and stabilization requirements from the USDA-FSIS were used to generate cooling curves to cool the product from 54.5 to 7.2°C with models developed by Amézquita et al. (1) and Nolan (9). Cooling profiles were programmed into the water baths by a commercial software package (NesCom Software, Portsmith, N.H.). Temperature changes in the water bath and the meat samples were simultaneously registered by the water bath thermometer and external data loggers (MKIII model, Temprecord, Modesto, Calif.) and were observed to follow the programmed temperature profiles. A set of two bags containing the inoculated meat for each treatment was subjected to heat shock and submerged in the programmable water bath; the cooling cycles were run individually. For each exponential cooling period, one set of samples was removed from the water bath once the temperature reached 54.4°C, and the second set was removed after a water bath temperature of 7.2°C was attained. C. perfringens spore populations in sample homogenates were enumerated by heat treating a 5-ml aliquot of the sample for 15 min at 75°C before plating. To determine the populations of vegetative cells, samples were plated without heat shock and enumerated as described below. Three independent replications were conducted for each cooling cycle.

Microbial enumeration. Following their removal from the water bath, each sample bag was submerged in alcohol, wiped with a clean paper towel, and aseptically opened to transfer the meat to a filter stomacher bag. The meat sample was homogenized with 20 ml of sterile 0.1% buffered peptone water in a stomacher lab blender (Stomacher 400, Seward Medical, London, UK) for 2 min. Serial dilutions were prepared with sterile buffered peptone water, and the sample was poured plated, spiral plated, or both onto petri plates containing a thin layer of tryptose-sulfite-cycloserine agar (Oxoid, Basingstoke, UK) without egg yolk. A modified plat-
ing technique was used (dual-layer pour plating) for pour plating. An aliquot (1 ml) of each sample was placed on petri plates containing a thin layer of tryptose-sulfite-cycloserine agar and subsequently mixed with about 15 ml of tempered tryptose-sulfite-cycloserine agar. After solidification, plates were overlaid with an additional 5 ml of tryptose-sulfite-cycloserine agar. All plates were incubated for 24 h at 37°C in an anaerobic jar (AnaeroGen, Oxoid), and typical black colonies were counted as CFUs.

Statistical analyses. Total *C. perfringens* populations (vegetative cells and spores) were converted to log CFU per gram of sample. Differences between treatments for each cooling cycle were analyzed by an analysis of variance of the General Linear Models procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, N.C.). Fisher’s least significant difference was used to separate the means of the residual *C. perfringens* populations (log CFU per gram) between the treatments.

RESULTS AND DISCUSSION

The pH of injected turkey containing calcium lactate (pH 5.26 to 5.65) (Fig. 1) was appreciably lower (*P* ≤ 0.05) than the pH of otherwise similarly injected turkey containing potassium lactate (pH 6.08 to 6.11) or sodium lactate (pH 6.04 to 6.10), while that of the untreated control meat sample was pH 5.84. Shelef and Potluri (13) reported that the addition of calcium lactate to liver sausage reduced meat pH to 5.8 from a pH of 6.4 for control samples (without lactate), whereas a minimal pH reduction was observed with the addition of sodium lactate (pH 6.3). While the meat pH was different in the present study from that reported by Shelef and Potluri (13), a similar reduction (of 0.6 pH units) was observed when calcium lactate (3.0%) was incorporated. The pH-lowering effect of calcium lactate could be because of the insufficient neutralization of lactic acid (with Ca(OH)$_2$) during manufacture. A similar reduction in meat pH was described by Aran (3) when calcium lactate was added to beef goulash, whereas a minimal reduction was observed when sodium lactate was incorporated.

The water activity (*a$_w$*) of the control product was 0.987 (Fig. 1). The incorporation of calcium lactate resulted in a reduction of the *a$_w$* by 0.009 when the concentration of the antimicrobial increased from 1 to 4.8%. As the concentration of potassium and sodium lactate increased from 1 to 4.8%, the *a$_w$* of the products decreased by 0.010 and 0.011, respectively. The slight decrease in the *a$_w$* with increasing concentrations of antimicrobial suggests that the greater antimicrobial effect at higher concentrations was not due to the *a$_w$*. The antimicrobial activity of lactates has been ascribed to their ability to reduce the *a$_w$* of the medium. A gradual decrease in meat *a$_w$* was observed with increasing the concentrations of calcium lactate (from 1.0 to 4.8%). A similar trend was observed with sodium and potassium lactates. Furthermore, the *a$_w$* of the meat with corresponding concentrations of the three species of lactates was higher for calcium lactate than for sodium and potassium lactates.

It is possible that the inhibitory activity of calcium lactate was more a result of the pH reduction in the meat compared to sodium and potassium lactates. Aran (3) also noted a slight decrease in the *a$_w$* of beef goulash to which 3% calcium lactate (from 0.974 to 0.970) and sodium lactate (from 0.974 to 0.967) had been added. In the same study, the author noted that this slight reduction in *a$_w$* might not have been sufficient to explain the antimicrobial effect of lactates, as the *a$_w$* was still within the range at which *C. perfringens* could grow.

The dual-layer pour plating method allowed a better enumeration of *Clostridium* colonies than did the direct pour plating method (Fig. 2). In the direct pour plating method, plates had black discoloration at the bottom by the end of 18 to 24 h of incubation, whereas use of the dual-layer pour plating method allowed easier counting.

The incorporation of sodium, potassium, and calcium lactates at a 1.0% concentration resulted in the inhibition of *C. perfringens* germination and outgrowth by 0.40, 1.50, and 1.47 log CFU/g compared with the control during a 21-h exponential cooling from 54.4 to 7.2°C (Fig. 3F). Increasing the concentrations of these antimicrobials to ≥3.0% resulted in an increase of <1.0 log CFU/g in *C. perfringens* germination and outgrowth, while a similar inhibition was observed for the inclusion of 2% calcium lactate. Reductions
in *C. perfringens* populations were observed in injected turkey containing 4.8% sodium or potassium lactate or ≥2.0% calcium lactate. The USDA-FSIS performance standard of <1.0 log CFU could be achieved in injected turkey by incorporating ≥3.0% sodium or potassium lactate or ≥2.0% calcium lactate during a 21-h exponential cooling.

While the *C. perfringens* population in injected turkey subsequent to exponential cooling within 18 h was similar to that at 21 h (5.84 versus 5.82 log CFU/g) (Fig. 3E), greater inhibition was observed with calcium and potassium lactates at a 1.0% concentration than with sodium lactate. Similar to the 21-h cooling, the incorporation of ≥2.0% calcium lactate or ≥3.0% sodium or potassium lactate was required to achieve <1.0 log CFU of *C. perfringens* growth.

Sodium and potassium lactate concentrations ≥3.0% and a calcium lactate concentration ≥2.0% were required to control *C. perfringens* spore germination and outgrowth to <1.0 log CFU during the exponential cooling of injected turkey within 15 h (Fig. 3D). However, lower concentrations (≥2.0%) of sodium, potassium, or calcium lactate were able to limit *C. perfringens* spore germination and outgrowth to <1.0 log CFU during the exponential cooling of injected turkey within 12 h (Fig. 3C). Thus, the type of antimicrobial (sodium, potassium, or calcium lactate), the concentration of the particular antimicrobial, and the cooling time dictated the extent of *C. perfringens* spore germination and outgrowth.

Cooling the injected turkey from 54.4 to 7.2°C within 9 or 6.5 h resulted in minimal increases (<1.0 log CFU) in *C. perfringens* populations (Fig. 3B and 3A, respectively). While the incorporation of antimicrobials (sodium, potassium, or calcium lactate) at 1.0% resulted in marginal differences in *C. perfringens* populations compared with injected turkey without any antimicrobials, faster cooling of the products can ensure that the spore germination and outgrowth is minimized. Although the 9-h exponential cooling rate used in this study is longer than the USDA-FSIS recommended cooling regime (6.5 h) for noncured, cooked RTE meat and poultry products, the *C. perfringens* germination and outgrowth observed were lower than the required performance standard (<1.0 log CFU). On the basis of the results obtained in this study, the USDA-FSIS recommended cooling regime (6.5 h) provides a margin of safety in limiting *C. perfringens* spore germination and outgrowth.

It is possible that the greater inhibition of *C. perfringens* spore germination and outgrowth in injected turkey containing calcium lactate (particularly at concentrations of up to 3.0%) was due to the lower pH (P = 0.05) compared to the product containing potassium and sodium lactates at comparable levels. Thippareddi et al. (15) suggested that even seemingly minor differences in pH values have a significant role in inhibiting the germination and outgrowth of *C. perfringens* spores. Aran (3) reported that the addition of calcium lactate (3%) to beef goulash reduced the pH of the product containing 3% calcium lactate to 5.5 from 6.0 for beef goulash without lactates, while there was no change in the pH of the product to which sodium lactate (3%) had been added. Similarly, Shelaf and Potluri (13) described the superior listeriostatic activity of calcium lactate and attributed it to the lowering of the pork sausage pH from 6.7 to 6.0 and the consequent increase in the amount of undissociated lactic acid compared to sodium lactate.

Calcium is a divalent ion, and thus, calcium lactate has two lactate ions, whereas potassium and sodium are monovalent and thus possess one lactate ion each. It is likely that
the greater antimicrobial effect of calcium lactate may be because of the higher number of lactate anions.

To better understand the role of the number of lactate ions, meat was prepared and lactates were added such that there were an equal number of lactate ions in all the products (equinormal concentrations). At similar lactate concentrations, calcium lactate (1 N) showed a greater inhibition ($P \leq 0.05$) than did potassium and sodium lactates at the same concentrations (Fig. 4). These findings suggest that lactate concentration was not the primary reason for the greater inhibitory effect of calcium lactate. The pH value of meat containing calcium lactate was 5.56, while the pH values of meat containing potassium and sodium lactates were 5.97 and 5.96, respectively. These data suggest that pH has a more important role in the greater antimicrobial efficacy of calcium lactate.

Shelef and Potluri (13) reported that, at similar concentrations, calcium lactate exhibited greater listeriostatic activity than sodium lactate. The authors stated that lactate, rather than the calcium ion, is the principal listeriostatic factor. At the concentrations used in the present study, the number of lactate ions added to the meat would be two times more for calcium lactate than for sodium lactate and potassium lactate. Shelef and Potluri (13) stated that, like other hydroxycarboxylic acids, lactic acid is known for its chelating properties, although these acids are weak in comparison to other chelating agents used in food products, such as polyphosphates, citrates, and EDTA. In addition, the authors speculated that the incorporation of lactates in the formulation, followed by heat treatment, resulted in the formation of stable complexes with polyvalent cations that rendered these ions unavailable for growth of the organisms. The incorporation of phosphate (calcium phosphate, dibasic) inhibited $L. monocytogenes$ growth, although the pH reduction in the substrate was significant (pH 5.0) compared to the control (pH 6.4). Phosphates are frequently used in food systems as sequestrants and chelators for metal ions, and the superior inhibitory effect could be because of both the sequestering and the pH reduction of the meat.

Thippareddi et al. (15) reported that the incorporation
FIGURE 4. Mean population (log CFU per gram) of C. perfringens in injected turkey containing equinormal (1 N) concentrations of calcium lactate (CL), potassium lactate (KL), and sodium lactate (NaL) after heat shock at 75°C for 20 min (HS) and after cooling from 54.4 to 7.2°C exponentially in 6.5 h (Chill). Different letters on the bars represent statistical differences (P ≤ 0.05) between the treatments.

of Ional (buffered sodium citrate) and Ional Plus (buffered sodium citrate containing sodium diacetate) at concentrations ≥1.0% into the formulations of roast beef or injected pork products can extend the cooling times (from 54.4 to 7.2°C) to 21 h after heat processing. The authors reported that the antimicrobial effects of sodium citrate were greater with longer cooling times (21 h), as this allowed a longer exposure of the cells to the antimicrobials at higher temperatures. Steele and Wright (14) compared the effect of three different cooling rates (6, 8, and 10 h) on the growth of C. perfringens in cooked, RTE turkey (without added antimicrobials). While the cooling turkey product within 6 or 8 h did not result in an outgrowth (<1 log) of C. perfringens, growth (>1 log) was observed when the cooling rate exceeded 8.9 h. Results of this study indicated that, at the same antimicrobial level, but at different cooling cycles, the growth of C. perfringens was greater during longer cycles than during shorter cycles and are in agreement with the data of Steele and Wright (14). At equinormal antimicrobial concentrations, a greater decrease with C. perfringens populations was observed with a 15-h cooling cycle than with a 12-h cooling cycle.

Sabah et al. (11) stated that buffered sodium citrate, sodium lactate, and sodium diacetate act as effective bacteriostatic treatments for the control of C. perfringens. The authors reported that 2.0 and 4.8% sodium lactate in restructured roast beef (products cooked from 54.4 to 4.4°C in 18 h) reduced populations of C. perfringens by 0.13 and 0.88 log CFU/g, respectively. The differences in the results could be due to differences in the product matrices (roast beef and injected turkey) or due to pH differences in the products used. Sabah et al. (11) adjusted the pH of sodium lactate (2.0 and 4.8%) to 7.3, whereas in the present study, the pH of meat containing sodium lactate (2.0 and 4.8%) was 6.10. Recently, Sánchez-Plata et al. (12) evaluated the effects of sodium lactate (2.5%), sodium lactate plus diacetate (2.5%), and buffered sodium citrate or buffered sodium citrate plus sodium diacetate (1.3%) and found them to be effective for inhibiting the germination and outgrowth of C. perfringens in injected roast beef.

Metal ion chelation has been suggested as an alternate mechanism for the antimicrobial activity of lactates on microorganisms. Bulgarelli and Shelef (5) reported that EDTA inhibited the outgrowth and multiplication of Bacillus cereus cells, and the incorporation of cations (Fe, Zn, and Ca) reversed the growth inhibitory activity. The authors also reported that EDTA did not affect the spore germination or release of Ca following heat activation and subsequent incubation, suggesting that the inhibitory activity of EDTA, and other chelators, is due to the inhibition of multiplication of the germinated spores. In our study, we could not recover C. perfringens spores subsequent to chilling the product. This could be because germinated spores are less heat resistant and because most of the inoculated spores have germinated by the heat shock. These germinated spores were not able to multiply and were destroyed by the heat treatment applied to inactivate the vegetative cells for the enumeration of spores in the chilled meat. Thus, the ability of the lactates to chelate metal ions seems to be a more plausible explanation for the inhibition of C. perfringens growth during the chilling of cooked meat products.

C. perfringens spores were not recovered from the injected turkey product subsequent to cooling, regardless of the presence, type, and concentration of lactates. Sánchez-Plata et al. (12) reported that C. perfringens spores were recovered from roast beef and injected pork products subsequent to exponential cooling in the presence of sodium lactate and buffered sodium citrate (≥1.0%). These differences could be because of variations in the inoculum size (initial spore concentration of 3.10 log CFU/g in the Sánchez-Plata et al. (12) study versus ca. 2.40 log CFU/g in the present study), heat resistance of the spores, or variations in the germination of the individual spores (10). It is possible that, with a greater initial spore population, a proportion of the spores did not germinate during the heat activation and subsequent cooling and that they were detected in the product subsequent to cooling, while with lower populations, the number of such spores will be lower and may not be detected.

Spore populations are known to be heterogeneous re-
garding germination; not all spores germinate at the same
time. Billon et al. (4) showed that the time to germination
was skewed (with a tail) and that the shape of the distribu-
tion depended on the germination temperature. The au-
thors speculated that this variability was because of a vari-
ability in the permeability of the spores or molecules, such
as lytic enzymes in the spore population. The trigger me-
chanism of spore germination (6) suggests that heat activates
a germinant receptor, which then binds to the germinant to
become an active enzyme that can hydrolyze the precursor
of a germination-specific lytic enzyme and that eventually
hydrolyzes the cortex, resulting in spore germination. Bil-
on et al. (4) stated that regardless of the type and nature
of the germinant molecule, its concentration in the dormant
spore determines how fast each spore germination is trig-
gerated, resulting in variations in the time to germination.
The authors stated that this biovariability could explain dif-
fferences in the time to spoilage or the time to toxin ob-
served with a low inoculum size of Clostridium botulinum
spores, when the population effect is not sufficient to over-
whelm the individual effects of low inoculum size. This
variability within a spore crop, and especially between
spore crops prepared at different times, could have a sign-
ficant effect on the germination of the spores and their
subsequent growth. This could explain the differences in
the survival of spore populations subsequent to the chilling,
as described by Sánchez-Plata et al. (12) and the present
study.

The findings of this study demonstrate that calcium,
potassium, and sodium salts of lactic acid are effective in
inhibiting the germination and outgrowth of C. perfringens
spores. Cooling of injected turkey within the USDA-FSIS
stabilization guidelines (6.5-h cooling cycle) resulted in a
growth of <1.0 log CFU of C. perfringens populations (0.5
log CFU/g), indicating that this would be a safe cooling
regime for injected turkey products that do not contain an-
timicrobial agents such as lactates. The role of pH in the
effectiveness of calcium lactate compared to potassium and
sodium lactates in controlling C. perfringens germination
and outgrowth needs to be further investigated. Further,
the impact of these antimicrobials on quality, such as moisture
retention (water binding), color, texture, and organoleptic
properties, should be evaluated. Incorporation of these an-
timicrobials provides an additional margin of safety to pro-
cessors when products do not meet USDA-FSIS stabiliza-
tion guidelines.

ACKNOWLEDGMENTS

This study was made possible by a contribution from the University
of Nebraska Agricultural Research Division, supported in part by funds
provided through the USDA-CSREES (Cooperative State Research, Ed-
ucation, and Extension Service) under agreement 25-6231-0078-001 and
the Hatch Act. The authors thank personnel in H. Thippareddi’s laboratory
for their assistance.

REFERENCES

modeling and experimental validation of cooling rates of large ready-
heat-treated enterotoxin-positive Clostridium perfringens and the im-
lications for safe cooling rates. J. Food Prot. 67:83–89.
3. Aran, N. 2001. The effect of calcium and sodium lactates on growth
from spores of Bacillus cereus and Clostridium perfringens in a “sous-
vide” beef goulash under temperature abuse. Int. J. Food Mi-
The effect of temperature on the germination of single spores of
netetraacetic acid (EDTA) on growth from spores of Bacillus cereus.
J. Food Sci. 50:661–664.
lyxanthaines and related compounds to enhance Clostridium perfrin-
gens sporation using Duncan and Strong medium. J. Rapid Meth-
Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness
tural comparison of spores from various strains of Clostridium perfrin-
gens and correlations with heat resistance parameters. Int. J.
2003. Use of organic acids for the control of Clostridium perfringens
in cooked vacuum-packaged restructured roast beef during an alter-
V. Juneja, and H. Thippareddi. 2005. Predictive model for Clostrid-
ium perfringens growth in roast beef during cooling and inhibition
of spore germination and outgrowth by organic acid salts. J. Food
Prot. 68:2594–2605.
in cooked liver sausage containing lactates. Food Microbiol. 12:221–
227.
14. Steele, F. M., and K. H. Wright. 2001. Cooling rate effect on out-
growth of Clostridium perfringens in cooked, ready-to-eat turkey
L. Kastner. 2003. Control of Clostridium perfringens germination
and outgrowth by buffered sodium citrate during chilling of roast
beef and injected pork. J. Food Prot. 66:376–381.
1999. Time/temperature guidelines for cooling heated products. FSIS
directive 7110.3, rev. 1. U.S. Department of Agriculture, Wash-
ington, D.C.
diacetate, sodium acetate, sodium lactate, and potassium lactate: di-
18. U.S. Food and Drug Administration, Center for Food Safety and
Applied Nutrition. 2005. Foodborne pathogenic microorganisms and
natural toxins handbook: the “Bad Bug Book.” U.S. Food and Drug
Administration, Center for Food Safety and Applied Nutrition,
Washington, D.C.
outgrowth of Clostridium perfringens spores in cooked ham and