CONTROL OF *Clostridium perfringens* GERMINATION AND OUTGROWTH BY BUFFERED SODIUM CITRATE DURING CHILLING OF ROAST BEEF AND INJECTED PORK

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Background

Clostridium perfringens continues to be a concern to the food industry, particularly to the retail food service industry and has been implicated in several large outbreaks (Bean et al., 1996). The organism and its spores are widely distributed in nature and are often contaminants in raw meat and poultry products. 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 (Bryan, 1988). Although *C. perfringens* vegetative cells do not survive the normal heat processing schedules employed in the meat industry, the spores can survive. Heat activated spores can germinate and grow rapidly if these products are improperly chilled. The time/temperature guidelines for cooling cooked products specifies that the maximum internal temperature should not remain between 54.4° and 26.7 °C for more than 1.5 h nor between 26.7° and 4.4° C for more than 5 h (USDA-FSIS, 2000). We hypothesize that incorporation of antimicrobial ingredients that can inhibit growth of *C. perfringens* will provide an additional measure of safety for cooked meat products in case of a chilling deviation. This will be particularly advantageous to small processors who cannot afford to invest in additional cooling equipment when the existing chilling power is inadequate.

Objective

Evaluate potential use of buffered sodium citrate (Ional™) alone and in combination with sodium diacetate (Ional Plus™) to control or inhibit germination and outgrowth of *C. perfringens* from spores in processed beef and pork products during extended chill situations.

Methods

C. perfringens cultures and spore production: *Clostridium perfringens* strains NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13) were used. The spore crop of each strain was prepared separately, and 3-strain *C. perfringens* spore cocktail was prepared immediately prior to experimentation. This spore mixture (0.75 mL) was then mixed with the beef and pork products (250 g of each product) for one minute, vacuum packaged (Model No. C-500, Multivac Inc., Kansas City, MO) and frozen until use.

Preparation of the meat and inoculation: Beef top rounds and boneless pork loins were obtained from a retail store (Athens, GA) and injected with minimal levels of salt (NaCl, 0.85% final concentration), potato starch (0.25%) and potassium tetra pyrophosphate (0.2%) at 12% pump rate. The products were diced (ca. 1 in^3) and ground through a 1/8" plate (Hobart, Troy, OH). The products (250 g portions) were mixed with buffered sodium citrate (BSC; Ional™) or BSC supplemented with sodium diacetate (Ional Plus™, BSC with 8.0% sodium diacetate) (WTI Inc., Kingston, NY) for 1 min in a mixer (Kitchen Aid, Troy, OH) and subsequently with the spore cocktail for 1 min to yield ca. 2.5 $\log_{10}$ spores/g. The product (roast beef or injected pork; 10 g) was distributed into 2" x 3" cook-in bags (Koch Supply Company, Kansas City, MO), and vacuum sealed at 12 mbar vacuum using a Multivac (Model A300/16, Multivac Inc., Kansas City, MO) packaging machine. Seven treatments [0.5%, 1.0% and 2.0% each of BSC (Ional™) or BSC with sodium diacetate (Ional Plus™), along with a control] were evaluated for each meat product (roast beef and injected pork).

***Heat shock, cooling and enumeration procedures*:** The bags containing product were submerged completely in a water bath set at 75.5° C (Exacal, Model RTE-221, NESLAB Instruments, Inc., Newington, NH), heat shocked for 20 min, removed, chilled immediately in an ice water bath, and plated as described. A second set of racks containing the product for each treatment were heat shocked and transferred to a water bath set at 54.5° C and chilled at an exponential rate from 54.5° to 7.2°C according to the desired chilling rates (18 and 21 h). After chilling to 7.2°C, each meat sample from the packages was transferred aseptically to a filter stomacher bag (Spiral Biotech, Bethesda, MD). Sterile peptone water (PW, 0.1%; 20 mL) was added and stomached for 2 min (Interscience, St. Nom, France). The samples were serially diluted in PW and plated on tryptose sulfite cycloserine (TSC) agar by pour or spiral plating methods and overlaid with an additional 10 ml of TSC and incubated at 37°Cfor 18-24 h anaerobiacally. Typical colonies were enumerated as *C. perfringens*.

***Statistical analysis*:** Three independent trials were performed for each of the exponential chilling rates (18 and 21 hour). The data were analyzed by analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC, 2000; Release 8.01). Fisher's Least Significant Difference (LSD) was used to separate means of the residual *C. perfringens* populations ($\log_{10}$ CFU/g) of the samples.

Results and Discussion

The products were chilled from 54.4° to 7.2° C following an exponential chill rate within 18 and 21 h. Both the 18 and 21 h temperature profiles represent extended chilling rates in view of the USDA-FSIS or the FDA stabilization requirements for chilling of cooked meat and poultry products. The pH of roast beef and injected pork corresponded to the normal pH of these products reported in the literature.

Chilling of control roast beef samples from 54.4° to 7.2 °C resulted in a 1.51- and 5.27-$\log_{10}$ CFU/g increase in *C. perfringens* populations when 18 and 21 h exponential chill rates were used, respectively (Table 1). Chilling control injected pork samples following similar chill rates resulted in 3.70 and 4.41 $\log_{10}$ CFU/g increases in *C. perfringens* populations. Higher levels of *C. perfringens* populations were observed in injected pork compared to roast beef for both 18 and 21 h chill rates. These differences in germination and outgrowth of *C. perfringens* could be due to the higher pH of the injected pork ($p \leq 0.05$) or the inherent differences in muscle food species (beef vs. pork).

Juneja et al. (1994) reported germination and outgrowth of *C. perfringens* from 1.5 $\log_{10}$ CFU/g to 6.0 $\log_{10}$ CFU/g during exponential cooling of autoclaved ground beef from 54.4° to 7.2°C. However, in our study, the growth was slower, resulting in increases of 1.51 units for roast beef following the 18 h exponential cooling rate. This minimal growth of *C. perfringens* in the roast beef system probably was due to differences in the meat substrate. Faster *C. perfringens* growth was observed from heat activated spores in the injected pork system, with increases of 5.27 $\log_{10}$ CFU/g from initial populations of ca. 2.75 $\log_{10}$ CFU/g. Thus, caution should be exercised when extending results of this and other studies for different meat systems such as poultry or in situations where the pH of the meat differs considerably from the meat systems evaluated herein. Results from our studies indicate that the chilling rates obtained using model systems such as autoclaved roast beef may not be applied to other products differing in composition (moisture, NaCl, phosphate, starch, etc.) and other intrinsic characteristics such as the product pH. Further, roast beef and injected pork were ground (1/8" grinder plate) in the present study, resulting in uniform distribution of the antimicrobials. Results obtained from such a ground meat systems will be conservative estimates of the antimicrobial

activity of the compounds since these compounds are generally concentrated on the product surface (purge and leak out of injected marinade) and the injection needle channels in whole-muscle injected products where the microbial contamination is expected in non-intact meat products.

Addition of Ional™ at 0.5% and subsequent chilling of ground roast beef and injected pork resulted in 0.98 and 0.21 $\log_{10}$ CFU/g *C. perfringens* reductions, respectively, when an 18-h exponential chill rate was followed. Extending the chill rate to 21 h resulted in 3.46 and 0.92 $\log_{10}$ CFU/g increases in *C. perfringens* populations in roast beef and injected pork, respectively. Addition of Ional Plus™ (0.5%) resulted in 0.37 and 1.10 $\log_{10}$ CFU/g *C. perfringens* increases in roast beef and injected pork, respectively following 18 h chill rate. Increases in *C. perfringens* populations were greater ($p \leq 0.05$) at the 21 h chill rate (3.84 and 1.19 $\log_{10}$ CFU/g for roast beef and injected pork, respectively).

Incorporation of Ional™ and Ional Plus™ at ≥1.0% into the meat formulation resulted in decreases ($p \leq 0.05$) in *C. perfringens* populations in both ground roast beef and injected pork products. The reductions were greater when a 21-h chill rate was followed, indicating that the antimicrobial activity of the sodium citrate was dependent on temperature, with longer exposures to higher temperatures following the 21 h chill rate. Although the increases in *C. perfringens* populations were <1.0 $\log_{10}$ CFU/g in both roast beef and injected pork when Ional Plus™ was incorporated into the formulation for 18 h chill rate, the same inhibition was not observed 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 when the product chilling rates extend beyond 18 h.

The USDA-FSIS stabilization guidelines for cooling of cooked meat products (6.5 h) can be extended by 14.5 h for roast beef and injected pork using either buffered sodium citrate (Ional™) individually or in combination with sodium diacetate (Ional Plus™; at >1.0% concentration). Presently, a maximum use level of 1.3% is approved for meat and poultry products for buffered sodium citrate (Ional™) for flavor retention and microbiological control (USDA-FSIS, 1996).

Conclusions

Incorporation of Ional™ and Ional Plus™ into product formulations can be an advantage to meat processors in situations where the product may not be cooled within the USDA-FSIS guidelines or to build safety into meat products to prevent potential germination and outgrowth of *C. perfringens* in case of cooling process deviations. Caution should be exercised when extrapolating data obtained from a model system to other food systems as these may result in germination and outgrowth to potentially hazardous levels of *C. perfringens* when spores are present in the raw meat ingredients.

Pertinent Literature

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Table

Table 1. Mean *Clostridium perfringens* populations in roast beef and injected pork immediately after heat shock and after chilling from 54.4° to 7.2° C in 18 or 21 h exponentially.

	18 h exponential chill rate				21 h exponential chill rate			
	Roast Beef		Injected Pork		Roast Beef		Injected Pork	
	Heat Shock	Chilled	Heat Shock	Chilled	Heat Shock	Chilled	Heat Shock	Chilled
Control	2.79^{Aa}	4.30^{Ab}	2.68^{Aa}	6.38^{Ac}	2.73^{Aa}	7.99^{Ad}	2.69^{Aa}	7.11^{Ae}
Ional[x] (0.5%)	2.80^{Aa}	1.83^{Bb}	2.64^{Aa}	2.43^{Bb}	2.66^{Aa}	6.13^{Bc}	2.73^{Aa}	3.65^{Bd}
Ional (1.0%)	2.73^{Aa}	0.86^{Bb}	2.76^{Aa}	0.98^{Cb}	2.65^{Aa}	0.46^{Cb}	2.76^{Aa}	0.75^{Cb}
Ional (2.0%)	2.75^{Aa}	0.28^{Bb}	2.63^{Aa}	1.00^{Cb}	2.75^{Aa}	0.41^{Cb}	2.75^{Aa}	0.32^{Cb}
Ional Plus[y] (0.5%)	2.83^{Aa}	3.21^{Cac}	2.80^{Aa}	3.90^{Dbc}	2.68^{Aa}	6.52^{Bb}	2.82^{Aa}	4.00^{Bbc}
Ional Plus (1.0%)	2.81^{Aa}	1.90^{Bab}	2.65^{Aa}	1.13^{CDb}	2.70^{Aa}	1.14^{Cb}	2.71^{Aa}	0.91^{Cb}
Ional Plus (2.0%)	2.68^{Aa}	0.61^{Bb}	2.66^{Aa}	0.63^{CDb}	2.71^{Aa}	0.31^{Cb}	2.81^{Aa}	0.68^{Cb}

[x]Ional: Buffered sodium citrate; [y]Ional Plus: Buffered sodium citrate supplemented with sodium diacetate (8.0%).

Different supercripts within a row (lower case) or a column (upper case) indicate significant differences ($p \leq 0.05$).