ANTIMICROBIAL ACTIVITY OF CETYLPYRIDINIUM CHLORIDE AGAINST LISTERIA MONOCYTOGENES IN READY-TO-EAT MEATS

Principal Investigators:

Harshavardhan Thippareddi, Ph.D.   hthippareddi2@unl.edu
Randall K. Phebus, Ph.D.   rphebus@oznet.ksu.edu
James L. Marsden, Ph.D.   jmarsden@oznet.ksu.edu
Manpreet Singh, GRA   manpreet@iastate.edu

Submitted To:

American Meat Institute Foundation
Washington, DC

2002

Submitted By:

Department of Animal Sciences & Industry
Kansas State University
Manhattan, KS 66506

ABSTRACT

Listeria monocytogenes has emerged as an important foodborne pathogen in the past decade resulting in a high rate of hospitalization and the highest fatality rate of all foodborne illnesses. The effects of cetylpyridinium chloride (CPC) on the inhibition and reduction of viable *L. monocytogenes* cells were studied in addition to effects on aerobic bacterial populations (APC), lactic acid bacteria (LAB), yeasts and molds, total coliforms, and *E. coli*. Frankfurters were inoculated with *L. monocytogenes* and sprayed with 1% CPC and CPC followed by a water spray treatment. Treatments were applied to the frankfurters using a spray cabinet with variable chemical spray parameters (25, 40, and 55 ºC spray temperatures; 20, 25, and 35 psi spray pressures; and 30, 40, and 60 s time of exposure). No differences ($p > 0.05$) were observed between the different chemical spray parameters. A 2.5 $\log_{10}$ CFU/ g reduction of *L. monocytogenes* was achieved by 1% CPC concentration.

Bacteriostatic effects of 1% CPC were observed as a result of inhibition ($p \leq 0.05$) of growth of *L. monocytogenes* on surfaces of frankfurters, polish sausages, and roast beef stored for up to 42 days at 0 ºC and 4 ºC. Similar effects were observed for APC, LAB, yeasts and molds, total coliforms, and *E. coli*. Spray treatment (1% CPC) did not ($p > 0.05$) affect the color (L^*, a^*, and b^*- values) of ready to eat meat products as a result of up to 42 days of storage at 0 ºC and 4 ºC. Hardness of 1% CPC treated frankfurters was significantly ($p \leq 0.05$) lower than non-treated frankfurters, but no effect ($p > 0.05$) of treatment was observed on the hardness of polish sausages and roast beef stored for 42 days at 0 ºC and 4 ºC.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

INTRODUCTION

Listeria monocytogenes has emerged as one of the most important and deadly foodborne pathogens resulting in a high rate of hospitalization (88%) and the highest fatality rate (20%) of all foodborne illnesses (Johnson et al., 1990; CDC, 1997). Although initial outbreaks of listeriosis were linked to consumption of coleslaw, raw vegetables, milk and Mexican-style cheese, the consumption of undercooked chicken and uncooked frankfurters has been strongly linked epidemiologically to an increased risk of listeriosis (Schwartz et al., 1988).

Trends in the food industry have been towards convenient, refrigerated foods with extended shelf life and consumer concerns about cholesterol, saturated fat, total calories, and sodium chloride have prompted food processors to modify product formulations to satisfy the demands of health conscious consumers. Although such foods may be readily accepted and greatly appreciated by consumers, the recent resurgence of *L. monocytogenes* as a processed meatborne pathogen has been attributed to processing techniques and ingredient changes that may have contributed to its higher incidence and survival in ready-to-eat (RTE) meat products (Borchert, 1999).

Thermal processing protocols for the manufacture of RTE meat and poultry products must be scientifically validated to completely eliminate *L. monocytogenes* under USDA HACCP regulations. However, the presence of this pathogen in processed meats at high frequency, the ubiquitous presence of this organism in the processing plant environment, and the high rate of asymptomatic human carriers suggests that the key issue with safety of cooked RTE meat and poultry products is post-process recontamination mainly during peeling, slicing, and packaging operations. This post-

1

process recontamination in RTE meat products is of great consequence, as these products are not routinely subjected to heating and further cooking at the point of consumption. This recontamination typically occurs infrequently and at very low levels. These very low *Listeria* levels however, can grow to problematic levels since *Listeria* is psychrotrophic and these RTE products have an extended shelf life.

Widespread distribution of these foods make it a more formidable problem as the foods are put into national distribution channels and may experience slight temperature fluctuations at any or all stages from production to consumption. Thus, precooked refrigerated food products that are free of microbial inhibitors and vacuum packaged present serious challenges to the food processors. It is necessary for food processors to build in as many barriers against potential pathogens, especially *L. monocytogenes*, as possible.

Although meat processors must operate under a Hazard Analysis Critical Control Point (HACCP) program to reduce, control and/or eliminate foodborne pathogens in meat products, it is essential to provide new and validated pathogen control systems to ensure the safety of these products. Approaches to ensure that RTE meats are free of *L. monocytogenes* are either to aseptically process and package these products under strict sanitation and/ or to surface pasteurize the unsliced meat products (in packages) to eliminate the pathogen.

Since the early 1980's, the predominant mindset by industry has been to achieve *Listeria* control by high-level plant sanitation. Obviously, this approach is crucial, however, the continued abundance of RTE meat and poultry recalls and disease outbreaks

2

indicate that sanitation alone is not sufficient to ensure complete absence of *L. monocytogenes* in these products.

In-package (post-process) pasteurization of RTE meat and poultry products is likely the only approach to systematically ensure the safety of such products. Such an approach requires scientific validation of the technology effectiveness. Currently, consideration is being given to thermal and chemical treatments as post-process pasteurization technologies, along with high pressure and irradiation. Thermal treatments should be designed carefully to attain a level of safety that not only defines the limits of pathogen reduction but also understands the potential of pathogen outgrowth in the RTE meat products during normal storage. Although heat has been traditionally relied upon to provide microbiological safety, its use for re-pasteurization of packaged RTE meat products can be limited due to adverse quality effects such as purge, color, and texture changes. Thus, it is necessary to evaluate other antimicrobial decontamination technologies that can provide a similar degree of microbiological safety for RTE meats.

Chemical agents that have been evaluated include organic acids, bioactive preservatives, and bacteriocins. Organic acids, their salts, and other agents that are commonly used in the manufacture of RTE meat products may not need regulatory approvals as they are traditional ingredients and most are listed as generally recognized as safe (GRAS). While these organic acids have the advantage of lower cost, they may not be suitable in some applications due to the low pH effects on product quality. Also, organic acids have been generally shown to effect only moderate reductions in pathogens on meat products.

Compounds with antilisterial properties that have no adverse effects on the organoleptic quality would be optimal for application to RTE meat products. Cetylpyridinium chloride (CPC) has been evaluated for its antimicrobial activity against the foodborne pathogens *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria* spp., and *Campylobacter* spp. and was found to have excellent antimicrobial activity in a variety of food matrices. CPC is a member of the quaternary ammonium (QAC) family of compounds and is an active ingredient in mouthwashes. Breen et al. (1995) evaluated the use of QACs, including CPC, and concluded that it is effective against *S. typhimurium*. Thus, CPC could potentially be used as a decontaminant in RTE meat products to reduce/ eliminate recontamination during slicing and packaging operations. At this time, CPC is not approved for use as meat decontaminant, however, it is being reviewed by the Food and Drug Administration (FDA).

LITERATURE REVIEW

Listeria spp. are gram positive, nonsporeforming, facultative anaerobic rods that are motile when cultured at 20 - 25 °C. While the optimal temperatures for growth are between 30 and 37 °C, *Listeria* are capable of growth over a temperature range of – 0.4 to 50 °C, making the organism a potential food safety concern in refrigerated foods (Johnson et al., 1990). It is catalase positive and oxidase negative and expresses a β-hemolysin, which produces zones of clearing on blood agar (Farber and Peterkins, 1991). Because *Listeria* can grow at low temperatures, it is classified as a psychrotroph (Petran and Zottola, 1989).

L. monocytogenes is ubiquitous in nature and is often found in plants, soil, and surface water. It has also been isolated from silage, sewage, slaughterhouse water, milk from normal and mastitic cows, and human and animal fecal matter (Farber and Peterkins, 1991). *L. monocytogenes* has been shown to occur in domestic animals intended for human consumption, raising the concern that fresh meats and poultry may also be contaminated with the organism, and hence a potential causative agent for human infections (Shelef, 1989). *L. monocytogenes* has been isolated from more than 35 mammalian species and at least 18 avian species including domestic chickens (Harrison and Carpenter, 1989). Since this organism is ubiquitous, psychrotrophic, and may be able to survive some degree of heating, *Listeria* may contaminate, survive, and proliferate on improperly processed products (Harrison and Carpenter, 1989).

Importance of *Listeria monocytogenes* in Meat Products

Although *Listeria monocytogenes* has emerged as one of the most important and deadly foodborne pathogens that results in a high rate of hospitalization and fatality

(Johnson et al., 1990; CDC, 1997), most of the cases of human listeriosis appear to be sporadic. It is estimated that there are approximately 1700 cases of listeriosis every year although the percentage of these cases transmitted by foods is unknown (Dickson, 1989). The source and route of infection are usually unknown, however, the recent association of *L. monocytogenes* with several large foodborne outbreaks suggests contaminated food may be the primary source of the organism. *L. monocytogenes* may be a common contaminant of meat and meat products, but little has been published about its incidence in these foods (Johnson et al., 1988). The majority of human cases of listeriosis occur in individuals who have an underlying condition which leads to suppression of their T-cell mediated immunity (Farber and Peterkins, 1991).

According to Johnson et al. (1988), because of the numerous opportunities for meat to become contaminated with listeriae and the psychrotrophic nature of this organism, knowledge of the fate of *L. monocytogenes* in a meat product such as ground beef is important. Brackett (1988), at that time, stated that although *L. monocytogenes* was occasionally found in foods in the past, it is only within the past few years that it has fully become established as a foodborne pathogen. There are, however, instances in which apparently normal healthy individuals have become ill with listeriosis in both foodborne epidemics and sporadic cases (Farber and Peterkins, 1991). The incidence of listeriosis appears to be on the increase worldwide, with number of cases rising especially in Europe. The annual endemic disease rate varies from 2 to 15 cases per million population (Farber and Peterkins, 1991). In a review by Cassiday and Brackett (1989) it was reported that within the past decade four outbreaks of human listeriosis in North America involving contaminated foods resulted in mortality rates of up to 33%. Ahamad

and Marth (1989) reported that confirmed outbreaks of human listeriosis have been associated with the consumption of contaminated foods from plant and animal sources. Dairy foods have received the most scrutiny as a vehicle for listeriosis because of several outbreaks and *L. monocytogenes* contamination has been reported in both pasteurized and raw milk (Brackett, 1988). It was further indicated that the abilities of *L. monocytogenes* to proliferate at refrigeration temperatures and hence contaminate refrigerated food could cause a significant health hazard in humans (Brackett, 1988). The virulence of *L. monocytogenes* is considered multifactorial because of its capacity for intracellular growth and production of iron compounds, catalase and superoxide dismutase, and hemolysins (Farber and Peterkins, 1991). Shelef (1989) in an independent study suggested that the presence of iron ions enhances the growth of *L. monocytogenes.*

It was further reported that temperature could affect the virulence of *L. monocytogenes*. At a reduced temperature (4 °C), an increase in virulence was observed in intravenously inoculated mice (Farber and Peterkins, 1991). Its ability to survive at refrigeration temperatures, where a mean generation time of 1.5 days was reported for several foods, raises the concern that foods may act as potential sources of infection (Shelef, 1989). Glass and Doyle (1989) at the time, reported that no outbreaks of listeriosis have been associated with the consumption of meat or poultry products, although a recent report of a population based case control study of risk factors for sporadic listeriosis suggested there is an epidemiological association between eating either uncooked hotdogs or undercooked chicken and human listeriosis. Foodborne 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 has been implicated in listeriosis in an immuno-compromised woman (Johnson et al., 1990). This was apparently the first documented case of foodborne transmission of *L. monocytogenes* by meat products. Additionally consumption of undercooked chicken and uncooked frankfurters has been epidemiologically linked to an increased risk of listeriosis (Johnson et al., 1990). More recently, outbreaks have been associated with contamination of RTE meat and poultry products. These outbreaks include the Sara Lee (Bilmar Foods) and Pilgrim's pride incidences in 1998 and 2002 respectively.

Zaika et al., in 1990 reported that listeriosis has been linked to the consumption of uncooked frankfurters and undercooked chicken, while a direct link between consumption of turkey frankfurters and listeriosis was reported as well. Frankfurters, having a moderate risk of post processing contamination and growth of *L. monocytogenes* during extended shelf life, are relatively low risk products if they are properly reheated, however, if they are not reheated the frankfurters are considered a high risk product (Glass et al., 2001). Wederquist et al. (1994) reported the death of a cancer patient after developing listerial meningitis, and the source of the *L. monocytogenes* was turkey frankfurters. It was also indicated in the same study that the same strain and isoenzyme type of *L. monocytogenes* was found in unopened packages of the same brand of frankfurters at a nearby retail store. Wang and Muriana (1993) further asserted concern especially in regards to frankfurters because of the common practice of eating nonreheated franks among children as well as adults, and the proclivity of frankfurters as "picnic" foods, which may likely undergo a period of temperature abuse. Johnson et al. (1990) had reported in a review that the consumption of *L. monocytogenes* contaminated

turkey frankfurters has been implicated in listeriosis in an immunocompromised woman, while consumption of undercooked chicken and uncooked frankfurters has been epidemiologically linked to an increased risk of listeriosis. It was also suggested in the same review that there is a tentative evidence of red meat linked to listeriosis.

Wederquist et al. (1994) indicated that processed meats with pH ≥ 6.0 enabled more growth of *L. monocytogenes* than meat products with a lower pH. It was further suggested that turkey and bologna of pH > 6.0 may provide enhanced potential for the survival and growth of *L. monocytogenes*. According to McKellar et al. (1994), thermal processing used in the manufacture of wieners should be sufficient to ensure the destruction of contaminating *L. monocytogenes*, however, recontamination may occur during the peeling and packaging stages. Harrison and Carpenter (1989) demonstrated survival of *L. monocytogenes* on thermally processed poultry cooked by a dry heat method. McKellar et al. (1994) further emphasized the capability of *L. monocytogenes* to grow on vacuum packaged, refrigerated, and ready to eat meat products.

Shelef (1989) indicated that on an average *L. monocytogenes* contamination occurred in 25% of the meats and 47% of the poultry samples tested. Shelef (1989) also suggested the presence of *Listeria* spp. in fresh meats is generally thought to indicate post-slaughter contamination. Harrison and Carpenter (1989) indicated that many animals that enter the abattoirs are either infected or contaminated with foodborne pathogens and further spread of contamination occurs during processing. Farber et al. (1989) conducted a survey of various foods for the presences of *Listeria* spp. and indicated that 30-40% of the raw minced meat samples, 60% of the raw poultry samples investigated, and 20% of dry-cured fermented sausage samples were positive for *L. monocytogenes*. It was cited by

Zaika et al. (1990) that the incidence in ground beef varied from 28% to 58%, 26% of the frozen minced beef steak samples had *L. monocytogenes*, whereas 22% of the ready to eat delicatessen products and 10% of the delicatessen products to be consumed after cooking were positive for *L. monocytogenes*.

Since 1989, the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) has conducted finished product testing for *L. monocytogenes* in several ready to eat meat and poultry product categories. Table 1 shows the results of the studies carried out by FSIS.

Table 1: *Listeria monocytogenes* in Food Safety and Inspection Service ready to eat sampling programs, 1989 to present (USDA-FSIS, 1999).

Product Category	Listeria monocytogenes		
	Tested	**Positive**	**Percent positive**
Jerky	575	4	0.7
Large Diameter Sausages	3099	51	1.6
Cooked Uncured Poultry	6055	148	2.4
Roast/ Corned/ Cooked Beef	4900	150	3.1
Salads and Spreads	3619	124	3.4
Small Diameter Sausages	4980	219	4.4
Sliced Ham/ Luncheon Meats	1360	78	5.7

Carcass contamination with *Listeria* spp. has long been attributed to fecal matter, but animal hides, human contact, and cutting and processing equipment may also be sources of listeriae (Johnson et al., 1990). Harrison and Carpenter (1989) further emphasized the recovery of *L. monocytogenes* from effluents of abattoirs and poultry

packaging plants, and raw and treated sewage. *Listeria* contamination of raw meats used to manufacture cured meat products makes it necessary to examine the effects of processing steps, such as salting, curing, heating, and drying employed in the manufacture of cured meat products (Zaika et al., 1990). Stringer et al. (1969) investigating the microbial profiles of fresh beef indicated that the growth of microbes on meat is one of the main factors that cause discoloration and spoilage. Shelef (1989) showed that the *L. monocytogenes* could be isolated for 15-20 d at 4 °C and 8 °C from minced and sausage meats, but could not be enumerated because of the difficulty in separating the organism from the background microflora.

Brackett (1987) indicated that approximately 70% of ground beef, 43% of pork sausage, and 48% of poultry was contaminated with *L. monocytogenes.* Brackett (1987) further reported that contamination might occur in processed meats such as fermented sausages, although the percentages of contamination appear to be lower than in fresh meats. Farber et al. (1989) reported that *L. monocytogenes* in fermented sausage products varied between 5-23% of the product examined. Johnson et al. (1990) indicated that the prevalence of listeriae in ground products and other products requiring cooking before consumption ranges from 8 to 92%, which is higher than that reported for fresh meat cuts suggesting additional processing steps and human contact contributed to the number of listeriae present in the final product. Johnson et al. (1990) in a review of the presence of *Listeria* spp. in meat products indicated 13-50% of the products sampled in Europe and Canada might be contaminated with *Listeria* spp. with about a third of the ready to eat products contaminated with *L. monocytogenes*. Johnson et al. (1990) reported that *Listeria* spp. were isolated from the interior cores of 3 of 50 beef roasts and 3 of 50 pork

roasts and suggested that environmental contamination during sampling was an unlikely source as the outer portions of the roasts were flamed and aseptically trimmed off. Johnson et al. (1990) further indicated that *L. monocytogenes* was isolated from 4.5% of the roasts in this study, with levels of ≤ 10 CFU/ g.

The isolation of *L. monocytogenes* from luncheon meats and hot dogs has lead many companies to recall products (Bernard and Scott, 1999). Moreover, both the number and magnitude of recalls of foods due to contamination with *L. monocytogenes* have risen dramatically in the last few years. In 1999 alone (Porto et al, 2002) the U.S. Department of Agriculture Food Safety Inspection Service issued 62 recalls of cooked meats, and 31 of these recalls were due to *L. monocytogenes*. Far less information, however, is available on the sources and levels of contamination in ready to eat foods, including frankfurters. Glass and Doyle (1989) determined that *L. monocytogenes*, when initially present at $>10^3$ CFU/g of sausage batter, can survive during the fermentation, drying, and refrigerated storage of hard salami, but at reduced levels. Glass and Doyle (1989) also showed that the initial invasion of microbes occurs at slaughter and continues throughout the various processes related to preparing the meat for consumption. In a study related to retail processing and packaging done by Stringer et al. (1969), it was reported that the meat trays, cooler walls, band saws, slicers, blocks, and knives were all highly contaminated. According to Kotula et al. (1975), the microbiological population on the surface of meat, prior to breaking, is as important as the sanitation procedures used during the fabrication of retail cuts of meat and meat products.

Glass and Doyle (1989) reported that *L. monocytogenes* can grow at temperatures of 4.4 °C, while the rate of growth depended largely on the pH and type of product. The

authors reported that luncheon meats sliced at delicatessens support a high growth rate of *Listeria* spp. during storage, but their relatively short shelf life decreases the risk of contamination. Sorrels et al. (1989) stated that refrigerated storage, however, is no guarantee of protection against growth of *L. monocytogenes* since it can grow at refrigeration temperatures. Grau and Vanderlinde (1990) reported that *L. monocytogenes* growth was minimal on chilled, raw vacuum packaged meat. Although growth was possible on sterile lamb at 8 °C, they observed no growth on sterile beef minced during 17 days of storage at this temperature. Post-process contamination of ready to eat products with *L. monocytogenes* has been identified as a hazard Johnson et al. (1990). Glass and Doyle (1989) observed growth of *L. monocytogenes* at 4.4 °C on ham, bologna, sliced chicken and turkey products, wieners, and fresh bratwurst, but little or no growth on summer sausage or roast beef. It was further shown by Samelis (2002) that the single use of antimicrobials like sodium lactate, sodium acetate, and sodium diacetate up to a concentration of 2% in meat products provided inhibition of surface inoculated (3 to 4 log CFU/ cm^2) *L. monocytogenes* ranging widely from 20 to 70 days between treatments of vacuum packaged frankfurters stored at 4 °C.

Bacterial contamination during poultry processing may occur at several processing steps, including scalding, picking, evisceration, and chilling which has lead to the proposal of using chemical or water spraying to control the contamination during processing (Xiong et al., 1998). The growth of bacteria in meats and meat products is controlled by the interaction of a number of environmental and nutritional parameters including temperature, pH, oxygen content, sodium chloride concentration, sodium nitrite concentration etc. (Buchanan et al., 1989). Grau and Vanderlinde (1992) reported a high

prevalence of listeriae on ready to eat meats obtained from retail stores, and, in addition a significant percentage of these meat samples contained more than 1000 CFU of *L. monocytogenes* per gram. Although the growth of *L. monocytogenes* in ready to eat meats has been previously described, uncured products such as sliced poultry products supports the most rapid growth and *Listeria*'s ability to grow on retail wieners varies considerably with reduction of pH, growth of competitive lactic acid bacteria, and the degree to which inhibitory smoke components are present (Glass et al., 2001). Petran and Zottola (1989) reported that *Listeria* will grow over a pH range of 5.5 to 9.6, but further investigations have proved the ability of the microorganism to demonstrate limited growth at pH values of 5.0 to 5.1 in cheddar cheese. Efforts to control *L. monocytogenes* contamination in raw and ready to eat foods have included low dose irradiation, modified atmosphere packaging in carbon dioxide, treatment with monoglycerides, inoculating foods with bacteriocin producing competitor strains, and organic acids or their salts (Farid et al., 1998).

Antimicrobial Agents in Food Systems

Meat and poultry processors are actively looking for reasonable interventions that minimize the risk of bacterial pathogens to processed meats from contaminated raw meat ingredients. The ability of *L. monocytogenes* to survive and grow in vacuum packaged, ready to eat meat and poultry products such as turkey frankfurters and bologna is of great concern to the food industry. Decontamination of carcasses with organic acids and other chemical sanitizers has been extensively investigated (Dorsa et al., 1995). One way to combat the problem of unsafe foods due to bacteria, including *L. monocytogenes*, is the use of antimicrobial washes or ingredients. While studies have shown the effectiveness of

14

hot water in significantly reducing aerobic bacterial counts, none have addressed the effects of various contamination levels found on carcass or efficacy of commercial spray cabinet application methods (Dorsa et al., 1995).

As development of effective chemical methods of food preservation has become critically important, the number of antimicrobial chemical preservatives approved for use in foods is limited due to low solubility and the deleterious effects on food sensory properties (Podolak et al., 1996). It has been indicated by Wederquist et al. (1994), that the incorporation of chemical preservatives in meat products may be important for control of *L. monocytogenes* when used in combination with other processing techniques. Several organic acids (acetic, lactic, citric, and propionic) have been used as antimicrobial agents in foods production (Timm et al., 2000). Podolak et al. (1996) proposed that among the chemicals used in long-term preservation of food, fumaric acid should be considered because of its unique advantages of cost effectiveness, strength, nontoxicity, and natural presence in fresh meats. Several studies have reported the efficacy of various washing procedures to reduce the microbial surface counts on carcasses (Johnson et al., 1979). The authors further stated that the benefit of these carcass-washing treatments is that they reduce the total bacterial counts on carcasses and in ground beef prepared from such carcasses. A reduction in the number of microorganisms is observed, a characteristic often viewed as an enhancement of quality and safety with an added benefit of continued antimicrobial action after acid treatment resulting in shelf life extension for these foods (Farid et al., 1998). Of the several acids (acetic, lactic, citric, and hydrochlorous acids) used to lower the pH of brain heart

infusion broth before using it as the growth medium for *L. monocytogenes* strains, acetic acid was the most effective growth inhibitor (Farber and Peterkin, 1991).

Okrend et al. (1986) attempted to reduce cross contamination in poultry scald water, keeping in view the constraints of safety, cost, and availability as well as effectiveness. It was further ascertained by Brackett (1987) that the use of chlorine dips or spray sometimes is an effective measure for controlling bacterial contamination of fruits and vegetables. Okrend et al. (1986) in their study had reviewed that acetic acid is a GRAS compound which fits the first and foremost requirement of safety of the meats and has historical use for controlling bacteria. Studies in 1997 by Bell et al., showed that mechanical spray washing of carcasses was not considered by the meat industry until 1981 when Tarpoff and Swientek focused on the savings it could generate in labor and time. Ahamad and Marth (1989) stated that although *L. monocytogenes* is inhibited by acetic, citric, and lactic acids, it would be difficult to extrapolate how the organism would behave in a food product or an environmental situation that might exist in a food-processing factory.

Studies by Conner et al. (1990) indicated that *L. monocytogenes* was more tolerant to acidic conditions than previously believed and demonstrated that *L. monocytogenes* grew in cabbage juice at pH 5.0 but died off at pH ≤4.6. They further ascertained that the growth of *L. monocytogenes* may be influenced not only by pH but other factors such as temperature and salt levels. According to Pickett and Murano (1996) *L. monocytogenes* has been isolated from raw and cooked products, as well as, from food contact surfaces in processing plants and the attachment of the cells to surfaces such as stainless steel, plastics, and rubber gaskets occurs by production of polysaccharides that

actually protect the organism from treatments with chemical sanitizers. Studies since the approval of acids as sanitizers have indicated that acetic acid is effective against *E. coli*, *L. monocytogenes*, and *S. typhimurium*, three organisms that are of major concern in the beef industry (Bell et al., 1997). However, Brackett (1987) recommended the use of solutions containing 60-80 mg of hypochlorite/ L to remove enteric pathogens from surfaces of fruits and vegetables, however, the effectiveness against *L. monocytogenes* was unknown. Kotula et al. (1975), in their study regarding the variability in the microbiological counts on beef carcasses, furnished a substantive description of a low microbial count on carcasses, and reported their research on the use of acids, steam, stannous chloride, and hydrogen peroxide for reducing bacterial numbers. According to Dickson (1988), a variety of methods like proper slaughter and dressing procedures in combination with good sanitation are effective in preventing initial contamination of carcasses with pathogens like *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Listeria*, while other researchers have investigated the use of washes or sprays to remove or destroy surface contamination on the animal carcasses.

Post processing application of antimicrobials may be more advantageous than their addition in the formulation, as the active compound is applied directly onto the product surface where contaminating *L. monocytogenes* cells usually attach following cooking and during slicing and packaging (Samelis et al., 2001). El-Shenawy and Marth (1989) evaluated inhibition/ inactivation of *L. monocytogenes* by sodium benzoate together with some organic acids. The authors reported that acetic acid and lactic acid in combination with sorbic acid inactivated *Salmonella typhimurium* in nutrient broth and *L. monocytogenes* in cheese. They further stated that one approach to control *L.*

monocytogenes is to employ acceptable food preservatives either singly or in combinations. Johnson et al. (1979) reported that the antibacterial action of organic acids such as acetic, tartaric, lactic, or citric is related to pH and the degree of dissociation. The authors stated that undissociated organic acids are more readily transported across the bacterial cell membrane, and therefore are more bactericidal than in the dissociated form. Timm et al. (2000) further stated that the use of peracetic acid (PAA) is very common because of its very strong oxidizing properties, which kill bacteria, fungi, yeasts, viruses, and spores. Conner et al. (1990) reported that the antimicrobial activity of many acids is attributed to the undissociated form of the acid molecule. Further studies by El-Shenawy and Marth (1989) helped to deduce that the inactivation, inhibition, and/ or growth of *L. monocytogenes* was affected by incubation temperature, pH, concentration of the preservatives, and the type of organic acid used. Buchanan et al. (1993) cited that acidic environments (pH < 5.4) do not support growth of *L. monocytogenes* and the organisms are inactivated. They further stated that the inhibition or inactivation of *L. monocytogenes* is enhanced when organic acids are used as acidulants.

Brackett (1987) studied antimicrobial effects of chlorine and found that the length of time to which *L. monocytogenes* is exposed to chlorine does not have a significant effect on survival of cells. Sorrells et al. (1989) found that acidity and/ or pH is an important preservative factor in many foods, either alone or in combination with other microbial barriers, such as refrigerated storage. Wang and Muriana (1993) in their study about the incidence of *L. monocytogenes* in packages of retail franks examined the exudates from vacuum packaged wieners for growth of *L. monocytogenes* and implicated those exudates with lower pH values and higher phenolic concentrations were most

inhibitory towards *L. monocytogenes*. Wang and Muriana (1993) further determined that the isoeugenol, a component of liquid smoke, and acetic acid were both effective and synergistic at inhibiting *L. monocytogenes*. Brackett (1987) showed that the sensitivity of *L. monocytogenes* to chlorine compounds was similar to other non-sporulating bacteria. It was also indicated that chlorine compounds were very effective for killing a wide variety of microorganisms. Dickson (1988) stated that high pressure washing with only water (no sanitizer) has been found to reduce the total aerobic and *Enterobacteriaceae* counts on beef by approximately 1 to 1.5 log cycles respectively. Further in this study it was said that the sanitizing compounds that have been evaluated include chlorine and organic acids, such as acetic and lactic, which demonstrated bactericidal effects on the microflora of meat. Wash treatments with sodium chloride (NaCl), potassium hydroxide (KOH), and sodium hydroxide (NaOH) with varying concentrations have been evaluated for reduction of *L. monocytogenes* on surfaces of meats and no significant differences between these compounds at different inoculum levels was reported (Dickson, 1988).

Several chemical or physical methods to reduce microorganisms in meat products have been proposed including ozone, trisodium phosphate, lactic acid, chlorine dioxide, hydrogen peroxide, and electrical stimulation (Breen et al., 1997). Xiong et al. (1998) reported that an increase in spray pressure for application of antimicrobials reduced the microflora on beef carcass surfaces, but was less effective as the increasing pressure forced the bacteria to physically penetrate into the tissues of the carcasses. It has been indicated that while research on the addition of lactates, acetates, and other chemicals to the formulation is available, the effectiveness of these preservatives or other antimicrobial compounds applied as dipping or spraying solutions has yet to be addressed

in the meat industry (Samelis et al., 2001). Xiong et al. (1998) showed that the exposure to the chemical spray, including spray time and setting time is an important factor for reducing bacteria on poultry carcasses. Breen et al. (1997) in the same study stated that such methods have been effective in a limited manner to reduce the microbial contamination and may affect the physical appearance of the meat products. Bernard and Scott (1999) further asserted that the extensive efforts to control *L. monocytogenes* can reduce the level of contamination, but it has not been possible to eradicate it from the processing environment or from all finished products. Studies by Wederquist et al. (1994) suggested that sodium acetate extended vacuum packaged beef steak shelf life in combination with other additives, while sodium bicarbonate was reported to have antimicrobial properties against pathogenic periodontal bacteria in the same study. Bedie et al. (2001) indicated that the antimicrobial effects of additives like acetates, lactates, and other chemical compounds, alone or in combination and in the presence of other antimicrobials, against *L. monocytogenes* depend on processing (pH, water activity, moisture, fat, nitrite, and salt content of the product) and storage (temperature and packaging atmosphere) conditions.

Samelis et al. (2001) stated that because of the non-approval for use of certain emerging technologies such as irradiation on packaged ready to eat meat products, interest in the incorporation of generally recognized as safe chemical (lactates, acetates, and sorbates) or biological (bacteriocins) antimicrobial compounds as safety barriers has been renewed. According to a recent study by Bedie et al. (2001), it was emphasized that the interest in use of lactates, mainly sodium lactate in its commercially available liquid (60% wt/ wt) form, and sodium acetate as additives in processed meat formulations is

associated with their potential to inhibit spoilage or pathogenic bacteria, especially *L. monocytogenes*. In a recent study by Samelis et al. (2002), it was emphasized that additional treatments, such as spraying or dipping of products in antimicrobial solutions before packaging and post packaging thermal pasteurization can be combined to enhance the effectiveness of chemical additives. Islam et al. (2002) indicated that application of certain antimicrobial chemicals to processed meat surfaces could provide a safeguard against contamination by *L. monocytogenes*. It was proposed in the same study that sodium benzoate is a generally recognized as safe (GRAS) preservative with antimicrobial properties that involve interference with the permeability of the microbial cell membrane. GRAS chemicals are exempted from the Food and Drug Administration's testing and approval process, however, the Food Safety and Inspection Service of the U.S. Department of Agriculture must approve any additive for specific use and specify acceptable levels in meat and poultry products.

Cetyl Pyridinium Chloride as an Antimicrobial Agent

Another antimicrobial being evaluated widely as a sanitizer/ disinfectant is cetylpyridinium Chloride (CPC). CPC is an antimicrobial, commercially known by the name of CECURE (Safe Foods Corporation, North Little Rock, Arkansas). CECURE is a 40% concentrate of the active ingredient CPC, which is an active ingredient in mouthwashes (Breen et al., 1995).

Structure of Cetyl Pyridinium Chloride

Cetyl pyridinium chloride is a quaternary ammonium compound (QAC), which can be classified as a heteroaromatic ammonium salt, that has one long-chain alkyl group

and the remaining is an aromatic system such that the quaternary nitrogen is part of an aromatic system like pyridine in the case of CPC.

$$CH_3(CH_2)_{14}CH_2 - N^{\oplus} \quad Cl^{\ominus}$$
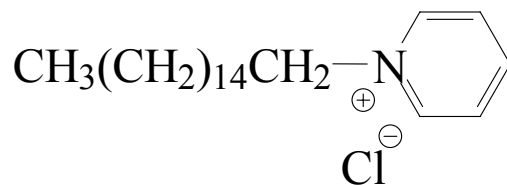
Figure 1: Structure of cetylpyridinium chloride (CPC)

The nitrogen plus the attached groups form the cation. The anion bound to nitrogen by an ionic bond is chloride. CPC is a water-soluble, colorless compound that has been used in toothpastes and throat lozenges (Cutter et al., 2000).

Mode of Action of Cetyl Pyridinium Chloride

The mechanism of action of CPC is similar to most QAC and the chemical properties of the cationic surfactants are directly related. These properties are reduction of surface tension, attraction to negatively charged materials such as bacterial proteins resulting in the destruction of bacteria, surface tension, solubility, and a denaturing effect on proteins (Petrocci, 1977). The possible modes of action of these compounds could be a direct effect on proteins, metabolic reactions, cell permeability, stimulatory effects on glycolysis reactions, and effects on enzymes, which maintain a dynamic cytoplasmic membrane (Cords, 1983). The most widely accepted mode of action is at the membrane of the cells, however, due to the large carbon chain its solubility in water is decreased hence causing a slightly lower sanitizing activity. The highest activity for QAC containing alkyl groups is in the range of C_{12}-C_{16}, with C_{14} being the maximum. The antimicrobial activity of QAC is directly affected by the time of contact with target

microorganisms, pH, temperature, and water hardness (Cords, 1983). CPC is a quaternary ammonium compound, which can be used in dipping or spraying to prevent attachment of *Salmonella* (Wang et al., 1997). CPC has been safely used for the past 30 years in oral hygiene products and has been shown to reduce *Salmonella* contamination on poultry tissues (Breen et al., 1995).

CPC is active and stable over a broad range of temperatures and is fairly tolerant to hard water. An added advantage of CPC is that it can form a residual antimicrobial film on surfaces leading to extended antimicrobial activity. The sanitizing activity of this compound is directly related to the length of the carbon side chain. Generally, the quaternary ammonium compounds adsorb to the bacterial cell surface, permeate and destroy the cell wall and cell membrane, and have a direct or indirect lethal effect on the cell. In the specific case of CPC and its closely related analogues, it has been shown that they interact strongly with the negatively charged surfaces, e.g., blastospores of *Candida albicans*, and their antimicrobial activity is related to their hydrophobicity (Cutter et al., 2000). In addition, CPC was also effective in preventing bacterial attachment, and thus having the potential to reduce the risk of cross-contamination, while not affecting the physical appearance of the poultry products (Breen et el., 1997). Results of this study showed that CPC could prevent and eliminate bacterial contamination. Nearly 5-log decontamination can be achieved by using CPC solutions for contact times as low as 3 minutes. In an investigation carried out by Kim et al. (1996), the effects of 0.1% CPC solution on *S. typhimurium* using both immersion and spraying treatments were evaluated, and 1.0 to 1.6 and 0.9 to 1.7 log of bacteria reductions were obtained respectively. Breen et al. (1995) showed that CPC was the only compound that produced

a notable reversal of attachment at a concentration as low as 10 μg/ ml. Xiong et al. (1998) reported the use of CPC spray on pre-chilled chicken carcasses with different setting times. A 0.1% CPC spray treatment at 138 kpa and 1 min. setting time reduced *Salmonella* by 0.7 logs, while reduction was increased to 1.1 log at 2 min. setting time and 1.7 log at 3 min. (Xiong et al., 1998). CPC affected a 1.9-log reduction of *Salmonella* spp. on chicken skins which was comparable to reductions associated with 10% trisodium phosphate (2.2 log) or 2% lactic acid (2.2 log) (Xiong et al., 1998). Breen et al. (1995) showed that some of the processes used for decontamination may adversely affect the appearance, color, flavor, and texture of the products, while some of the existing technologies are not completely effective in removing all attached microorganisms from poultry and meat tissues. In their study it was also stated that investigation of the effects of surface-active agents (surfactants) on bacterial attachment could provide information regarding more effective agents for removal of attached organisms or for prevention of attachment, as well as chemical probes for studying biochemical mechanisms.

MATERIALS AND METHODS

Preparation of Bacterial Cultures

A five-strain cocktail of *Listeria monocytogenes* [101M, 109, 108M, serotype 4c American Type Culture Collection (ATCC), and serotype 3 ATCC] was used. The cultures were maintained separately on Tryptic Soy Agar (TSA; Difco, Detroit, MI) slants at 4 °C. Transfers of the cultures were made every 30 days to maintain viability. The inoculum was prepared by first inoculating the cultures into 10 ml Tryptic Soy Broth (TSB; Difco, Detroit, MI) and incubating at 35 °C for 24 hours. Cultures (1 ml) were then transferred into 100 ml TSB contained in 250 ml centrifuge bottles and further incubated at 35 °C for 20 hours. These cultures were centrifuged at 15,600 x g for 10 min. at 4 °C (Beckman J2-21 M/E centrifuge, JA-14 rotor, Palo Alto, CA). The cell pellets were resuspended with 50 ml of 0.1% sterile peptone water (PW; Difco, Detroit, MI) and recentrifuged. The resultant pellet was resuspended with 10 ml of PW. A cocktail was prepared by mixing the five cultures in a sterile bottle to get a final volume of 50 ml of the inoculum.

Inoculation of Frankfurters and Polish Sausage

Frankfurters made of beef and pork ("8 in a pack" size) and Polish sausage made of beef, pork, and chicken ("16 in a pack" size) were obtained from a local grocery store and stored at 4 °C before removal from the packages. Prior to inoculation, the frankfurters and polish sausages were removed from packages and placed onto butcher paper. Each frank or sausage was individually dried with blotting paper. To avoid contamination while handling, one end of each frankfurter and polish sausage was wrapped with 2 in. wide parafilm (Laboratory film; American National Can™, Chicago, IL 60631). The

inoculum was sprayed onto the frankfurters and polish sausage by "misting" inside a "bio-containment" chamber. The products after inoculation were held for 30 min. in a sterile laminar flow cabinet (SterilGARD II [®], The Baker Company, Sanford, ME) to allow attachment of *L. monocytogenes* on the surface of the products. For the microbiological shelf life evaluation of reduction of *L. monocytogenes* over time, and qualitative analysis (general microbiological analysis, color and hardness), the products were inoculated at either high (7 to 8 $\log_{10}$ CFU/ g) or low (2 to 3 $\log_{10}$ CFU/ g) inoculum levels.

Application of Treatment on Frankfurters and Polish Sausage

According to the company recommendation regarding the concentration of cetylpyridinium chloride (CPC; CECURE[®], Safe Foods Corporation, North Little Rock, AR) to be used on the surfaces of food products a 1 % solution of the CPC was made by adding 25 ml of 40% concentrated CPC to 1000 ml of deionized (DI) water. The pH of the CPC solution was recorded using a pH meter (Model SA 520; Orion research Inc.). The average pH of the CPC was 7.0 and the temperature of water used to prepare the solution was 25 °C. A laboratory model spray washer (Figure 2; KSU, Manhattan, KS) was used to apply the treatments onto the product. The treatments were applied for 30, 40, and 60 seconds.

Three frankfurters were assigned to each treatment to test for reduction of *L. monocytogenes*. The treatments were; spray temperature (25, 40, and 55 °C), spray pressure (20, 25, and 35 psi), and time of exposure to CPC (30, 40, and 60 seconds). The two types of treatments for each set of parameter combinations were; 1) CPC only and 2) CPC followed by a water wash.

Figure 2: Picture of the spray wash cabinet used for the application of treatments onto frankfurters and Polish sausages

For the evaluation of efficacy of 1% CPC solution to reduce *L. monocytogenes* over time, sets of three frankfurters and/ or polish sausages constituted one sample. The inoculated product intended for microbiological shelf life evaluation was vacuum packed (Multivac, Kansas City, MO) in sets of three per package (8" x 10", 3 MIL standard barrier, nylon/ PE vacuum pouch) after treating them with 1% CPC at 20 psi, 25^0C, and 30 s time of exposure in a spray washer (using 1.6 L per min. at 20 psi). The treated frankfurters and polish sausages for shelf life evaluation were stored at 0 °C and 4 °C for 3, 7, 14, 21, 28, and 42 days.

Non-inoculated product were treated similarly as the inoculated product and stored for 3, 7, 14, 21, 28, and 42 days at 0 °C in a walk-in cooler and 4 °C in a retail

display cabinet simulating the conditions of light and temperature found in a retail store. General microbiological, color and hardness evaluations were made at each sampling time. For each treated sample a parallel control (non-treated) sample was also stored under similar conditions. The frankfurters and polish sausages were aseptically removed from their packages for microbial analysis to determine residual *L. monocytogenes* population. Shelf life samples were analyzed for *L. monocytogenes*, Aerobic Plate Counts (APCs), Total Coliform Counts (TCC), *Escherichia coli* Counts (ECC), Yeast and Mold Counts (YM), and Lactic Acid Bacteria Counts (LAB).

Color evaluation was performed using the Hunter D54 spectrophotometer (Ultra Scan SpecWare, version 1.20) to determine the lightness, redness, and yellowness (L^*, a^*, and b^* values) of frankfurters and polish sausages. A Texture Profile TA.TX2 Analyzer (TPA; Stable Micro Systems, Haslemere, England) was used to determine the hardness of the product. The texture analysis of frankfurters and Polish sausages was done by using a p-75 compression probe with 100 g force at a test speed of 2 mm/ s penetrating 20 mm into the surface of the products.

Inoculation of Roast Beef

Roast beef was obtained commercially and stored in a meat cooler at 32 °F. The roast beef was removed from the meat cooler, the casing aseptically removed and the roast beef sliced manually to provide 6.5 in. long, 4 in. wide, and 2 in. thick slices. Slices of roast beef were individually placed on a tray and mist inoculated in a bio-contaminant chamber. The roast beef slices were held for 30 min. to allow surface attachment of *L. monocytogenes* in a sterile laminar flow cabinet. For the microbiological shelf life evaluation of reduction of *L. monocytogenes* over time, roast beef was inoculated with

two levels of inoculum viz. high (7 to 8 $\log_{10}$ CFU/ cm^2) and low (2 to 3 $\log_{10}$ CFU/ cm^2), whereas noninoculated roast beef was used for the qualitative analysis (general microbiological analysis, color and hardness).

Application of Treatment on Roast Beef

The inoculated product was treated with a 1% CPC solution by dipping in a plastic bowl for one min. The slices were vacuum packaged in cryovac bags (Cryovac Sealed Air Corporation, Duncan, SC) and placed into both a 0 °C cooler and a 4 °C retail display case for 3, 7, 14, 21, 28, and 42 days to evaluate the antimicrobial effects of CPC on *L. monocytogenes*. For enumeration of general microbiological populations of *E. coli*, coliforms, lactic acid bacteria, total plate counts, and yeast and mold over time, and to evaluate the effect of 1% CPC treatment on the textural properties and color of the roast beef, non-inoculated product was treated and stored identically. Each treated sample had a corresponding control (non-treated) sample that was identically stored and analyzed. Color analysis of the surface and cut side of roast beef was done independently. A Hunter miniscan spectrophotometer (Model MS/ S-4000S; Hunter Associated Lab., Inc., Reston, VA) was used to determine the lightness, redness, and yellowness (L, a*, and b* values) of roast beef. A p/ 0.5S stainless steel spherical ½$^{"}$ diameter ball probe with a 100 g force and 1.7 mm/s test speed penetrating 8.9 mm into the surface of the product was used for the textural analysis of roast beef.

Microbial Sampling of the Products

Frankfurters: To determine reductions of *L. monocytogenes*, the treated sets of three frankfurters were removed from the spray cabinet and two frankfurters were placed into a sterile stomacher bag with 100 ml of pre-poured 0.1% sterile peptone diluent to make a

1:1 dilution. Before sampling, the parafilm wrapping from one end was aseptically removed. Each sample was homogenized in a stomacher (Tekmar Co., Cincinnati, OH) for two minutes. For the shelf life study both the treated and non-treated samples were vacuum packaged ("four in a pack"). The samples from the shelf life evaluation were aseptically removed from vacuum packages and 2 frankfurters were placed into a sterile stomacher bag with 100 ml of 0.1% sterile PW and homogenized in a Stomacher 400 lab blender for two minutes. The remaining two frankfurters from each treatment set of the low level inoculum were held in the cooler for subsequent enrichment procedures in the event that no residual *L. monocytogenes* was recovered by direct plating after CPC treatment.

Polish Sausage: Treated sets of three Polish sausages were removed from the spray cabinet and the parafilm wrapping from one end was aseptically removed. A single Polish sausage from each set was put into a sterile stomacher bag and diluted with 100 ml of 0.1% sterile peptone diluent to make a 1:1 dilution. Each diluted sample was then homogenized in a stomacher for two minutes. The samples for the shelf life evaluation were aseptically removed from vacuum packages and one Polish sausage was placed into a sterile stomacher bag with 100 ml of 0.1% sterile PW and homogenized in a Stomacher 400 lab blender for two minutes. The remaining 2 Polish sausages from each treatment set of the low level inoculum were put back into the cooler for enrichment procedures if necessary.

Roast Beef: The treated roast beef samples were taken out of the 1% CPC solution and cored. Two cores (8.5 cm^2) each were taken from the surface and cut side of the slice (to a depth of 0.5 cm) and put into a stomacher bag separately. The cores were diluted with

25 ml of 0.1% sterile PW and homogenized in a stomacher 400 lab blender for two minutes. The samples from the shelf life evaluation were aseptically removed from vacuum packages and cored identically. The cores from the surface and cut side were separately placed in a sterile stomacher bag with 25 ml of 0.1% sterile PW and homogenized for two minutes.

Microbiological Enumeration

Samples were serially diluted in sterile PW and spiral plated onto Modified Oxford Agar (MOX; Oxoid ltd., Basingstoke, Hampshire, England) and Tryptose Phosphate Agar (TPA; Difco, Detroit, MI) using a Whitley automatic spiral plater (Don Whitley Scientific ltd., Shipley, West Yorkshire, England). The colony forming units were enumerated manually using a spiral plate dark field Quebec colony counter (Model 330; American optical company, Buffalo, NY). The plates were incubated at 37 °C for 24 h. Counts were recorded as log Colony Forming Units per gram (log_{10} CFU/g) for the frankfurters and Polish sausages. The counts for the roast beef were reported as log_{10} CFU/ cm^2).

Five typical *L. monocytogenes* colonies from duplicate plates of MOX and TPA were isolated and confirmed as *L. monocytogenes* using Gram staining procedures, and a primary catalase test. Further verification was done using fermentation of glucose, xylose, mannitol, and reduction of nitrite. The presence or absence of *L. monocytogenes* in the products inoculated with low levels of inoculum (2 to 2.5 log_{10} CFU/ g) was done by *Listeria* enrichment procedures (FDA; Bacteriological Analytical Manual, 8th Ed., Revision A/ 1998) using modified *Listeria* enrichment broth (LEB; Difco, Detroit, MI). The LEB was incubated at 35 °C for 24-48 hrs. followed by streaking onto MOX agar.

The streaked MOX plates were then incubated at 35 °C for 24-48 hrs. Typical colonies of *L. monocytogenes* were then confirmed as stated earlier.

Aerobic plate count petrifilm^{TM} (APC; 3M, St. Paul, MN) was used for APCs, *E. coli* petrifilm^{TM} (ECC; 3M, St. Paul, MN) was used for *E. coli* and TCCs, Potato Dextrose Agar (PDA; Difco, Detroit, MI)) was used for YM evaluation, and deMan, Rogosa, and Sharpe agar (MRS; Difco, Detroit, MI) was used for the enumeration of LAB. The APC and ECC petrifilm^{TM} were incubated at 35 °C for 24-48 h. PDA was incubated at room temperature (25 °C) for 48 h, and MRS was incubated anaerobically in an anaerobic jar with an Anaerobic system envelope which contained Palladium catalyst (H_2 + CO_2) hydrated with 10 ml of distilled water (GasPak Plus^{TM}, Becton Dickinson Microbiology Systems, Sparks, MD) at 35 °C for 24-48 h.

<u>**Color and Hardness Evaluation of the Products**</u>

The non-inoculated frankfurters and Polish sausages were removed from their respective vacuum packages and evaluated firmness. Products at 4 °C were stored in a display case, which simulated lighting conditions of retail stores. The intensity of light used in the display case was 1070 lux and this was measured on weekly intervals. The samples were rotated at 3-day intervals for proper distribution of light on the surface of the products. The surface hardness and cut side hardness of roast beef were evaluated separately. A Texture Profile Analyzer (TPA; Stable Micro Systems, Haslemere, England) was used to determine the hardness of the product. The probes used for the evaluation of hardness were a p-75 compression probe for the frankfurters and Polish sausage, and p/ 0.5S stainless steel spherical ½" diameter ball probe for the roast beef. The size of the load cell used was a 5 Kg weight, which was penetrated into the surface

of the frankfurters and Polish sausages at 2 mm/ s up to a depth of 20 mm, whereas a test speed of 1.7 mm/ s up to a depth of 8.9 mm was used for roast beef.

Evaluation of color of the products was done while in the package. A Hunter Miniscan Spectrophotometer was used to evaluate the color of the surface and cut side of roast beef, whereas a Hunter Lab D54 Spectrophotometer was used for analysis of color of frankfurters and Polish sausage. The L, a*, and b* values were determined to evaluate the color of the products.

Experimental Design

A randomized block design of three temperatures, three spray pressures, and three times of exposure was used to evaluate the efficacy of 1% cetylpyridinium chloride (CPC) to reduce *L. monocytogenes* on surfaces of ready to eat meat products. A similar design was used to evaluate the efficacy of 1% CPC to inhibit or inactivate the growth of *L. monocytogenes*, aerobic plate count, lactic acid bacteria, *E. coli*, total coliforms, yeasts and mold, along with color and hardness of ready to eat meat products stored at 0 °C and 4 °C with repeated measures on days 0, 3, 7, 14, 21, 28, and 42. To compare the reductions of *L. monocytogenes* as a result of a 1% CPC or a 1% CPC followed by water treatment on frankfurters the data was analyzed using a three-way analysis of variance (ANOVA) using SAS PROC GLM procedure (SAS Institute, 1998). For the evaluation of efficacy of 1% CPC on the shelf life of the frankfurters, polish sausage, and roast beef analysis of variance was done using SAS PROC MIXED procedure and comparison of the LSmeans.

RESULTS AND DISCUSSION

I. **Effects of Spray Temperature, Spray Pressure, and Time of Exposure of 1% CPC on destruction of _Listeria monocytogenes_**

Recovery of _L. monocytogenes_ on frankfurters treated with combination of different spray parameters (spray temperature, spray pressure, and time of exposure of the product to CPC) of 1% CPC was evaluated. Analysis of Variance (ANOVA) of mean $\log_{10}$ CFU/ g of _L. monocytogenes_ recovered from the product following treatment with 1% CPC or 1% CPC followed by water treatment did not show any significant differences ($p > 0.05$) when selective and non-selective media (MOX and TPA) were used. Figures 3 and 4 depict the _L. monocytogenes_ recoveries on MOX and TPA respectively, when the inoculated frankfurters were treated with CPC or CPC followed by water wash at 25 ºC spray temperature and varying combinations of exposure times (30, 40, and 60 seconds) and spray pressures (20, 25, and 35 psi).

Although approximately 2 $\log_{10}$ CFU/ g reductions of _L. monocytogenes_ populations were observed, there were no differences ($p > 0.05$) among the different spray pressures and times of exposure of the inoculated product to 1% CPC. The probable reason for no additional increase in destruction of _L. monocytogenes_ with increased spray pressure can be attributed to the foaming of CPC on the surface of frankfurters which caused excessive run off, lesser amounts of CPC solution being sprayed onto the surface of the frankfurters, and lower contact time for the CPC to the product surface. These results were consistent with a study done by Brackett (1987), which suggested that the length of time to which _L. monocytogenes_ was exposed to chlorine did not have a significant effect on the destruction of the cells.
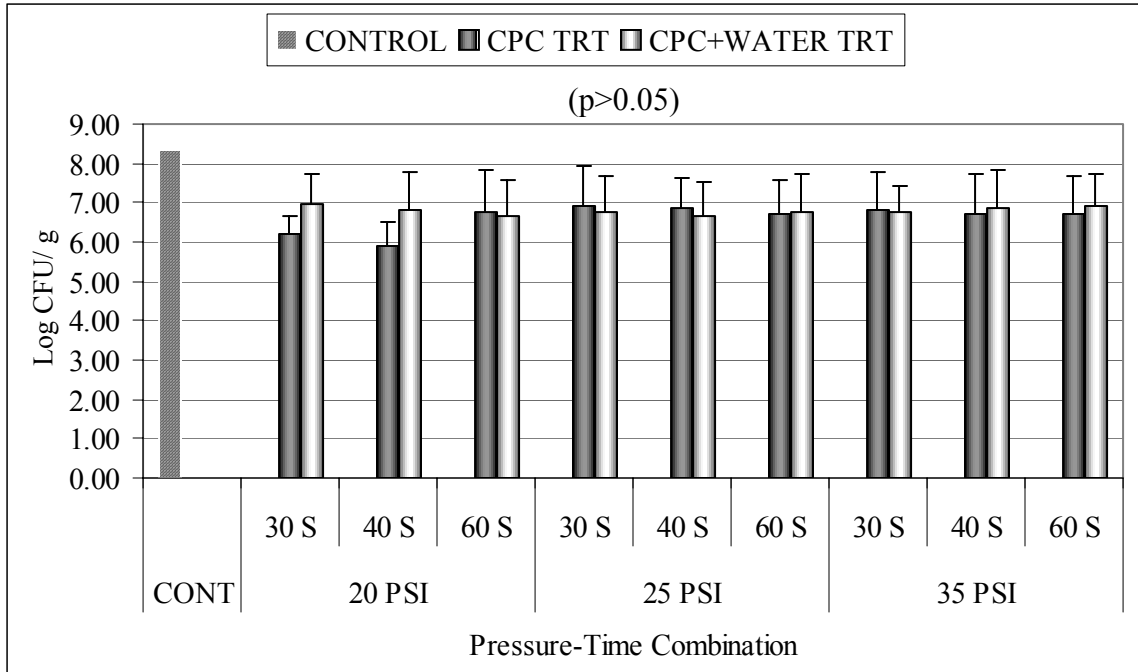
Figure 3: *L. monocytogenes* recovery on MOX from frankfurters treated with 1% cetylpyridinium chloride at 25 ºC
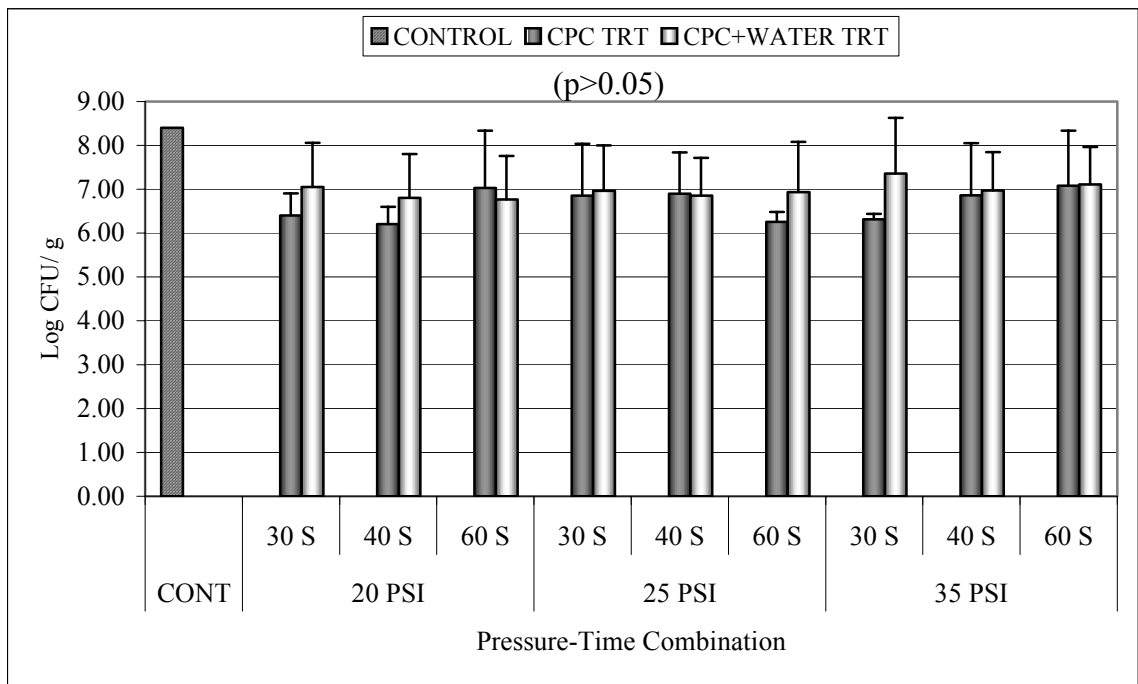
Figure 4: *L. monocytogenes* recovery on TPA from frankfurters treated with 1% cetylpyridinium chloride at 25 ºC

The results obtained were according to studies done by Xiong et al. (1998), which concluded that the most effective temperature for destruction of *S. typhimurium* by 0.1% CPC spray was 40 ºC as compared to 55 ºC. This was due to increased foaming when CPC sprays were used at or above 55 ºC, which might have prevented the CPC from closely contacting the surfaces of the product and thus, weakening any bactericidal effect. But at the same time, these observations were contrary to studies done by Breen et al. (1997), who suggested that nearly a 5 log cycle decontamination of *Salmonella typhimurium* may be achieved by treating poultry tissues with CPC solutions for contact times as low as 3 min.
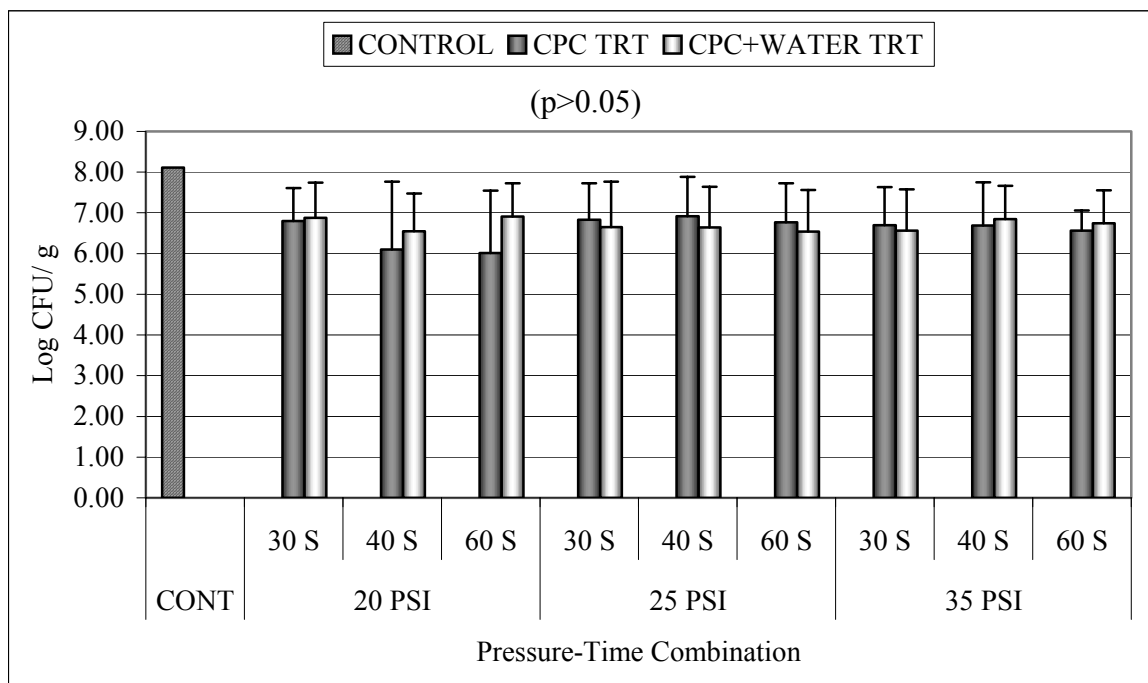
Figure 5: *L. monocytogenes* recovery on MOX from frankfurters treated with 1% cetylpyridinium chloride at 40 ºC

Breen et al. (1997) reported a 3.4 log reduction of *Salmonella* cell numbers after a 90 s spraying of chicken carcasses with a 5 mg/ ml solution of CPC. The high levels of decontamination achieved by Breen et al. could be due to the longer times

of exposure of the inoculated products to CPC in addition to a higher concentration of the CPC solution. Figures 3 and 4 further summarize the populations of *L. monocytogenes* recovered in the treated product on MOX and TPA at 40 ºC spray temperature.
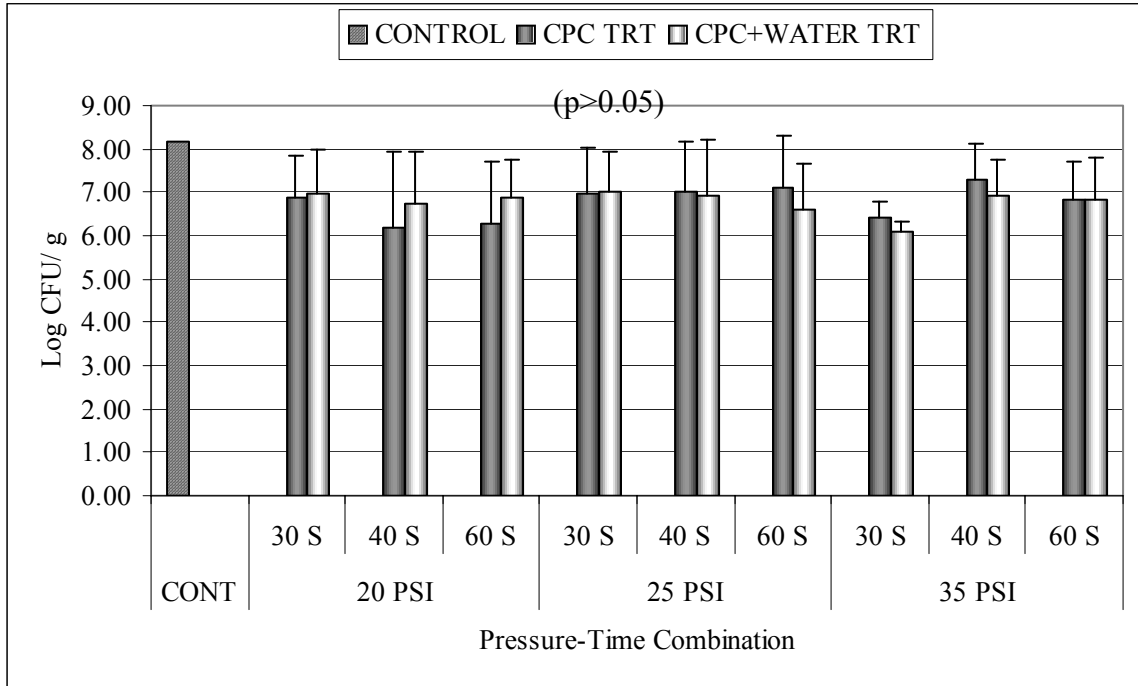
Figure 6: *L. monocytogenes* recovery on TPA from frankfurters treated with 1% cetylpyridinium chloride at 40 ºC

As evident from Figures 5 and 6, there was no difference ($p > 0.05$) in log reductions between the CPC and CPC followed by water treatments irrespective of the time of exposure of the inoculated product to 1% CPC and pressure of spraying 1% CPC on the surface of the product at 40 ºC. Wang et al. (1997) concluded that spray pressures at 10 ºC on chicken skin did not play an important role in reduction of *Salmonella* using 0.1 % CPC. However, when the solution was heated to 60 ºC, the bactericidal effects became much weaker by increasing the spray pressure.
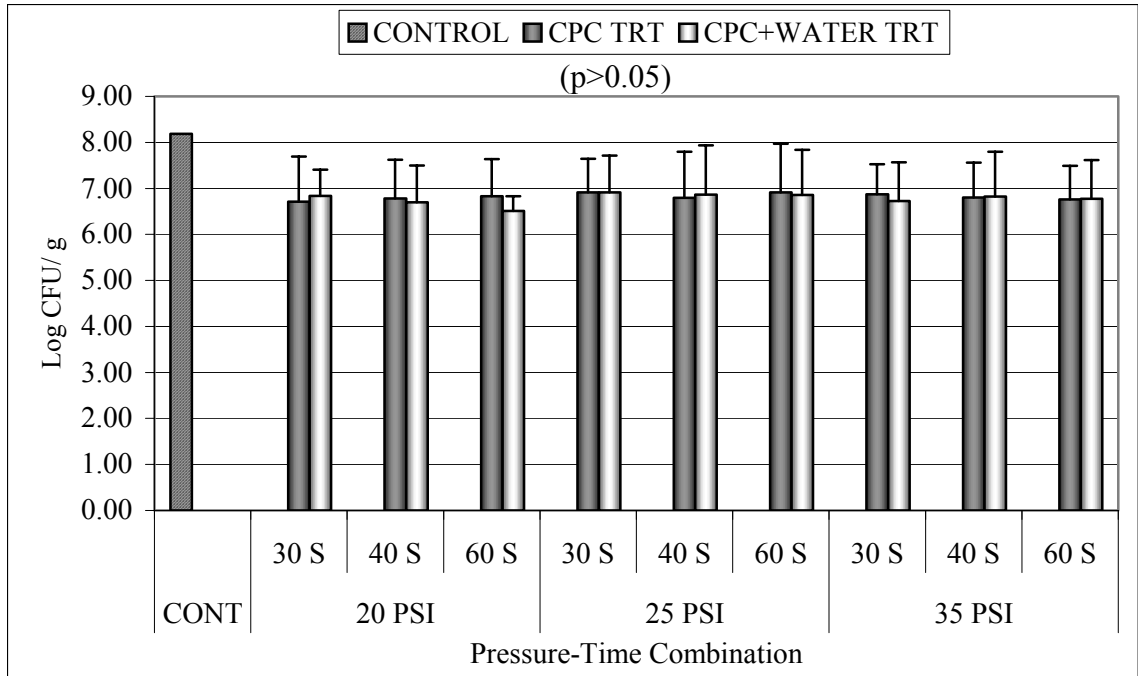
Figure 7: *L. monocytogenes* recovery on MOX from frankfurters treated with 1% cetylpyridinium chloride at 55 ºC
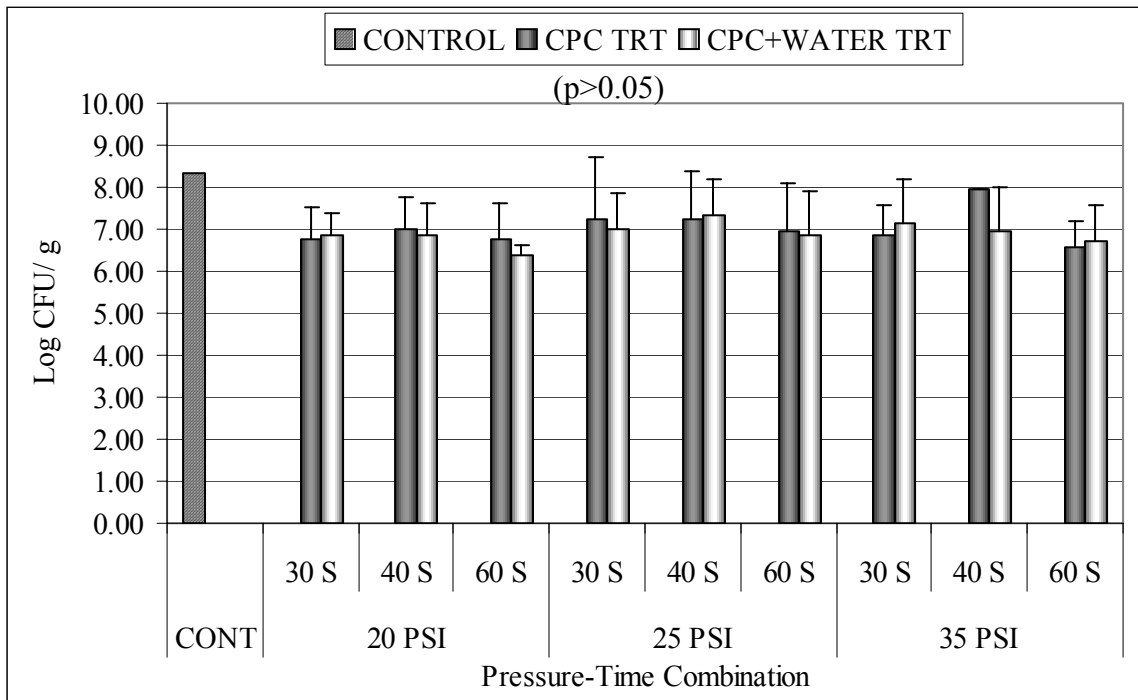
Figure 8: *L. monocytogenes* recovery on TPA from frankfurters treated with 1% cetylpyridinium chloride at 55 ºC

This weakening of the bactericidal effects of CPC at higher temperatures and pressures are shown in Figures 7 and 8. As discussed earlier, the formation of foam at higher temperatures and pressures is likely the main cause of decreased effect of CPC.

Increasing the spray pressure of 1% CPC from 20 psi up to 35 psi, extending the time of exposure of the product to 1% CPC from 30s to 60s, and increasing the temperature of spraying 1% CPC onto the surface of meat from 25 ºC to 55 ºC did not result in greater (p>0.05) destruction of *L. monocytogenes* on the surface of frankfurters. As a result, a spray temperature of 25 ºC, spray pressure of 20 psi, and time of exposure of 1% CPC to the ready to eat meat products were selected to conduct further shelf life studies.

II.   Effects of 1% CPC on Shelf Life of Frankfurters

a.   Frankfurters inoculated with *Listeria monocytogenes*

Figure 9 summarizes the $\log_{10}$ CFU/ g of *L. monocytogenes* recovered from the frankfurters when a low level ($\log_{10}$ 2.5 CFU/ g) of the inoculum was inoculated onto the product. Initial bacterial loads of the product were determined on the day of inoculation (day 0) and subsequently on days 3, 7, 14, 21, 28, and 42 of storage at 0 ºC and 4 ºC.

The ANOVA indicated that a combination of treatment and day had a significant effect ($p \leq 0.05$) on the recovery of *L. monocytogenes* from the frankfurters. It was observed that temperatures (0 and 4 ºC) of storage of the product, treated and non-treated products, and days of storage of the product had a significant effect ($p \leq 0.05$) on the recovery of *L. monocytogenes*. Ahamad and Marth (1989) reported that lower temperatures permitted growth of *L. monocytogenes*, indicating the psychrotrophic character of both strains of the pathogen, but the lag times were significantly longer as

the temperature of incubation was decreased. Thus, generation times were always higher at the lower temperature of incubation. In the current study, days 14, 21, 28, and 42 were significantly different ($p \leq 0.05$) from days 0 and 3, although no significant difference ($p > 0.05$) was observed between days 0 and 7, days 3 and 7, days 14 and 21, and days 28 and 42 irrespective of the temperature of storage and 1% CPC treatment.
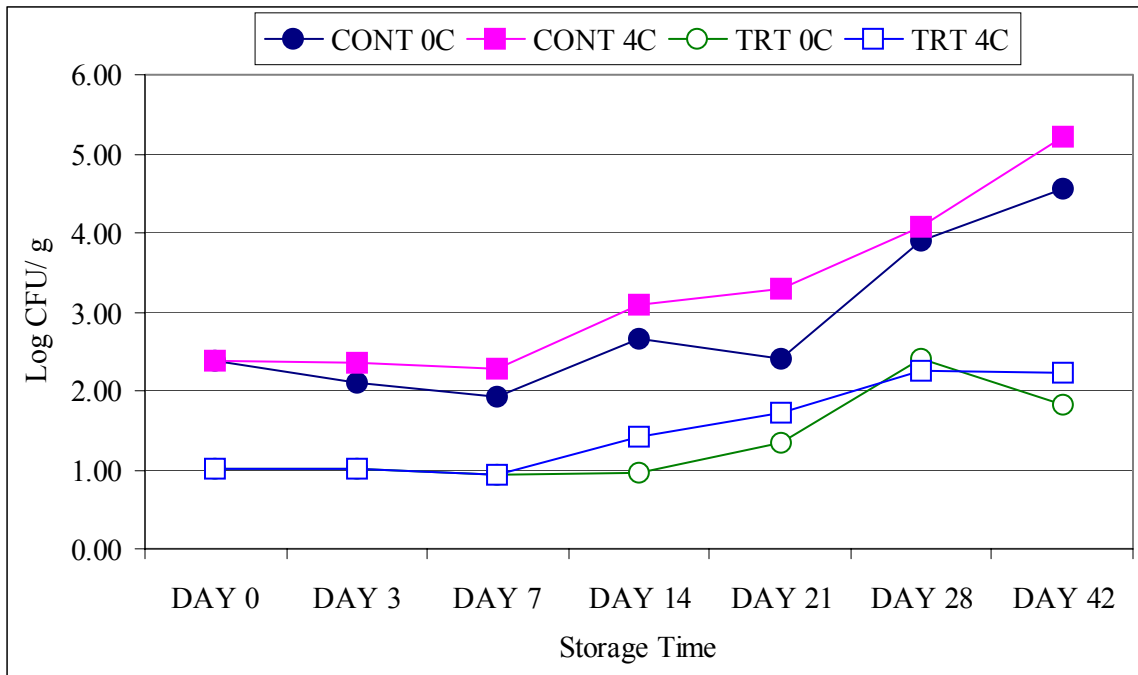
Figure 9: *L. monocytogenes* growth on non-treated and 1% cetylpyridinium chloride treated frankfurters inoculated with low levels (ca. $\log_{10}$ 2.5 CFU/ g) and stored over time at 0 and 4 ºC

These results suggest a bactericidal and bacteriostatic effect of 1% CPC for up to 21 days of storage. Samelis et al. (2002) reported complete inhibition (bacteriostatic effect) of *L. monocytogenes* at 4 ºC up to 120 days on frankfurters against an initial inoculum of approximately 3 $\log_{10}$ CFU/ cm^2 as a result of the use of a combination of 0.25% sodium acetate, 0.25% sodium diacetate, and 1.8% sodium lactate. In the current study, 1% CPC did not indicate this level of effectiveness as a bacteriostatic agent. However, CPC did provide a 1.4 log cycle bactericidal effect immediately after

application. It is important to note that after initial day 0 lethal effect of CPC, *L. monocytogenes* populations only increased by 0.8 and 1.2 log cycles over 42 days of storage at 0 and 4 ºC respectively, whereas the magnitude of increase in non-treated controls increased up to 2.2 (reaching 4.55 $\log_{10}$ CFU/ g) and 3.0 (reaching 5.22 $\log_{10}$ CFU/ g) log cycles at 0 and 4 ºC respectively.

ANOVA of *L. monocytogenes* populations recovered from frankfurters inoculated with a high level of inoculum (7.5 $\log_{10}$ CFU/ g) and treated with 1% CPC and stored over a period of 42 days at 0 and 4 ºC indicated that a combination of treatment and storage day had a significant ($p \leq 0.05$) effect on the growth pattern of the organism irrespective of the temperature at which the product was stored (Figure 10). This result was in agreement with studies done by Porto et al. (2002), who concluded that treatment of frankfurters with 2% or 3% potassium lactate (an antimicrobial) lowered the pathogen numbers appreciably regardless of the level of inoculum and temperature of storage of the product.

It can be observed from the figure 10 that although there was an initial bactericidal effect of 1% CPC on *L. monocytogenes*, the bacteriostatic effect persisted for 14 days. Comparisons of *L. monocytogenes* populations recovered from frankfurters over time indicated no significant ($p \geq 0.05$) differences between counts recovered on days 0 and 3, 0 and 7, 3 and 7, 3 and 14, 7 and 14, 21 and 28, 21 and 42, and 28 and 42 of storage irrespective of the temperature at which the product was stored. These results suggest that 1% CPC has both bactericidal and bacteriostatic effects on products inoculated with high levels of the *L. monocytogenes*. Islam et al. (2002) conducted a study on the control of *L. monocytogenes* on turkey frankfurters by GRAS preservatives

41

and reported growth of the organism on all frankfurters not treated with preservative and stored at either 4, 13, or 22 °C. Further conclusions from their study indicated a continuous decline in the populations of *L. monocytogenes* during refrigerated storage at 4 °C during a 14 day period. The inhibitory effect of the preservatives was predominantly dependent upon the concentration used in the frankfurters.
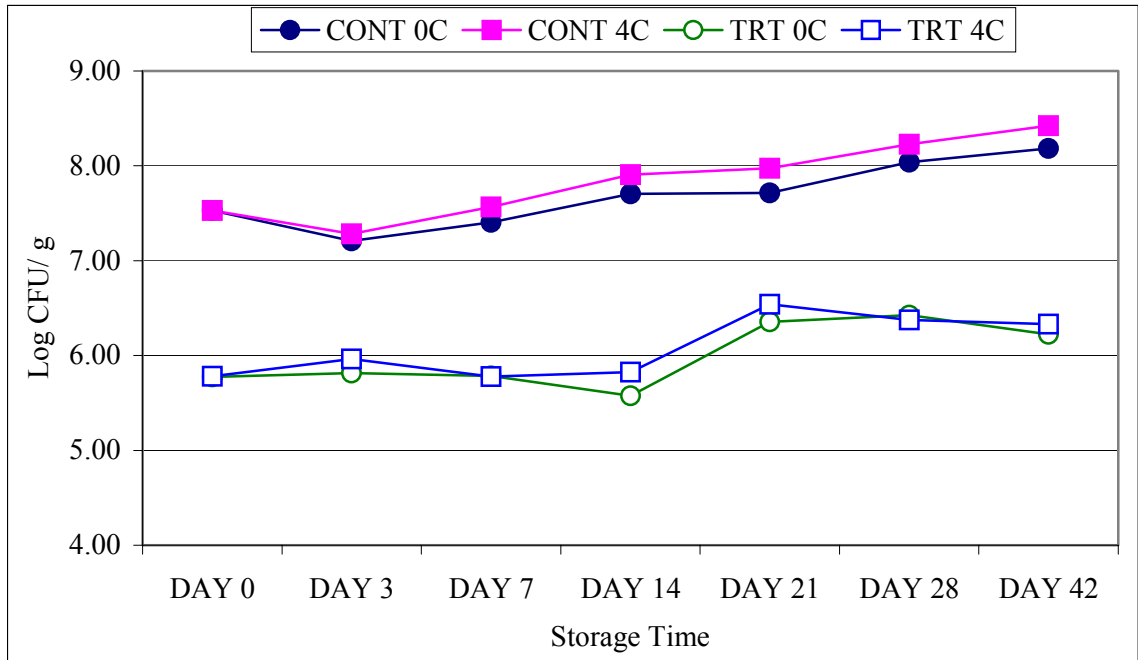
Figure 10: *L. monocytogenes* growth on non-treated and 1% cetylpyridinium chloride treated frankfurters inoculated with high levels (ca. $\log_{10}$ 7.5 CFU/ g) and stored over time at 0 and 4 °C

The inhibitory effects of 1% CPC on low levels of inoculum in this study were in agreement to studies done by Bedie et al. (2001), who reported an inhibition period for the pathogen ranging widely from 20 to 70 days when inoculated at levels of 3 to 4 $\log_{10}$ CFU/ cm^2 on the surface of peeled frankfurters stored at 4 °C in vacuum packages post-treatment with antimicrobials. Ahmad and Marth (1989) reported that the type and concentration of acid as well as temperature of incubation affected the behavior of *L. monocytogenes*. Although a higher bacteriostatic effect of 1% CPC was observed when

frankfurters were inoculated with low levels of inoculum as compared to high levels of inoculum, it can be concluded that treatment of frankfurters with 1% CPC resulted in an initial bactericidal effects and subsequently, slowed the growth of *L. monocytogenes* up to 42 days. This finding would indicate an improved microbiological safety associated with CPC treated frankfurters over extended storage. Studies by Glass and Doyle in 1989 revealed that substantial growth of *L. monocytogenes* (> 4 log_{10} CFU/ g in 9 weeks) occurred at refrigeration temperatures of 4.4 ºC. Hence, if post processing contamination with *L. monocytogenes* occurs with low numbers in the product, the organism will not be able to grow when treated with 1% CPC and stored at refrigeration temperatures. These results are in agreement with a study done by Shelef (1989) who found that *L. monocytogenes* was not able to grow in ground beef stored at 4 ºC for 2 weeks in either oxygen permeable or oxygen impermeable bags. Samelis et al. (2002) reported bactericidal effects of antimicrobial combinations of sodium lactate, sodium acetate, and sodium diacetate on *L. monocytogenes* over 35 to 50 days of storage. In contrast to the inhibitory effects of antimicrobials, Samelis et al. (2002) reported the ability of *L. monocytogenes* to recover and grow significantly by 20 to 30 days of storage following treatment with hot water for 30, 60, or 90 seconds despite initial reductions which were proportional to immersion times.

Bactericidal and bacteriostatic results obtained from this experiment were further supported by a study done by El-Shenawy and Marth (1989) suggesting that the inactivation, inhibition, and/ or growth of *L. monocytogenes* were affected by incubation temperature, pH, concentration of antimicrobial, and the type of organic acid used to adjust the pH. Bedie et al. (2001) reported ca. 3 log_{10} CFU/cm^2 increase of *L.*

monocytogenes in frankfurters without antimicrobials within 20 days and eventually by 5 $\log_{10}$ CFU/ cm^2 increase with prolonged storage at 4 ºC. Islam et al. (2002) suggested that higher storage temperature allowed the growth of *L. monocytogenes*, but the effect of temperature was minimal in retarding the growth of listeriae compared with the more extensive growth repressive effect of a preservative. Glass et al. (2002) reported that the populations of *L. monocytogenes* on wieners treated with 6% sodium lactate increased by 1.1 $\log_{10}$ CFU per package at 30 days and by additional 0.6 $\log_{10}$ CFU per package at 45 days. Further studies (Glass et al. 2002) indicated no inhibition of the growth of *L. monocytogenes* when the exposure time was reduced from 2 min. to 5 s irrespective of the type of chemical additive and their concentrations.

b.   **Non-inoculated frankfurters**

A comparison of the non-treated and 1% CPC treated frankfurters indicated that treatment and the number of days for which the product was stored had a significant effect ($p \leq 0.05$) on the aerobic plate count (APC) and lactic acid bacteria (LAB) count of the product irrespective of the temperature of storage (Figures 11 and 12). This result was contrary to the studies done by Johnson et al. (1979), which indicated no significant interactions of treatment of beef carcasses with hypochlorous acid and days of storage as a measure of controlling APC and LAB. APC increased up to 4 $\log_{10}$ CFU/ g from undetectable levels (detection limit <2 $\log_{10}$ CFU/ g) in the 1% CPC treated frankfurters, whereas in the non-treated frankfurters, APC growth was observed up to 5 $\log_{10}$ CFU/ g after a period of 42 days (Figure 11). Comparison of the least squares means indicated no significant difference in the APC of the product between days 7 and 14, whereas all the remaining days of storage (3, 21, 28, and 42) were significantly different ($p \leq 0.05$) from

44

each other, while the temperature of storage was not of significance (p>0.05). Breen et al. (1997) reported up to 6 $\log_{10}$ CFU/ g reduction of APC on lean beef surfaces when treated with 1% CPC, while results from the present study indicated a 1.5 $\log_{10}$ CFU/ g reduction of APC over 42 days of refrigerated storage. This variability in the results could possibly be due to the interference of the fat in the frankfurters leading to reduced bactericidal effects of 1% CPC. Glass et al. (2002) reported a 7.4 $\log_{10}$ CFU per package gradual increase in the aerobic bacteria in 6% sodium lactate and 3% sodium diacetate treated wieners after 60 days of storage.
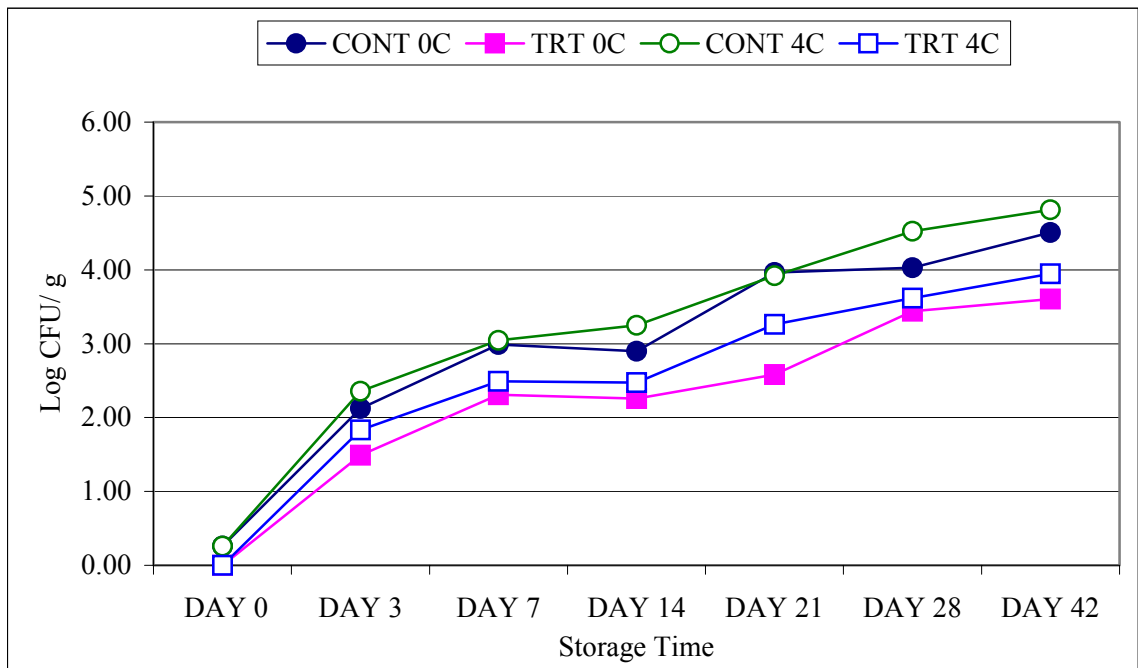
Figure 11: Aerobic Plate Count ($\log_{10}$ CFU/ g) of non-treated and 1% cetylpyridinium chloride treated frankfurters stored over time at 0 and 4 ºC

LAB showed similar growth patterns as APC and increased up to 3.2 $\log_{10}$ CFU/ g from undetectable levels (detection limit<2 $\log_{10}$ CFU/ g) post-treatment over 42 days storage period. Cutter et al. (2000) stated the presence of residual CPC in beef tissues is probably sufficient to inhibit remaining bacterial populations over the 50 days of

refrigerated, vacuum packaged storage. Contrary to results from this experiment, Porto et al. (2002) reported that levels of LAB increased by 4.7 $\log_{10}$ CFU per package over 90 days at 4 °C, whereas the levels of LAB increased by 10.5 $\log_{10}$ CFU per package over 60 days at 10 °C indicating that the temperature of storage had a significant effect on the growth of LAB. Days 14, 21, and days 28, 42 were not significantly different ($p>0.05$) from each other upon analysis for LAB irrespective of the temperature of storage.
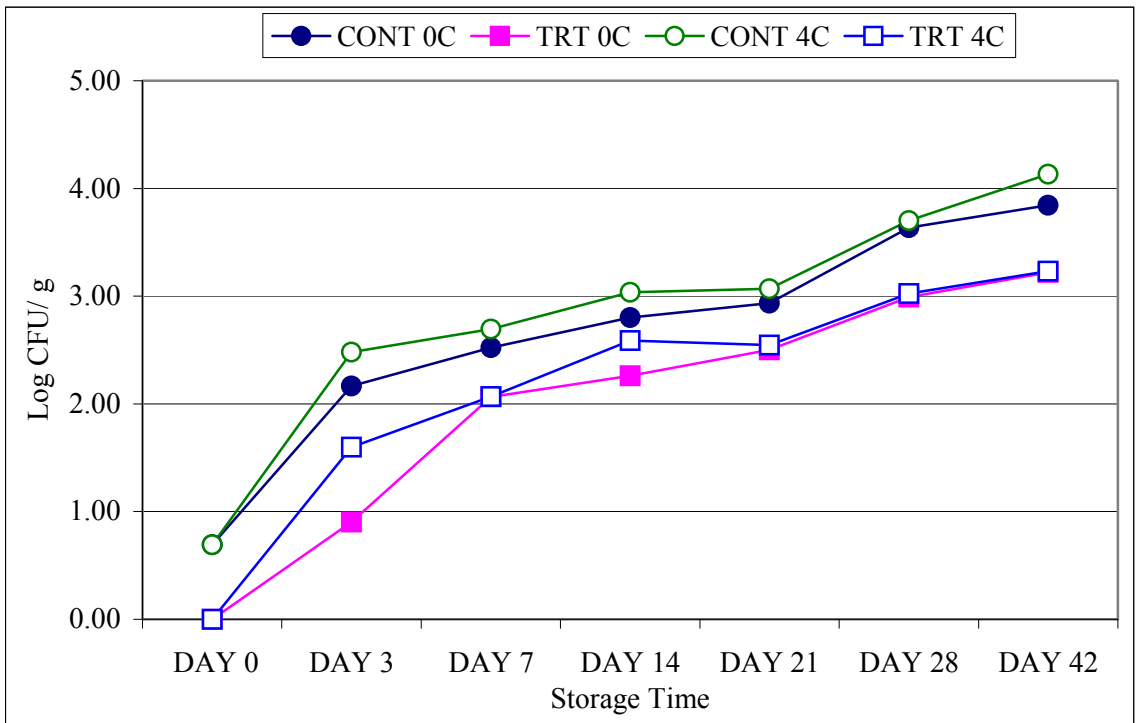
Figure 12: Lactic acid bacteria (LAB) count ($\log_{10}$ CFU/ g) of non-treated and 1% cetylpyridinium chloride treated frankfurters stored over time at 0 and 4 °C

The analysis of efficacy of 1% CPC on controlling growth of yeasts and molds in frankfurters suggested that storage day was of significant ($p\leq0.05$) importance. Furthermore, the analysis indicated that there was no difference ($p>0.05$) between day 3 and 7, 3 and 14, 7 and 14, and 14 and 21, while temperature of storage, and treatment did not have a significant ($p>0.05$) effect on the yeast and mold populations. However, 1%

CPC treatment increased the lag phase for the growth of yeasts and molds up to 21 days compared to non-treated frankfurters when stored at 0 ºC.

Enumeration of non-treated and 1% CPC treated frankfurters did not yield any growth of coliforms or *E. coli* in the product (detection limit < 2 CFU/ g). Breen et al. (1997) reported an insignificant growth of *E. coli* after 35 days of refrigerated storage.

c.   Color of frankfurters

ANOVA of color of frankfurters treated with 1% CPC and non-treated frankfurters stored up to 42 days at 0 ºC and 4 ºC indicated that the L^*- values (measure of lightness) of the frankfurters were not (p>0.05) affected by treatment whereas the interaction effects of temperature of storage and days of storage had a significant (p≤0.05) effect. In addition to this interaction, the days of storage alone also had a significant (p≤0.05) effect on the lightness values of frankfurters irrespective of treatment (Table 2). At 0 ºC, days 0 and 7, 0 and 21, 0 and 42, 3 and 7, 3 and 14, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 21, 21 and 28, and 21 and 42 were significantly different (p≤0.05). At 4 ºC, day 0 was significantly different (p≤0.05) from days 7, 28, and 42. Statistical differences (p≤0.05) were also observed between days 3 and 28, 3 and 42, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 28, 14 and 42, 21 and 28, 21 and 42, whereas days 28 and 42 were not significantly different (p>0.05). The variation in the lightness values of the frankfurters can be due to the bleaching action caused by CPC spray treatments.

Table 2: Mean (SD) lightness, redness, and yellowness values of non-treated (control) and 1% cetylpyridinium chloride treated frankfurters stored for 42 days at 0 ºC.

St. Time (Days)	L^*-Values ‡		a^*-Values ‡		b^*-Values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	48.67 ± 0.31	48.27 ± 0.15	13.40 ±0.26	14.30 ± 1.39	14.73 ± 0.25	15.47±0.45
3	48.70 ± 0.61	49.27 ± 1.31	13.93 ±0.06	16.97 ± 4.16	15.00 ±0.36	13.63±3.15
7	47.43 ± 0.50	47.63 ± 0.64	15.13 ±1.08	14.20 ± 0.98	16.30 ±0.50	15.77±0.46
14	49.53 ± 0.55	48.37 ± 0.47	14.63 ±0.47	14.13 ± 1.42	15.80 ±0.00	15.63±0.29
21	49.63 ± 0.75	50.17 ± 0.35	17.23 ±3.87	16.20 ± 4.81	13.87±3.35	13.73±3.24
28	48.57 ± 0.55	49.03 ± 0.29	15.07 ±0.76	13.83 ± 0.98	15.60±0.44	15.47±0.35
42	48.40 ± 0.50	49.03 ± 0.71	14.17 ±1.03	14.50 ± 0.60	15.57±0.06	16.53±0.81

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 0.38, a^*-Values = 1.35, and b^*-Values = 0.97

Farid et al. (1998) studied the effects of organic acid dipping of catfish fillets on color and concluded that the L^*- values of the fillets increased as a result of the reduction in the surface pH. In support of the effects of storage temperature on the color of the frankfurters Kropf and Hunt (1984) suggested that the lighting in display conditions could effect the appearance as a result of temperature elevation on the surface of meat, photochemical effects, and/ or light rendition due to different spectral energy distribution patterns. Hence, it can be speculated that the lighting used in this experiment and the 1% CPC treatment had a synergistic effect on the color of frankfurters when stored for 42 days at 0 and 4 ºC.

ANOVA of color of frankfurters treated with 1% CPC and non-treated frankfurters stored up to 42 days at 0 and 4 ºC indicated that the storage temperature of

frankfurters had a significant effect on the a^*- values (measure of redness) of the product. Tables 2 and 3 summarize the changes in the redness of frankfurters over a 42 day period of storage at 0 and 4 ºC respectively. It is evident that the product stored at 4 ºC had a lesser a^*- value (redness) as compared to the product stored at 0 ºC irrespective of the 1% CPC treatment. This suggests that storage temperature and not the treatment of frankfurters with 1% CPC is a possible cause for the deterioration in the redness. This storage temperature effect on the product's a^*- value can be attributed mostly to the fact that the product stored at 4 ºC was displayed under light in a simulated retail display case, whereas, the 0 ºC product was not exposed to light. It can also be inferred from the tables 2 and 3 that redness of the treated frankfurters is lower compared to the non-treated frankfurters possibly due to the increase in the lightness of the product as a result of bleaching action of the antimicrobial spray treatment. The significance of temperature on L^*- values and a^*- values could also have been possible due to the depressed metabolic activity of microorganisms at lower temperatures, hence inhibiting oxidation and minimizing the color changes. However, the most logical explanation is that at 4 ºC, the retail lighting negatively influenced the product color.

ANOVA of b^*- values (measure of yellowness) of the 1% CPC treated and non-treated frankfurters suggested no significant difference ($p > 0.05$) of the 1% CPC treatment, time of storage, and temperature of storage on yellowness of the product (Table 2 and 3). Farid et al. in 1998 studied the effects of organic acid dipping of catfish fillets on color and revealed that the extent of pink color loss and muscle lightness increased, while the organic treatment also lead to significantly yellower (with high b^*- values) fillets as a result of dip treatment with organic acids. Tables 2 and 3 illustrate the

49

comparison of yellowness of the frankfurters treated with 1% CPC and non-treated frankfurters stored over 42 days at 0 and 4 ºC. The yellowness of the treated frankfurters is slightly higher than the non-treated frankfurters when stored at 0 ºC whereas there is no difference when stored at 4 ºC. This slight increase in the yellowness of 1% CPC treated frankfurters could possibly be due to increased purge in the packages. Again the difference in the lighting in the 0 and 4 ºC storage units make these differences in yellowness mostly a function of light as opposed to temperature.

Table 3: Mean (SD) lightness, redness, and yellowness values of non-treated (control) and 1% cetylpyridinium chloride treated frankfurters stored for 42 days at 4 ºC.

St. Time (Days)	L^*-Values ‡		a^*-Values ‡		b^*-Values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	48.7 ± 0.30	48.33 ± 0.31	13.47 ± 0.32	14.27 ± 1.07	15.2 ± 0.62	15.5 ± 0.62
3	48.13 ± 0.12	48.43 ± 0.59	12.93 ± 0.67	13.67 ± 1.15	15.13 ± 0.21	15.33 ± 0.45
7	47.5 ± 0.53	48.07 ± 0.45	13.5 ± 0.10	13.33 ± 2.00	15.3 ± 0.20	15.33 ± 0.29
14	48.73 ± 0.70	48.7 ± 1.30	13.1 ± 0.60	13.13 ± 0.85	14.93 ± 0.35	15.97 ± 1.07
21	48.07 ± 0.59	49.33 ± 0.85	12.97 ± 1.94	12.5 ± 1.08	15.63 ± 0.71	15.5 ± 0.56
28	49.6 ± 0.53	49.47 ± 1.00	12.73 ± 0.06	16 ± 4.96	15.47 ± 0.06	13.97 ± 3.46
42	49.63 ± 1.14	49.33 ± 1.66	13.4 ± 1.00	14 ± 1.47	16.07 ± 0.25	16.07 ± 0.49

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 0.52, a^*-Values = 1.08, and b^*-Values = 0.67

Kropf (1980) in a study on effects of retail display conditions on meat color suggested that microbial growth over time might bring about discoloration of meat. Another important reason for enhanced discoloration at 4 ºC could probably be due to the

higher microbial populations (APC and LAB), which contribute to oxidative conditions encouraging loss of color. Lighting of the retail simulated display case (4 ºC) was likely a major factor for the discoloration of the frankfurters. Light causes accelerated oxidative changes and could possible enhance discoloration of meat products when stored under light in retail display cases.

d. Hardness of frankfurters

Table 4 summarizes the effects of treatment, storage temperature, and time of storage on the hardness of frankfurters.

Table 4: Mean (SD) firmness values of non-treated (control) and 1% cetylpyridinium chloride treated frankfurters for 42 days at 0 and 4 ºC.

St. Time (Days)	Control (0 ºC) ‡	Treated (0 ºC) ‡	Control (4 ºC) ‡	Treated (4 ºC) ‡
0	2854.59 ± 296.25	2873.55 ± 130.62	2751.61 ± 203.15	2924.62 ± 138.22
3	2715.45 ± 93.77	2726.60 ± 91.50	2698.82 ± 174.67	2832.19 ± 196.16
7	2772.51 ± 333.67	2691.11 ± 179.13	2628.61 ± 203.21	2671.78 ± 340.05
14	3159.00 ± 613.32	2820.51 ± 150.72	2918.11 ± 222.67	2535.57 ± 226.05
21	2612.90 ± 303.99	2594.80 ± 167.19	2797.81 ± 220.16	2687.13 ± 111.67
28	2724.47 ± 197.27	2611.05 ± 166.13	2862.70 ± 272.85	2757.37 ± 167.53
42	2703.50 ± 59.53	2810.58 ± 206.76	2935.71 ± 146.56	2876.10 ± 297.19

‡ Means ± Standard Deviation
LSD values for firmness = 121.99

The combination of 1% CPC treatment and day, temperature of storage and day, and day alone had a significant ($p \leq 0.05$) effect on the hardness of frankfurters. Significant differences in the hardness of the frankfurters were observed between the

product stored for days 0 and 21, 3 and 14, 7 and 14, 14 and 21, 14 and 28, 14 and 42 when stored at 0 ºC. Furthermore differences (p≤0.05) were observed between 0 and 7, 7 and 42, and 14 and 42 days at 4 ºC storage temperature. It is evident that treatment in combination with the days of storage tends to make the product softer. However the 1% CPC treated product stored at 0 ºC was slightly harder than that stored at 4 ºC but not significantly different.

III. Effects of 1% CPC on Shelf Life of Polish Sausages

a. Polish sausages inoculated with *Listeria monocytogenes*

In the product inoculated with low levels ($\log_{10}$ 3 CFU/ g) of *L. monocytogenes*, treatment with 1% CPC, temperature of storage of the product, days for which the product was stored, and a combination of treatment with days of storage had a significant effect (p≤0.05) on the growth pattern of the organism over a period of 42 days. Figure 13 summarizes the growth pattern of *L. monocytogenes* when stored at 0 and 4 ºC for up to 42 days post-treatment with 1% CPC. The statistical analysis indicated no significant differences (p>0.05) in the recovery of *L. monocytogenes* from non-treated polish sausages stored for days 3 and 7, 7 and 14, 14 and 28, 21 and 28, 21 and 42, and 28 and 42. Treatment with 1% CPC did not have a significant effect (p>0.05) on the recovery of *L. monocytogenes* from the product stored for days 3 and 14, 7 and 21, 21 and 28, 21 and 42, and 28 and 42 irrespective of the storage temperature. Except for days 3 and 7, all other days of storage treatment had a significant effect (p≤0.05) on the growth of *L. monocytogenes* on the product. Another important observation from the results was that storing the product at 0 ºC and 4 ºC had a significantly effect (p≤0.05) on the behavior of *L. monocytogenes* over a 42 day storage period.

52

Although an increase in the $\log_{10}$ CFU/g of *L. monocytogenes* was observed after 14 days of storage, this effect can be explained by the higher resistance of gram-positive, non-spore forming organisms to the presence of high concentrations of undissociated acids (Podolak et al., 1996). Wederquist et al. (1994) suggested that the presence of antimicrobials/ chemical additives such as sodium acetate prolonged the lag phase, and hence significantly reduces the growth of *L. monocytogenes*. No listerial growth was observed on bratwurst treated with lactates and diacetates stored at 3 ºC for 14 days but by day 28 the populations of *L. monocytogenes* on uncured, unsmoked sausages had increased up to initial inoculum levels of 7.1 $\log_{10}$ CFU per package (Glass et al., 2002).
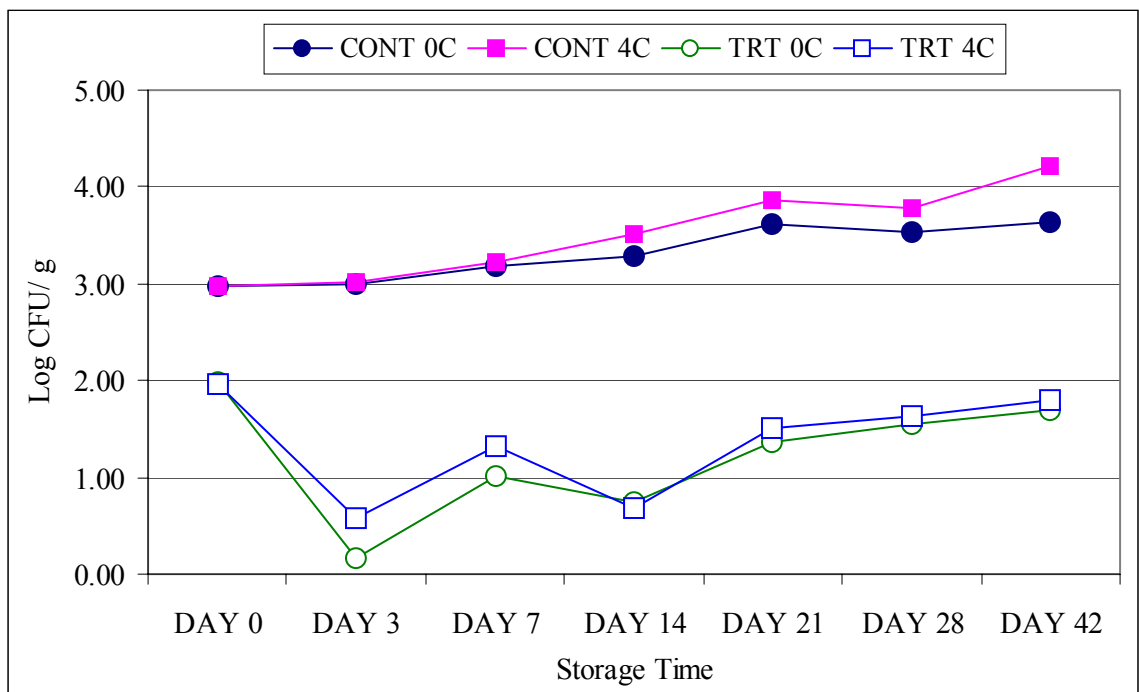
Figure 13: *L. monocytogenes* recovery on non-treated and 1% cetylpyridinium chloride treated Polish sausages inoculated with low levels (ca. $\log_{10}$ 3 CFU/ g) and stored over time at 0 and 4 ºC

ANOVA of $\log_{10}$ CFU/ g of *L. monocytogenes* recovered from polish sausage inoculated with high levels of inoculum ($\log_{10}$ 7 CFU/ g) and treated with 1% CPC and

stored over a period of 42 days at 0 ºC and 4 ºC indicated a significant effect (p≤0.05) of combination of treatment and storage day and combination of treatment and temperature of storage. In addition individual significant (p≤0.05) effects of temperature of storage, 1% CPC treatment and days of storage of polish sausage were observed on the pattern of growth of the organism (Figure 14). These results were in accordance to studies done by Wederquist et al. in 1996, indicating significant reduction of *L. monocytogenes* in vacuum packaged refrigerated turkey bologna by incorporation of chemical additives such as lactates, acetates, and sorbates.

Comparison of least squares means of the main effects (storage time, 1% CPC treatment, and temperature of storage) indicated no significant differences (p>0.05) in the growth pattern of *L. monocytogenes* on non-treated polish sausages stored for day 3 and 7, 21 and 28, and 28 and 42 irrespective of the temperature. Additionally no significant (p>0.05) differences were observed for 1% CPC treated polish sausages stored for days 3 and 7, 3 and 14, 3 and 21, 7 and 14, 7 and 21, 14 and 21, 12 and 28, and 28 and 42 irrespective of the temperature of storage. These results of inhibitory effects of 1% CPC treatment were according to studies done by Podolak et al. (1996), which confirmed an increasing inhibitory effect of fumaric acid at concentrations of 0.5, 1.0, 1.5, and 2.0% on *L. monocytogenes* with storage time up to 7 days. While 1% CPC treatment had bacteriostatic effects for up to 42 days, their studies indicated no effects of fumaric acid irrespective of the concentration on *L. monocytogenes* after 7 days. Growth of *L. monocytogenes* on non-treated polish sausages at 4 ºC indicates that refrigeration itself cannot prevent the growth of food borne pathogens and emphasizes the importance of

preventing post-process contamination of RTE meats by incorporating the use of antimicrobials.

Glass and Doyle (1989) recommended from the results of their study on the fate of *L. monocytogenes* in processed meat products during refrigerated storage that meat processors can no longer rely entirely on refrigerated storage at 4 to 7 ℃ to be assured of pathogen control. Novel, non-traditional approaches, such as the use of antimicrobials, reduced temperature (<2 ℃) storage, or post-package pasteurization of products may need to be considered for the control of *L. monocytogenes*. A lower temperature enhances the antilisterial activity of lactate and diacetate on unsmoked bratwurst (Glass et al., 2002).

Figure 14: *L. monocytogenes* recovery on non-treated and 1% cetylpyridinium chloride treated Polish sausages inoculated with high levels (ca. $\log_{10}$ 7 CFU/ g) and stored over time at 0 and 4 ℃
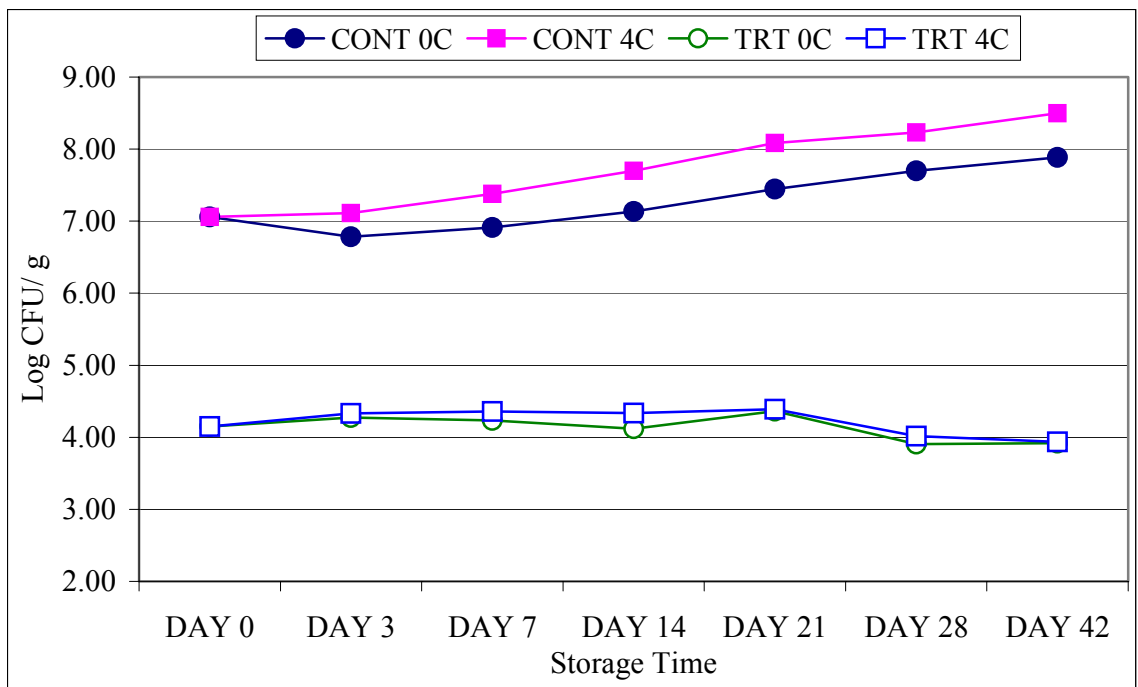
b. **Non-inoculated Polish sausages**

Application of 1% CPC treatment had a significant effect ($p \leq 0.05$) on the aerobic plate count (APC) and lactic acid bacteria (LAB) of polish sausages irrespective of the temperature (0 ºC and 4 ºC) at which the product was stored. In addition to this the days for which the product was stored also had a significant ($p \leq 0.05$) influence on the APC and LAB, hence leading to a significant ($p \leq 0.05$) synergistic effect of 1% CPC treatment and days of storage on the APC (Figure 15) and LAB (Figure 16).
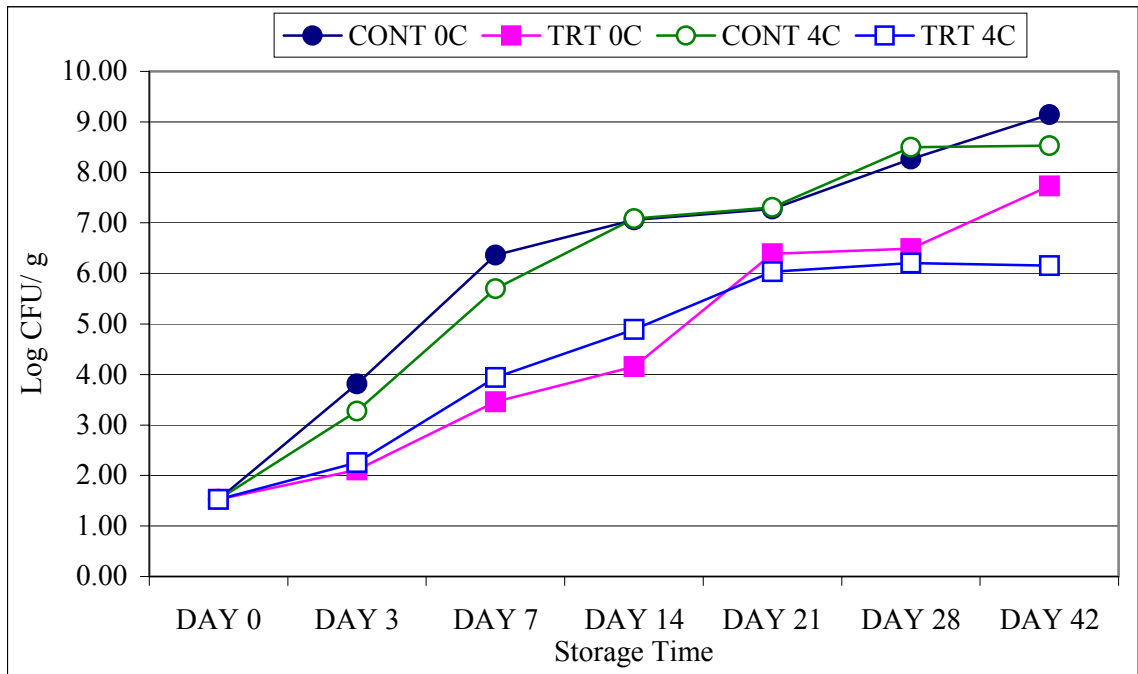
Figure 15: Aerobic Plate Count ($\log_{10}$ CFU/ g) of non-treated and 1% cetylpyridinium chloride treated Polish sausages stored over time at 0 and 4 ºC

The results from the ANOVA further indicated that an interaction between the temperature of storage and days for which the polish sausage was stored had a significant ($p \leq 0.05$) effect on the growth of LAB. A significant difference ($p \leq 0.05$) in the APC of the treated and non-treated polish sausage was observed between 3 and 7, 7 and 21, 7 and 28, 14 and 42, 21 and 42, and 28 and 42 days of storage irrespective of the temperature at

which the product was stored. APC increases from 1.5 $\log_{10}$ CFU/ g to 8.5 $\log_{10}$ CFU/ g on non-treated polish sausages while the growth on treated polish sausages was from 1.5 $\log_{10}$ CFU/ g to 6 $\log_{10}$ CFU/ g at 4 ℃ after 42 days of storage. Cutter et al (2000) suggested that concentration of CPC used for treatment of lean surfaces of beef did not have statistical difference from each other and reported no significant differences in the APC of 1% CPC treated adipose beef tissue after long term refrigerated, vacuum packaged storage up to 35 days. Although a significant difference in the APC was reported by Cutter et al. (2000) between non-treated and 1% CPC treated beef tissues after 2 days of storage. Furthermore, temperatures of 0 and 4 ℃ did not have a significantly different ($p > 0.05$) effect on the APC of polish sausage.
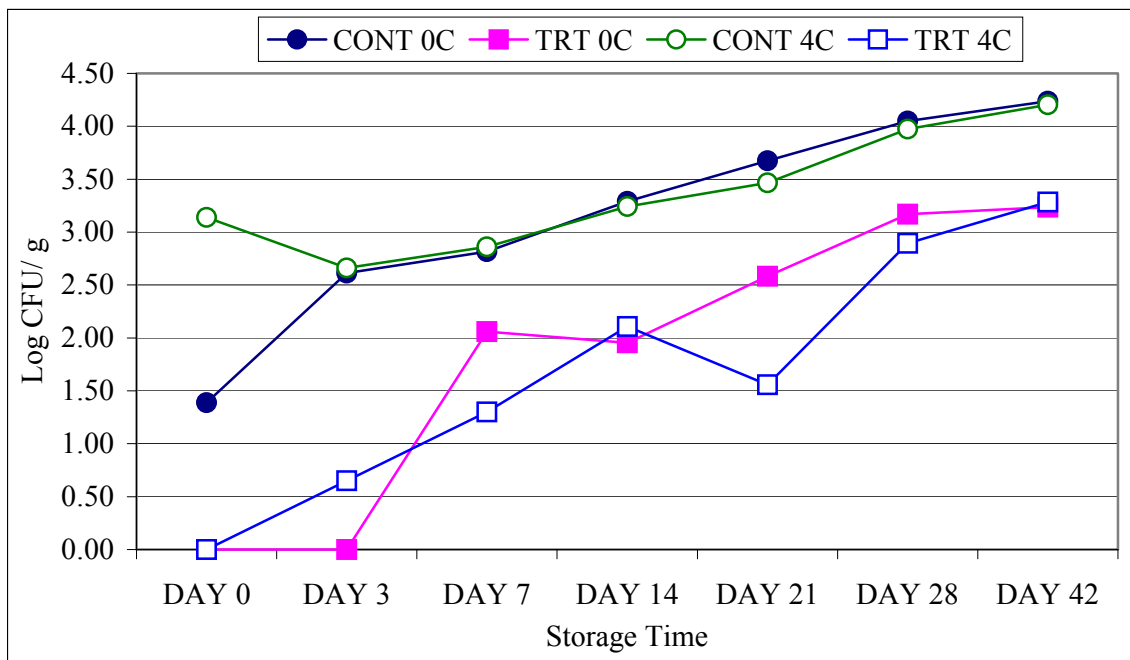
Figure 16: Lactic acid bacteria (LAB) counts ($\log_{10}$ CFU/ g) of non-treated and 1% cetylpyridinium chloride treated Polish sausages stored over time at 0 and 4 ℃

LAB showed similar ($p > 0.05$) growth patterns on the non-treated polish sausages stored for days 3 and 7, 14 and 21, and 28 and 42 irrespective of the temperature of

storage. At 3 and 28, 7 and 28, 7 and 42, 14 and 28, and 14 and 42 days of storage of the polish sausage 1% CPC treatment did not show any significant (p>0.05) difference in the LAB growth at 0 and 4 ºC. No significant differences (p>0.05) in the growth of LAB were observed in the product at days 0, 3, 7, and 14.

Growth of yeasts and molds on polish sausages was significantly (p≤0.05) affected by the combined effects of 1% CPC treatment, days of storage of the product, and the temperature at which the product was stored. Treatment of polish sausages with 1% CPC inhibited the growth of yeasts and molds to <0.5 $\log_{10}$ CFU/ g as compared to a 1.75 $\log_{10}$ CFU/ g growth observed in the non-treated polish sausages irrespective of the temperature of storage by the end of a 42-day storage period. Treatment of polish sausages with 1% CPC reduced the total coliform counts and *E. coli* in the product to below detectable limits (detection limit < 2 CFU/ g).

c. <u>Color of Polish sausages</u>

ANOVA of color of polish sausages indicated that there was no significant effect (p>0.05) of 1% CPC treatment, storage time, and temperature of storage on the L^*- values (lightness) of the product. L^*- values of the treated product were consistently lower as compared to the non-treated product (Tables 5 and 6). The non-treated Polish sausages stored at 4 ºC had a lower L^*- value than the product stored at 0 ºC. These differences in the L^*- values of the polish sausage due to temperature could be due to the fact that the product stored at 4 ºC was stored in a display case under light, which might have an influence on the color of the product.

Statistical analysis of the a^*- values indicated that 0 ºC and 4 ºC temperatures of storage had a significant effect (p≤0.05) on the redness of the product. In addition to

temperature, days of storage, interaction of temperature of storage with treatment, and combination of days of storage and treatment had a significant ($p \leq 0.05$) effect on the a^*-values of polish sausage. It is evident in the tables 5 and 6 that there is a significant decrease in the redness of the polish sausages irrespective of the 1% CPC treatment and temperature at which the product is stored. The maximum decrease in redness of polish sausage was seen in the product treated with 1% CPC and held at 4 ℃ for 42 days. This observation is in agreement with other studies (Farid et al., 1998). Farid et al. (1998) reporter increase in muscle lightness and loss of pink color in catfish fillets dipped in organic acids.

ANOVA of b^*- values of polish sausage following treatment with 1% CPC and non-treated polish sausages (Tables 5 and 6) indicated that there was no significant effect ($p \leq 0.05$) of temperature of storage (0 and 4 ℃), time of storage (up to 42 days), and 1% CPC treatment on yellowness of the product. It was observed that the yellowness of the product stored at 4 ℃ was higher than that of the product stored at 0 ℃ irrespective of the treatment. A sudden decrease in the yellowness of the treated polish sausage was observed on day 7 at 0 ℃, which could possibly have been due to loss of vacuum in the packages leading to an increase in the purge inside the package.

Table 5: Mean (SD) lightness, redness, and yellowness values of non-treated (control) and 1% cetylpyridinium chloride treated Polish sausages stored for 42 days at 0 ºC.

St. Time (Days)	L^*- Values ‡		a^*- Values ‡		b^*- Values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	36.74 ± 0.83	34.90 ± 1.51	12.36 ± 0.72	12.74 ± 1.09	11.00 ± 0.47	10.81 ± 0.31
3	37.03 ± 0.90	34.20 ± 0.31	11.35 ± 1.20	12.98 ± 0.05	10.81 ± 2.12	11.06 ± 0.21
7	36.32 ± 0.59	34.74 ± 1.50	11.23 ± 0.79	11.90 ± 0.82	10.72 ± 0.68	11.38 ± 0.13
14	37.58 ± 1.58	34.70 ± 1.46	11.80 ± 0.34	12.09 ± 0.64	10.90 ± 0.50	10.99 ± 0.38
21	34.56 ± 1.05	34.59 ± 0.41	11.48 ± 1.06	12.82 ± 0.75	11.11 ± 0.27	8.51 ± 5.75
28	34.04 ± 1.18	34.67 ± 2.73	11.90 ± 1.01	12.36 ± 1.49	11.97 ± 0.26	11.64 ± 0.99
42	36.21 ± 1.40	35.80 ± 1.46	11.45 ± 0.83	11.08 ± 1.10	11.17 ± 0.14	10.95 ± 0.31

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 0.85, a^*-Values = 0.58, and b^*-Values = 1.07

Table 6: Mean (SD) lightness, redness, and yellowness values of non-treated (control) and 1% cetylpyridinium chloride treated Polish sausages stored for 42 days at 4 ºC.

St. Time (Days)	L^*- Values ‡		a^*- Values ‡		b^*- Values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	36.74 ± 0.83	34.90 ± 1.51	12.36 ± 0.72	12.74 ± 1.09	11.00 ± 0.47	10.81 ± 0.31
3	34.85 ± 1.45	34.27 ± 0.57	12.47 ± 0.62	12.22 ± 0.90	10.49 ± 0.29	10.38 ± 1.11
7	35.57 ± 0.80	36.10 ± 1.94	11.64 ± 0.68	10.89 ± 1.52	12.02 ± 0.73	11.34 ± 0.51
14	36.39 ± 2.08	35.67 ± 1.35	10.83 ± 0.81	10.74 ± 1.57	10.98 ± 0.40	11.20 ± 0.31
21	34.79 ± 1.35	34.56 ± 0.33	10.43 ± 0.37	9.94 ± 0.60	11.00 ± 0.62	11.07 ± 0.27
28	34.06 ± 2.88	35.25 ± 0.64	10.38 ± 0.82	8.79 ± 0.63	10.66 ± 0.79	10.32 ± 0.49
42	35.52 ± 1.29	35.48 ± 2.94	8.43 ± 0.45	8.19 ± 0.67	11.79 ± 0.23	10.44 ± 1.26

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 1.03, a^*-Values = 0.56, and b^*-Values = 0.40

d. **Hardness of Polish sausages**

Analysis of variance of fixed effects indicated that an interaction between days of storage and treatment had a significant effect ($p \leq 0.05$) on the hardness of the product. In addition to this interaction effect, the time for which the product was stored also had a significant ($p \leq 0.05$) effect on the hardness of the polish sausages irrespective of the temperature of storage. Comparison of the differences in least squares means indicated that there was a significant difference ($p \leq 0.05$) in the non-treated product stored for days 3 and 7, 7 and 14, 7 and 21, 7 and 28, 7 and 42, and 28 and 42 irrespective of the temperature at which the product was stored. Gimeno et al. (1999) in a study on influence on partial replacement of NaCl with KCl and $CaCl_2$ on texture and color of dry fermented sausages reported a significant reduction in the hardness in modified sausages. The probable reason for reduction in hardness of modified sausages or sausages treated with antimicrobials could be the denaturation of proteins resulting in weakening of the cross-linked structure of the processed muscle foods. According to Hachmeister and Herald (1997) fat content of the sausages is an important factor that governs the hardness of processed meats.

Table 7 shows the hardness of polish sausages stored at 0 and 4 ºC over a time period of 42 days post-treatment with 1% CPC. It can be observed from table 7 that the non-treated polish sausages were consistently harder than the 1% CPC treated polish sausages within a similar storage temperature (i.e. non-treated product stored at 0 ºC was harder than 1% CPC treated product stored at 4 ºC). Significant difference ($p \leq 0.05$) in hardness of 1% CPC treated and non-treated polish sausages was observed for days 3, 7, 3 and 7, 3 and 14, 3 and 21, 3 and 28, 3 and 42, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14

and 28, while 1% CPC treated polish sausages differed significantly (p≤0.05) at 3 and 7, 3 and 14, 3 and 21, 3 and 28, 3 and 42, 7 and 14, 7 and 21, 7 and 28, and 7 and 42 days of storage irrespective of the temperature of storage.

Table 7: Mean (SD) firmness values of non-treated (control) and 1% cetylpyridinium chloride treated Polish sausages for 42 days at 0 and 4 ºC.

St. Time (Days)	Control (0 ºC) ‡	Treated (0 ºC) ‡	Control (4 ºC) ‡	Treated (4 ºC) ‡
0	2702.57 ± 239.05	2984.53 ± 185.88	2702.57 ± 239.05	2984.53 ± 185.88
3	3088.28 ± 239.48	2631.02 ± 251.19	2812.55 ± 217.10	2545.16 ± 259.79
7	2723.01 ± 181.47	2532.98 ± 240.22	2638.18 ± 301.56	2535.76 ± 252.70
14	3042.06 ± 245.07	2830.18 ± 150.97	3062.78 ± 247.12	2970.01 ± 284.12
21	3182.99 ± 226.50	3014.45 ± 240.68	2942.34 ± 376.43	2976.92 ± 384.18
28	3153.86 ± 318.99	3060.24 ± 375.91	3034.86 ± 235.54	3005.84 ± 324.98
42	2901.62 ± 146.08	2856.69 ± 278.21	2916.72 ± 241.48	2975.07 ± 296.14

‡ Means ± Standard Deviation
LSD values for firmness = 132.67

From table 4 it is evident that there is a significant decrease in the hardness of frankfurters, which can be due to the increase in moisture content of the product as a result of exposure to 1% CPC for a period of 42 days. Salt being a major ingredient in frankfurters as well as polish sausages can possibly be another factor contributing to reduced hardness in these products. Hand et al. (1987) conducted a study on the effects of preblending, reduced fat and salt levels on frankfurter characteristics and revealed that low fat frankfurters required greater shear force than high fat frankfurters. Although, a contradicting result was reported by Gimeno et al. (2000), that there was very little

correlation between the fat and certain textural parameters such as hardness and gumminess. Salt according to Hand et al. (1987) also had a significant effect on the hardness of frankfurters and suggested an increase in the shear force with increase in salt concentrations in the product. Barrett et al. (1998) suggested a complex, non-linear relationship between glycerol levels and texture of meat emulsion systems (frankfurters) and showed firmness of products attributed in part to glycerol enhanced emulsion stability.

IV. Effects of 1% CPC on Shelf Life of Roast Beef

a. Roast Beef inoculated with *Listeria monocytogenes*

Analysis of variance of $\log_{10}$ CFU/ cm^2 of *L. monocytogenes* recovered from sliced/ cut side and surface of roast beef inoculated with low levels of inoculum ($\log_{10} 4$ CFU/ cm^2) following treatment with 1% CPC and stored over a period of 42 days at 0 and 4 °C indicated a significant ($p \leq 0.05$) interactive effects of storage temperature, days of storage, and 1% CPC treatment. In addition to this interactive effect, the individual main effects of storage temperature, storage time, and 1% CPC treatment significantly ($p \leq 0.05$) affected the growth pattern of *L. monocytogenes* on the surface as well as on the cut side of roast beef.

Figure 17 shows the growth pattern of *L. monocytogenes* on sliced/ cut side of roast beef treated with 1% CPC and non-treated roast beef inoculated with low levels (ca. $\log_{10} 4$ CFU/ cm^2) and stored up to 42 days at 0 and 4 °C. It can be observed that 1% CPC treatment of the roast beef had an immediate bactericidal effect on *L. monocytogenes* and did not allow the organism to grow up to higher levels with time, hence exhibiting bacteriostatic characteristics as well. It was indicated from figure 17 that the non-treated

inoculated roast beef stored at 0 ºC supported lesser growth of *L. monocytogenes* as compare to those stored at 4 ºC over a period of 42 days.
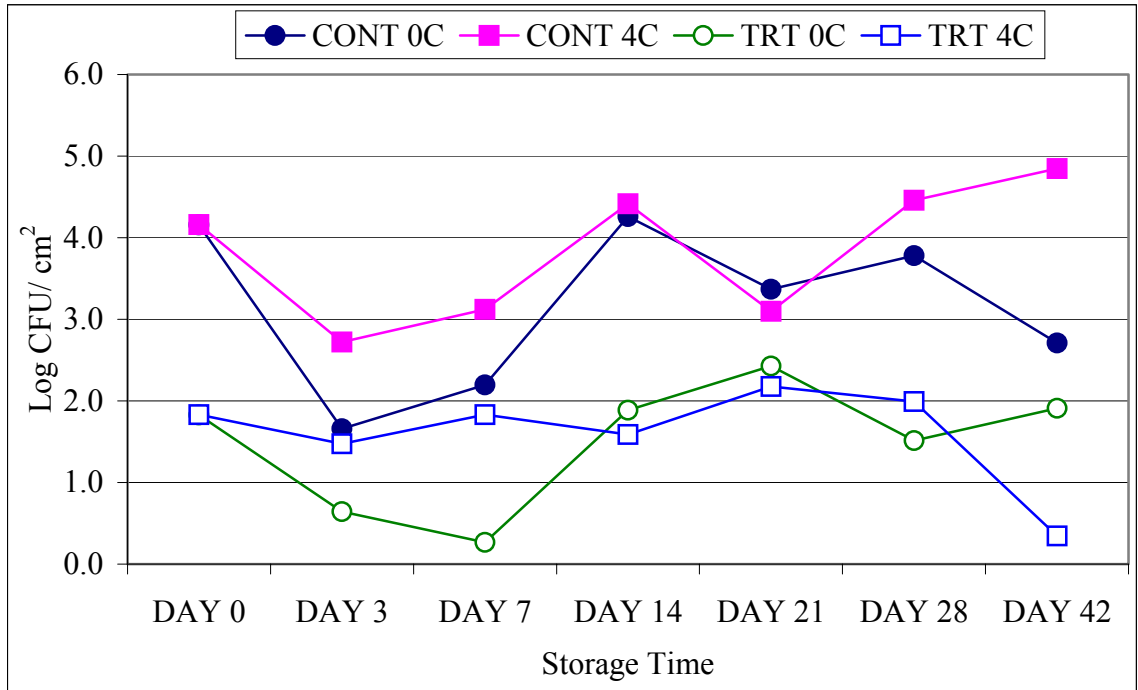
Figure 17: *L. monocytogenes* recovery on sliced/ cut side of non-treated and 1% cetylpyridinium chloride treated roast inoculated with low levels (ca. $\log_{10}$ 4 CFU/ cm^2) and stored over time at 0 and 4 ºC

Figure 18 shows the growth pattern of *L. monocytogenes* on the surface of roast beef treated with 1% CPC and non-treated roast beef inoculated with low levels (ca. $\log_{10}$ 4 CFU/ cm^2) and stored for 42 days at 0 and 4 ºC. It can be clearly observed that 1% CPC had a bactericidal as well as a bacteriostatic effect on the growth of the organism on surfaces of roast beef. Storage temperature of 4 ºC consistently supported higher growth of *L. monocytogenes* as compared to 0 ºC for 42 days. These results were in consistency with a study done by Grau and Vanderlinde (1990), which suggested that the populations of *Listeria* spp. reached in 2 weeks storage at 5.3 ºC were greater than those reached in 11 weeks at 0 ºC. Shelef (1989) offered an explanation for the ability of *L. monocytogenes* to

endure prolonged refrigerated storage in meat without an increase in viable populations. It was suggested that perhaps meat lacks a nutrient required for the growth of the organism. However, the exact reason for the persistence but absence of growth of *L. monocytogenes* in meats is yet to be explained.
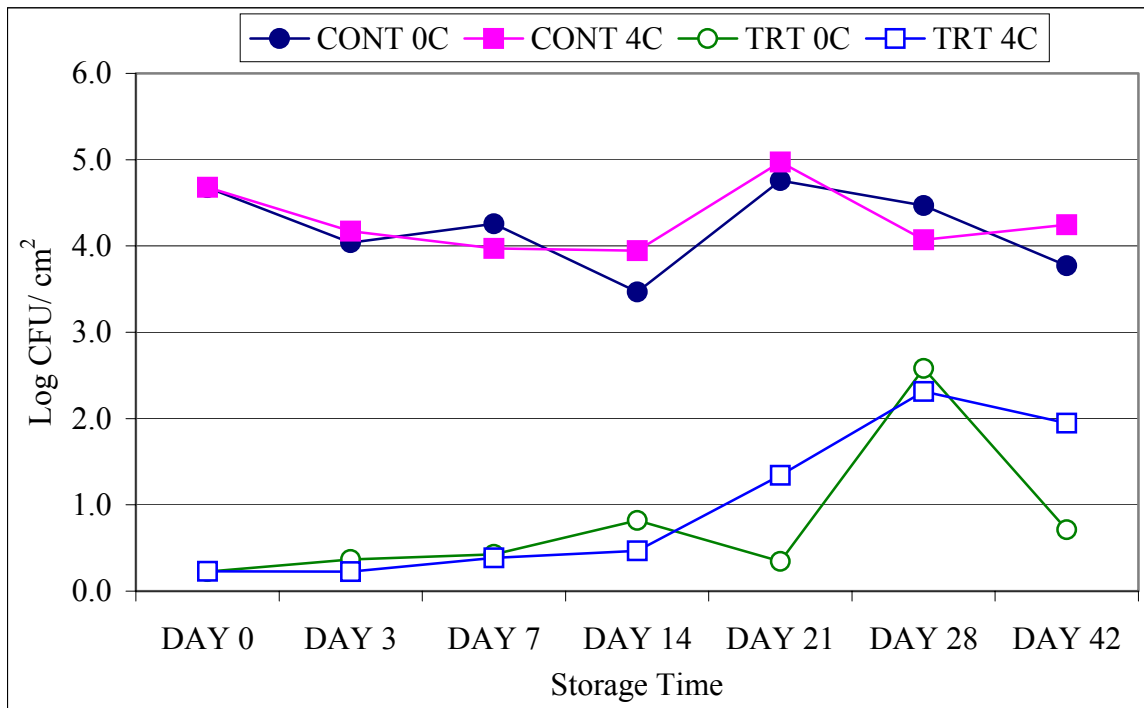
Figure 18: *L. monocytogenes* recovery on surface of non-treated and 1% cetylpyridinium chloride treated roast beef inoculated with low levels (ca. $\log_{10}$ 4 CFU/ cm^2) and stored over time at 0 and 4 ºC

Results from the analysis of variance of $\log_{10}$ CFU/ cm^2 of *L. monocytogenes* recovered from the sliced/ cut side of roast beef inoculated with high levels (ca. $\log_{10}$ 7 CFU/ cm^2) indicated that the storage temperature, days up to which the product was stored, and 1% CPC treatment had a significant ($p \leq 0.05$) effect on the growth behavior of the organism. Figure 19 summarizes the growth pattern of *L. monocytogenes* on the sliced/ cut side of roast beef treated with 1% CPC and non-treated roasts stored for 42 days at 0 and 4 ºC. The recovery of *L. monocytogenes* was higher on the sliced/ cut side

of non-treated roast beef stored at 4 ºC as compared to 0 ºC and 1% CPC treated product

had significantly lower recovery of *L. monocytogenes* than the non-treated product.
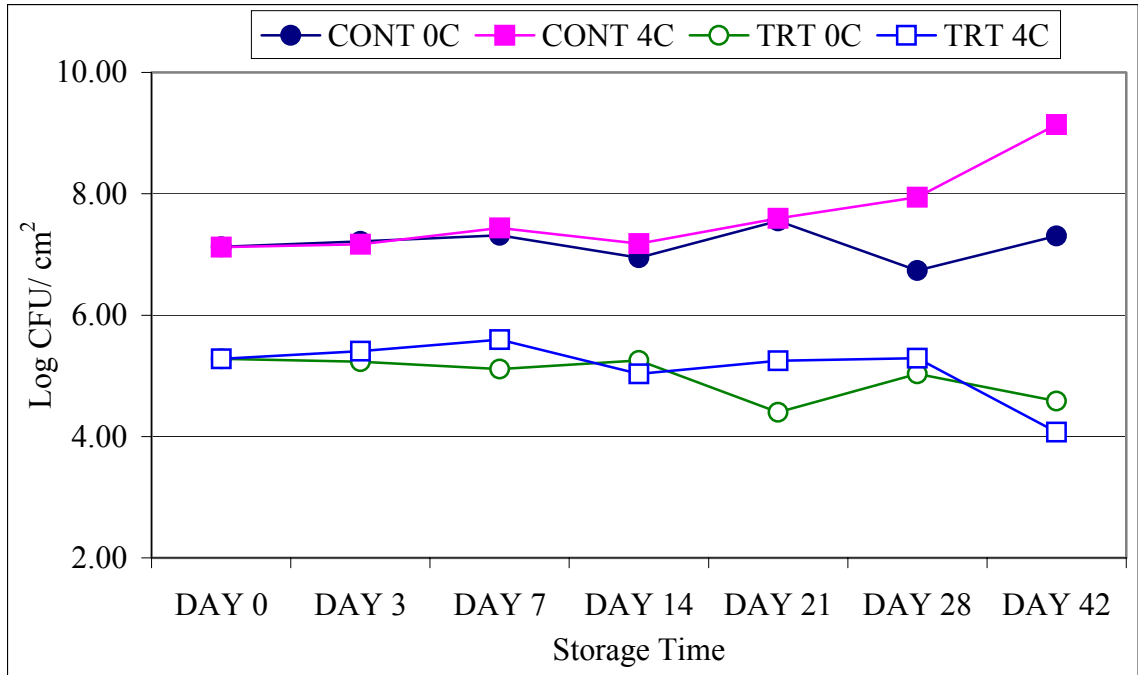
Figure 19: *L. monocytogenes* recovery on sliced side of non-treated and 1% cetylpyridinium chloride treated roast beef inoculated with high levels (ca. $\log_{10}$ 7 CFU/ cm^2) and stored over time at 0 and 4 ºC

Analysis of variance of fixed effects indicated that an interaction between days of

storage and 1% CPC treatment had a significant effect ($p \leq 0.05$) on the recovery of *L.*

monocytogenes from the surface of 1% CPC treated and non-treated roast beef inoculated

with high levels (ca. $\log_{10}$ 7 CFU/ cm^2) of the organism over 42 days at 0 and 4 ºC. An

interaction of storage temperature and days of storage also had a significant effect

($p \leq 0.05$) on the growth pattern of the organism. Further analysis of the fixed effects

indicated that temperature of storage, 1% CPC treatment, and days of storage each had a

significant ($p \leq 0.05$) individual effect on the growth of *L. monocytogenes* on the surface

of roast beef up to 42 days. Comparison of the differences in least squares means

revealed a significant difference ($p \leq 0.05$) in the growth patterns of the organism on days

3 and 21, 7 and 21, 14 and 21, 14 and 28, and 21 and 42 irrespective of the temperature of storage and treatment with 1% CPC.
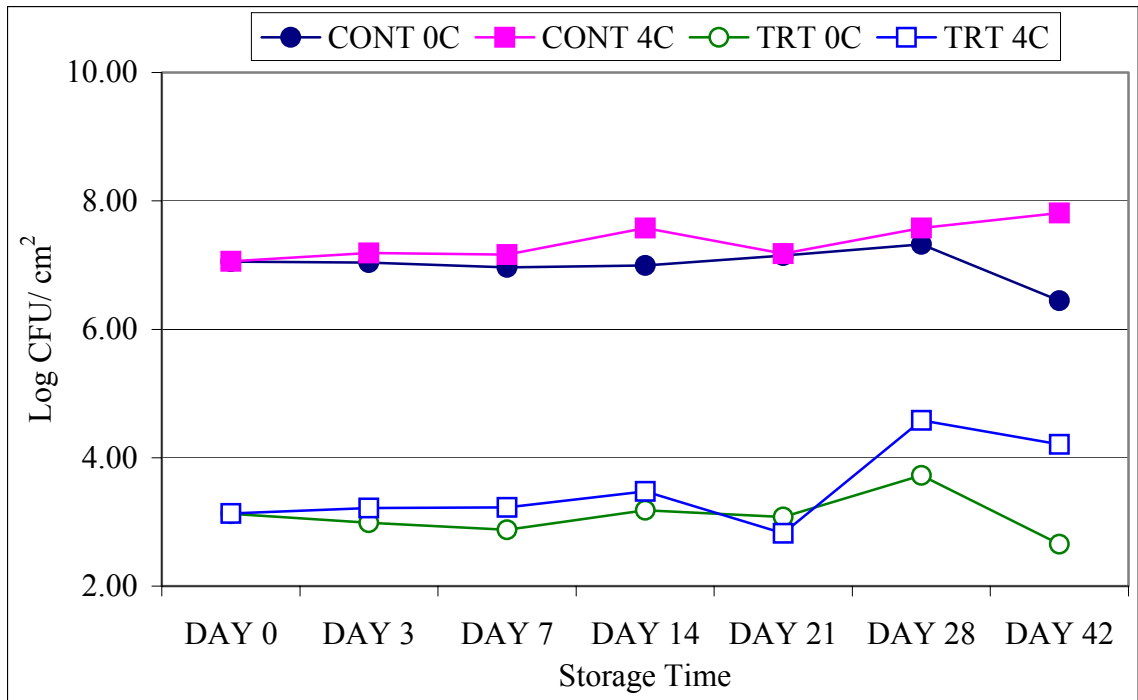
Figure 20: *L. monocytogenes* recovery on surface of non-treated and 1% cetylpyridinium chloride treated roast inoculated with high levels (ca. log10 7 CFU/ cm2) and stored over time at 0 and 4 ºC

From figure 20 it is evident that treatment of roast beef with 1% CPC had a significant bactericidal as well as a bacteriostatic effect on the growth of *L. monocytogenes*. Although the non-treated roasts also did not show any growth over time under refrigeration, the 1% CPC treated roast beef stored at 0 ºC consistently showed lesser recovery as compared to when stored at 4 ºC. This result is in agreement to a study done by Johnson et al. (1988), which revealed that the viable population of *L. monocytogenes* in inoculated ground beef remained relatively constant during a 14-day storage period at 4 ºC. The results from our study were also in agreement with a study

done by Glass and Doyle (1989), which suggested that little or no growth of *L. monocytogenes*, occurred on precooked roast beef during refrigerated storage (4.4 ºC).

b. <u>Non-Inoculated roast beef</u>

Combination of 1% CPC treatment and days of storage had a significant effect ($p \leq 0.05$) on the growth of aerobic plate count ($\log_{10}$ CFU/ cm^2 of APC) on the sliced/ cut side as well as on the surface of roast beef irrespective of the temperatures (0 and 4 ºC) at which the product was stored. Further analysis of main effects indicated that day of storage and 1% CPC treatment also had a significant ($p \leq 0.05$) on the growth of APC at 0 and 4 ºC storage temperature on both the sliced/ cut side and surface of roast beef. Comparison of least squares means revealed that 1% CPC treatment did not have any significant ($p > 0.05$) effect on APC of the sliced/ cut side at day 0 and between days 0 and 3, 3 and 14, 3 and 21, 3 and 28, 3 and 42, 7 and 14, 7 and 28, 7 and 42, 14 and 42, 28 and 42 irrespective of the temperature at which the product was stored. Whereas the comparison of least squares means of growth of APC on the surface of roast beef indicated no significant differences ($p > 0.05$) between days 0 and 3, 3 and 14, 3 and 21, 3 and 28, 3 and 42, 7 and 28, 7 and 42 irrespective of the storage temperature of the product.
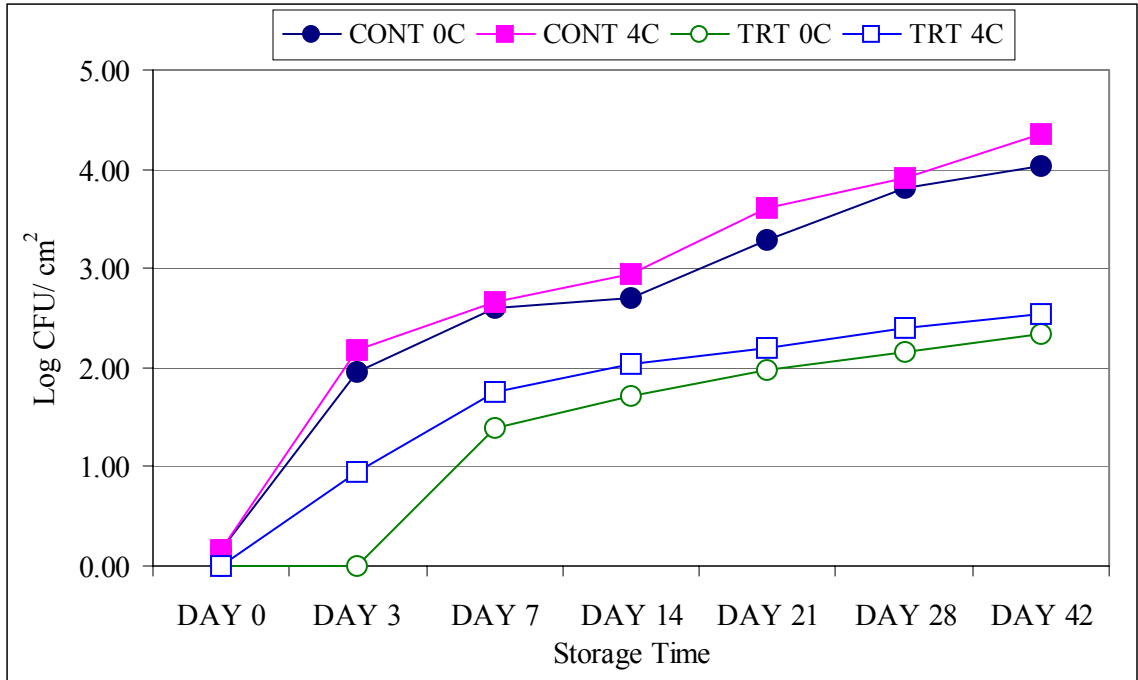
Figure 21: Aerobic Plate Count ($\log_{10}$ CFU/ cm^2) on sliced side of non-treated and 1% cetylpyridinium chloride treated roast beef stored over time at 0 and 4 ºC
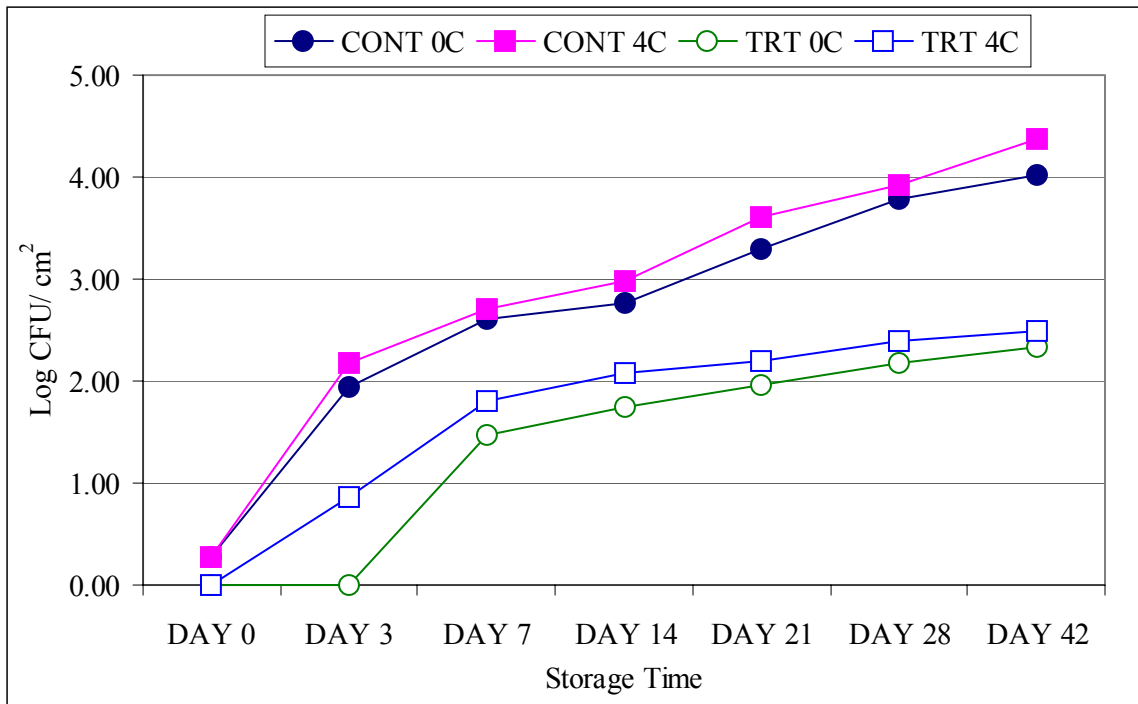
Figure 22: Aerobic Plate Count ($\log_{10}$ CFU/ cm^2) on surface of non-treated and 1% cetylpyridinium chloride treated roast beef stored over time at 0 and 4 ºC

Aerobic plate count on the sliced/ cut side (Figure 21) and surface (Figure 22) of non-treated roast beef increases up to 4.5 $\log_{10}$ CFU's/ cm^2 as compared to a 2.5 $\log_{10}$ CFU's/ cm^2 increase on 1% CPC treated roast beef over 42 days indicating bacteriostatic effects of CPC. The likely reason for reduction in numbers on whole muscle tissue is due to the bactericidal action of antimicrobial compounds (Dickson, 1988). Results from our study were according to results reported by a study done by Cutter et al. (2000), in which reductions in the populations of APC on beef surfaces were observed when treated with 1% CPC immediately after spray treatments and after extended, refrigerated, vacuum packaged storage. Analysis of variance of $\log_{10}$ CFU's/ cm^2 of lactic acid bacteria (LAB) recovered from the sliced/ cut side (Figure 23) and surface (Figure 24) of roast beef indicated that combination of storage day and 1% CPC treatment had a significant (p≤0.05) effect on the growth of LAB at 0 and 4 ºC. Additionally individual significant (p≤0.05) effects of storage day, 1% CPC treatment, and temperature of storage of roast beef were observed on the sliced/ cut side of the product. While storage temperature did not have a significant effect (p>0.05) on the growth of LAB on the surface of roast beef.

A comparison of the least squares means indicated that there were no significant (p>0.05) differences in the growth pattern of LAB between non-treated and 1% CPC treated roasts at day 0 and 7, 3 and 14, 3 and 21, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 42 on the sliced/ cut side of the product. While 1% CPC treatment did not have a significant difference (p>0.05) on LAB on the cut side at days 14 and 21, 14 and 42, 21 and 28, 21 and 42, 28 and 42 irrespective of the temperature at which the product was stored.

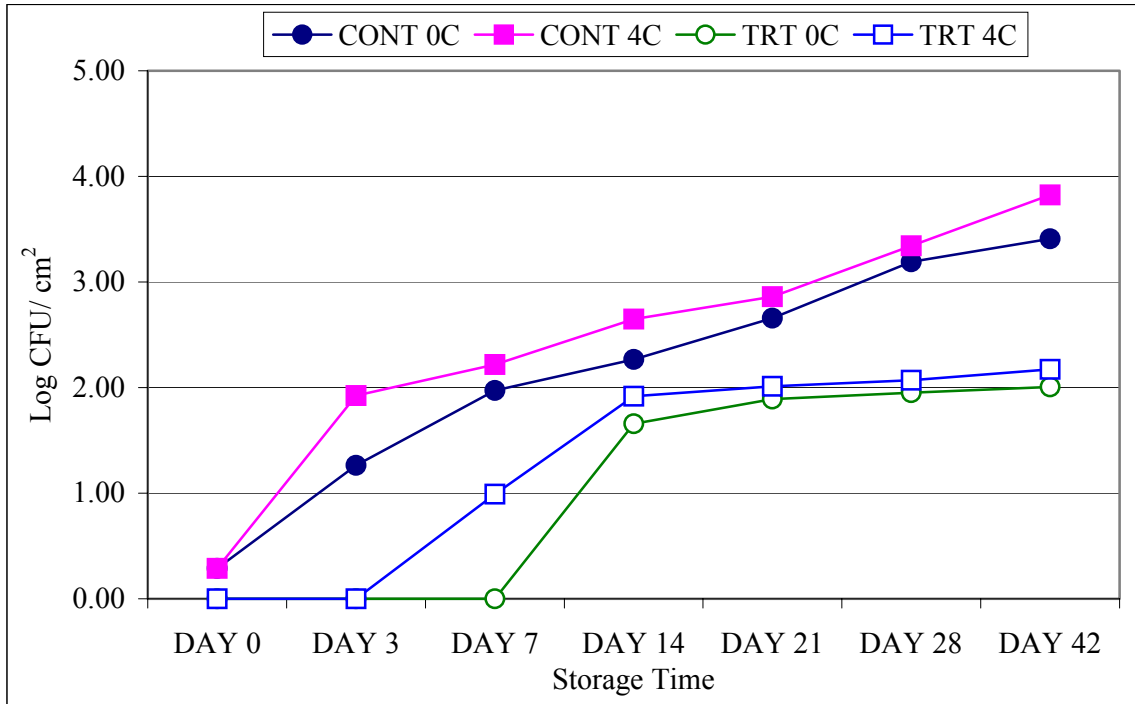**Figure 23:** Lactic acid bacteria (LAB) count ($\log_{10}$ CFU/ cm^2) on sliced side of non-treated and 1% cetylpyridinium chloride treated roast beef stored over time at 0 and 4 ºC
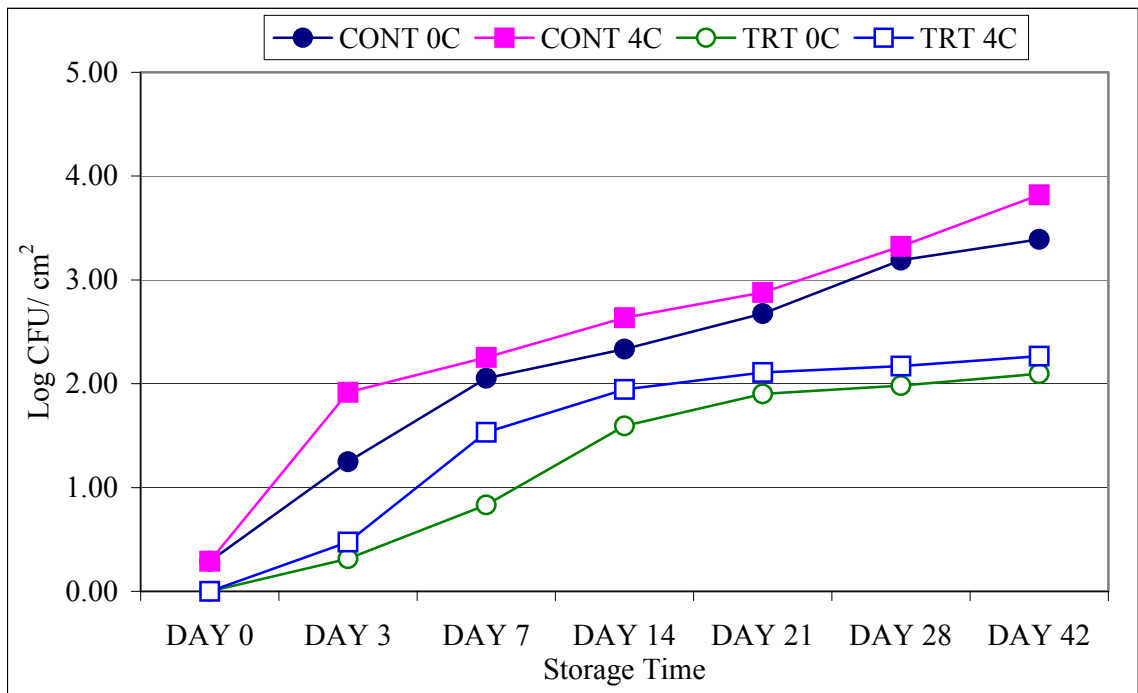

Figure 24: Lactic acid bacteria (LAB) counts ($\log_{10}$ CFU/ cm^2) on surface of non-treated and 1% cetylpyridinium chloride treated roast beef stored over time at 0 and 4 ºC

Comparison of least squares means of growth of LAB on the surface of roast beef indicated no significant (p>0.05) effect of 1% CPC treatment between days 0 and 3, 3 and 7, 3 and 14, 3 and 21, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 28, 14 and 42 irrespective of the temperature of storage of the product. Treatment of roast beef with 1% CPC suppressed the growth of LAB at either temperatures of storage up to 2.2 $\log_{10}$ CFU/ cm^2 on the sliced/ cut side as well as on the surface compared to a growth of up to 3.8 $\log_{10}$ CFU/ cm^2 in non-treated product. These results were in agreement with a study done by Johnson et al. (1979), in which an approximately 2.8 $\log_{10}$ CFU/ g growth of LAB was observed in ground beef over a period of 13 day at 2 ºC when treated with hypochlorous acid. Although no citable literature is available on the effects of 1% CPC on growth of yeasts and molds, treatment of roast beef with 1% CPC reduced the numbers of yeast and mold, total coliform counts (TCC) and *E. coli* below detectable limits (< 0.75 CFU/ cm^2) on the sliced/ cut side as well as on the surface in the product over a period of 42 days irrespective of the temperature of storage.

c. <u>Color of roast beef</u>

Tables 8, 9, 10, and 11 summarize the influences of 1% CPC treatment, storage temperature and storage days on the L^*- values (lightness), a^*- Values (redness), and b^*- values (yellowness) of sliced/ cut side and surface of roast beef stored at 0 and 4 ºC.

Significant (p≤0.05) interactive effects of storage temperature and days of storage were observed on the lightness of sliced/ cut side of roast beef (Tables 8 and 9). Temperature and days of storage individually had a significant effect (p≤0.05) on the L^*- value of the cut side of the product. A comparison of least squares means indicated no significant difference (p>0.05) on the lightness of the cut side of roast beef between days

3 and 14, 3 and 28, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 28, 14 and 42, and 28 and 42 of storage at 0 ºC. Whereas at 4 ºC temperature of storage no significant differences (p>0.05) in the lightness of cut side of roast beef were observed between days 3 and 21, 3 and 28, 14 and 21, 14 and 28, 14 and 42, 21 and 28, 21 and 42, and 28 and 42 of storage.

Table 8: Mean (SD) lightness, redness, and yellowness values of sliced side of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 0 ºC.

St. Time (Days)	L^* -values ‡		a^* -values ‡		b^* -values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	31.74 ± 3.98	34.28 ± 1.41	4.36 ± 1.21	3.16 ± 0.42	7.03 ± 1.56	5.01 ± 0.83
3	35.58 ± 0.55	37.26 ± 1.73	3.13 ± 0.29	2.88 ± 0.07	3.96 ± 0.51	5.85 ± 1.89
7	40.05 ± 6.69	41.98 ± 3.24	2.50 ± 0.77	2.06 ± 0.38	5.47 ± 1.51	4.42 ± 0.98
14	37.21 ± 1.53	39.95 ± 5.91	1.70 ± 0.18	2.00 ± 0.53	4.32 ± 0.27	4.81 ± 1.15
21	43.53 ± 1.50	43.05 ± 0.54	3.02 ± 0.75	2.95 ± 0.35	5.42 ± 0.82	4.62 ± 1.33
28	36.14 ± 0.65	40.06 ± 1.21	1.94 ± 0.43	2.04 ± 0.24	4.04 ± 1.32	4.09 ± 0.77
42	39.66 ± 1.85	38.44 ± 4.21	2.54 ± 0.56	2.03 ± 0.39	5.21 ± 1.52	3.92 ± 0.34

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 1.99, a^*-Values = 0.35, and b^*-Values = 0.73

Table 9: Mean (SD) lightness, redness, and yellowness values of sliced side of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 4 ºC.

St. Time (Days)	L^* -values ‡		a^* -values ‡		b^* -values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	31.74 ± 3.98	34.28 ± 1.41	4.36 ± 1.21	3.16 ± 0.42	7.03 ± 1.56	5.01 ± 0.83
3	37.23 ± 1.20	40.72 ± 3.72	3.98 ± 0.24	2.67 ± 1.02	5.09 ± 0.74	5.83 ± 1.14
7	51.45 ± 3.66	49.72 ± 2.62	4.02 ± 0.67	3.52 ± 0.10	8.69 ± 1.79	7.64 ± 1.09
14	42.08 ± 0.64	46.13 ± 5.06	2.43 ± 0.22	3.48 ± 0.95	5.06 ± 0.42	7.49 ± 2.23
21	43.84 ± 3.01	39.53 ± 1.96	2.66 ± 0.51	2.03 ± 0.36	4.99 ± 1.38	3.31 ± 0.78
28	41.20 ± 3.55	40.69 ± 0.38	2.34 ± 0.35	2.03 ± 0.08	3.69 ± 1.46	3.29 ± 0.21
42	40.95 ± 1.32	46.48 ± 4.87	2.61 ± 0.75	3.22 ± 1.09	4.81 ± 0.98	6.66 ± 2.79

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 1.93, a^*-Values = 0.43, and b^*-Values = 0.89

Analysis of variance of L^*-values with three-way effects indicated that days of storage had a significant (p≤0.05) effect on the lightness of the surface of the product irrespective of 1% CPC treatment and temperature (0 and 4 ºC) of storage (Tables 10 and 11). Whereas temperature of storage and 1% CPC treatment did not have a significant effect (p>0.05) on the lightness of roast beef stored for a period of 42 days. Further comparisons of least squares means of L^*- values of the surface of roast beef suggested significant differences (p≤0.05) between day 0 and the rest of the days of storage (3, 7, 14, 21, 28, and 42), days 3 and 7, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 21 and 28, and 28 and 42 irrespective of 1% CPC treatment and temperatures (0 ºC and 4 ºC) of storage.

Table 10: Mean (SD) lightness, redness, and yellowness values of surface of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 0 ºC.

St. Time (Days)	L^* -values ‡		a^* -values ‡		b^* -values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	31.91 ± 1.01	32.27 ± 4.14	2.96 ± 0.34	2.72 ± 0.30	6.98 ± 0.27	6.79 ± 0.50
3	36.90 ± 1.18	36.54 ± 0.00	3.26 ± 0.36	1.99 ± 0.00	5.35 ± 1.38	5.48 ± 0.00
7	50.36 ± 8.89	48.64 ± 8.23	2.72 ± 1.91	2.32 ± 1.50	7.66 ± 4.75	6.90 ± 3.46
14	37.96 ± 3.48	41.47 ± 3.14	2.04 ± 0.57	1.87 ± 0.50	5.86 ± 0.38	5.85 ± 2.14
21	43.73 ± 3.14	42.96 ± 4.52	2.53 ± 0.44	2.72 ± 1.10	4.90 ± 1.12	4.42 ± 2.57
28	37.79 ± 5.99	36.75 ± 3.80	2.13 ± 0.15	1.64 ± 0.32	5.24 ± 0.59	4.94 ± 0.75
42	41.89 ± 5.01	41.25 ± 0.61	2.30 ± 0.49	1.80 ± 0.26	6.22 ± 0.94	4.94 ± 1.79

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 2.90, a^*-Values = 0.50, and b^*-Values = 1.25

Analysis of variance of a^*- values (redness) of cut side and surface of roast beef indicated a significant ($p \leq 0.05$) interactive effect of temperature and days of storage, treatment and days of storage, along with individual effects of storage day and temperature of storage on the redness of cut side of roast beef. Tables 8 and 9 indicate the patterns of redness of cut side of roast beef stored for 42 days at 0 and 4 ºC. Comparison of least squares means of a^*- values of cut side of roast beef indicated a no significant ($p > 0.05$) differences between days 3 and 7, 7 and 21, 7 and 42, 14 and 21, 14 and 28, 14 and 42, 21 and 42 of storage of the product irrespective of 1% CPC treatment.

75

Table 11: Mean (SD) lightness, redness, and yellowness values of surface of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 4 ºC.

St. Time (Days)	L^*-values ‡		a^*-values ‡		b^*-values ‡	
	Control	Treated	Control	Treated	Control	Treated
0	31.91 ± 1.01	32.27 ± 4.14	2.96 ± 0.34	2.72 ± 0.30	6.98 ± 0.27	6.79 ± 0.50
3	40.30 ± 2.91	42.52 ± 0.99	3.55 ± 1.22	2.06 ± 0.07	6.27 ± 0.58	6.23 ± 0.61
7	47.95 ± 8.93	53.42 ± 3.65	2.55 ± 0.42	3.59 ± 0.40	6.89 ± 0.62	10.6 1 ± 2.24
14	43.55 ± 7.56	41.44 ± 4.35	2.29 ± 1.14	2.01 ± 0.74	6.48 ± 3.15	5.25 ± 1.76
21	43.00 ± 8.02	37.60 ± 2.20	2.01 ± 1.25	1.41 ± 0.55	4.67 ± 4.10	2.35 ± 1.36
28	38.22 ± 0.19	39.19 ± 2.96	1.75 ± 0.19	1.63 ± 0.38	3.15 ± 0.35	3.80 ± 1.01
42	43.18 ± 2.64	43.13 ± 4.48	2.63 ± 0.49	2.50 ± 0.49	5.33 ± 1.39	5.41 ± 1.90

L^*-Values = lightness, a^*-Values = redness, and b^*-Values = yellowness
‡ Means ± Standard Deviation
LSD values for L^*-Values = 2.93, a^*-Values = 0.43, and b^*-Values = 1.13

1% CPC treatment and days for which the roast beef was stored had a significant (p≤0.05) effect on the a^*-values of surface of the product stored at 0 ºC and 4 ºC. Tables 10 and 11 summarize the changes in the redness of the surface of roast beef treated with 1% CPC and non-treated roast beef stored for 42 days at 0 and 4 ºC. Comparison of differences of least square means indicated significant differences (p≤0.05) in the a^*-values of roast beef surface between days 0 and 14, 0 and 21, 0 and 28, 3 and 14, and 7 and 28 irrespective of storage temperature.

Tables 8 and 9 summarize the effects of 1% CPC on the b^*- values of the sliced/ cut side of roast beef stored at 0 and 4 ºC for 42 days. Analysis of variance with tests of

fixed effects indicated significant (p≤0.05) interactive effects of treatment and days of storage, and storage temperature and storage day on the b^*- values of the cut side of roast beef. Furthermore, storage day also had a significant effect (p≤0.05) on the b^*- values of the cut side of the product. From table 9 it is evident that there was an increase in the b^*- value of the cut side of roast beef when treated with 1% CPC and stored at 4 ºC up to 42 days storage time. Comparisons of differences in least squares means of storage days individually suggested a significant (p≤0.05) difference in the b^*- values of cut side of the product between days 0 and 21, 0 and 28, 3 and 7, 3 and 28, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 28, and 28 and 42 irrespective of the temperature of storage and treatment of the product.

Analysis of variance indicated a significant (p≤0.05) effect of storage days on the b^*- values (yellowness) of surface of roast beef irrespective of storage temperature and 1% CPC treatment. Whereas no significant (p>0.05) effects of storage temperature (0 ºC and 4 ºC) and 1% CPC treatment were observed on the yellowness of cut side of the product (Tables 10 and 11). A comparison of the differences in the least squares means indicated a significant (p≤0.05) difference between days 0 and 21, 0 and 28, 0 and 42, 3 and 7, 3 and 21, 3 and 28, 7 and 14, 7 and 21, 7 and 28, 7 and 42, 14 and 21, 14 and 28 irrespective of the storage temperature and 1% CPC treatment on the surface yellowness of roast beef. From table 11 it can be observed that the surface yellowness of roast beef treated with 1% CPC and stored at 4 ºC fluctuated over 42 days which could possibly be due to the excessive CPC that might have been present on the vacuum packaged product.

Shao et al. (1999) reported a decrease in the lightness, redness and yellowness of restructured beef steaks as a result of treatment with treating the steaks with sodium

alginate lipid encapsulated lactic acid/ calcium lactate, sodium tripolyphosphate/ sodium chloride, and fibrinogen/ thrombin. Barrett et al. (1998) reported a decrease in the lightness of processed Beefsticks as a result of treatment with glycerol over a period of 4 weeks. Barrett et al. (1998) attributed decrease in lightness of processed beef sticks due to reduced water activity with storage time and nonenzymatic browning in meat due to reactivity of glycerol with proteins. Results from our studies were in accordance with Shao et al. (1999) indicated similar decreases in yellowness, redness, and no changes in the lightness on sliced/ cut side and surface of the roast beef treated with 1% CPC and stored for 42 days at 0 and 4 ºC.

d. <u>Hardness of roast beef</u>

Tables 12 and 13 summarize the influences of 1% CPC treatment on the firmness of sliced/ cut side and surface of roast beef over a period of 42 days at 0 and 4 ºC.

Analysis of variance indicated a significant difference ($p \leq 0.05$) in the sliced/ cut side hardness of roast beef stored for 42 days at 0 ºC and 4 ºC. Whereas a significant ($p \leq 0.05$) interactive effect of storage temperature and 1% CPC treatment, in addition to a significant ($p \leq 0.05$) individual effect of storage temperature was observed on the surface hardness of roast beef. Comparison of differences in least squares means suggested significant ($p \leq 0.05$) differences between the effects of surface hardness of non-treated and treated roast beef when stored at 0 ºC, treated roast beef stored and non-treated product at 4 ºC, and 1% CPC treated roasts stored at 0 ºC and 4 ºC.

It was observed (Table 12) that the cut side hardness of non-treated roasts is consistently higher than that of 1% CPC treated roast beef. Also, a sharp increase in the hardness of cut side of non-treated roast beef on day 42 at 4 ºC was observed. Results

from our study were contradictory to studies done by Barrett et al. (1998), which suggested a decrease in the firmness of whole muscle meat due to increased water and glycerol. Barrett et al. (1998) also suggested it that the textural characteristics of all processed beefsticks declined throughout accelerated storage up to 4 weeks, but glycerol did not augment the rate of softening. Table 13 suggests that 1% CPC treated roast beef stored at 0 ºC had consistently softer surface as compared to the treated roast beef stored at 4 ºC as well as the non-treated roast beef.

Table 12: Mean (SD) firmness values of sliced side of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 0 and 4 ºC.

St. Time (Days)	Control (0 ºC) ‡	Treated (0 ºC) ‡	Control (4 ºC) ‡	Treated (4 ºC) ‡
0	15937.74 ± 1929.24	14607.83 ± 1566.41	12851.73 ± 4634.85	12879.28 ± 3062.81
3	15275.70 ± 5189.05	15651.87 ± 774.40	14998.58 ± 616.15	14936.18 ± 1799.86
7	16815.74 ± 438.76	14857.20 ± 825.45	15548.79 ± 1476.60	16480.49 ± 2942.20
14	14902.63 ± 378.78	15112.06 ± 1155.91	15392.78 ± 1619.55	16610.16 ± 1093.46
21	17965.83 ± 4407.07	15168.93 ± 1778.92	15801.33 ± 842.56	11575.00 ± 2894.55
28	17962.42 ± 2183.78	14463.85 ± 5227.43	16084.78 ± 1267.05	13347.60 ± 4422.27
42	15986.87 ± 1706.71	16014.47 ± 271.94	25836.42 ± 11071.33	11935.07 ± 3970.86

‡ Means ± Standard Deviation
LSD values for firmness = 2727.6

Table 13: Mean (SD) firmness values of surface of non-treated (control) and 1% cetylpyridinium chloride treated roast beef stored for 42 days at 0 and 4 ºC.

St. Time (Days)	Control (0 ºC) ‡	Treated (0 ºC) ‡	Control (4 ºC) ‡	Treated (4 ºC) ‡
0	14638.37 ± 2296.94	12857.87 ± 1355.81	13397.42 ± 1709.68	14509.56 ± 1190.45
3	16609.82 ± 1875.08	12038.4 ± 1253.34	15524.74 ± 2055.78	17473.15 ± 3407.55
7	13693.34 ± 1274.30	12399.58 ± 1240.30	14312.35 ± 3145.75	13413.19 ± 1702.64
14	14900.73 ± 629.75	13312.94 ± 659.64	15163.94 ± 1841.08	16557.94 ± 3201.28
21	14309.48 ± 2003.45	11936.68 ± 1383.92	13894.69 ± 971.34	15194.26 ± 830.26
28	14816.77 ± 1982.81	12060.79 ± 1042.31	14600.95 ± 1809.35	15246.77 ± 1103.25
42	14102.3 ± 1535.08	12320.89 ± 1203.55	15269.54 ± 1639.04	14521.28 ± 610.06

‡ Means ± Standard Deviation
LSD values for firmness = 1439.4

SUMMARY

1. Treatment of RTE meat products (frankfurters) with 1% cetyl pyridinium chloride (CPC) resulted in up to 2.5 $\log_{10}$ CFU/ g surface destruction of *Listeria monocytogenes* irrespective of the temperature of CPC spray, time of exposure of the products to CPC and pressure at which the CPC is sprayed onto the surface of the products.

2. Treatment of ready to eat meats with 1% CPC had a bactericidal as well as a bacteriostatic effect on the surface of products stored at 0 ºC and 4 ºC up to 42 days.

3. Application of 1% CPC on the surfaces of ready to eat meats suppressed the growth of APC, LAB, total coliforms, *E. coli*, and yeasts and molds for up to 42 days at 0 ºC and 4 ºC temperatures of storage.

4. Treatment of ready to eat meats with 1% CPC did not effect the lightness, redness, and yellowness of frankfurters and roast beef stored for up to 42 days at 0 ºC and 4 ºC. Whereas the redness of polish sausages tends to decrease post-treatment under identical storage conditions.

5. Application of 1% CPC did not have an effect on the hardness of polish sausages and roast beef, but frankfurters stored at 0 ºC and 4 ºC appeared to be softer as a result of 1% CPC treatment over time (42 days).

6. CPC may provide the foundation on sanitizing formulations that can improve the microbiological quality and safety of meat products. But it still remains to be established if enhancement of microbiological safety/ quality will result in the products having acceptable sensory quality.

CONCLUSIONS

Overall, from this study it can be concluded that an integrated approach would be an ideal solution to the control of *L. monocytogenes* in processed RTE meat and poultry products. Some of the factors that must be considered for prevention of contamination in the food industry are to understand the importance of sanitation standard operating procedures (SSOP), have a validated cooking process for the RTE products, addition of a critical control point (CCP) which can effectively repasteurize the surfaces of cooked RTE products (post-process pasteurization), treatment with chemical antimicrobials which can act as bactericidal and bacteriostatic agents to inhibit low-level outgrowths during the intended shelf life of these products. In case of certain sliced products like roast beef a validated, controlled mini-environment/ clean room could be helpful to prevent re-contamination during slicing and/ or packaging. Verification of all these steps by microbiological testing would enhance the overall effectiveness of this integrated approach in the food industry.

REFERENCES

1. Ahamad N. and E.H. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21, and 35°C in Tryptose Broth Acidified with Acetic, Citric, or Lactic acid. J. Food Prot. 52(10) 688-695.

2. Barrett A.H, J.Briggs, M.Richardson, and T.Reed. 1998. Texture and Storage Stability of Processed Beefsticks as Affected by Glycerol and Moisture Levels. J. Food Sci. 63(1) 84-87.

3. Bedie G.K., J.Samelis, J.N.Sofos, K.E.Belk, J.A.Scanga, and G.C.Smith. 2001. Antimicrobials in the Formulation To Control *Listeria monocytogenes* Postprocessing Contamination on Frankfurters Stored at 4C in Vacuum Packages. J. Food Prot. 64(12) 1949-1955.

4. Bell K.Y., C.N.Cutter, and S.S.Sumner. 1997. Reduction of Foodborne Microorganisms on Beef Carcass Tissue using Acetic Acid, Sodium Bicarbonate, and Hydrogen Peroxide Spray Washes. Food Microbiol.14: 439-448.

5. Bernard D.T. and V.N.Scott. 1999. *Listeria monocytogenes* in Meats: New Strategies Are Needed. Food Technol. 53(3) 124.

6. Borchert L.L. 1999. Technology Forum: *Listeria monocytogenes* interventions for ready-to-eat meat products. American Meat Institute, Washington D.C.

7. Brackett R.E. 1987. Antimicrobial Effect of Chlorine on *Listeria monocytogenes*. J. Food Prot. 50(12) 999-1003.

8. Brackett R.E. 1988. Presence and persistence of *Listeria monocytogenes* in Food and Water. Food Technol. 162-164.

9. Breen P.J., C.M.Compadre, E.K.Fifer, H.Salari, D.C.Serbus, and D.L.Lattin. 1995. Quaternary Ammonium Compounds Inhibit and Reduce the Attachment of Viable *Salmonella typhimurium* to Poultry Tissues. J. Food Sci.60 (6) 1191-1196.

10. Breen P.J., H.Salari, and C.M.Compadre. 1997. Elimination of *Salmonella* Contamination from Poultry Tissues by Cetylpyridinium Chloride Solutions. J. Food Prot.60 (9) 1019-1021.

11. Buchanan R.L., H.G.Stahl, and R.C.Whiting. 1989. Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the growth of *Listeria monocytogenes*. J. Food Prot. 52(12) 844-851.

12. Buchanan R.L., M.H.Golden, and R.C.Whiting. 1993. Differentiation of the Effects of pH and Lactic or Acetic Acid Concentration on the Kinetics of *Listeria monocytogenes* Inactivation. J. Food Prot. 56(6) 474-478.

13. Cassiday P.K, and R.E.Brackett. 1989. Methods and Media to Isolate and Enumerate *Listeria monocytogenes*: A Review. J. Food Prot. 52(3) 207-214.

14. CDC. The Food borne Diseases Active Surveillance Network, 1996. Morbidity and Mortality Weekly Report. 1997. 46: 258-261.

15. Conner D.E., V.N.Scott, and D.T.Bernard. 1990. Growth, Inhibition, and Survival of *Listeria monocytogenes* as Affected by Acidic Conditions. J. Food Prot. 53(8)652-655.

16. Cords B.R. 1983. Sanitizers: Halogens and Surface-Active Agents. In Antimicrobials in Foods, edited by A.L Branen and P.M.Davidson. Marcel Dekker, Inc., New York. Pp. 279-290.

17. Cutter C.N., W.J.Dorsa, A.Handie, S.Rodriguez-Morales, X.Zhou, P.J.Breen, and C.M.Compadre. 2000. Antimicrobial Activity of Cetylpyridinium Chloride Washes against Pathogenic Bacteria on Beef Surfaces. J. Food Prot. 63(5) 593-600.

18. Dickson J.S. 1988. Reduction of Bacteria Attached to Meat Surfaces by Washing with Selected Compounds. J Food Prot. 51(11) 869-873.

19. Dickson J.S. 1989. Survival and Growth of *Listeria monocytogenes* on Beef Tissue Surface As Affected by Simulated Processing Conditions. J. Food Safety.10: 165-174.

20. Dorsa J. W., C.N.Cutter, G.R.Siragusa, and M.Koohmaraie. 1995. Microbial Decontamination of Beef and Sheep Carcasses by Steam, Hot Water Spray Washes, and a Steam Vacuum Sanitizer. J. Food Prot. 59(2) 127-135.

21. El-Shenawy M.A. and E.H.Marth. 1989. Inhibition or Inactivation of *Listeria monocytogenes* by Sodium Benzoate together with some Organic Acids. J. Food Prot. 52(11) 771-776.

22. Farber J.M. and P.I Peterkin. 1991. *Listeria monocytogenes*, a Food-Borne Pathogen. Microbiological Reviews 55(3) 476-511.

23. Farber J.M., G.W. Sanders, and M.A.Johnston. 1989. A Survey of Various Foods for Presence of *Listeria* Species. J Food Prot. 52(7) 456-458.

24. Farid M., A.Bal'a, and D.L.Marshall. 1998. Organic Acid Dipping of Catfish Fillets: Effect on Color, Microbial Load, and *Listeria monocytogenes*. J. Food Prot. 61(11) 1470-1474.

25. FSIS. *Listeria* Guidelines for Industry, 1999. [Internet, WWW], ADDRESS: http://www.fsis.usda.gov/oa/topics/lmguide.htm.

26. Gimeno O., I.Astiasaran, and J.Bello. 1999. Influence of partial replacement of NaCl with KCl and $CaCl_2$ on texture and color of dry fermented sausages. J. Agri. and Food Chem. 47(3): 873-877.

27. Gimeno O., D.Ansorena, I.Astaisaran, and J.Bello. 2000. Characterization of chorizo de Pamplona: instrumental measurements of color and texture. Food Chem. 69(2): 195-200.

28. Glass K.A. and M.P.Doyle. 1989. Fate of *Listeria monocytogenes* in Processed Meat Products during Refrigerated Storage. App. Environ. Microbiol.55: 1565-1569.

29. Glass K.A., D.A.Granberg, A.L.Smith, A.M.McNamara, M.Hardin, J.Mattias, K.Ladwig, and E.A.Johnson. 2001. Inhibition of *Listeria monocytogenes* by Sodium Diacetate and Sodium Lactate on Weiners and Cooked Bratwurst. J. Food Prot. 65(1) 116-123.

30. Grau F.H. and P.B.Vanderlinde. 1990. Growth of *Listeria monocytogenes* on Vacuum-packaged Beef. J. Food Prot. 53(9) 739-741.

31. Grau F.H. and P.B.Vanderlinde. 1992. Occurrence, Numbers, and Growth of *Listeria monocytogenes* on some Vacuum Packaged Processed Meats. J. Food Prot. 55(1) 4-7.

32. Hachmeister K.A. and T.J.Herald. 1997 Thermal and Rheological Properties and Textural Attributes of reduced-fat Turkey Batter. Poultry Science (57): 23-29.

33. Hand L.W., C.A.Hollingsworth, C.R.Calkins, and R.W.Mandigo. 1987. Effects of Preblending, Reduced Fat, and Salt Levels on Frankfurter Characteristics. J. Food Sci. 52(5) 1149-1151.

34. Harrison M.A. and S.L.Carpenter. 1989. Survival of Large Populations of *Listeria monocytogenes* on Chicken Breasts Processed Using Moist Heat. J. Food Prot. 52(6) 376-378.

35. Islam M., J.Chen, M.P.Doyle, and M.Chinnan. 2002. Control of *Listeria monocytogenes* on Turkey Frankfurters by Generally Recognized as Safe Preservatives. J. Food Prot. 65(9) 1411-1416.

36. Johnson J.L., M.P.Doyle, and R.G.Cassens. 1988. Survival of *Listeria monocytogenes* in Ground Beef. Int. J. Food Microbiol.6: 243-247.

37. Johnson J.L., M.P.Doyle, and R.G.Cassens. 1990. Incidence of *Listeria* spp. in Retail Meat Roasts. J. Food Sci. 55(2) 572-574.

38. Johnson J.L., M.P.Doyle, and R.G.Cassens. 1990. *Listeria monocytogenes* and other spp. in Meat and Meat Products: A Review. J. Food Prot. 53(1) 81-91.

39. Johnson M.G., T.C.Titus, L.H.McCaskill, and J.C.Action. 1979. Bacterial Counts on Surfaces of Carcasses and In Ground Beef From Carcasses Sprayed or Not Sprayed with Hydrochlorous Acid. J. Food Sci. 44(1) 169-172.

40. Kim J.W., M.F.Slavik, and F.G.Bender. 1996. Cetylpyridinium Chloride (CPC) Treatment on Poultry Skin to Reduce Attached *Salmonella*. J. Food Prot. 59:322-326.

41. Kotula A.W., W.R.Lusby, and J.D.Crouse. 1975. Variability in Microbiological Counts on Beef Carcasses. J. Ani. Sci. 40(5) 834-837.

42. Kropf D.H. 1980. Effects of Retail Display Conditions on Meat Color. Proc. 33rd Ann. Recip. Meat Conf.: 15-32.

43. Kropf D.H., and M.C.Hunt. 1984. Effect of Display Conditions on Meat Products. Proc. of the Meat Ind. Res. Conf.: 158-176.

44. Kusumaningrum H.D., M.M.V.Putten, F.M.Rombouts, and R.R.Beumer. 2002. Effects of Antibacterial Dishwashing Liquid on Foodborne Pathogens and Competitive Microorganisms in Kitchen Sponges. J Food Prot. 65(1) 61-65.

45. Lee C.Y., D.Y.C.Fung, and C.L.Kastner. 1982. Computer-Assisted Identification of Bacteria on Hot-Boned and Conventionally Processed Beef. J. Food Sci. 47:363.

46. McKellar R.C., R.Moir, and M.Kalab. 1994. Factors Influencing the Survival and Growth of *Listeria monocytogenes* on the Surface of Canadian Retail Weiners. J. Food Prot. 57(5) 387-392.

47. Okrend A.J., R.W.Johnston, and A.B.Moran. 1986. Effect of Acetic Acid on the Death Rates at 52 °C of *Salmonella newport*, *Salmonella typhmurium*, and *Campylobacter jejuni* in Poultry Scald Water. J. Food Prot. 49(7) 500-503.

48. Petran R.L. and E.A.Zottola. 1989. A Study of Factors Affecting Growth and Recovery of *Listeria monocytogenes* Scott A. J. Food Sci. 54(2) 458-460.

49. Petrocci A.N. 1977. Quaternary ammonium compounds. In disinfection, sterilization, and preservation, edited by S.S.Block. Lea and Febiger, Philadelphia. Pp. 325-347.

50. Pickett E.L. and E.A.Murano. 1996. Sensitivity of *Listeria monocytogenes* to Sanitizers after Exposure to a Chemical Shock. J. Food Prot. 59(4) 374-378.

51. Podolak R.K., J.F.Zayas, C.L.Kastner, and D.Y.C.Fung. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on Beef by Application of Organic Acids. J. Food Prot. 59(4) 370-373.

52. Porto A.C.S., B.D.G.M.Franco, E.S.Sant'anna, J.E.Call, A.Piva, and J.B.Luchansky. 2002. Viability of a Five-Strain Mixture of *Listeria monocytogenes* in Vacuum Sealed Packages of Frankfurters, Commercially Prepared with and without 2.0 or 3.0% Added Potassium Lactate, during Extended Storage at 4 and 10C. J. Food Prot. 65(2) 308-315.

53. Samelis J., G.K.Bedie, J.N.Sofos, K.E.Belk, J.A.Scanga, and G.C.Smith. 2002. Control of *Listeria monocytogenes* with Combined Antimicrobials after Postprocess Contamination and Extended Storage of Frankfurters at 4 °C in Vacuum Packages. J. Food Prot. 65(2) 299-307.

54. Samelis J., J.N.Sofos, M.L.Kain, J.A.Scanga, K.E.Belk, and G.C.Smith. 2001. Organic Acids and Their Salts as Dipping Solutions To Control *Listeria monocytogenes* Inoculated following Processing of Sliced Pork Bologna Stored at 4 °C in Vacuum Packages. J. Food Prot. 64(11) 1722-1729.

55. Schwartz H., Y.B.Li, M.Slavik, and J.Walker. 1998. Chemical Spray Conditions for Reducing bacteria on Chicken Skins. J. Food Sci. 63: 699-701.

56. Shao C.H., J.S.Avens, G.R.Schmidt, and J.A.Maga. 1999. Functional, Sensory, and Microbiological Properties of Restructured Beef and Emu Steaks. J. Food Sci. 64(6) 1052-1054.

57. Shelef Leora A. 1989. Survival of *Listeria monocytogenes* in Ground Beef or Liver During Storage at 4 and 25 °C. J. Food Prot. 52(6) 379-383.

58. Sorrells K.M., D.C.Enigl, and J.R.Hatfield. 1989. Effect of pH. Acidulant, Time, and Temperature on the Growth and Survival of *Listeria monocytogenes*. J. Food Prot. 52(8) 571-573.

59. Stringer W.C., M.E.Bilskie, and H.D.Naumann.1969. Microbial Profiles of Fresh Beef. Food Technol. 23:97-102.

60. Timm, R. R., M. N. Hajmeer, and J. L. Marsden. 2000. Efficacy of Peracetic Acid against *Listeria monocytogenes* inoculated on commercial sausages. The Food Safety Consortium report. Fayetteville, Ar. 83-90.

61. Wang C. and P.M.Muriana. 1993. Incidence of *Listeria monocytogenes* in Packages of Retail Franks. J. Food Prot. 57(5) 382-386.

62. Wang W.C., Y.Li, M.F.Slavik, and H.Xiong. 1997. Trisodium Phosphate and Cetylpyridinium Chloride Spraying on Chicken Skin to Reduce Attached *Salmonella typhimurium*. J. Food Prot. 60(8)992-994.

63. Wederquist H.J., J.N.Sofos, and G.R.Schmidt. 1994. *Listeria monocytogenes* Inhibition in Refrigerated Vacuum Packaged Turkey Bologna by Chemical Additives. J. Food Sci. 59(3) 498-500, 516.

64. Xiong H., Y.Li, M.F.Slavik, and T.J.Walker. 1998. Spraying Chicken with Selected Chemicals to Reduce Attached *Salmonella typhimurium*. J. Food Prot. 61:272-275.

65. Xiong H., Y.Li, M.Slavik, and J.Walker.1998. Chemical Spray Conditions for Reducing Bacteria on Chicken Skins. J. Food Sci. 63(4) 699-701.

66. Zaika L.L., S.L.Palumbo, J.L.Smith, F.D.Corral, S.Bhaduri, C.O.Jones, and A.H.Kim. 1990. Destruction of *Listeria monocytogenes* during Frankfurter Processing. J. Food Prot. 53(1) 18-21.

APPENDIX

Table 14. Mean log CFU/ g reduction of *Listeria monocytogenes* (p>0.05) following combinations of spray treatment parameters of 1% cetylpyridinium chloride on frankfurters (initial level of 8.20 log CFU/g) at different temperatures

Temperature (ºC)	Exposure Time (Sec)	20 psi		25 psi		35 psi	
		CPC	CPC+W	CPC	CPC+W	CPC	CPC+W
25	30	2.10	1.36	1.39	1.55	1.49	1.55
25	40	2.39	1.51	1.47	1.65	1.63	1.43
25	60	1.53	1.64	1.61	1.57	1.61	1.37
40	30	1.31	1.23	1.27	1.46	1.41	1.54
40	40	2.01	1.56	1.19	1.47	1.42	1.27
40	60	2.09	1.20	1.34	1.57	1.54	1.36
55	30	1.47	1.34	1.27	1.27	1.52	1.45
55	40	1.40	1.48	1.39	1.32	1.38	1.36
55	60	1.35	1.67	1.27	1.32	1.42	1.41
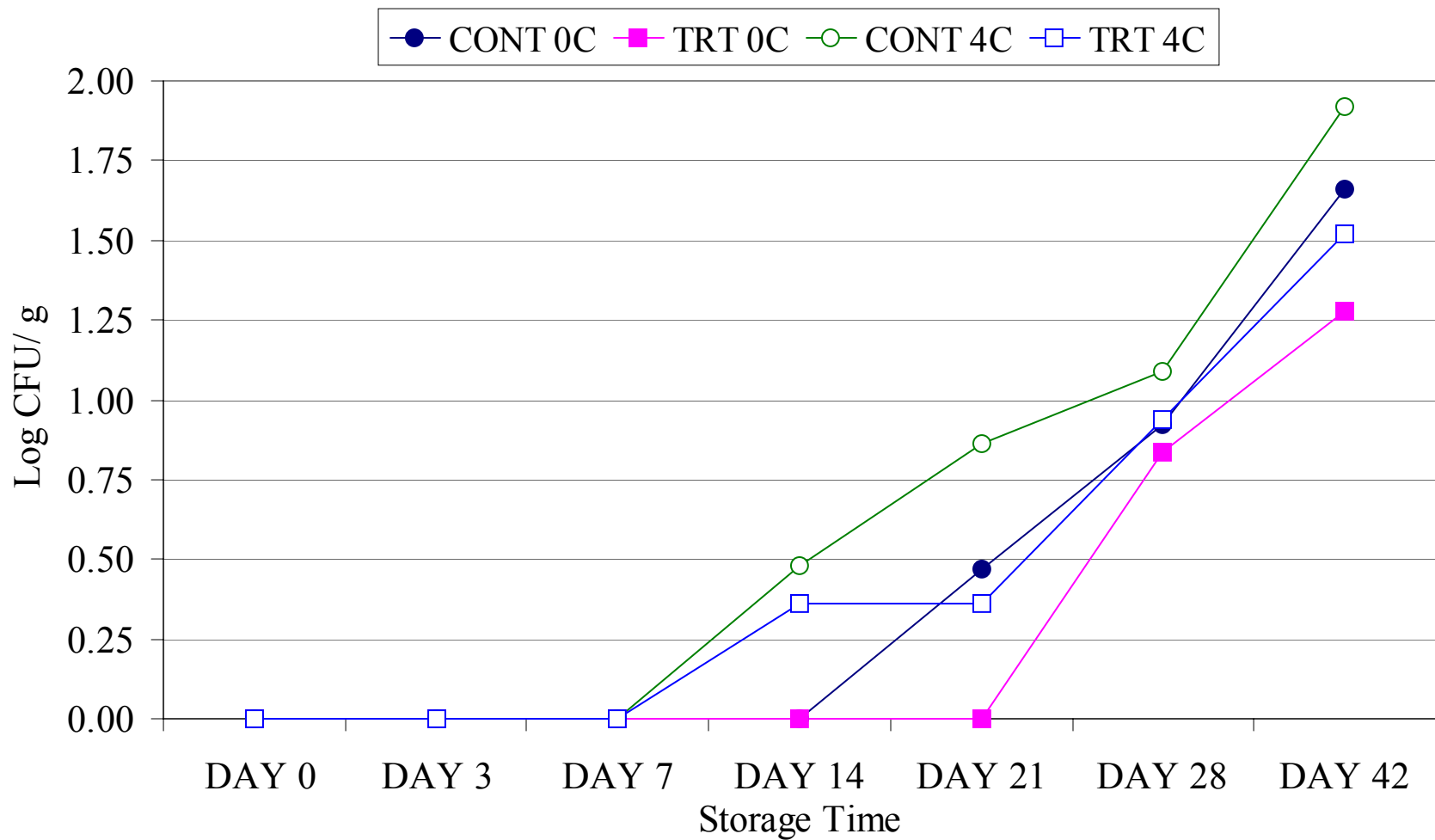
Figure 25: Yeast and Mold Count ($\log_{10}$ CFU/ g) of frankfurters treated with 1% cetylpyridinium chloride and non-treated frankfurters stored over time at 0 and 4 ℃
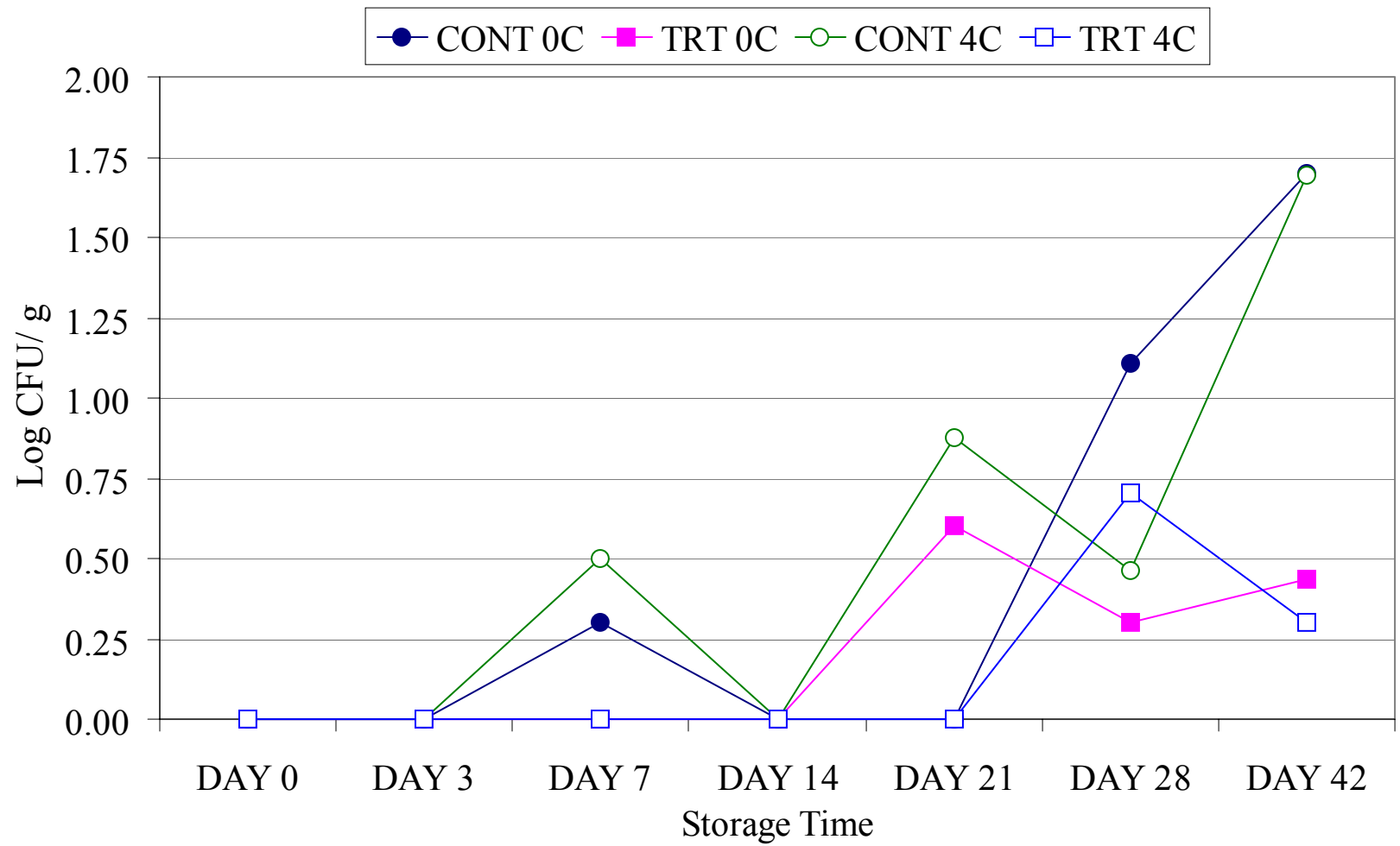
Figure 26: Yeast and Mold Count (log$_{10}$ CFU/ g) of polish sausages treated with 1% cetylpyridinium chloride and non-treated polish sausages stored over time at 0 and 4 ºC