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Control of Listeria Monocytogenes in ready-to-eat meats using cetyl pyridinium chloride

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Abstract
Cetyl Pyridinium Chloride (CPC) spray using variable application temperatures, pressures, and times was evaluated for its effectiveness in reducing Listeria monocytogenes inoculated on the surfaces of commercial frankfurters and Polish sausage. Frankfurters and Polish sausage were inoculated with a five-strain cocktail of L. monocytogenes (101M, 109, 108M, serotype 4c ATCC, and serotype 3 ATCC) and subjected to no treatment, CPC treatment, and CPC followed by water treatment. CPC (1%) was applied to the frankfurters and Polish sausage by spraying in a cabinet using all combinations of 77, 104, and 131°F spray temperatures; 20, 25, and 35 psi spray pressures; and 30, 40, and 60 second times of exposure. No individual effect ($P>0.05$) of any particular application temperature, pressure, or time on the reduction of L. monocytogenes was observed. Hardness and color of the product was not affected when treated with 1% CPC. From initial inoculum levels of 8.20 log colony forming units (CFU)/gram, 1% CPC reduced L. monocytogenes by 1.19 to 2.39 log CFU/gram.

Keywords
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CONTROL OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT MEATS USING CETYL PYRIDINIUM CHLORIDE

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Summary

Cetyl Pyridinium Chloride (CPC) spray using variable application temperatures, pressures, and times was evaluated for its effectiveness in reducing *Listeria monocytogenes* inoculated on the surfaces of commercial frankfurters and Polish sausage. Frankfurters and Polish sausage were inoculated with a five-strain cocktail of *L. monocytogenes* (101M, 109, 108M, serotype 4c ATCC, and serotype 3 ATCC) and subjected to no treatment, CPC treatment, and CPC followed by water treatment. CPC (1%) was applied to the frankfurters and Polish sausage by spraying in a cabinet using all combinations of 77, 104, and 131°F spray temperatures; 20, 25, and 35 psi spray pressures; and 30, 40, and 60 second times of exposure. No individual effect ($P>0.05$) of any particular application temperature, pressure, or time on the reduction of *L. monocytogenes* was observed. Hardness and color of the product was not affected when treated with 1% CPC. From initial inoculum levels of 8.20 log colony forming units (CFU)/gram, 1% CPC reduced *L. monocytogenes* by 1.19 to 2.39 log CFU/gram.

Introduction

Listeria monocytogenes has emerged as an important and deadly food-borne pathogen that causes a high rate of hospitalization and death. Food-borne transmission of *L. monocytogenes* has been implicated in human outbreaks of listeriosis involving consumption of coleslaw, raw vegetables, milk, and Mexican style cheese. Consumption of *L. monocytogenes* contaminated turkey frankfurters was implicated in listeriosis in an immunocompromised woman. Consumption of undercooked chicken and uncooked frankfurters has been epidemiologically linked to an increased risk of listeriosis.

Listeria species are gram positive, asporogenous coccobacilli that are motile when cultured at 68 to 77°F. While their optimal temperatures for growth are between 86 and 99°F, *Listeria* can grow over a temperature range of 34 to 113°F, which makes the organism a potential food safety concern in refrigerated foods. Organic acids such as acetic, lactic, citric, and propionic have been used as antimicrobial agents in food products. Another antimicrobial used as a sanitizer/disinfectant is Cetyl Pyridinium Chloride (CPC), commercially known by the name of CECURE (Safer Foods Corporation, North Little Rock, Arkansas). CECURE is 40% active CPC. CPC is an active ingredient in mouthwashes and is a quaternary ammonium compound.

This study was designed to examine the efficacy of CPC as a post-process decontaminant for ready-to-eat meats and to optimize its application parameters for use in frankfurters and Polish sausage.

Experimental Procedures

A five-strain cocktail of *L. monocytogenes* (101M, 109, 108M, serotype 4c ATCC, and serotype 3 ATCC) was used. Cultures were maintained separately on Tryptic Soy Agar (Difco, Detroit, MI) slants at 39°F. Fresh cultures for the inoculum were prepared by inoculating the cultures into Tryptic Soy Broth

(Difco, Detroit, MI) and incubating at 95°F for 24 hours. Fresh cultures (1 ml) were transferred into centrifuge bottles containing 100 ml Tryptic Soy Broth and further incubated at 95°F for 20 hours. Cultures were then centrifuged, resuspended with 50 ml of 0.1% peptone water (Difco, Detroit, MI), and recentrifuged. The resultant pellet was resuspended with 10 ml of peptone water. A cocktail was prepared by mixing the five cultures in a sterile bottle.

Product preparation and inoculation. Frankfurters (8 in a pack) and Polish sausage (16 in a pack) obtained from a local grocery store were stored at 39°F before removal from the packages. They were placed onto butcher paper and individually dried with blotting paper. The top of each frankfurter and Polish sausage was wrapped with parafilm to avoid contamination while handling. The inoculum was sprayed onto the surface of the wrapped product by "misting" in a "bio-containment" chamber. After inoculation, products were held for 30 minutes in a laminar flow cabinet to allow attachment of *L. monocytogenes*.

Three frankfurters/Polish sausages were assigned to each treatment. The treatments included all combinations of three levels of each application parameter; spray temperature (77, 104, 131°F), spray pressure (20, 25, and 35 psi), and time of exposure to CPC (30, 40, and 60 seconds). For the microbiological shelf life evaluation, the products were inoculated with two different inoculum levels; high (10^9 cfu/ml) and low (10^2 to 10^3 cfu/ml).

CPC was prepared to a concentration of 1% by adding 25 ml of concentrated CPC to 1 liter of distilled water. The pH of the CPC was 5.2, and the temperature of water used to prepare the solution was 77°F. A spray washer (Kansas State University, Manhattan) was used to apply the treatments (1.6 L per minute at 20 psi) onto the product. Sets of three frankfurters/Polish sausages were considered as one sample. The treatments were applied for 30, 40, or 60 seconds. Two types of treatments for each set of spray combinations were used: 1) CPC only and 2) CPC followed by water wash. The inoculated product intended for microbiological shelf life evaluation was vacuum packed in sets of three per package after treating them with 1% CPC at 20 psi, 77°F, and 30 seconds of exposure to CPC. The treated product for shelf life evaluation was stored at 32°F and 39°F for 1, 2, 3, 4, and 6 weeks. Non-inoculated product was treated similarly as the inoculated product and stored for 1, 2, 3, 4, and 6 weeks at 32°F and 39°F in a simulated retail display. For each treated sample, a parallel control (non-treated) sample was also stored under similar conditions. The frankfurters/Polish sausages were then removed for microbial analysis to determine residual *L. monocytogenes* population. A Texture Profile Analyzer was used to determine the hardness of the shelf life samples.

Microbial sampling. Treated sets of three frankfurters/Polish sausages were removed from the spray cabinet and placed into sterile stomacher bags that contained pre-poured 1% peptone diluent to make a 1:1 dilution. Before sampling, the top parafilm wrapping was removed. Each sample was homogenized in a stomacher (Tekmar Co., Cincinnati, OH) for 2 minutes. The samples from the shelf life evaluation were removed from vacuum packages and placed into sterile stomacher bags for homogenizing in a stomacher for 2 minutes.

Microbiological enumeration. Samples were serially diluted in peptone water and spiral plated onto Modified Oxford Agar (Oxoid Ltd., Basingstoke, Hampshire, England) and Tryptose Phosphate Agar (Difco, Detroit, MI). The plates were incubated at 100°F for 24 hours. Enumeration was performed by counting black colonies on Modified Oxford Agar

and white colonies on Trypose Phosphate Agar (used for recovery of injured cells).

Results and Discussion

Treatment of frankfurters/Polish sausages with 1% CPC and 1% CPC followed by water wash resulted in reductions of 1.19 and 2.39 log CFU/gram of *L. monocytogenes*, respectively, from initial levels of 8.20 log CFU/gram when enumerated on Modified Oxford Agar. Increasing the spray pressure of CPC from 20 psi to 35 psi, or extending the time of exposure from 30 seconds to as long as 60 seconds did not result in additional reductions ($P>0.05$) of *L. monocytogenes*. Similarly, spray temperature did not affect numbers of *L. monocytogenes*.

The main objective of this study was to optimize parameters of application of CPC onto the surface of ready-to-eat meat products like frankfurters. Exposure time, spray pressure, and temperature of spray used in this study did not influence reduction of *L. monocytogenes*. However, the effectiveness of 1% CPC against *L. monocytogenes* and its potential use as an antimicrobial rinse on frankfurters was documented.

Because no difference ($P>0.05$) was observed between treatment parameters; 20 psi, 77°F, and 30 seconds of exposure to CPC was selected for treating the product for shelf life studies. These treatments with 1% CPC did not affect the hardness of the frankfurters stored for 6 weeks.