Research Note

Inhibition of *Clostridium perfringens* Growth by Potassium Lactate during an Extended Cooling of Cooked Uncured Ground Turkey Breasts

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ABSTRACT

The U.S. Department of Agriculture’s Food Safety and Inspection Service compliance guideline known as Appendix B specifies chilling time and temperature limits for cured and uncured meat products to inhibit growth of spore-forming bacteria, particularly *Clostridium perfringens*. Sodium lactate and potassium lactate inhibit toxigenic growth of *Clostridium botulinum*, and inhibition of *C. perfringens* has been reported. In this study, a cocktail of spores of three *C. perfringens* strains (ATCC 13124, ATCC 12915, and ATCC 12916) were inoculated into 100-g samples of ground skinless, boneless turkey breast formulated to represent deli-style turkey breast. Three treatment groups were supplemented with 0 (control), 1, or 2% potassium lactate (pure basis), cooked to 71°C, and assayed for *C. perfringens* growth during 10 or 12 h of linear cooling to 4°C. In control samples, populations of *C. perfringens* increased 3.8 to 4.7 log CFU/g during the two chilling protocols. The 1% potassium lactate treatment supported only a 2.5- to 2.7-log increase, and the 2% potassium lactate treatment limited growth to a 0.56- to 0.70-log increase. When compared with the control, 2% potassium lactate retarded growth by 2.65 and 4.21 log CFU/g for the 10- and 12-h cooling protocols, respectively. These results confirm that the addition of 2% potassium lactate inhibits growth of *C. perfringens* and that potassium lactate can be used as an alternative to sodium nitrite for safe extended cooling of uncured meats.

An estimated 1,000,000 cases of food poisoning caused by *Clostridium perfringens* occur annually in the United States; this infection is currently the third most common foodborne illness in the country (18). Uncured meat and poultry products are favorable substrates for *C. perfringens* growth (1). *C. perfringens* is an anaerobic endospore-forming bacterium, and although classified as an anaerobe, *C. perfringens* is extremely aerotolerant (19). The spores can withstand normal cooking procedures in poultry and will germinate if given enough time and an optimal temperature (10). Although the temperature limits for growth of *C. perfringens* are 12 to 50°C, the optimal temperature range has been reported as 43 to 47°C (109 to 117°F) (4, 7, 20), a temperature range through which all cooked meats must pass during cooling (10).

The U.S. Department of Agriculture’s Food Safety and Inspection Service (FSIS) requires federally inspected production facilities to address *C. perfringens* risk via controlled chilling after cooking products as indicated in 9 CFR 417.5(a)(2) with guidelines published in a document known as Appendix B (20). For uncured products, Appendix B states, “During cooling, the product’s maximum internal temperature should not remain between 130°F (54.4°C) and 80°F (26.7°C) for more than 1.5 hours nor between 80°F (26.7°C) and 40°F (4.4°C) for more than 5 hours . . . [and] all product[s] should be chilled from 120°F (48°C) to 55°F (12.7°C) in no more than 6 hours.’’ Cooling requirements for cured meats are less stringent (from 54.4 to 26.7°C in 5 h) because the nitrate present in them is an effective inhibitor of spore germination and bacterial growth, particularly for *Clostridium botulinum* (15, 20). These guidelines are in place to ensure that less than a 1-log increase in *C. perfringens* occurs (20). However, the majority of outbreaks of *C. perfringens* infections occur in facilities such as hospitals, schools, and other institutions where it is common practice to prepare and heat food hours before serving, whereas no outbreaks have been associated with commercially prepared products that comply with the stringent cooling requirements (21).

The addition of sodium or potassium lactate can impede the growth of *C. perfringens* depending on the amount added, the species of meat or poultry, moisture, pH, salt or adjunct spice levels, and storage temperature or cooling profile (1, 8, 11, 16, 17, 22, 23). The necessary range of sodium lactate falls between 1% in marinated (pH 5.0) turkey breasts (12) and 3% in ‘sous-vide’ beef goulash (1). Growth inhibition of *C. perfringens* also has been noted in beef patties at a level of 2 or 3% potassium lactate with no
found that the addition of lemon juice and vinegar
potassium lactate
syrup 0.0 1.67
2.0
% Formulations of poultry products supplemented with 0 (control), 1, or 2
16.67
spore germination and
ethanol. The spore-ethanol suspension was stored at 4
manufacturers of such products might gain
% g
76.2
C.

and growth. By
for 20 min, the
% supernatant was
free
2
2.0
strains. The fresh ground turkey, water mixture, and
and incubated
% (14)
C. perfringens
solution. The aqueous portion of the solution was compensated for by reducing the
20.0
3.33
germination and growth. By
for 20 min, the
% spores. Sodium or potassium
C. perfringens
potassium lactate (anhydrous basis)
% % % %

Commercial potassium lactate comes as a 60% solution. The aqueous portion of the solution was compensated for by reducing the amount of water added.

negative effects on the product’s sensory aspects (6). Li et al. (13) found that the addition of lemon juice and vinegar products inhibited C. perfringens spore germination and outgrowth. This research suggests that either organic acids or their neutralized salts have an effect on the germination and growth of C. perfringens spores. Sodium or potassium lactate also are used to inhibit growth of C. botulinum in uncured ready-to-eat (RTE) meat and poultry products (14).

In light of these previous studies, we postulated that common levels of potassium lactate added to formulations of uncured RTE turkey breast meat products would extend the time that the product can be cooled after cooking without risk of C. perfringens germination and growth. By taking advantage of growth inhibiting properties against C. perfringens, manufacturers of such products might gain efficiency and save energy by chilling uncured products in a manner similar to that for conventionally chilled items. However, unlike cured products (which have a cooling rate prescribed in Appendix B), the efficacy of alternative antimicrobials during extended cooling must be validated to address specific conditions of meat type, salt, moisture, pH, and cooling rate. This study was conducted to validate two such chilling protocols in combination with potassium lactate as a bacterial growth inhibitor.

MATERIALS AND METHODS

Spore preparation. Three C. perfringens strains, ATCC 13124, ATCC 12915, and ATCC 12916, were individually grown under conditions to induce sporulation using procedures modified from those described by Juneja et al. (9). Each stock culture was grown in 10 ml of cooked meat medium (Difco, BD, Sparks, MD) under anaerobic conditions for 24 h at 35°C. This culture was transferred to freshly steamed fluid thioglycollate medium (10 ml per tube) and incubated anaerobically at 35°C for 24 h, and the cycle was repeated seven times to improve the spore crop. Five milliliters of the fluid thioglycollate culture was inoculated into 500 ml of modified Duncan-Strong medium (5) and incubated anaerobically at 40°C for 24 h to achieve greater than 60% free spores as determined by phase-contrast microscopy. Spore cultures were centrifuged at 3,700 \( \times g \) for 20 min, the supernatant was discarded, and the pellet was suspended in approximately 1 to 2 ml of 95% ethanol. The spore-ethanol suspension was stored at 4°C for 2 h and then centrifuged at 3,700 \( \times g \) for 20 min, the supernatant was discarded, and the pellet was rinsed twice with 0.85% saline and vortexed thoroughly each time before centrifuging. Spores were enumerated by heat shocking an aliquot of each strain at 75°C for 20 min to kill vegetative cells, serially diluting the aliquot in peptone water (0.1% peptone, 0.5% NaCl), and plating onto tryptose-sulfite-cycloserine (TSC) plates with a thin agar overlay of TSC. The culture was incubated for 24 h at 35°C under anaerobic conditions. Spore crops were stored in 25 ml of 0.85% saline at −20°C for up to 6 weeks before preparing spore cocktails for inoculation. For each meat inoculation and cooling trial, fresh inoculum of the cocktail of spores was prepared by adding approximately equal levels of the three strains to yield approximately 2 log CFU/g of poultry.

Poultry preparation and inoculation. Fresh boneless turkey breasts were obtained from a commercial source within 5 days of animal harvest and stored at 2.2 to 4.4°C (36 to 40°F) until use. Turkey was ground through a 3.175-cm (0.125-in.) plate on a grinder (model 4732, Hobart Corp., Troy, OH). Three treatment formulations were designed to contain no antimicrobial (control) or 1 or 2% potassium lactate (Table 1). All dry ingredients were dissolved into the appropriate amount of water before they were added to the ground poultry. To ensure adequate dissolving of all additives, the nonpoultry ingredients were added in the following order: phosphate, salt, starch, and then the appropriate amount of potassium lactate. Each treatment was then inoculated with an approximately 2-log CFU/g cocktail of spores of the three C. perfringens strains. The fresh ground turkey, water mixture, and inoculum were then mixed for 5 min in a mixer (model AS 200, Hobart Corp.). For each sample, 100-g inoculated portions were vacuum sealed in boilable oxygen- and moisture-impermeable bags (high-barrier ethylene vinyl alcohol pouches, 3 mm thick, 13 by 13 cm; Deli 1 material: oxygen transmission of 2.3 cm²/cm²/24 h at 24°C, water transmission of 7.8 g/cm²/24 h at 37°C and 90% relative humidity; WinPak, Winnipeg, Manitoba, Canada) using a Multivac vacuum packaging machine (AGW 1578, Wolfertschwenken, Allgäu, Germany). Samples were stored overnight at 4°C before cooking and cooling.

Cooking, cooling, and sampling. Packages were immersed in a 73.3°C water bath and heated with agitation until the internal temperature of representative packages reached 71°C, which heat shocked the spores and killed any vegetative cells. Internal temperature for each product type was monitored with a digital thermometer (traceable thermometer and type K probe, Thermo Fisher Scientific, Waltham, MA) inserted into one package through a rubber septum. The time to target cook temperature and temperatures of the product and water bath were manually recorded. Cooked samples were then placed into an air incubator to follow either a 10- or 12-h linear cooling procedure (from 70 to 7°C, with approximately 5 and 7 h between 58 and 27°C for the two cooling protocols, respectively; Fig. 1). The incubator temperatures were manually adjusted hourly, and the internal temperatures of the turkey breast samples were recorded every 3

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>1% potassium lactate*</th>
<th>2% potassium lactate*</th>
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</thead>
<tbody>
<tr>
<td>Skinless, boneless turkey breast meat</td>
<td>76.2%</td>
<td>76.2%</td>
<td>76.2%</td>
</tr>
<tr>
<td>Watera</td>
<td>20.0%</td>
<td>18.33%</td>
<td>16.67%</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.4%</td>
<td>1.4%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Sodium tripolyphosphate</td>
<td>0.4%</td>
<td>0.4%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Modified waxy maize starch</td>
<td>2.0%</td>
<td>2.0%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Potassium lactate, 60% syrup</td>
<td>0.0%</td>
<td>1.67%</td>
<td>3.33%</td>
</tr>
</tbody>
</table>

* Calculated on an anhydrous wt/wt basis.

a Commercial potassium lactate comes as a 60% solution.
hour to confirm that targets were reached. Triplicate samples from each treatment group were removed at 0, 2, 5, 7, and 10 h and at 0, 3, 6, 9, and 12 h for the two cooling protocols, respectively. From each 100-g sample, a representative 50-g portion was diluted with 50 ml of Butterfield’s phosphate buffer and homogenized with a stomacher for 3 min. Homogenates were serially diluted, plated onto TSC plates with an 8- to 10-ml TSC agar overlay, and anaerobically incubated at 35°C for 24 h (10). The 10- and 12-h cooling protocols were run on different days; each set of experiments was replicated three times using different batches of meat and spore inocula.

Proximate analysis. Triplicate samples of each formulation were taken for proximate analysis after cooking. Moisture was determined with the vacuum oven method 950.46 (2) after 5 h at 100°C. The pH was determined by diluting a 10-g homogenized portion 1:10 in distilled water and measuring the pH of the slurry with Accumet Basic pH meter and Orion 8104 combination electrode (Thermo Fisher Scientific). NaCl was measured as the percentage of Cl− using AgNO3 potentiometric titration (model DL22 food and beverage analyzer, Mettler, Columbus, OH), and water activity was determined with a water activity meter (AquaLab TE4, Decagon, Pullman, WA).

Statistical analysis. Enumeration data were log transformed (n = 9 for each sampling interval) and analyzed using a single factor analysis of variance to determine statistical significance of growth inhibition achieved with potassium lactate compared with that in the no-antimicrobial control group for each cooling protocol (Excel 2010, Microsoft, Redmond, WA).

RESULTS AND DISCUSSION
Measurements for salt, pH, moisture, and water activity for the turkey treatment groups are summarized in Table 2. All three treatments had essentially the same NaCl content (1.54 to 1.59%) and water activity (0.973 to 0.976), but the pH of the control was 0.1 units lower than that of either lactate treatment and the moisture content was 0.9 and 1.8% higher than that of the 1 and 2% lactate treatments, respectively. Changes in populations of C. perfringens over time versus temperature are presented in Figure 1. The mean
level of inoculum recovered in the 10-h cooling trials was 1.96 ± 0.50 log CFU/g. During the 10-h linear cooling protocol, C. perfringens populations remained unchanged in the three treatments until 7 h, when the internal temperature of the poultry reached 21.5 °C (70.7 °F) (Fig. 1A). After 7 h of cooling (i.e., by the time the temperature reached 21 °C), populations of C. perfringens had increased by 1.97 ± 1.22 log CFU/g in the control and by 1.33 ± 1.11 and 0.47 ± 0.40 log CFU/g in the 1 and 2% potassium lactate treatment groups, respectively (P > 0.05). At the end of the 10-h cooling period, populations of C. perfringens had increased by 3.82 ± 0.8, 2.53 ± 1.42, and 0.70 ± 0.45 log CFU/g for the 0, 1, and 2% lactate treatments, respectively. Thus, 2% potassium lactate significantly delayed growth of C. perfringens in uncured deli-style turkey breast compared with the control or the 1% lactate treatment at the 10-h cooling mark (P < 0.05) and limited growth to <1 log CFU/g at all time points for the duration of the 10-h cooling experiment.

Similar to the 10-h samples, the 12-h cooling protocol resulted in little growth and no differences among treatment samples until the 6-h sampling time (Fig. 1B). Initial populations of C. perfringens recovered at the beginning of chilling were 2.20 ± 0.36 log CFU/g, and no growth was observed in any treatments at 3 h. At the 6-h sampling point (internal temperature of the poultry was 37.5 °C), 1.94 ± 0.22-log and 0.7 ± 0.28-log increases were noted for the control and 1% lactate treatments, respectively; in contrast, no significant changes in C. perfringens populations were observed in the 2% potassium lactate treatment (–0.01 ± 0.19 log change; P < 0.05). At the end of the 12-h cooling period, C. perfringens populations increased by 4.66 ± 1.04 log CFU/g in the control without antimicrobials but by only 2.75 ± 0.37 log CFU/g in the 1% potassium lactate samples. As in the 10-h cooling protocol, 2% potassium lactate limited growth to <1 log CFU/g, with a mean 0.56 ± 0.10-log increase at the end of the 12-h cooling period. Both the 1 and 2% lactate treatments significantly inhibited growth of C. perfringens compared with the control at 6, 9, and 12 h (P < 0.05); the 1 and 2% lactate samples were not significantly different from each other at 6 h, but 2% lactate was significantly more inhibitory than 1% lactate at the 9- and 12-h sampling times.

These results differ slightly from those reported by Juneja and Thippareddi (12), who found that addition of 1% sodium lactate was sufficient to control C. perfringens germination and outgrowth (<1.0 log CFU/g growth) after 15 h of exponential chilling. Variation in growth inhibition by 1% lactate may be attributed to differences in chilling rates; more rapid cooling occurred in the exponential cooling protocol than in our linear cooling protocol. Differences also could be attributed to the product formulation, strains, or use of sodium versus potassium lactates, which were used at similar weight percentages but had different molar additions (89.2 mM/liter for sodium lactate versus 78.0 mM/liter for potassium lactate). Food products are almost exclusively formulated based on a weight basis, including antimicrobial additions. However, because sodium and potassium salts have different molecular weights, one antimicrobial may appear to be more effective when in actuality the differences in efficacy are more likely due to the molarity differences. Velugoti et al. (22) also reported that potassium lactate treatment resulted in inhibition of spore germination and outgrowth in turkey products, but when used at equivalent percentages the sodium lactate treatment slightly delayed growth of C. perfringens compared with the potassium lactate treatment during 9, 12, and 15 h of exponential cooling.

Regardless of the exact numbers, potassium lactate provides a practical level of protection against C. perfringens growth during extended cooling. We observed a <1-log increase in C. perfringens growth in high-moisture uncured turkey with 2% potassium lactate during both the 10- and 12-h linear cooling protocols. Compared with the control, 2% potassium lactate inhibited growth by 2.65 and 4.21 log CFU/g in the 10- and 12-h cooling protocols, respectively. The control group growth results were similar to predictions from a ComBase predictive model for C. perfringens growth (3). With this model, the user can input the meat pH, salt concentration, and amount of sodium nitrite, but the model does not take into account brine ratio (salt-in-moisture concentration) or the addition of other antimicrobials.

Although the 10- and 12-h cooling curves revealed a similar trend for potassium lactate inhibition of the germination and growth of C. perfringens, each curve was unique because of the different rates of cooling. However, both curves reveal the effectiveness of 2% potassium lactate for inhibiting the growth of C. perfringens. The addition of 2% potassium lactate satisfies the requirements of the FSIS stipulated in Appendix B (20), which states that germination and outgrowth of C. perfringens should not exceed 1.0 log CFU/g, despite the fact that both cooling procedures lasted longer than 6.5 h. Therefore, the addition of 2% potassium lactate provides meat and poultry processors with the potential to safely extend the cooling of uncured RTE turkey products by 3.5 to 5.5 h.

TABLE 2. Analytical values for salt content, pH, moisture content, and water activity measured for poultry samples from control, 1% potassium lactate, and 2% potassium lactate treatments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1% potassium lactate</th>
<th>2% potassium lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>% salt</td>
<td>1.54 ± 0.03</td>
<td>1.59 ± 0.11</td>
<td>1.54 ± 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>6.18 ± 0.12</td>
<td>6.27 ± 0.10</td>
<td>6.28 ± 0.05</td>
</tr>
<tr>
<td>% moisture</td>
<td>76.4 ± 0.2</td>
<td>75.6 ± 0.3</td>
<td>74.6 ± 0.5</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.976 ± 0.000</td>
<td>0.973 ± 0.000</td>
<td>0.973 ± 0.001</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations of nine samples for each parameter.
Under the conditions of this study, the addition of 2% potassium lactate kept the growth of *C. perfringens* to less than 1 log CFU/g in both the 10-h and 12-h linear cooling protocols and thus would be a suitable approach to providing safe RTE cooked uncured turkey breast produced under these chilling conditions. The results of this study provide a scientific basis for the meat and poultry industry to extend their current cooling times beyond those currently permitted under the FSIS Appendix B guidelines.

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**REFERENCES**


