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Influence of Chlorine Dioxide Ice on Pathogen Survival and Recovery on Chilled Pork and Turkey Subprimals

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Influence of Chlorine Dioxide Ice on Pathogen Survival and Recovery on Chilled Pork and Turkey Subprimals

By

Justin Robert Ransom

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ABSTRACT

Influence of Chlorine Dioxide Ice on Pathogen Survival and Recovery on Chilled Pork and Poultry Subprimals

By
Justin Robert Ransom

Chlorine dioxide (ClO_2) has been investigated as an antimicrobial in meat washing systems and brine chillers. This study was designed to observe the antimicrobials effectiveness of ClO_2 applied in an ice form. An ice machine with a ClO_2 generator produced ice containing 0 or 20 ppm inactivated ClO_2 and 5 or 10 ppm activated ClO_2. Subprimals were inoculated with *E. coli* 0157:H7, *S. typhimurium*, *L. monocytogenes* or *Y. entercolitica*. Inoculated subprimals were placed in ice treatments for 24 hours, then stored (2°C) for 7 days. Samples were taken for bacterial enumeration before and after ice and during storage. The main effect of ClO_2 concentration did not reduce ($P>0.05$) the microbial load of pathogens on subprimals. The interaction of temperature during ice application and day of storage did reduce ($P<0.05$) the microbial load. Ice applied to pork at 26°C reduced *L. monocytogenes,* and at 2°C, *E. coli* 0157:H7 and *S. typhimurium* were reduced after 7 days of storage. Neither temperature during ice application reduced *Y. entercolitica*. In turkey, ice treatments applied at the environmental temperature of 26°C reduced *E. coli* 0157:H7 and *S. typhimurium*. This reduction may be attributed to the release of more ClO_2 during melting. The largely activated ice-melt in the bottom of the cooler contained 2.5-3.0 log_{10}CFU/ml fewer pathogens compared to the control ice-melt. The use of ClO_2 ice as an antimicrobial should be further investigated for potential use in retail meat display cases or in ice-packed meat transportation systems.

This thesis is dedicated to everyone who has encouraged me to go beyond my perceived limits.

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Table of Contents

List of Tables

List of Figures

INTRODUCTION

Recent illnesses associated with new strains of microorganisms, such as *Salmonella typhimurium* and virulent strains of *Escherichia coli* 0157:H7 have increased interest in pathogen intervention strategies and technologies within the food industry. The Centers for Disease Control and Prevention consider *Escherichia coli* 0157:H7, *Salmonella* and *Listeria monocytogenes* to be of greatest concern because of the severity and number of illnesses they cause (Richardson, et al., 1998). To address these concerns, pathogen intervention strategies such as meat and poultry carcass washing systems have been tested extensively in laboratories and in processing plants. Incorporation of various organic acids and chlorine compounds as part of these washing systems are critical to effectively reduce these pathogenic organisms.

Chlorine is a disinfectant that has been used for the last 100 years, but has drawn concern because of the toxic mutagens (chloroforms) generated during the reaction with organic matter (Tsai et al., 1997). Lillard (1979) found chlorine dioxide (ClO_2) to be seven times more effective than chlorine and it did not generate toxic chloroform upon reaction with organic matter. Several studies have reported the effectiveness of ClO_2 as an antimicrobial applied as a liquid (Cutter and Dorsa, 1995). Coupling the bactericidal effectiveness of ClO_2 and the temperature reducing/stabilizing ability of ice could offer a unique pathogen intervention strategy to reduce the growth and survivability of pathogenic organisms.

The purpose of this study was to observe the effectiveness of ClO_2 applied in an ice form on the survival of pathogens on fresh pork and poultry subprimals. The hypothesis was that the bactericidal effect of the ClO_2 and the low temperature of the ice would result in multiple hurdles, averting pathogen survival and growth on the meat product.

There were two primary objectives in this study. The first objective was the development of an ice machine prototype that accurately generated ClO_2 ice at 20 ppm inactivated ClO_2 ice, 20 ppm marginally activated ClO_2 ice (5 ppm activated ClO_2, 15 ppm inactivated ClO_2), 20 ppm largely activated ClO_2 ice (10 ppm activated ClO_2, 10 ppm inactivated ClO_2) or control (no ClO_2). The second objective was the determination of the ClO_2 ice application, concentrations and storage parameters that reduced pathogen survival and growth during retail storage. The long-term objective was to transfer knowledge gained into recommendations for the pork and poultry industries.

CHAPTER 1

Review of Literature

I. Overview of Food Safety:

Current Status of Food Safety

Meat and meat by-products are a significant part of the diet for much of the world's population. Meat is an important contribution to the economy of domestic and global agriculture. Due to the "presumed" increased incidence of virulent pathogens in the food supply, foodborne illness has become a major topic of both public and scientific debate. Despite the high level of concern, there is much to be learned about the nature of food poisoning. The safety of meat can be compromised in various ways, including chemical residues (pesticides, antibiotics), physical hazards (glass, metal) and most importantly, microbiological contamination. Although researchers have made significant advances in food safety in recent years, it continues to be an evasive problem.

Food safety is defined as those measures that are taken to ensure that foods can be eaten without adversely affecting the consumer (IDEX, 1998). This includes minimizing the contamination of foods, killing microbial contaminates, denaturing toxins and inhibiting the growth of pathogenic organisms on food sources. In order for this to be accomplished, researchers, producers, packers, and consumers must work together to minimize microbiological contamination from "farm to fork" (Smith, 1999).

Despite current efforts being made, more than half of the current foodborne outbreaks reported remain of unknown origin. The World Health Organization predicts that there are 300 to 350 percent more outbreaks than what are reported (Saucier, 1999). Some estimates suggest that hundreds of millions of people around the world suffer due to the incidence of foodborne illnesses. In addition, it is predicted that at least five to ten percent of the population is affected annually (Saucier, 1999).

Although the estimates of cases of microbial foodborne disease vary, it is suggested that world wide, 6.5 to 33 million annually become sick and 525 to 9,000 die (CAST Report, 1994).

Federal Regulation

Unsanitary conditions in meat-packing plants and the use of poisonous preservatives and dyes in foods were the major problems leading to the enactment of food laws (FSIS, 1999). By the end of the 19th century many countries had created laws that prevented the sale of diseased and spoiled meats; however, the USA fell far behind. Because of adulteration, misrepresentation and threat to consumers, European countries prohibited the import of American food into their domestic markets. The result of this caused U.S. officials to rethink their lack of regulation.

In 1906, the United States Congress passed two laws: 1.) The Pure Food and Drug Act (1906) and 2.) The Meat Inspection Act (1906). These laws were administrated by the United States Department of Agriculture's Food Safety and

Inspection Service (USDA-FSIS) and extended to all segments of the food and beverage industries. FSIS has jurisdiction over food that contains more than 2-3 percent meat or poultry. The Department of Health, Education and Welfare through its agency, the Food and Drug Administration (FDA), has jurisdiction over all food products other than those inspected by FSIS (FDA Backgrounder, 1999).

Many multinational agreements for international transport and marketing of food and beverages are based on the 1906 food laws. The problems with these laws were insufficient standards and lack of strict enforcement. This was due to inadequate inspection. In 1938, the 1906 Pure Food and Drug Act and the Meat Inspection Act were revised. The law is presently called the Food, Drug and Cosmetic Act of 1938. This serves as the foundation for the laws that regulate the food and beverage industry today. In 1954, the Food, Drug and Cosmetic Act of 1938 was amended to include the regulation of pesticides in food crops. This new law was the Miller Pesticide Amendment. The Food Additives Amendment of 1958 specifies the kinds and quantities of additives industry can add to food without compromising human safety. The 1959 Poultry Inspection Act and the 1972 Fair Packaging and Labeling Act were two of the last regulations administered (FDA Consumer, 1981).

Secretary of Agriculture Espy directed FSIS in 1993 to initiate rulemaking to require all inspected meat and poultry slaughter facilities to develop and maintain a Hazard Analysis and Critical Control Point (HACCP) system (Manis, 1995). HACCP would supplement, not replace FSIS inspection. FSIS

inspectors would maintain a daily presence in these establishments to determine whether products were being produced under the direction of the HACCP system and in adherence to regulations set forth by the Food, Drug and Cosmetic Act. It was not until July of 1996, that FSIS handed down the final rule of the long-awaited modernization of the meat and poultry inspection system (Manis, 1995).

II. Food Safety Regulations

Pathogen Reduction

The FSIS proposal to require the implementation of HACCP in meat and poultry establishments was supported by both large and small businesses, scientific and public health officials, consumers and public interest organizations. Supporters believed the system should systematically build science-based food safety measures into their production systems (Federal Register, 1996). FSIS stated the goal of its food safety strategy and proposed Pathogen Reduction/ HACCP regulations as follows:

> "FSIS believes its food safety goal should be to reduce the risk of foodborne illness associated with the consumption of meat and poultry products to the maximum extent possible by ensuring that appropriate and feasible measures are taken at each step in the food production process where hazards can enter and where procedures and technologies exist or can be

developed to prevent the hazard or reduce the likelihood it will occur (60 FR 6785) (Federal Register, 1996)."

Through establishing this goal, FSIS realized no single solution or technology could solve the problems facing the food industry. However, FSIS believed through continuous efforts to improve hazard identification and prevention, this goal could be attained.

From a food safety standpoint, the most important objective is to create effective measures to reduce and control harmful bacteria on raw meat and poultry products. HACCP, combined with appropriate food safety performance standards, and science based technology, is the most effective means available for controlling and reducing harmful bacteria on raw meat and poultry products (Manis, 1996).

HACCP System Final Rule

The USDA/FSIS mandated the requirements to reduce the occurrence and numbers of pathogens on meat and poultry products, to reduce the incidence of foodborne illness associated with consuming these products, and to provide a framework for modernization of the meat and poultry inspection service on July 25, 1996 (IDEXX, 1998). Four new programs were the basis of new regulations. The first of these programs required the establishment of Sanitation Standard Operation Procedures (SSOPs). Secondly, a regular microbial testing program for slaughter establishments for the removal of fecal

contamination and associated bacteria. The third program required slaughter and grinding plants to meet pathogen reduction performance standards for *Salmonella*. The fourth and final program mandated all meat and poultry plants to develop and implement HACCP programs.

By January 27, 1997 all federally inspected establishments were expected to implement SSOPs and test for generic *E. coli* as an indicator of the adequacy of the plant's process control for fecal contamination. At the same time, tests for generic *Salmonella* were mandated because it is the leading cause of foodborne illness among enteric pathogens (FSIS Backgrounders, 1996). Plants that employed 500 people or more had to have HACCP program implementation by January 26, 1998. Smaller establishments with 10 to 499 employees had to comply by January 25, 1999. Finally, plants with fewer than 10 employees or less than $2.5 MM were forced to comply by January 25, 2000. *Salmonella* pathogen reduction performance standards had to begin at the same time HACCP implementation took place (Federal Register, 1996).

Impact of HACCP

HACCP is not an inspection system; it is an industry process control system. The FSIS inspector's role changed from a command and control regulator, to a performance standard monitor. Inspectors determine if each plant's SSOPs and HACCP plan conforms to regulatory requirements. Some of the duties include microbial verification, preparation of written material to document failure and to meet regulatory requirements and enforcement when a

plant is not in conformance with the established requirement (Nunes, 1997) FSIS is now working on the development of measures that can be used on the farm to reduce food safety hazards, although it does not mandate production practices at this stage.

Six studies have been planned over the next three years to evaluate HACCP Systems (FSIS, Nov 1999). Some of the questions that these studies hope to answer are: Do HACCP processing control systems reduce foodborne illness? Do HACCP systems make inspection more effective? Do HACCP systems increase consumer confidence? Do HACCP systems control production safety hazards? The final question is, Do HACCP systems provide an opportunity for increased productivity?

In addition to these studies, the International HACCP Alliance has been created as a proactive step to assist the meat and poultry industry in preparing and regulating HACCP. The mission of the alliance is to promote international public health and food safety by facilitating uniform development and implementation of HACCP programs from farm to table. The founders of the HACCP Alliance realized the need to standardize the training for those implementing HACCP systems. The Alliance works with USDA's FSIS and other government agencies worldwide to enhance communication between industry and government (International HACCP Alliance Homepage)

In addition, other programs such as the Food Safety Virtual University, the State HACCP Network, USDA/FDA HACCP Training Program and Resource

Database, the USDA/FDA Foodborne Illness Education Information Center and the FDA Bad Bug Book were set up to help industry establish HACCP systems.

III. Organisms and symptoms associated with food borne pathogens

Escherichia coli 0157:H7

Escherichia is of the *Enterobacteriaceae* family of which it shares common characteristics. *E. coli* is one of six species in the genus *Escherichia*. Most of the *Escherichia* species ferment lactose and produce gas (Varnam, 1991). In addition, they are facultative, non-spore forming, rod-shaped anaerobes (Kraft, 1992). Currently there are five known strains of *E. coli*: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enteroadherent-aggregative (EA-AggEC) and Enterohemoraggic (EHEC). The four main types, EPEC, ETEC, EIEC and EHEC are distinctly different in epidemiology, pathogenicity and O:H serovar (Varnam, 1991).

MacConkey and violet red-bile agars are selective agars used when isolating *E. coli*; however, these media will not differentiate pathogenic serogroups from non-pathogenic serogroups. Serological and genetic methods have been developed for differentiating *E. coli* strains. Reliable methods such as polymerase chain reaction (PCR) and pulsed field gel electrophoresis (PFGE) have been devised to distinguish different enteroinvasive and enterohaemorrhagic *E. coli* strains (Buchanan and Doyle, 1997)

In the past 20 years *E. coli* 0157:H7 has emerged as a pathogen of public health concern (Riemann and Cliver, 1998). Although most *E. coli* are normal

flora and reside in the colons of healthy people and other warm-blooded animals, several strains are capable of causing significant human threat. EHEC has been transmitted via food and caused disease ranging from hemorrhagic colitis (bloody diarrhea) to Hemolytic Uremic Syndrome (HUS) (Buchanan and Doyle, 1997). EHEC exhibit disease symptoms similar to *Shigella* dysentery, and is most commonly found in developed countries (Riemann and Cliver 1998).

In 1975, *E. coli* 0157:H7 was first isolated from an infected woman in California (Merritt 1998); however, it was not until 1982 that it was considered a human pathogen following two hemorrhagic colitis outbreaks. The first outbreak took place in Oregon, where of 26 cases reported, 19 were hospitalized. In Michigan a second outbreak occured, 21 cases were reported and 14 were hospitalized. In both cases, undercooked hamburgers testing positive for *E. coli* 0157:H7 were the cause (Buchanan and Doyle, 1997). Many food items have been found to be a vehicle for *E. coli.* These foods include unpasteurized milk and apple juice; ham, turkey and cheese sandwiches; ground beef patties; dry fermented sausage; salad; and non-chlorinated water (Riemann & Cliver 1998).

Fortunately, there are several factors that prevent, hinder and stop the growth of *E. coli.* A pH less than 4.4 or greater than 9.0, a water activcity (a_w) of less than 0.95 and a 3KGy dose of irradiation are sufficient for the inhibition or destruction of *E. coli* (Varnam, 1991). In addition, the pathogen is unable to grow at temperatures less than 7 °C or greater than 48°C. *E. coli* cannot withstand temperatures at 55°C for 5 minutes or 60°C for 0.1 minute. There is

no evidence that *E. coli* can survive after pasteurization or after proper cooking has been achieved (Varnam, 1991).

Salmonella typhimurium

The genus *Salmonella* is a member of the *Enterobacteriaceae*, and are classified on the basis of being gram-negative, non-sporeforming, motile bacilli. They are facultative anaerobes, consisting of three kinds of antigens (Ekperigin and Nagaraja, 1998). There are more than 2200 serovars in the genus, of which 50 to 150 have been implicated in disease outbreaks (Merritt, 1998).

From the late 1800's to 1949, typhoid fever caused by *Salmonella typhi* was the predominant strain to cause infections in humans (Tauxe, 1991). However as technology and antimicrobials advanced, typhoid fever was nearly eliminated in many industrialized nations. At the same time the non-typhoid (*Salmonella typhimurium*) strain began to become reported more frequently as a leading cause of gastroenteritis (Tauxe, 1991).

Salmonella typhimurium, known as Definitive Type 104 (*S. typhimurium* DT104) can affect not only man, but also a wide range of warm-blooded animals (Merritt, 1998). In 1888, it was first isolated from a man who had consumed a moribund cow, however, the strain did not appear to become prevalent until the middle of the 20th century (Tauxe, 1991). Today, it is the leading cause of Salmonellosis in the United States (Tauxe, 1991 and Vela, 1997).

S. typhimurium may be present in the gastrointestinal tract of poultry and large meat animals that show no symptoms of sickness. Healthy animals may carry the deadly pathogen in their feces, lymph nodes, spleen, liver, kidneys and

gall bladder (Merritt, 1998). In pork processing, Saide-Albonoz, et. al., (1995) noted the stress of transportation and feed deprivation from farm to slaughterhouse increases the likelihood for pathogen shedding via fecal material, which ultimately passes *S. typhimurium* onto the slaughter floor and throughout the packing plant. Ekperigin and Nagaraja, (1998) suggest that when beef, pork or poultry are infected with *S. typhimurium* prior to slaughter their carcasses test positive for the pathogen 0% to 90% of the time.

One of the factors that make *S. typhimurium* so unique is the ability to withstand antibiotic treatments of Ampicilin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracyclines and more recently Trimethoprim and Fluoroquinolones (Ekperigin and Nagaraja, 1998). In recent years, there has been an observed association between antibiotic therapy and increased susceptibility to infection by the antibiotic-resistant strain. In a clinical study, patients who had been on antibiotic therapy, then exposed to *S. typhimurium* were fivefold more likely to show symptoms of infection. It was hypothesized that the antimicrobials increased host susceptibility by depleting the host's normal microflora and creating favorable conditions for S. *typhimurium* (Varnam, 1991).

Although *S. typhimurium* is resistant against several antimicrobial agents that other pathogens are not, there are ways the pathogen can be eliminated. Salmonella grows over a range of temperatures from 5°C to 47°C, with an optimum temperature of 37°C. Growth rates at below 10°C are very slow, but are significant if the shelf life of the product is prolonged. *Salmonella* is

destroyed at temperatures of 74°C. Irradiation at 3KGy has proven to be effective in controlling *Salmonella* in poultry, shellfish and frog legs. In addition, it grows over a range of pH from 4.5 to 9.0, with an optimum pH of 6.5 to 7.5. At pH levels lower than 4.1, inactivation and death will occur. Succinic acid and chlorine have proven to be effective in eliminating *Salmonella* loads in chicken carcasses (Varnam, 1991). Using multiple steps to eliminate the pathogen load, *S. typhimurium* can be controlled.

Yersinia entercolitica

The genus *Yersinia* is composed of a collection of gram-negative, facultatively anaerobic bacilli that share morphological, biochemical and serological features with other genera in the *Enterobacteriaceae* family. There are three species that are recognized as human pathogens: *Y. pestis, Y. pseudotuberculosis* and *Y. entercolitica* (Karib et. al., 1995). *Y. entercolitica* are psychrotrophic in nature, which presents a unique problem in the food industry. They can multiply at temperatures from –2° to 45°C (Varnam, 1991).

Y. entercolitica was first recognized as a human pathogen in 1939; however, it's significance to foodborne illness was not realized until the mid 1970's (Kraft, 1992) Y. *entercolitica* causes a variety of human infections. It is the cause of 1-3% of the gastroenteritis cases reported annually. The foodborne transmission *of Y. entercolitica* was established in 1976, when 222 people became sick after consuming contaminated chocolate milk. An outbreak

of 87 cases occurred in 1981, when tofu was packed in untreated spring water, which contained the same serotype as the infected patients (Kraft, 1992).

The incidence of *Y. entercolitica* in fresh meat is very high, yet few foodborne outbreaks of yersiniosis have been reported due to the high number (millions) of cells needed to cause disease (Smulders, 1987). The symptoms of the infection include abdominal pain and diarrhea, which may vary from loose stools to ulcerated lesions. When the bacteria invade the intestinal wall and enter the blood system, secondary pathological effects such as arthritis or meningitis can result (Frank, 1992).

Swine are the major reservoir for the pathogen, which results in human infections. Although isolations of *Yersinia* have been made from poultry, the importance of yersiniosis spreading in poultry is questionable (Kraft, 1992). The epidemiology of yersiniosis is complex and has not been fully established. Modes of transmission are contact with infected animals, person-to-person transmission within infected families or consumption of contaminated foods (Karib et. al., 1995).

Y. entercolitica will not survive heat treatments of 62.8°C for 0.7 to 17.0 seconds. Irradiation has proven to be effective in eliminating the pathogen load at 0.7 to 1.2 KGy. In addition, it is considered to be sensitive to a low pH range. However, the pH value alone has little effect on its growth and survival. *Y. entercolitica* can be controlled by 0.1 to 0.2% sorbate at pH 5.5 (Varnam, 1991).

Listeria monocytogenes

The genus *Listeria* is composed of small motile, nonspore-forming, facultative anaerobic, gram-positive rods. These organisms are catalase positive and oxidase negative and ferment glucose (Cooper and Walker, 1998). There are seven species of *Listeria*. *L. monocytogenes* is the only one associated with human foodborne disease (Vela, 1997). It is most commonly found in the soil, but can also be found in animal food and organs as well as on the skin of infected animals (Frank, 1992).

L. monocytogenes is capable of growing at temperatures from 3° to 45°C. It is resistant to freezing, thawing and drying. It can survive for years at a neutral (7.0) pH. The organism resides in the intestinal tract of vertebrae and invertebrate animals (Cooper and Walker, 1998). It is estimated that 5 to 10% of the general population are carriers of *L. monocytogenes* (Notermans, et. al., 1998).

L. monocytogenes is capable of existing as both a plant and animal pathogen. It enters the body by penetrating the epithelial barrier in the intestine and multiplies in the hepatic and splenic macrophages. It also enters through damaged mucosal surfaces, by inhalation or conjuctival contamination, and then invades the central nervous system via the neural sheath of peripheral nerve endings (Cooper and Walker, 1998).

Even with the frequency of human exposure to *L. monocytogenes*, research suggests that listeriosis is a relatively rare disease. Studies conducted by the Center for Disease Control have established a incidence of listeriosis of 7.1 cases per one million people (Notermans, et. al, 1998). Historically,

listeriosis is not contagious and only occurs in sporadic cases worldwide (Cooper and Walker, 1998).

In 1929, it was first reported as a human pathogen, but had been known as an animal pathogen since 1911 (Kraft, 1992). The first major outbreak of listeriosis occurred in Boston MA, in 1979. Although the total number of cases was not definitely established, 5 people died after consuming raw vegetables that had been fertilized with fecal material from infected dairy cattle (Vela, 1997). In December 1998, *L. monocytogenes* was responsible for the largest meat recall ever. Thirty-five million pounds of ready to eat meat were recalled; ultimately, 101 people were sickened and 21 people died (Young, 1999). The people who are the most susceptible to listeriosis are the immunocompromised, elderly and infants (Kraft, 1992). Some scientists suggest that the unique characteristics of *L. monocytogenes* make it a problem of great concern, but most healthy people are not at risk to listeriosis, thus outbreaks have not been more widespread (Doyle, 1988).

There are three main clinical disease symptoms associated with *L. monocytogenes* infections in human: (1) neural (circling disease), (2) visceral (flu-like symptoms followed by neural or reproductive tract infections), and (3) reproductive (causing abortions, stillbirths or pre-mature births). The onset of serious complications depends upon the health of the person and the amount of bacteria ingested. Penicillins such as ampicillin or amoxicillin in combination with an aminoglycoside have been shown to be effective in the treatment of infected people (Cooper and Walker, 1998).

In 1983 it was thought that pasteurization was adequate for the destruction of the pathogen, but when the milk was harvested from cows infected with *L. monocytogenes*, pasteurization did not kill all of the deadly bacteria (Vela, 1997). It was determined that the bacteria were being protected by the bovine leukocytes in the milk. In view of this, pasteurization procedures became scrutinized and thermal processing parameters for milk were reconsidered. New requirements were established to modify the pasteurization process to include thermal processing at 71.7°C for 15 s, resulting in a 5.2D reduction to inactivate *L. monocytogenes* in milk. The safety margin is greater in low-temperature short-time pasteurization (62.8°C for 30 min, resulting in a 39D reduction) (Varnam, 1991). Previous research concluded that *L. monocytogenes* growth is compromised at a pH of 5.6 or less; however, scientists have found that a pH of 4.42 is considerably more limiting. As with other pathogens, *L. monocytogenes* is vulnerable at irradiation doses of 3KGy (Varnam, 1991).

IV. Pathogen Intervention Strategies

Overview

The muscle tissue of healthy animals is usually free from microorganisms (Sammarco et al., 1997). It has been well documented that microbial contamination of animal carcasses during the slaughter process is both undesirable and yet unavoidable (Dickinson and Anderson, 1991, Podolak et al., 1996, Sirgusa, 1996, Tamblyn and Conner, 1997). Advances in engineering technology have allowed the meat industry to become modernized, streamlined,

and more efficient (Saucier, 1999). Although technology enhances productivity, the delicate balance between efficiency and food safety cannot be compromised.

Carcass decontamination refers to the ability to reduce pathogen loads by sprays, washes, steam-vacuuming, hot-fat-trimming and quick-chilling, or a combination of treatments. It is not possible to reduce the level of microbial contamination on a carcass to zero using a single decontamination method (Siragusa, 1996).

Antimicrobials

As part of the FSIS Pathogen Reduction/HACCP regulation, FSIS recommended that all beef, pork, lamb and poultry slaughter establishments apply at least one antimicrobial treatment prior to carcass chilling. Proposed treatment recommendations included any antimicrobial compounds previously approved by FSIS, as well as hot water and chlorine compounds (Federal Register, July, 1996). There have been a wide variety of antimicrobials used to reduce the pathogen load on meat and poultry carcasses. Short chain organic acids have been identified as the most logical agents to spray on carcasses. Acetic, lactic, citric, formic and propionic acids have all been used for this purpose (Siragusa, 1996). Acetic and lactic acids are inexpensive, environmentally friendly and occur naturally in nature. In general, organic acids reduce aerobic plate counts by 1 to 2 log CFU/area of tissue surface, regardless of the acid type (Siragusa, 1996). The acid concentration appears to be an important factor in the degree of pH decline and antimicrobial effect (Podolak et

al., 1996, Tamblyn & Conner, 1997, Dorsa et al, 1997, Cutter and Siragusa, 1994). However it is important to consider that high acid concentrations could have adverse affects on meat quality (color and flavor).

Chlorine (Cl_2) water has also been used to decontaminate carcasses. Most researchers agree that chlorinated water has little or no effect unless sprayed frequently on the carcass over a long period of time (2- 4 hours) (Yang et al., 1998, Skelly et al., 1985, Cutter and Siragusa 1995, Siragusa, 1996). It is assumed that greater pathogen reductions did not occur because of the inactivation of chlorine by the organic and nitrogenous compounds associated with meat (Cutter and Dorsa, 1995).

Chlorine Dioxide (ClO_2) is a chlorinated compound that has been widely used throughout Europe for the disinfection of public water supplies (Latshaw, 1994). Lilliard (1979) was the first to use ClO_2 in the reduction of pathogens in poultry chiller water. Results from his studies showed that ClO_2 was effective at reducing pathogens at ClO_2 of 20 parts per million (ppm). Other studies showed similar differences when used as a soaking solution or brine chiller (Cutter and Dorsa, 1995, Theissen et al., 1984, Villarreal et al., 1990). However when used as a wash or spray, it was proven to be ineffective (Cutter and Dorsa, 1995).

Delivery Systems

A variety of antimicrobial delivery systems have been used to reduce the levels of pathogens on meat surfaces (Cutter et. al., 1997). Inconsistencies in the literature suggest the terms "acid sprays", "acid washes" and "acid rinses" are synonymous and are used interchangeably. These systems utilize

antimicrobial agents to spray the meat surface in order to reduce microbial loads (Yang, et al., 1998, Dickens, 1995, Brackett, et al., 1994, Dorsa, et al., 1997a 1997b, Conner, et al.,1997, Woolthuis, et al., 1985, Prasai, et al., 1997). "Acid spray" will be the term used throughout this literature review to signify the use of these technologies.

Another way of applying acids and chlorine compounds to the meat surface is to place the meat product in a container containing the antimicrobial (Dickens, et al., 1994; El-Khatteib, et al., 1992; Fratamico, et al., 1996; Tamblyn, et al., 1997; and Podolak, 1996) . "Acid dip", "acid bath", "acid rinse" are terms used to describe this method. "Acid dip" will be the term used throughout this literature review to signify the use of these technologies.

Treating the brine chiller water for poultry carcasses is another way of utilizing antimicrobials agents. Organic acids, chlorine and chlorine dioxide have all been added to poultry chilling systems to aid in the reduction of pathogens (Tsai, et al., 1991; Lillard, 1979, 1980; Dickens and Whittemore, 1995; Rathgeber and Waldroup, 1995; Villarreal, et al., 1990).

Acid Spray

Numerous studies have investigated spraying organic acids and chlorine compounds on carcasses and meat products in order to reduce undesirable bacteria (Cutter et. al., 1997). Organic acids and chorine compounds are effective on those organisms that are in contact with the acid for a period of time (> 60 s) that is sufficient to be lethal to that organism. However, the acid does not affect organisms that have become entrapped or protected by the carcass

surface, because the acid is not able to penetrate the carcass surface (Cutter and Siragusa, 1994). Dickens, (1995) found that spraying the carcass preevisceration not only sanitized the carcass, but also reduced the stickiness of the carcass. Wetting the carcass surface reduces the stickiness; therefore, pathogens have less opportunity to adhere to the carcass. Marshal et al. (1977) observed acid spray systems were most effective in reducing the bacterial load when application of acid was at the highest pressure, and when the highest volume of acid was applied for the longest period of time. Therefore, in order to decontaminate a carcass, a combination of pressure, volume and concentration must be used. The high-pressure spray will cause detachment of the bacteria and the acid will then cause inactivation (Siragusa, 1996).

Woolthuis and Smolders (1995) used lactic acid (LAC) in a pilot model washing system. They noted that LAC in concentrations of 2.0% discolored the beef carcasses. Nonetheless, LAC was effective in reducing the aerobic plate counts (APC) by 0.8 and 1.3 $\log_{10}$ CFU/cm^2. Cutter and Siragusa (1994) used acetic acid (AAC), citric acid (CAC) and LAC at concentrations of 1, 3, and 5% to inoculate beef carcass tissue with E. coli 0157:H7. The researchers observed no pathogen reduction differences in the types of acids used; however, the 5% concentrations were the most effective in reducing the pathogen load (1 to 2 $\log_{10}$ CFU/cm^2). It was noted that use of 5% acid concentration did reduce the pathogen load to zero levels. Three years later, Cutter and Siragusa (1997) performed another study analyzing the effectiveness of spraying LAC, AAC and trisodium phosphate (TSP) acid on beef carcass tissue. The product was

vacuum packaged and stored at 5°C. Samples were removed from vacuum packaging at 0, 7, 14, and 21 days for bacterial enumeration. The acids proved to be effective in reducing the pathogen load by 1.3 to 2.0 $\log_{10}$ CFU/cm^2. Yang et al. (1998), observed a 1.7 to 2.0 $\log_{10}$ CFU/cm^2 reduction in S. typhimurium when poultry carcasses were sprayed with either LAC, TSP, cetylpyridinium chloride (CPC) or sodium bisulfate (SBS). Although no reduction greater than 2.0 $\log_{10}$ CFU/cm^2 were observed, other researchers found similar results (Prasai et al., 1997, Conner et al., 1997).

Brackett and Doyle (1994) and Cutter et al. (1997) heated LAC AAC to approximately 55°C and observed heat treatments had no significant advantages over acids held at room temperature. They noted that this method was not effective for practical use.

Acid Dip

Acid dip is another method used by researchers to reduce the pathogen load on meat products. Instead of spraying the product with nozzles, they dip the product in antimicrobial agents. Dickens et al. (1994) observed less than 1 $\log_{10}$ CFU/cm^2 reductions in the poultry carcasses dipped in 0.6% AAC. Podolak, et al. (1996) reported using a 1% concentration of fumaric acid as a bactericidal dip was effective in reducing the pathogen load of *L. monocytogenes* and *E. coli* 0157:H7 by 1 and 1.3 $\log_{10}$ units respectively. Tamblyn and Conner (1997) showed that the bactericidal activity of organic acids increased linearly with increased acid concentrations. They noted that in

general, greater than 4% acid is need to kill greater than 2 $\log_{10}$ number of cells of *S. typhimurium* attached to broiler skin.

Fratamico, et al. (1996) found that when TSP is used as a dip, TSP reduced the levels of *E. coli* attached to the tissue up to 3.2 $\log_{10}$ units. These researchers suggested that TSP could be effective for reducing *E. coli* on beef carcass tissue. Although not all organic acids are effective in reducing the pathogen levels, it is suggested that greater pathogen reduction is observed at higher the acid concentrations.

Quick Chill

Quick chilling is used throughout the U.S. in pork and beef processing plants. The carcasses come off of the kill floor and go straight into chilling rooms at temperatures ranging from -1 to 2°C. The air velocity is 2 to 4 meters/second (Bem and Hechelmann, 1995).

The rate of chilling meat product has a significant effect on bacteria. The leading factor in the cause of foodborne illness is improper holding temperatures for food (ASHRAE, 1994).

The growth of bacteria takes place in four phases: latent phase, (lag phase), log phase (exponential growth), stationary phase and organism reduction phase (Bem and Hechelmann, 1995). The lag phase is the period of time when the organisms are adapting to the environment. In the food industry, it is most ideal to lengthen the lag phase as long as possible, not allowing organisms to enter the log phase. The log phase is when the organisms experience the most growth. Once the organisms enter this phase, it is difficult

to control the growth without cooking or irradiation. At this point the organisms can double every 20 minutes or less. Toxin production usually occurs at the end of this phase. The stationary phase is the phase where the organisms begin to run out of nutrients to continue to reproduce. Finally, the reduction phase is the phase where organisms die off (ASHRAE, 1994).

Control of pathogens in the food industry has typically depended on temperatures below 5°C (Palumbo, et al., 1995). Fung et al., (1981) found that the initial chilling rate of beef carcasses is important in ensuring the microbiological quality of hot-boned beef. Lee, et al. (1985) showed that hot-boned beef chilled to 21°C in 6 hours had less pathogen growth than beef chilled to 21°C in 11.3 h. The microflora at the time of packaging ultimately affected the number of microflora isolated after storage. Proper chilling of meat will significantly reduce pathogen propagation and increase the shelf life of meat products (Smith, 1976).

Brine Chill

There are a variety of quick chilling methods that are implemented by the poultry industry. Brine chilling or "continuous immersion chilling", is the most common method. Carcasses are submerged in water and tumbled by mechanical agitation through several chill tanks. Ice is placed in the first tank; thus, the ice and water flow continuously from one tank to another (Kraft, 1992). Although it is a commonly used method, it has drawn criticism because of the

bacterial load sometimes associated with the chiller water. Lillard (1979) showed that fecal coliform counts in the water were as high as 3.4 $\log_{10}$ CFU/ml.

Although the primary goal is to reduce the temperature of the poultry carcasses, the secondary goal became to reduce the microbial load in the brine chillers (Lillard, 1979). The use of chlorine was earlier suggested by Gorselin et al. (1951) and chlorine and chlorine compounds are now used to control spoilage bacteria in the brine chillers. Lillard was the first to try and generate chlorine dioxide (ClO_2) on-site. Lillard hypothesized that because ClO_2 did not react with nitrogenous compounds, including simple amino acids, it would be effective in reducing the bacterial load in the brine chiller. Five ppm of ClO_2 was proven to be as equally as effective and less corrosive than 34 ppm of Cl_2 (Lillard, 1979). Lillard (1980) published work that showed the ClO_2 and Cl_2 extended the shelflife on 95% of the poultry carcasses in excess of 20 days.

Villarreal et al. (1990) reported that 1% slow release ClO_2 ice-slurry in the brine eliminated any recoverable *Salmonella* from turkey carcasses. Tsai et al. (1992) found in laboratory tests that Cl_2 at 100 to 150 ppm would reduce the bacterial load of the brine chiller water by 99% in 3 to 5 minutes. Increased exposure resulted in increased reductions.

In less confounding evidence, Dickens and Whitmore (1995) showed AAC added to the brine water reduced the Salmonella incidence by 0.32 to 1.4 $\log_{10}$ CFU/cm^2. Rathgeber and Waldroup (1995) used up to 1.5% Brifisol KTM (a commercial blend of sodium acid pyrophosphate and orthophosphoric acid)

during the chilling of poultry carcasses in a brine chiller. The solution was effective in reducing the bacterial load by approximately 1 $\log_{10}$.

Hurdle Technology

A combination of the previously mentioned prevention strategies in food processing could result in the inhibition or destruction of microorganisms. With this in mind, the implementation of these strategies may prevent negative effects of processing the product as well as decrease the likelihood of foodborne disease (Frank, 1992).

There are four basic methods used to inhibit or destroy microorganisms (Leistner, 1978). Those methods are sterilization, freezing, chilling and adjustment of pH and a_w. The hurdle effect is the process of controlling growth, metabolic activity, resistance and survival of microorganisms in food. Each hurdle represents a method used for controlling microbial growth (Frank, 1992). In 1991 the idea of combining the hurdle effect with HACCP systems offered a new dimension to food safety. The implementation of this technology has become known as hurdle technology (Leistner, 1994).

Until 1998, few studies had attempted to determine the long-term viability of bacterial populations found in ground beef when the beef carcasses were subjected to hurdle technology. Dorsa et al (1998) found that meat treated with antimicrobial compounds in a hurdle technology system had residual efficacy and resulted in lower or no detectable levels of pathogenic organisms 21 days later.

Dickson and Anderson (1991) found that washing with distilled water before evisceration and using 2% AAC to sanitize after washing decreased the pH of the carcass surface and ultimately reduced the microbial population by 2 log_{10}. Although not significant, slight reduction trends were observed when AAC was heated to 55°C prior to washing.

The combined treatments of a water wash, trimming, hot water washes and acid washes reduced the pathogen load on beef carcass tissue by 4.0 to 4.9 log CFU/cm^2. Individual treatments alone observed less reduction than the combined treatments (Castillo et al., 1998).

Castillo et al. (1999) studied the magnitude of microbial reduction when inoculated carcasses were steam vacuumed or steam vacuumed combined with sanitizing hot water or lactic acid sprays. The results showed that a combination of the treatments were more effective in reducing the pathogen load. The combination treatment reduced the pathogen load by 3 log_{10}.

V. Chlorine Dioxide

Overview

The bacteriacidal properties of chlorine dioxide (ClO_2) have been known since the early 1900's (Wei, et al., 1985). The first commercial use of ClO_2 as a biocide was in the early 1940's when the city of Niagara Falls added it to the drinking water to control the odor and taste of the water (Latshaw, 1994).

Chlorine dioxide is soluble in water (Latshaw, 1994), it is a yellow-green gas at low concentrations, and is similar in appearance and odor to chlorine (Cl_2). (Kim, 1997). Due to its highly explosive nature, ClO_2 cannot be

compressed and bottled, therefore it must be generated on site, (Latshaw, 1994, Kim, 1997, Lillard, 1979).

Some of the attributes that make ClO_2 unique is its effectiveness in killing microorganisms by interrupting their protein synthesis at pH levels from 4-10 (Latshaw, 1994). Unlike chlorine, ClO_2 does not react with ammonia or organic nitrogen, therefore there is no harmful chloramine or trihalomehtane formation (Kim, 1997). When ClO_2 reacts with organics it is reduced to chlorite anions (ClO_2^-): $ClO_2 + e^- \rightarrow ClO_2^-$. In addition, ClO_2 has a greater oxidizing capacity than Cl_2 (Kim et al., 1998).

The bactericidal effect of ClO_2 has proven to be seven times more effective than Cl_2 in poultry chiller water (Lillard, 1979). In addition, ClO_2 maintains it bactericidal activity longer than Cl_2 (Wei, et al., 1985). ClO_2 kills microorganisms that are resistant to Cl_2 treatment. Also, ClO_2 has been effective in killing microorganisms and extending shelflife for fresh produce, fish, beef and poultry (Richardson, et al., 1998).

In Europe, chlorine dioxide is widely used as an alternative to chlorine for drinking water disinfection (Kim, 1997). ClO_2 is used for treating drinking water in more than 500 U.S. cities (Richardson, et al., 1998), including such cities as Philadelphia, PA, Shreveport, LA, El Paso, TX, and Galveston, TX (Kim, 1997).

Chemical Mechanisms

Theoretically, ClO_2 is a mixed anhydride of chlorus acid ($HClO_2$) and chloric acid ($HClO_3$). It can be produced by the following reduction process (Kim, 1997):

$$2\ ClO_2 + H_2O \rightarrow HClO_2 + HClO_3$$

Most commercial operations utilize this process of reduction to optimize ClO_2 production. (Kim, 1997).

To date, little is known about the mechanisms ClO_2 has on microorganisms. Bernarde et al. (1967) showed how ClO_2 disrupts protein synthesis, resulting in the inactivation of the bacteria. Roller et al., (1980) suggested that ClO_2 caused damage to the cell membrane of the bacteria resulting in the inhibition of key enzymes that ultimately change the permeability of the cell. More recently, Berg et al. (1986) noted cell membrane damage resulting in massive leakage of intracellular macromolecules does not occur. Berg et al., (1996) believed ClO_2 caused the efflux of potassium, caused the loss of permeability control and was the primary cause of cell death.

Delivery Systems

ClO_2 has been proven to be effective in the reduction of microorganisms on a variety of foods (Richardson, et al., 1998). The effect of ClO_2 on the organic matter and microorganisms in poultry chiller water has proven to be successful. Lillard (1980) observed that ClO_2 was seven times more effective than Cl_2 in reducing the aerobic bacteria in the poultry chiller water. In addition, Lillard (1980) found ClO_2 was less corrosive on processing equipment. Thiessen et al., (1984), Tsai, et al., (1995) found ClO_2 was effective in reducing the microbial load in poultry chiller water. In 1996, FDA and the USDA approved its use for poultry chiller water (Tsai, et al., 1997).

Villarreal, et al., (1990) showed when chlorine dioxide was slowly released as an additive to turkey rinse and chiller water, the incidence of *Salmonella* contaminated carcasses was reduced an average of 70% after evisceration to 25% after chilling.

Cutter and Dorsa (1995) observed that ClO_2 spray-treatments were no more effective than water spray treatments in reducing the microbial load on contaminated beef carcass tissue. It was suggested that ClO_2 could be more effective if the meat was: (a) exposed to the ClO_2 spray-treatments for more than one minute or (b) sprayed with ClO_2 intermittently during the 24 hour chilling period.

Murano, et al., (1999) showed polyethylene film could be impregnated with up to 1.8% ClO_2. Meat was wrapped in the ClO_2 impregnated polyethylene film. The film proved to be effective in reducing the microbial load on beef cuts by 90%. Unfortunately, the ClO_2 film had deleterious effects on the meat color, causing it to turn from red to a dark green. This would ultimately result in decreased consumer appeal.

VI. Economic Implications

Costs Associated with Food Poisoning

Although difficult to estimate total direct and indirect costs, foodborne poisoning causes significant economic loss to all involved in the food processing chain (Varnam, 1991). For the people directly affected by foodborne poisoning, there are economic impacts associated with loss of earnings and job

productivity, as well as possible loss of human life or life-long side effects. In addition, there are medical costs associated with treatments for recuperation.

USDA's Economic Research Service (ERS) estimated the value of lifetime medical costs and lost productivity costs to generate the estimated direct annual costs of illness caused by pathogens. FSIS took this figure, and then multiplied it by the percentage of illnesses attributed to meat and poultry. It was estimated that foodborne illness in meat and poultry costs consumers $1.1 to $4.1 billion annually (Roberts, et al., 1996).

Varnam (1991) suggests the company involved in the foodborne poisoning will incur economic losses as well. Destruction of stock, loss of production, loss of sales, and brand rehabilitation are some of the more obvious losses. In addition, the company may have to retrain staff, perform an in-house investigation, renovate facilities and possibly compensate victims.

The economic impact to the company varies from situation to situation. In 1998, BilMar Foods of Zeeland, MI encountered the largest meat (processed) recall ever (35 million pounds), resulting in a direct economic loss of $76 million. After the recall, sales dropped for the following six months, resulting in an estimated $200 million economic loss (Young, 1999).

Costs Associated with Food Safety

The Food Safety Research Workgroup reports 21 federal agencies spend $200 million per year on food safety research. State and industry officials match those funds, resulting in a total of at least $400 million spent on food safety

research each year. This amount is less than 0.1% of the $647 billion spent on food in the U.S. each year (Forsythe, 1996, 1997).

The ability to put a dollar amount on food safety is difficult, but can be helpful in identifying critical control points that are both risk-effective and cost-effective. (Morales, 1998). Food safety has been given a value by estimating consumers' willingness to pay, behavior costs and costs incurred for medical care and labor productivity (Roberts, et al., 1996).

The ERS estimates the cost of the federally mandated HACCP regulations will cost the meat and poultry industry $1.0 to $1.2 billion per year ($0.18 per pound) over the next twenty years. However, it is anticipated that HACCP implementation will reduce the prevalence of pathogens in meat and poultry products, resulting in public health savings/benefit of $7.13 to $26.59 billion over the next twenty years. (Roberts, et al., 1996).

VII. Summary

Food safety is not a new problem. The establishment of HACCP is proving to be effective from both a food safety and an economic perspective. Through science, pathogen intervention strategies have been established in meat production systems to effectively minimize the threat of foodborne disease. Pathogen intervention strategies such as carcass washing/rinsing, steam vacuuming, and rapid chilling are proving to be effective in reducing the microbial load on fresh meat and poultry products. Scientists continue to search for new antimicrobial compounds and intervention technologies that offer

maximum bactericidal effects. Realistically, more research is needed to determine all possible pathogen intervention strategies to ensure the safety of the food supply. The findings of this research address the efficacy of ClO_2 in the form of ice, and its application as a new intervention strategy that may be implemented in the food industry to prevent the proliferation of pathogens commonly found in meat and meat products.

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CHAPTER 2

Materials and Methods

Development of Ice Machine

A Scottsman® ice machine (Fairfax, SC) was assembled in the meat science laboratory at Michigan State University. An Aqua Pure (Meriden, CT) water filter was used to filter the tap water used for the ice production. Two Valcor Scientific (Springfield, NJ) pumps were attached to the ice machine to pump the Oxine® and phosphoric acid (J.T. Baker, Pittsburgh, NJ) into the water reservoir of the ice machine. Two Idec (Sunnyvale, CA) timers were used to regulate the rate (time) and amount (volume) of Oxine® and phosphoric acid pumped. The ice machine drained the contents of the water reservoir into the ice-mixing chamber where ice was made.

Concentrations

Oxine® is 2% ClO_2 and 98% inert ingredients. It was not further diluted for this study. Eighty-six percent phosphoric acid (PA) was diluted and then used for the activation of marginally and largely activated ClO_2 ice. Activating the ClO_2 generates "free" ClO_2, which is the most likely to reduce the pathogen load. A ratio of one part PA to two parts water was used in the activation of marginally activated (5 ppm activated ClO_2, 15 ppm inactivated ClO_2) ClO_2 ice. The largely activated (10 ppm activated ClO_2, 10 ppm inactivated ClO_2) ClO_2 ice was generated using a one to one ratio (PA:H_2O). When making either marginally or largely activated ClO_2 ice, PA and ClO_2 are pumped into the water reservoir

every 41 s. The ice machine drains the contents of the water reservoir into the ice-mixing chamber where the ice is made. When making inactivated ClO_2 (20 ppm), only ClO_2 is pumped into the water reservoir. Control ice is made using no ClO_2 or PA.

Titration

The Burette Titration Method was used for the determination of total available chlorine dioxide (Bio-Cide International, 1999). The reagent 0.6N hydrochloric acid (HCl) solution was prepared by slowly adding 60 ml of 31.45% hydrochloric acid to 1 l of deionized water. The 6% potassium iodide (KI) solution was made by adding 65 grams of KI to 1 liter of deionized water, while mixing on a magnetic stir plate. After the KI has dissolved in the water, 20 g of sodium bicarbonate was added to adjust the pH of the solution to between 7.5 and 8.5. The reagents 0.00564N Phenylarsine Oxide (PAO) (Fischer, Pittsburgh, PA) and 0.5% Starch Solution (SS) (Lab Chem Inc., Pittsburgh, PA) were purchased.

The 6% KI solution (25 ml) and 50 ml of the 0.6N HCl were mixed in a 125 ml Erlenmeyer flask on a magnetic stir plate. Ice (25 g) was added to the KI and HCl mixture. After the ice completely melted in the mixture, it was titrated with PAO until the mixture turned pale yellow. At this point, 1 ml of the SS was added to the mixture, turning the color to dark blue. Slowly, PAO was added to result in a clear end point. The milliliters of PAO used was recorded. The following equation was used to determine total available ClO_2:

$$\frac{(\text{ml of PAO}) \times 76.083}{} = \text{total ppm available } ClO_2 \text{ (wt/wt)}$$

sample weight in grams

Product Procurement

In the pork study, Boston Butts (BB) (406, NAMP Meat Buyers Guide) were purchased from DeVries Meats Inc., Coopersville, MI. The BB were taken from the right side of the pork carcasses 24 h after slaughter. The BB were split in half (laterally) at the slaughter plant then kept together in a plastic bag. The BB were placed in an insulated ice chest for transport to the meat microbiology lab at MSU. Subsamples were excised from each BB, and bacterial enumeration was conducted to determine the microbial quality prior to inoculation. In the turkey study, birds were slaughtered at the MSU meat laboratory in the morning then chilled to 4°C. Once properly chilled, the turkeys were fabricated. Leg-thigh combinations were removed from the birds then transported in an ice chest to the meat microbiology lab. Because of the surface area needed (350 cm^2) to collect adequate samples throughout the study, four leg-thigh combinations were required for one sample. Subsamples were excised from the leg-thigh combinations to determine initial baseline microflora.

Test Microorganisms

For inoculation of the BB, isolates of *E. coli* 0157:H7 (AB317), Salmonella *typhimurium* (G01074), *Listeria monocytogenes* (DUP1907-11038) and *Yersinia entercolitica* (Michigan State University isolate) were used. *E. coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* were transferred from stock cultures to 10 ml sterile tryptic soy broth (Difco, Detroit, MI). *Yersinia entercolitica* was transferred into brain-heart infusion broth (Difco, Detroit, MI).

All pathogens were incubated at 35°C for 20 to 22 hours. For the turkey study *E. coli* 0157:H7 (AB317), Salmonella *typhimurium* (G01074), *Listeria monocytogenes* (DUP1907-11038) were used.

Inoculation of Test Microorganisms

Each subprimal (pork) or quad of subprimals (turkey) were placed in a sterile tin-foil tray to prevent cross contamination and to minimize handling of inoculated product. On each subprimal, 12 5 cm^2 areas were inoculated with either *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* or *Yersinia entercolitica.* Immediately after inoculation, each 5 cm^2 area was marked with green food coloring to assist in the identification of the inoculated area. During the 12 to14 h attachment time, the tinfoil trays were wrapped in tinfoil then placed in a 2°C cooler .

Sampling Method

Using a sterilized scalpel, 5 cm x 5 cm x 2 mm subsamples were excised for bacterial enumeration. A total of 14 subsamples were excised from the subprimals (2 excised prior to inoculation, 4 excised after inoculation, 4 excised after ice treatment and 4 excised after 7 d of 2°C storage).

Bacterial Enumeration

Subsamples were placed in a 400 ml Stomacher® bag (Seward Medical, London, UK) and weighed. The subsamples were serially diluted using 1% buffered peptone water (Difco, Detroit, MI). The subsamples were stomached for 90 s in a 400 ml masticator (IUL Instruments, Cincinnati, OH). From the bag, 0.1 ml was extracted then serially diluted to 10^{-3}, 10^{-5} and 10^{-7}. The dilutions

were plated in duplicate for determination of most probable number. *E. coli* 0157:H7 was serially diluted and plated on MacConkey Sorbitol Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN). *Salmonella typhimurium* was serially diluted and plated on Bismuth Sulfite Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN). *Yersinia entercolitica* was plated on Yersinia Selective Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN). *Listeria monocytogenes* was plated on Oxford medium with antimicrobic supplement added (Difco Laboratories, Detroit, MI) and 3M™ aerobic plates (3M, St. Paul, MN).

Meat Storage Containers

Eight 96-quart ice chests were used to store meat and ice treatments in one of two temperatures (2° or 26°C). A 120 cm x 44 cm x 4 mm sheet of expanded steel was placed 9.5 cm from the bottom of the cooler to allow draining of the melted ice. Each ice chest was partitioned into five sections, using 4 mm-thick plexi-glass. To prevent cross contamination between samples, the plexi-glass was stabilized by commercial sealing caulk.

Vacuum Packaging

After subsamples were excised following ice treatment, they were vacuum packaged. They were packaged using 4-mil thick heat seal polyethylene bags (Diagger, Lincolinshire, IL). The bags were sealed using an impulse heat sealer (Diagger, Lincolinshire, IL). A sterile 20 gauge needle was attached to a 15 mm-diameter rubber hose that was attached to a Welch vacuum pump (Welch

Vaccum, Skokie, IL). The vacuum pump was used to extract the air from the bag. After air extraction, the bag was sealed again to prevent airflow from the needle insertion point.

pH Determination

The pH of the ice was measured with a Fischer Scientific pH meter (Pittsburgh, PA). Ice (250 ml) was placed in a 300 ml beaker. The pH was measured by inserting the pH probe into the middle of the ice filled beaker. In order to observe pH decline over time, 22 readings were taken over a 4-hour period.

TBA Analysis

Thiobarbituric acid analysis was conducted to measure the effect of ice treatments on the rate of rancidity. Only samples that were not inoculated, but exposed to ice treatments were analyzed. TBA analysis was conducted on samples after ice treatments and after 7 d of storage according to the methods established by Tarladgis et al., (1960) and Zipser et al., (1962)

Statistical Analysis

Statistical analysis was conducted using SAS, Version 7.0 (SAS Institute Inc., Cary, NC,) For all meat studies, the mixed procedure to analyze least square means was used for each ice x temperature x day with all possible interactions. All treatments in the study were replicated twice.

CHAPTER 3

Development and Evaluation of an Ice Machine that Generates Chlorine Dioxide Ice at Various Levels of Activation

Abstract

Chlorine Dioxide (ClO_2) ice was generated by injecting varying amounts of Oxine® (Bio-Cide International) and phosphoric acid (PA) into the water reservoir of a commercial ice machine. The ice machine accurately (± 3 ppm) generated inactivated ClO_2 ice (20 ppm), marginally activated ClO_2 ice (5 ppm activated ClO_2, 15 ppm inactivated ClO_2), largely activated ClO_2 ice (10 ppm activated ClO_2, 10 ppm inactivated ClO_2) and a control (no ClO_2). Burette titration method was used for the determination of total available ClO_2. When titrated, there was a linear correlation (R^2 = .99) between the amount of Phenylarsine Oxide (PAO) needed to titrate and the amount of ClO_2 present in the ice. Over time, the pH of the ice declined from 4.8 to 2.6 as the ClO_2 and PA was released from the ice crystal during melting and tended to stabilized after 4 hour.

Introduction

Leistner (1978, 1994, 1995) has shown the effectiveness of hurdle technology in food safety. Hurdles such as low pH and temperature inhibit pathogen growth and possibly kill pathogens. The Federal Register (1996) reported that as part of the FSIS Pathogen Reduction/HACCP regulation, all beef, pork, lamb and poultry slaughter establishments should apply at least one antimicrobial treatment prior to carcass chilling. Proposed treatment

recommendations included any antimicrobial compounds previously approved by FSIS, as well as hot water and chlorine compounds

Chlorine dioxide (ClO_2) is a chlorinated compound that has been widely used throughout Europe for the disinfection of public water supplies (Latshaw, 1994). In 1979 Lilliard was the first to use ClO_2 in the reduction of pathogens in poultry chiller water. Results from his studies showed that ClO_2 at 5 ppm was effective at reducing pathogens. Other studies showed similar differences when used as a soaking solution or brine chiller (Cutter and Dorsa, 1995, Theissen et al., 1984, Villarreal et al., 1990).

Phebus et. al., (1997) showed the most effective reduction of bacteria on beef carcass tissue was observed when multiple hurdles in the pathogen intervention strategy were introduced. When using a combination of steam pasteurization, knife trimming, water washing, hot water/vacuum spot cleaning and spraying with 2% vol/vol lactic acid, pathogens were reduced by 4.2 to 5.3 $\log_{10}$ CFU/cm^2. When used individually, knife trimming, steam vacuuming or steam pasteurization reduced pathogen load from 2.5 to 3.7 $\log_{10}$ CFU/cm^2.

The purpose of this research was to develop an ice machine that could generate ClO_2 ice at various targeted concentrations that could be used as an added pathogen intervention strategy to ultimately decrease the likelihood of foodborne illness. The antimicrobial properties of the ClO_2 ice offer a new dimension in pathogen intervention strategies for the reduction of harmful bacteria. The bactericidal effect of ClO_2 in the ice could prevent the growth and possibly kill pathogens on the meat surface. Bem and Hechelmann (1995)

suggested that the simplest application of refrigeration is the direct contact between the substance being chilled and melting ice. Coupling the antimicrobial effectiveness of ClO_2 with the temperature reduction ability of ice could be an effective pathogen intervention strategy. When using ClO_2 ice prior to and during product shipment, the pathogens on the product would be exposed to the treated ice for an extended period of time. During treated ice application, the ClO_2 would be released from the ice and could inhibit pathogen survival. Also, during ice application, the ClO_2 ice would lower the temperature and pH, diminishing the ideal environment for pathogen survival and growth. When produced, this ice could be proven beneficial as an antimicrobial control mechanism in the pork and poultry industries.

Materials and Methods

Ice Machine and Pump Assembly

A Scottsman® ice machine (Fairfax, SC) was assembled in the meat science laboratory at Michigan State University. An Aqua Pure (Meriden, CT) water filter was used to filter the tap water that was used for the ice production. Two Valcor Scientific (Springfield, NJ) pumps were attached to the ice machine to pump the Oxine® and phosphoric acid (J.T Baker, Pittsburg, NJ) into the water reservoir of the ice machine. The two separate pumps were used in order to vary the concentration of each chemical. Two Idec® (Sunnyvale, CA) timers were used to regulate the rate(time) and amount(volume) of Oxine® and phosphoric acid pumped. The ice machine drained the contents of the water

reservoir into the ice-mixing chamber, where the ice was made. Figure 1.1 demonstrates the development of the chlorine dioxide generator that is used to pump the Oxine and PA into the water reservoir. The set up of the ice machine and pumps was relatively easy and took 10 hours.

Concentrations

Oxine® is 2% ClO_2 and 98% inert ingredients. It was not further diluted for this study. 86% phosphoric acid (PA) was diluted and then used for the activation of marginally and largely activated ClO_2 ice. Activating the ClO_2 generates "free" ClO_2, which is the most effective in the reduction of the pathogen load. A ratio of one part PA to two parts water was used in the activation of marginally activated (5 ppm free ClO_2, 15 ppm inactivated ClO_2) ClO_2 ice. The largely activated (10 ppm free ClO_2, 10 ppm inactivated ClO_2) ClO_2 ice was generated using a one to one ratio (PA:H_2O). When making either marginally or largely activated ClO_2 ice, PA and ClO_2 are pumped into the water reservoir every 41 seconds. The ice machine drains the contents of the water reservoir into the ice-mixing chamber where the ice is made. When making inactivated ClO_2 (20 ppm), only Oxine® (ClO_2) is pumped into the water reservoir. Control ice is made using no Oxine® or PA.

Titration

The burette titration method (Bio-Cide International, 1999) was used for the determination of total available chlorine dioxide. The reagents, 0.6N

hydrochloric acid (HCl) solution, and 6% potassium iodide (KI) solution were prepared. The 0.00564N Phenylarsine Oxide (PAO) (Fischer, Pittsburg, PA) and 0.5% Starch Solution (SS) (Lab Chem Inc., Pittsburg, PA) were purchased.

Twenty-five ml of the 6% KI solution and 50 ml of the 0.6N HCl were mixed in a 125 ml Erlenmeyer flask on a magnetic stir plate. Ice (25 g) was added to the KI and HCl. After the ice melted in the mixture, it was titrated with PAO until the mixture turned pale yellow. At this point, 1 ml of the SS was added to the mixture, turning the color to dark blue. Slowly, PAO was added until a clear end point was reached. The milliliters of PAO used were recorded. The following equation was used to determine total available ClO_2:

$$\frac{\text{(ml of PAO)} \times 76.083}{\text{sample weight in grams}} = \text{Total ppm available } ClO_2 \text{ (wt/wt)}$$

To determine amount of activated ClO_2 the following equation is used:

$$\frac{\text{(ml PAO)} \times 320}{\text{sample weight in grams}} = \text{ppm of activated } ClO_2 \text{ (wt/wt)}$$

Results and Discussion

The results of this study showed that chlorine dioxide ice could be produced at various targeted concentration levels. The difficult part of generating ClO_2 ice was determining the frequency and length of pump stroke, which regulated the amount of Oxine® and PA that was injected into the water reservoir. The first goal was to establish the most ideal length of pump stroke. Through trial and error, using only half of the possible pump stroke length proved to be ideal. It is important that the Oxine® and PA are pumped into the

water reservoir continuously to attain maximum consistency. It was found that a pump stroke every 41 s was adequate to maintain the appropriate level of Oxine® and PA in the water reservoir for consistent generation of targeted concentrations of ClO_2. The ClO_2 ice contains approximately 0.002 - 0.0025% ClO_2. The level of activation of ClO_2 was controlled by the (PA:H_2O) ratio that was injected. A ratio of one part PA to two parts water was used in the activation of marginally activated ClO_2 ice. The largely activated ClO_2 ice was generated using a (PA:H_2O) ratio. Only Oxine® (ClO_2) was used in the generation of inactivated ClO_2. For the generation of control ice, no Oxine® (ClO2) or PA were used (Figure 1.2). One of the drawbacks of the ice generator was that only one ice treatment could be made at a time. In order to generate different kinds of ice for this pathogen reduction study, 10 h was necessary for manufacturing of each ice treatment to produce sufficient quantities of ice.

Figure 1.3 shows the linear correlation between PAO and total ppm of ClO_2, which predicts the accuracy that ClO_2 ice can be made. A R^2 value of 0.99 indicates the ability to predict the ppm of ClO2 to be generated at a certain volume (ml) of PAO. Increasing the mls of PAO used to titrate the dark blue color that was activated by the starch indicator solution, indicated there was more ppm of ClO_2 in the melted ice. One thing to be considered with each titration is that there are slight differences in levels of total ppm of ClO_2, due to differences in ice crystal shape and size. Therefore, the ability to acheive the targeted ClO_2 ppm varied ± 3 ppm (Figure 1.2).

The pH decline (Figure 1.4) in the ice over time explains that as the ice melts, the ClO_2 and PA is released from the ice crystal, which causes a significant ($P > 0.05$) drop in pH from 4.89 to 2.56. Ultimately, the pH returned to 2.97. Due to the fact that many pathogens cannot withstand a low pH or low temperatures, applying this treated ice in a pathogen intervention strategy could prove to be effective in the reduction of pathogens.

When an economic analysis was conducted after the initial fixed (depending on size and kind of ice machine and ClO_2 generator) cost of approximately $4,000.00, the cost of producing the ClO_2 ice is approximately $ 0.0017 per kg ($0.38 per 100 pounds) of ice. This antimicrobial ice delivery system could possibly serve as a potential intervention strategy for poultry slaughter facilities in brine chillers. Due to lack of refrigeration in some meat transportation and holding facilities in third-world countries, the ClO_2 ice could serve as both an antimicrobial and as a temperature control mechanism. The ice also could be used in ice-packed meat transportation systems or in fresh poultry and seafood retail case displays.

Figure 1.1 Chlorine Dioxide Ice generator

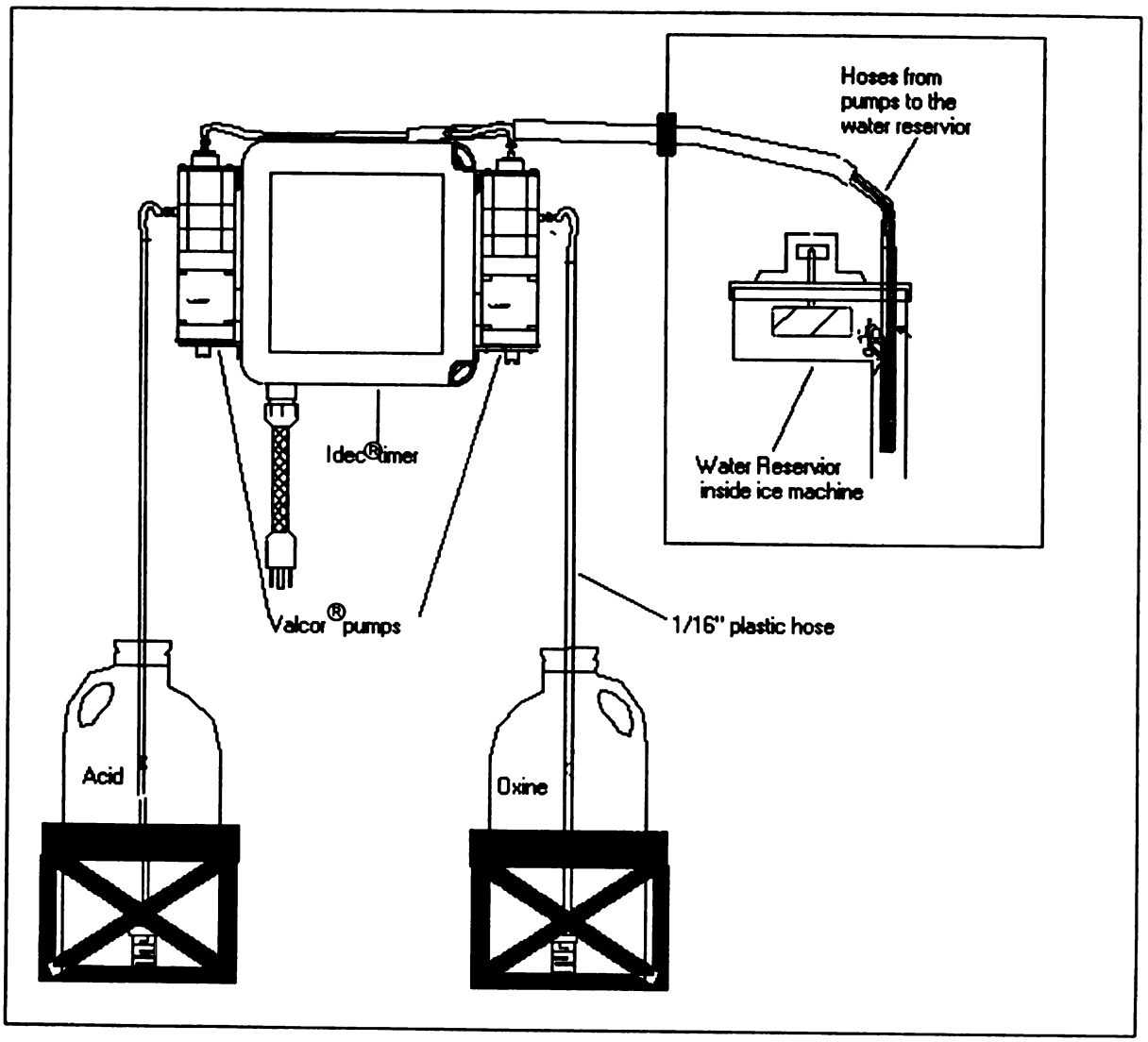


Figure 1.2 Ratio of Phosphoric Acid and Water used to generate targeted levels of activated or inactivate ClO_2 Ice

Ice	Ratio		Targeted ppm	Actual ppm
	Phosphoric Acid	H_2O		
Control	0	0	0	0
Inactivated	0	0	20	23
Marginally activated	1	2	20	20
Largely Activated	1	1	20	21

Figure 1.3 Linear correlation between PAO and total available ClO_2

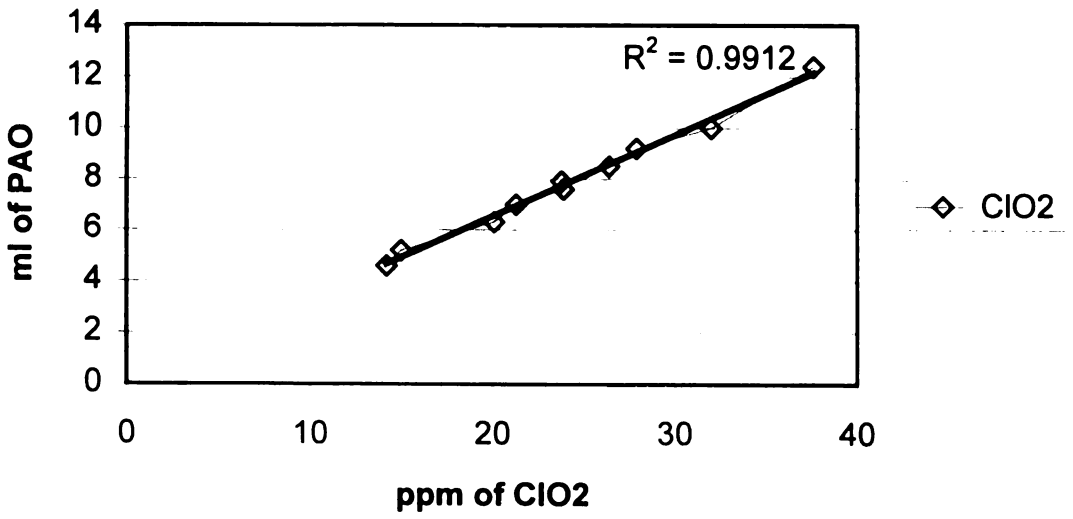
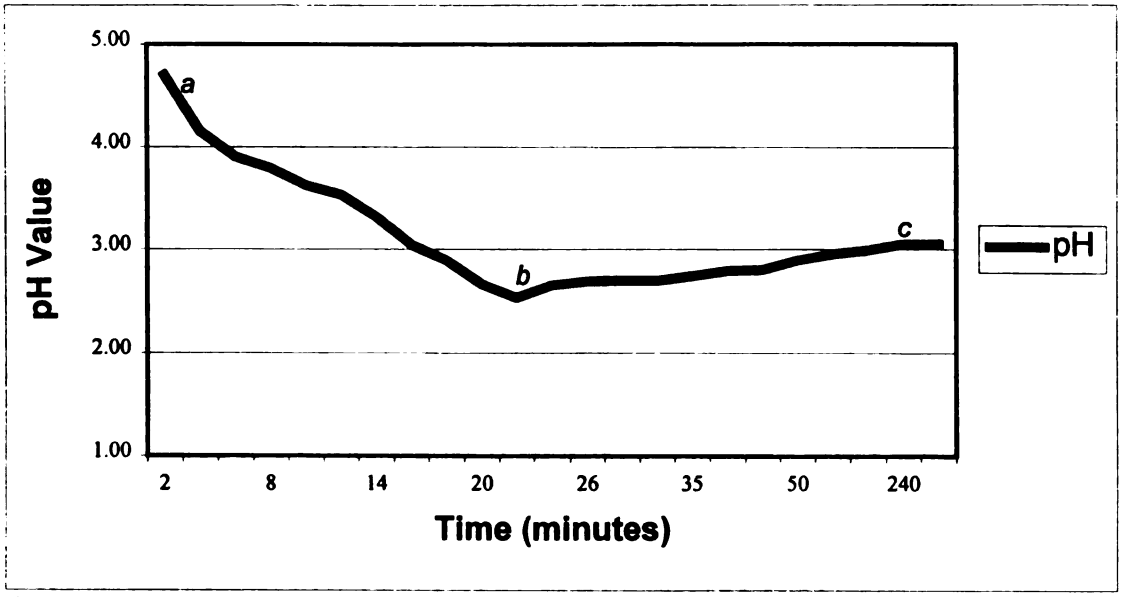


Figure 1.4 Ice pH decline over 4-hour observation period



a, b, c Means with the same letter are not significantly different ($P > 0.05$)

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CHAPTER 4

Influence of Chlorine Dioxide Ice on Pathogen Survival and Recovery on Chilled Pork and Turkey Subprimals

Abstract

Chlorine dioxide (ClO_2) solutions have been investigated as an antimicrobial to reduce pathogens in meat washing/dipping systems and brine chillers. This study investigated various concentrations of ClO_2 solutions incorporated as an antimicrobial ice treatment for pork and turkey subprimals. An ice machine with a ClO_2 generator produced ice containing 0 or 20 ppm inactivated ClO_2 and 5 or 10 ppm activated ClO_2. Subprimals were inoculated with *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia entercolitica* (pork) or *Escherichia coli* 0157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* (turkey). Inoculated subprimals were placed in partitioned ice chests with one of four ice treatments for 24 h at either refrigerated (2°C) or room temperature (26°C). Subprimals were removed from ice, vacuum packaged, and stored (2°C) for 7 days. For bacterial enumeration, samples were excised from the subprimals prior to and after ice treatment and during storage (2°C; 7 days). The main effect of ClO_2 concentration did not reduce ($P>0.05$) the microbial load of pathogens on subprimals. The interaction of temperature during treated ice application (2° and 26°C) and day of storage (day 1 and 7) did significantly reduce ($P<0.05$) the microbial load. Treated ice applied to pork at 26°C reduced *Listeria monocytogenes,* and at 2°C, *E. coli* 0157:H7 and *Salmonella typhimurium* were reduced after 7 days of storage.

Neither temperature during ice application reduced *Yersinia entercolitica*. In turkey, ice treatments applied at the environmental temperature of 26°C reduced *E. coli* 0157:H7 and *Salmonella typhimurium*. This reduction may be attributed to the release of more ClO_2 during melting. The largely activated ice-melt in the bottom of the cooler contained 2.5-3.0 log_{10}CFU/ml fewer pathogens compared to the control ice-melt.

Introduction

Chlorine is a disinfectant that has been used for the last 100 years, but has drawn concern because of the toxic mutagens generated during the reaction with organic matter (Tsai et al., 1997). Another chlorinated compound that is currently used in more than 600 water treatment facilities in the US is chlorine dioxide (ClO_2) (Latshaw, 1994). Lillard (1979) found ClO_2 to be seven times more effective than chlorine and did not generate the toxic chloroform when it reacted with organic matter. Several studies have reported the effectiveness of ClO_2 as an antimicrobial applied as a liquid (Cutter and Dorsa, 1995). Bem and Hechelmann (1995) suggested that the simplest application of refrigeration is the direct contact between the substance being chilled and melting ice. Coupling the bactericidal effectiveness of ClO_2 and the temperature reducing/stabilizing ability of ice would offer a pathogen intervention strategy that has never been investigated.

The purpose of this study was to observe the effectiveness of ClO_2 applied in an ice form on the survival of pathogens on fresh pork and turkey subprimals. The hypothesis was that the bactericidal effect of the ClO_2, the low

temperature and the low pH of the ice would result in multiple hurdles, preventing pathogen survival and growth on the meat product.

Materials and Methods

A Scottsman® ice machine (Fairfax, SC) was assembled in the meat science laboratory at Michigan State University. An Aqua Pure (Meriden, CT) water filter was used to filter the chlorinated tap water that was used for the ice production. Two Valcor Scientific (Springfield, NJ) pumps were attached to the ice machine to pump Oxine® and phosphoric acid (J.T Baker, Pittsburg, NJ) into the water reservoir of the ice machine. The ice machine accurately (within 3 ppm) generated inactivated ClO_2 ice (20 ppm), marginally activated ClO_2 ice (5 ppm free ClO_2, 15 ppm inactivated ClO_2), largely activated ClO_2 ice (10 ppm free ClO_2, 10 ppm inactivated ClO_2) or control (no ClO_2). Burette titration method (Bio-Cide International, 1999) was used for the determination of total available ClO_2.

Fresh pork and turkey subprimals were used in the model ice application. Twelve 25 cm^2 areas were inoculated on each subprimal with approximately 5 log_{10} of *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia entercolitica* (pork, Study I) or *Escherichia coli* 0157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* (turkey, Study II). Each subprimal was inoculated with a different pathogen. To allow attachment, inoculated subprimals were stored at 2°C for 12 to 14 h prior to ice treatment. Samples were excised after inoculation and prior to ice treatments

for baseline bacterial enumeration. Methods described by the USDA's Bacteriological Analytical Manual (BAM, 1995) were used.

Eight Commercial sized (96-quart) ice chests were partitioned into five (Study I) or four (Study II) sections with 4 mm-thick sheets of plexiglass. Expanded steel was placed 9.5 cm from the bottom of the ice chest to allow the melted ice to drain off of the product. Each ice chest was assigned one of four ice treatments and an environmental temperature of either 2°C (refrigerated) or 26°C (room temperature). In every ice chest, 1 Kg of one of the ice treatments was placed in each partitioned section. Next, inoculated subprimals were placed on top of the ice, then an additional 2 Kg of ice was placed on top of the subprimal (3 Kg ice total).

After 24 h of ice application, the subprimals were removed and subsamples were excised for bacterial enumeration and TBA analysis. To test the effect of ice treatments on shelflife, the subprimals were vacuum packaged and stored in refrigeration (2°C) for 7 days. Subsamples were then removed and bacterial enumeration and TBA analysis (Tarladgis, G. G. et. al., 1960 and Zipser, M. W. et. al., 1962) were conducted.

Results and Discussion

The ice was successfully generated at targeted ppm of ClO_2 for each ice treatment. In both studies, the main effect of ClO_2 concentration did not significantly reduce ($P>0.05$) the microbial load of pathogens on either pork or turkey subprimals (Tables 1 and 2). This could be due to a number of reasons. If the pathogens were exposed to ClO_2 for a longer period of time, it could have had more of a detrimental effect on the pathogen load. Cutter and Dorsa, (1995)

suggested the longer the exposure time (> 60 seconds), a higher pathogen reduction would occur. Also, if the total ppm of activated ClO_2 was increased, the ClO_2 ice could prove to have a greater antimicrobials effect.

The interaction of temperature during treated ice application (2° and 26°C) and day of storage (day 1 and 7) did significantly reduce ($P<0.05$) the microbial load. The increase in the room temperature (26°C) environment could have caused more ice melt, resulting in an increase of ClO_2 being released from the ice as well as a decrease in the pH of the ice melt. When using similar concentrations of ClO_2, Lillard (1980) found greater reductions of total aerobic bacteria on chicken carcasses (1.2 $\log_{10}$ CFU/g) when chilled in ClO_2 treated versus untreated poultry chiller water. No significant difference was observed between 34 ppm of Cl_2 and 5ppm of ClO_2 treatments on the poultry carcasses or the chiller water in that study. In all observations, the chicken breast skin had a higher total aerobic plate count than the chiller water they were in. In correlation with Lillard's findings, in this study, the pathogen load was higher on the meat product than in the ice melt.

In this study, *E. coli* 0157:H7 and *Salmonella typhimurium* on pork subprimals were slightly reduced after 7 days of storage when the ice treatment was applied at an environmental temperature of 2°C (Figures 1 and 2 respectively). Although both pathogens are facultative anaerobes, the vacuum packaged storage at 2°C could have caused the reduction. In addition, maintaining the environmental temperature at 2°C immediately after inoculation, during ice application and through the 7 day storage period, could have

prevented the proliferation of pathogens. In past research, Lillard et al., (1980), showed that Cl_2 and ClO_2 had a residual effect which extended the shelf-life of a product by more than 20 days.

In both the pork and turkey studies, treated ice applied to subprimals at the environmental temperature of 26°C reduced *Listeria monocytogenes,* (Figures 3 and 7). Considering the fact that *L. monocytogenes* is psychrotrophic, the increased temperature (26°C) during ice application could have contributed to this observed reduction in numbers.

Neither temperature during ice applications reduced *Yersinia entercolitica* (Figure 4). Y. entercolitica was tested in this study because of its prevalence in the pork industry. The ineffectiveness of ClO_2 on *Y. entercolitica* is not well documented. It could be postulated that *Y. entercolitica* contains a cellular-barrier mechanism that inhibits the antibacterial effectiveness of ClO_2.

In turkey, ice treatments applied at the environmental temperature of 26°C reduced *E. coli* 0157:H7 and *Salmonella typhimurium* (Figures 5 and 6). This reduction may be attributed to the release of more ClO_2 due to increased ice melt during exposure at 26°C. Another theory suggested by Wei et al., (1985) was that the bactericidal activity of ClO_2 decreases as application temperatures decreases, thus, when the ClO_2 is at room temperature it is more effective.

To observe the viability of pathogens that could have been rinsed off the product during ice melt, samples were taken from the bottom of the ice chests. The largely activated ice-melt in the bottom of the ice chests contained a mean

average of 1.8 $\log_{10}$ CFU/ml fewer ($P<0.05$) microorganisms compared to the control ice-melt (Figure 8). In a follow-up study, the largely activated ice-melt contained 3.6 $\log_{10}$ CFU/ml fewer ($P<0.05$) total aerobic counts than the control ice. The ice melt in the bottom of the cooler contained up to 100% higher ppm than the original ice melt. Biocide International engineers (Khanna, 2000) speculated that the ions in the ice crystal leach out the ClO_2. The ClO_2 then becomes heterogeneous and localizes in the bottom of the ice chest, which causes a higher reduction of pathogens. In additon, during ice melt, the pH declines and could aid in the reduction of pathogenic orgaisms.

Using 5 ppm of ClO_2, Lillard (1979) found less of a reduction in the poultry chiller water than what was found in the 20 ppm ClO_2 ice melt in this study. Although exact ppm of ClO_2 were not reported, Thiessen et al., (1983) observed *Salmonella* and coliforms could be reduced in the chiller water from 3.14 $\log_{10}$ to 0 CFUs when ClO_2 was added at 1.39 mg of ClO_2/liter of water. However, on the poultry carcasses, coliforms were only slightly reduced (<0.5 $\log_{10}$). When using Cl_2, Tsai, et al., (1991) showed that 100 to 150 ppm was needed to reduce poultry chiller water by 2 $\log_{10}$.

On beef carcass tissue, Cutter and Dorsa, (1995) showed that bacterial populations were not reduced by more than 0.93 log CFU/cm^2, regardless of the liquid ClO_2 concentration. In addition, they did observe ClO_2 length of application period (15, 30 or 60 seconds) did not affect amount of reduction. They concluded that ClO_2 was no more effective than water when used as a spray.

After data analysis in this study, it was hypothesized that the extended time (12 to 14 hour) that was allowed for the pathogens to attach to the meat surface was to long. An attachment study was conducted to determine if a shorter attachment time would increase the effectiveness of the ice treatments. Poultry subprimals were inoculated with *E. coli* 0157:H7, and then ice was applied at either 2, 4, 6, 8, 10 or 12 hours after inoculation. Figure 9 shows there were significant differences ($P<0.05$) between attachment times. However, it is important to note that the maximum differences in pathogen reduction over different time periods were no more than 0.20 $\log_{10}CFU/cm^2$. It can be postulated that the attachment time in the initial study had little to no affect on the effectiveness of the ice treatments.

In addition to studying the effect of ClO_2 ice on the survival of pathogenic organisms, the effect of ClO_2 on the product shelf life was observed as well. A thiobarbituric acid (TBA) test was conducted to determine if the application of ClO_2 ice treatments had an affect on the shelf life of the pork and turkey subprimals. In both the pork and turkey study (Table 3) there was a significant main effect ($P<0.05$) of day. In the pork study, none of the samples contained more than 0.33 mg of malonaldehyde/1000 g sample. In the turkey study, greater lipid oxidation was observed, especially on day 7. This increase was likely due to the higher level of polyunsaturated fatty acids in the turkey skin. In the both the pork and turkey studies, no significant differences were observed between ClO_2 ice treatments or ice application at either environmental temperature (2°C or 26°C).

Conclusions

A model ClO_2 ice application protocol was developed to simulate ice-packed meat transportation systems or meat retail case displays. On fresh pork and turkey subprimals, the interaction of temperature during treated ice application (2° and 26°C) and day of storage (day 1 and 7) significantly reduced ($P<0.05$) the microbial load on *E. coli* 0157:H7, *S. typhimurium* and *Listeria monocytogenes*. *Y. entercolitica* was not reduced. In this study, relatively low concentrations of ClO_2 were used to prevent the leaching of the chlorine odor associated with chlorine compounds onto the meat product and to prevent discoloration of the meat product. It is also important to note that the ice treatments used had no deleterious effects on the shelf life of the subprimals. Increasing the ppm of ClO_2 in the ice would likely enhance the effectiveness of the ice in a pathogen reduction system. Furthermore, increasing the ice melt by using warm (body temperature immediately after slaughter) meat will increase the ClO_2 released from the ice, which will offer more of an antimicrobial effect. The ClO_2 in the ice melt in the bottom of the ice chest was more localized and thus was more effective at reducing the pathogens in the melted ice water. In addition, the pH of the ice declines as it melts, potentially decreasing pathogen numbers. Due to lack of refrigeration in some meat transportation and holding facilities in developing and third-world countries, the ClO_2 ice could serve as both an antimicrobial and as a temperature control mechanism. This ClO_2 ice could also be used in ice-packed meat transportation systems or in a meat retail case.

Table 1 Survival of pathogens on pork subprimals submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

Plates	Baseline	2°C		26°C		SEM[d]
		Day		Day		
Eschericia coli 0157:H7		1	7	1	7	
APC[1]	4.76[a]	4.50[a]	4.31[a]	3.93[b]	4.30[a]	0.34
Coliform[2]	4.61[a]	3.97[a]	4.20[a]	3.94[a]	3.92[a]	0.38
MaConkeys[3]	4.56[a]	4.01[a]	3.94[a]	3.99[a]	4.13[a]	0.35
Salmonella typhirmirium						
APC[1]	5.01[a]	4.96[a]	4.40[b]	4.71[a]	4.80[a]	0.15
Coliform[2]	4.62[a]	4.77[a]	3.92[b]	4.36[a]	4.56[a]	0.21
Bismuth[3]	4.80[a]	4.65[a]	4.13[b]	4.30[b]	4.51[a]	0.20
Listeria monocytogenes						
APC[1]	3.87[a]	4.13[a]	4.28[a]	3.79[a]	4.25[a]	0.27
Oxford[3]	3.15[a]	3.07[a]	3.05[a]	3.21[a]	2.74[a]	0.42
Yersinia entercolitica						
APC[1]	5.39[a]	5.41[a]	6.87[b]	5.34[a]	6.90[b]	0.23
Coliform[2]	5.46[a]	5.37[a]	6.73[b]	5.18[a]	6.67[b]	0.23
Yersinia[3]	5.45[a]	5.40[a]	6.76[b]	5.24[a]	6.74[b]	0.22

[1] 3M® Aerobic Plates Count petri film

[2] 3M® Coliform perti film

[3] Selective Agar specific to the pathogen

[a, b] Means having the same superscript within columns are not significantly different (P>0.05)

[d] Standard Error of the Mean

Table 2 Survival of pathogens on turkey subprimals submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

Plates	Baseline	2°C		26°C		SEM[d]
		Day		Day		
Eschericia coli 0157:H7		1	7	1	7	
APC[1]	4.07[a]	4.94[b]	3.86[a]	5.40[b]	3.55[a]	0.39
Coliform[2]	4.30[a]	4.81[a]	3.38[a]	5.16[b]	2.75[bc]	0.45
MaConkeys[3]	3.70[a]	4.98[b]	3.56[a]	5.72[b]	2.91[a]	0.52
Salmonella typhirmirium						
APC[1]	4.97[a]	5.82[b]	4.74[a]	6.35[b]	4.23[b]	0.27
Coliform[2]	4.90[a]	5.50[b]	4.58[a]	6.19[b]	4.48[a]	0.30
Bismuth[3]	4.29[a]	5.80[b]	4.70[a]	5.43[b]	3.95[a]	0.44
Listeria monocytogenes						
APC[1]	4.46[a]	5.29[b]	4.90[bc]	5.38[b]	4.89[bc]	0.15
Oxford[3]	3.54[a]	3.95[a]	4.17[a]	4.17[a]	3.76[a]	0.60

[1] 3M® Aerobic Plates Count petri film

[2] 3M® Coliform perti film

[3] Selective Agar specific to the pathogen

[a, b, c] Means having the same superscript within columns are not significantly different (P>0.05)

[d] Standard Error of the Mean

Table 3 Milligrams of malonaldehyde/1000 g sample in pork and turkey subprimals submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, at day 1 and day 7 (vacuum packaged storage at 2°C).

Study	Day		SEM[c]
	1	7	
Pork	0.231[a]	0.283[b]	0.02
Turkey	0.685[a]	2.096[b]	0.20

[a, b] Means having the same superscript within columns are not significantly different (P> 0.05)
[c] Standard Error of the Mean

Figure 1. Two-way interaction (P<0.05) of the growth of *E. coli* 0157:H7 on pork sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

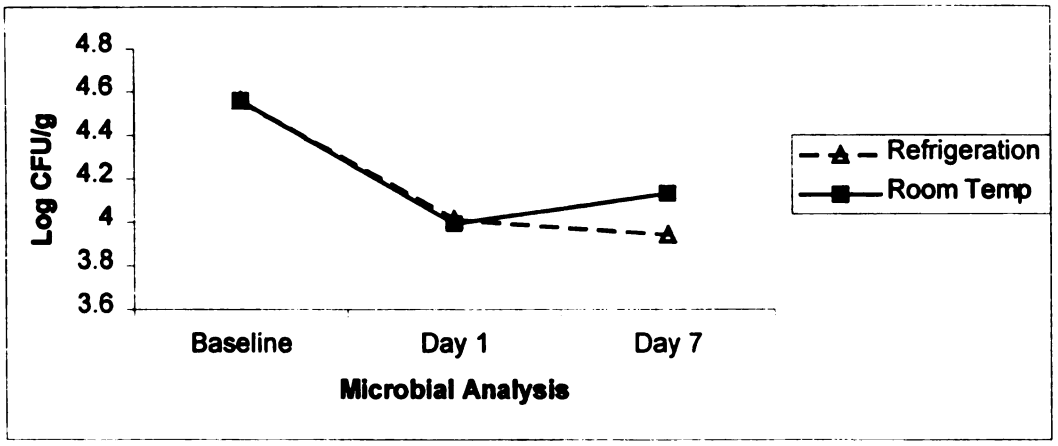


Figure 2. Two-way interaction (P<0.05) of the growth of *Salmonella typhimurium* on pork sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

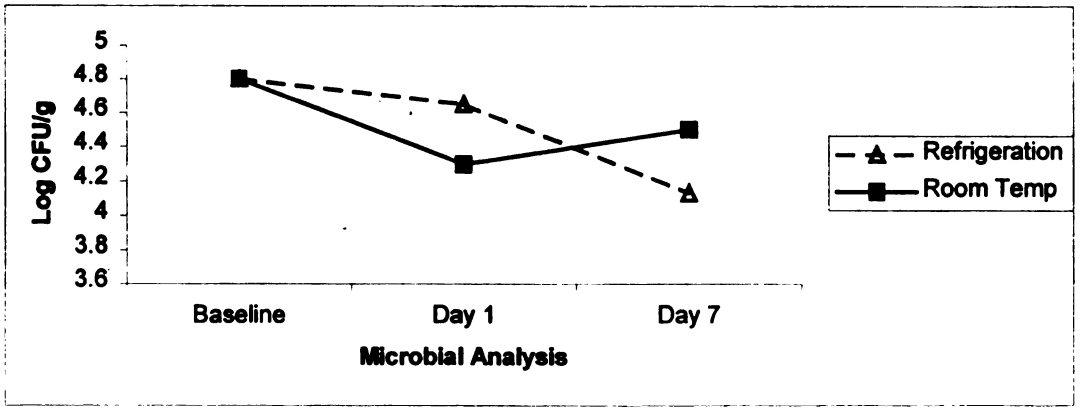


Figure 3. Two-way interaction (P<0.05) of the growth of *Listeria monocytogenes* on pork sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

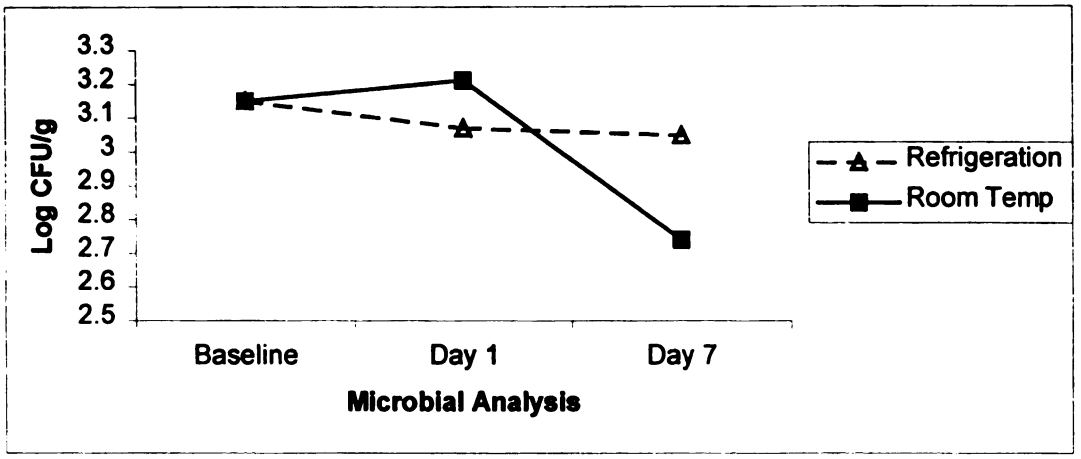


Figure 4. Two-way interaction (P<0.05) of the growth of *Yersinia entercolitica* on pork sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

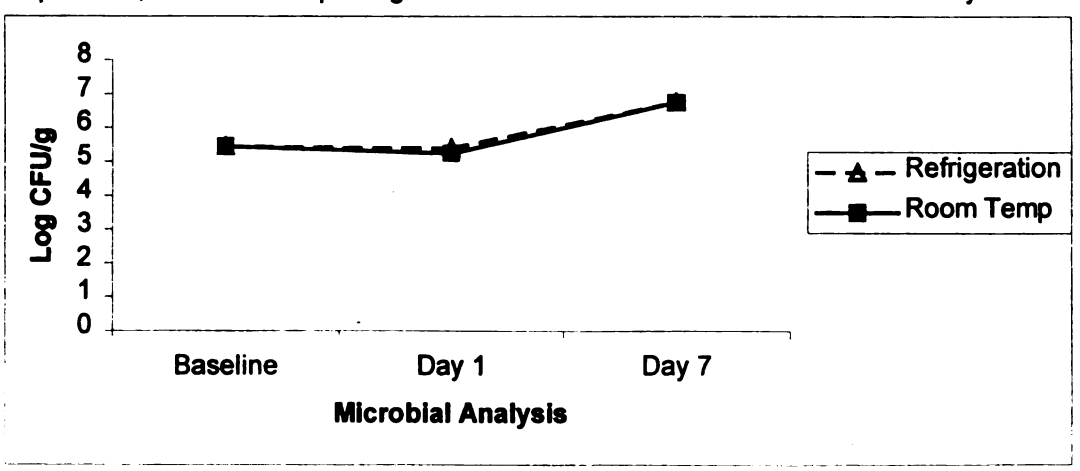


Figure 5. Two-way interaction *(P<0.05)* of the growth of *E. coli* 0157:H7 on turkey sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

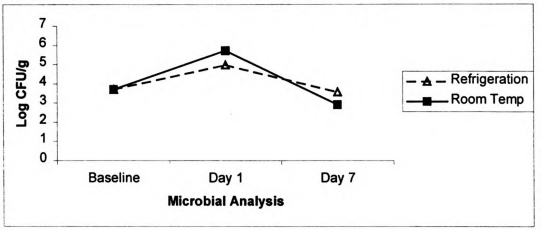


Figure 6. Two-way interaction (P<0.05) of the growth of *Salmonella entercolitica* on turkey sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

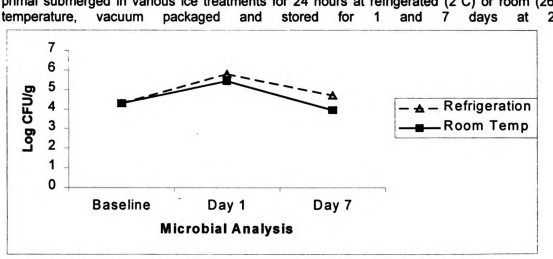


Figure 7. Two-way interaction of the growth of *Listeria monocytogenes* on turkey sub-primal submerged in various ice treatments for 24 hours at refrigerated (2°C) or room (26°C) temperature, vacuum packaged and stored for 1 and 7 days at 2°C.

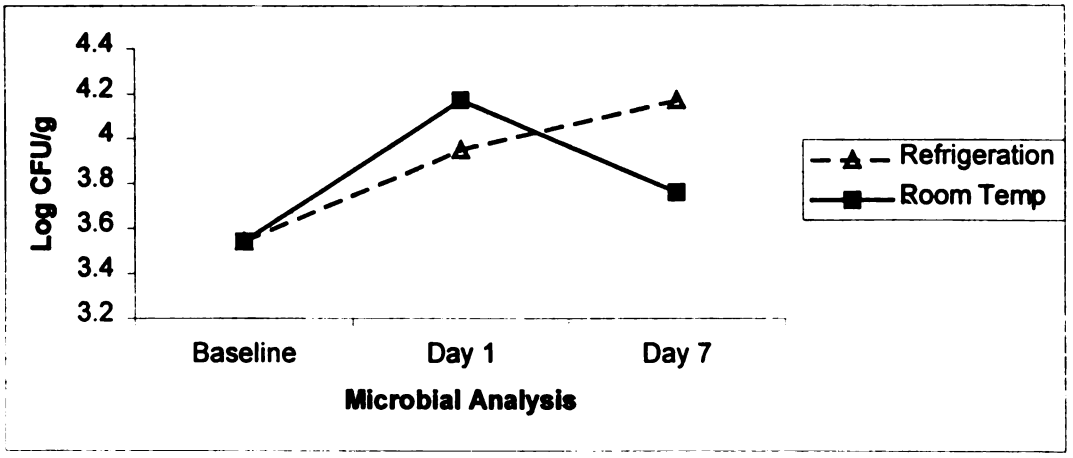
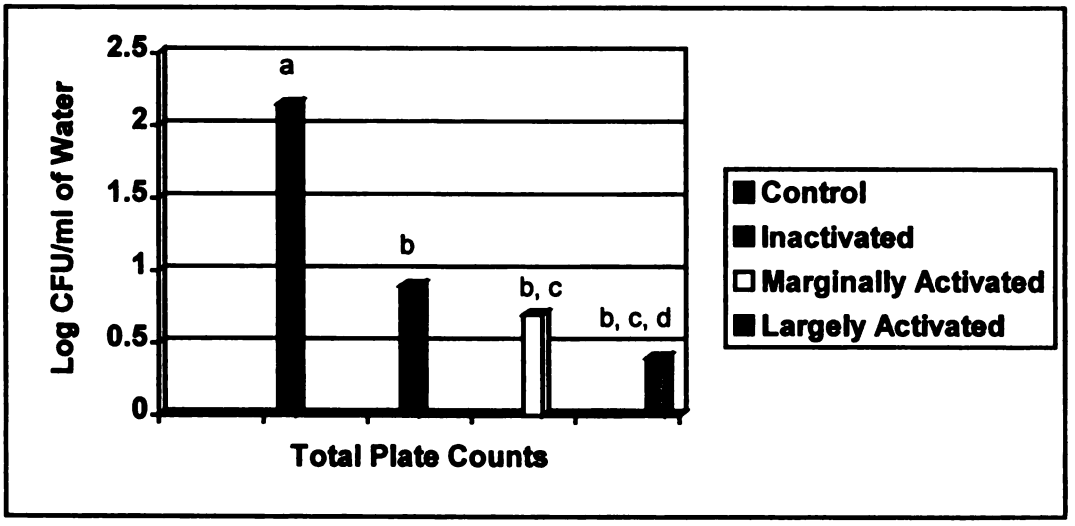
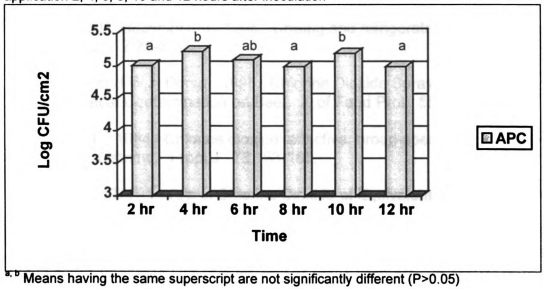


Figure 8. Effect of chlorine dioxide ice-melt on pathogen survival in the bottom of the ice chests.



[a, b, c, d] Means having the same superscript are not significantly different (P>0.05)

Figure 9 Differences in the $\log_{10}$ CFU/cm^2 of *E. coli* 0157:H7 that were recovered after ice application 2, 4, 6, 8, 10 and 12 hours after inoculation



[a, b] Means having the same superscript are not significantly different (P>0.05)

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APPENDICES

APPENDIX

Procedures and Protocols

APPENDIX 1: Development of Chlorine Dioxide Generator

1. A Scottsman® ice machine (Fairfax, SC) was assembled in the meat science laboratory at Michigan State University. An Aqua Pure (Meriden, CT) water filter was used to filter the tap water used for the ice production.

2. Two Valcor Scientific (Springfield, NJ) pumps were attached to the ice machine to pump the Oxine® and phosphoric acid (J.T. Baker, Pittsburgh, NJ) into the water reservoir of the ice machine.

3. Two Idec (Sunnyvale, CA) timers were used to regulate the rate (time) and amount (volume) of Oxine® and phosphoric acid pumped. The ice machine drained the contents of the water reservoir into the ice-mixing chamber where ice was made.

APPENDIX 2: Concentrations of Chlorine Dioxide

1. Oxine® (Bio-Cide International, Norman, OK) is 2% ClO_2 and 98% inert ingredients. It was not further diluted for this study.

2. Phosphoric acid (PA) (86%) was diluted and then used for the activation of marginally and largely activated ClO_2 ice.

3. A ratio of one part PA to two parts water was used in the activation of marginally activated ClO_2 ice.

4. Oxine® and PA were each in 500 ml Erlenmeyer flasks attached to the side of the ice machine.

5. Using the Chlorine Dioxide Generator, the Oxine® and PA were pumped into the water reservoir, which was then drained into the ice-mixing chamber where the ice was made.

6. When making inactivated or marginally/largely activated ClO_2 ice, PA and Oxine® are pumped into the water reservoir every 41 seconds, using only half of a pump stroke each time.

7. The largely activated ClO_2 ice was generated using a PA ratio of one to one.

8. When making inactivated ClO_2 ice only Oxine® was pumped in the water reservoir. If PA were added, it would cause the ClO_2 to become activated.

9. Control ice is made using no ClO_2 or PA.

APPENDIX 3: Burette Titration Method for Determination of Total Available Chlorine Dioxide

1. The Burette Titration Method was used for the determination of total available chlorine dioxide (Bio-Cide International, 1999).

2. The reagent 0.6N hydrochloric acid (HCl) solution was prepared by slowly adding 60 ml of 31.45% hydrochloric acid to 1 liter of deionized water.

3. The 6% potassium iodide (KI) solution were made by adding 65 grams of KI to 1 liter of deionized water, while mixing on a magnetic stir plate. After the KI has dissolved in the water, 20 grams of sodium bicarbonate was added to adjust the pH of the solution to between 7.5 and 8.5.

4. The reagents 0.00564N Phenylarsine Oxide (PAO) (Fischer, Pittsburgh, PA) and 0.5% Starch Solution (SS) (Lab Chem Inc., Pittsburgh, PA) were purchased.

5. The 6% KI solution (25 ml) and 50 ml of the 0.6N HCl were mixed in a 125 ml Erlenmeyer flask on a magnetic stir plate.

6. Ice (25 g) was added to the KI and HCl mixture.

7. After the ice completely melted in the mixture, it was titrated with PAO until the mixture turned pale yellow.

8. At this point, 1 ml of the SS was added to the mixture, turning the color to dark blue.

9. Slowly, PAO was added to result in a clear end point.

10. The milliliters of PAO used was recorded.

The following equation was used to determine total available ClO_2:

$$\frac{(\text{ml of PAO}) \times 76.083}{\text{sample weight in grams}} = \text{total ppm available } ClO_2 \text{ (wt/wt)}$$

The following equation was used to determine activated ClO_2:

$$\frac{(\text{ml of PAO}) \times 320}{\text{sample weight in grams}} = \text{activated } ClO_2 \text{ (wt/wt)}$$

Theory behind calculation:

$$\frac{ml \times N \times e \times 10^6}{sw} = \text{ppm available } ClO2 \text{ (wt/wt)}$$

Where:

ml = PAO volume in millimeters
N = normality of PAO (0.00564N)
e = Milliequvalent weight of chlorine dioxide (0.01349)
sw = sample weght
10^6 = factor to convert milligrams/ml to ppm

APPENDIX 4: Product Procurement

1. In the pork study, Boston Butts (BB) (406, NAMP Meat Buyers Guide) were purchased from DeVries Meats Inc., (Coopersville, MI).

2. The BB were taken from the right side of the pork carcasses 24 hours after slaughter.

3. The BB were split in half (laterally) at the slaughter plant then kept together in a plastic bag.

4. The BB were placed in an insulated ice chest for transport to the meat microbiology lab at MSU.

5. Subsamples were excised from each BB and bacterial enumeration was conducted to determine the microbial quality prior to inoculation.

6. In the turkey study, birds were slaughtered at the MSU meat laboratory in the morning then chilled to 4°C.

7. Once properly chilled, the turkeys were fabricated. Leg-thigh combinations were removed from the birds then transported in an ice chest to the meat microbiology lab.

8. Because of the surface area needed (350 cm^2) to collect adequate samples throughout the study, four leg-thigh combinations were required for one sample.

9. Subsamples were excised from the leg-thigh combinations to determine initial baseline microflora.

APPENDIX 5: Test Microorganisms

1. For inoculation of the BB, isolates of *E. coli* 0157:H7 (AB317), Salmonella *typhimurium* (G01074), *Listeria monocytogenes* (DUP1907-11038) and *Yersinia entercolitica* (Michigan State University isolate) were used.

2. For inoculation of the turkey subprimals, isolates of *E. coli* 0157:H7 (AB317), Salmonella *typhimurium* (G01074), *Listeria monocytogenes* (DUP1907-11038) were used.

3. *E. coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* were transferred from stock cultures to 10 ml sterile tryptic soy broth (Difco, Detroit, MI).

4. *Yersinia entercolitica* was transferred into brain-heart infusion broth (Difco, Detroit, MI).

5. All pathogens were incubated at 35°C for 20 to 22 hours.

APPENDIX 6: Inoculation of Test Microorganisms

1. Each subprimal (pork) or quad of subprimals (turkey) were placed in a sterile tin-foil tray to prevent cross contamination and to minimize handling of inoculated product.

2. On each subprimal, 12 5cm^2 areas were inoculated with either *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* or *Yersinia entercolitica.*

3. Immediately after inoculation, each 5cm^2 area was marked with green food coloring to assist in the identification of the inoculated area.

4. During the 12-14 hour attachment time, the tin-foil trays were wrapped in tin-foil then placed in a 2°C cooler .

APPENDIX 7: Sampling Method

1. Using a sterilized scalpel, 5cm x 5cm x 2mm subsamples were excised for bacterial enumeration.

2. A total of 14 subsamples were excised from the subprimals (2 excised prior to inoculation, 4 excised after inoculation, 4 excised after ice treatment and 4 excised after 7 days of 2°C storage).

APPENDIX 8: Bacterial Enumeration

1. Subsamples were placed in a 400 ml Stomacher® bag (Seward Medical, London, UK) and weighed.

2. The subsamples were serially diluted using 1% buffered peptone water (Difco, Detroit, MI).

3. The subsamples were stomached for 90 seconds in a 400 ml masticator (IUL Instruments, Cincinnati, OH).

4. From the bag, 0.1ml was extracted then serially diluted to 10^{-3}, 10^{-5} and 10^{-7}.

5. The dilutions were plated in duplicate for determination of most probable number.

6. *E. coli* 0157:H7 was serially diluted and plated on MacConkey Sorbitol Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN).

7. *Salmonella typhimurium* was serially diluted and plated on Bismuth Sulfite Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN).

8. *Yersinia entercolitica* was plated on Yersinia Selective Agar (Difco Laboratories, Detroit, MI) and 3M™ coliform and aerobic plates (3M, St. Paul, MN).

9. *Listeria monocytogenes* was plated on Oxford medium with antimicrobic supplement added (Difco Laboratories, Detroit, MI) and 3M™ aerobic plates (3M, St. Paul, MN).

APPENDIX 9: Meat Storage Containers

1. Eight 96-quart ice chests were used to store meat and ice treatments in one of two temperatures (2° or 26°C).

2. A 120 cm x 45 cm x 4mm sheet of expanded steel was placed three inches from the bottom of the cooler to allow draining of the melted ice.

3. Each ice chest was partitioned into five sections, using 4 mm-thick plexi-glass.

4. To prevent cross contamination between samples, the plexi-glass was stabilized by commercial sealing caulk.

APPENDIX 10: Vacuum Packaging

1. After subsamples were excised after ice treatment, they were vacuum packaged.

2. They were packaged using 4 mil thick 51 cm x 76.5 cm heat seal polyethylene bags (Diagger, Lincolinshire, IL).

3. The bags were sealed using an impulse heat sealer (Diagger, Lincolinshire, IL).

4. A sterile 20 gauge needle was attached to a 1.5 cm-diameter rubber hose that was attached to a Welch vacuum pump (Welch Vaccum, Skokie, IL).

5. The vacuum pump was used to extract the air from the bag.

6. After air extraction, the bag was sealed again to prevent airflow from the needle insertion point.

APPENDIX 11: pH Determination

1. The pH of the ice was measured with a Fischer Scientific pH meter (Pittsburgh, PA).

2. Ice (250 ml) was placed in a 300 ml beaker.

3. The pH was measured by inserting the pH probe into the middle of the ice filled beaker.

4. In order to observe pH decline over time, 22 readings were taken over a 4-hour.

APPENDIX 12: TBA Analysis

Tarladgis, G. G., Watts, B. M., Younathan, M. T., and Dugan, J. Jr., J. Am. Oil Chemists, 37:44-48, (1960).
Zipser, M. W., Watts, B.M., Food Technology, 16 (7): 102, (1962).

1. TBA Reagent

Prepare the amount of TBA Reagent needed for your samples according to the table below.

Thiobarbituric Acid	Distilled Water	Total VolumeH_2O with Acid
1.4416 g	50 ml	500 ml
0.7208 g	25 ml	250 ml
0.5766 g	20 ml	200 ml
0.2883 g	10 ml	100 ml
0.1442 g	5 ml	50 ml

Dissolve the Thiobarbituric Acid (Sigma Chemical Co., St. Louis, MO) in the distilled. Place the flask in sonic cleaner (5 minutes) and shake occasionally until TBA is dissolved. Allow reagent to come to room temperature then bring to volume. Store in cooler, may be kept for two days. Reclaim unused reagent as described above.

2. HCl Solution

Make volume as needed; 1:2, HCl:H_2O (v/v).

3. Antifoam (Thomas®, Swedeboro, NJ)

The use of antifoam may or may not be needed, depending on the product. Fish and eggs require antifoam. In this study, antifoam was used.

Procedure:

1. Assemble connecting tube (spouts) and graduated cylinders
2. Turn on condenser water
3. Into 500 ml extraction flasks, add 2 4mm glass beads (Fisher Scientific, Pittsburgh, PA) 2.5 ml HCl solution and antifoam.
4. Weigh 10 g sample directly into homogenizer flasks
5. Add 50 ml distilled water plus antioxidant solution
6. Homogenize 1 minute
7. Quantitatively transfer homogenate into 500 ml extracting flask and rinse with distilled water to bring total volume to 100 ml
8. Connect extraction flasks to distilling tubes and tighten heating mantels in place
9. Turn power on and heat flasks rapidly.
10. Distill and collect 50 ml of the distillate.
11. Transfer distillate to culture tubes, cap and hold in refrigeration for TBA reaction.

TBA Reaction / Spechtrophotometric Determination

12. Invert each test tube containing the 50 ml distillate and pipet 5 ml into each of 2 blanks. Pipet 5 ml of distilled water into another blank.
13. Add 5ml of TBA Reagent into each tube containing 5 ml of sample. Thoroughly mix each tube on the vortex Genie shaker.
14. Immerse tube support containing tubes into boiling water bath for 35 minutes.
15. Turn spectrophotometer on to warm up (10 minutes).

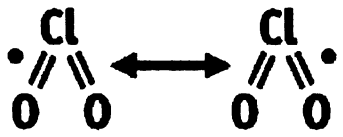
16. When the tubes are done heating in the water bath, cool them in cold water for 10 minutes.

17. Read with spectrophotometer within 1 hour.

18. Fill spec cell and read % transmittance of the sample against the Blank. Using 1 ml volumetric pipet, transfer 1 ml of solution to 5 ml volumetric flask. Bring to volume with distilled water. Mix and Read sample. Calculation: 7.8 x 5 = 39; 39 is the constant to use in the calculations to get the TBA number.

19. Convert % T to optical density and multiply by the constant 7.8 to convert to mg malondehyde/1000 g of sample, i. e. TBA number

APPENDIX 13: Structure and Reaction of Chlorine Dioxide



$2\ ClO_2 + H_2O \rightarrow HClO_2 + HClO_3\ (2H^+ + ClO_2^- + ClO_3^-)$

Recommendations for Future Research

The results of this microbial project have indicated that ClO_2 ice can be accurately generated at various ppm. After the initial fixed costs of the ice machine and generator, the production of ClO_2 ice is relatively inexpensive. In this research, a model ClO_2 ice application protocol was developed to simulate ice-packed meat transportation systems or meat retail case displays. The ClO_2 ice could be used as a new hurdle in a pathogen intervention strategy already in place in meat processing, packaging and transportation systems. Some developing and third-world countries lack proper refrigeration and pathogen intervention strategies to guard against foodborne illness. Using ClO_2 ice as antimicrobial and as a temperature control mechanism in unrefrigerated meat transportation and temporary storage systems could prove to be beneficial.

Chlorine dioxide ice was proven to be effective in the reduction of *E. coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* on fresh pork and turkey subprimals by up to 0.5 $\log_{10}$ CFU/cm^2. Although not effective in the reduction of *Yersinia entercolititca*, future research could investigate the mechanisms that allows *Y. entercolitica* to be resistant to ClO_2 treatment. Results of this research indicated that increasing the level of ClO_2 and more importantly activated ClO_2 up to 100 to 200 ppm could prove to be effective in the reduction if not elimination of the pathogen load. Currently, USDA has approved 1200 ppm of ClO_2 in meat washing pathogen reduction systems. When exposure times up to 24 hours are used, excessive levels (> 200 ppm) of ClO_2 ice should be used with extreme caution to prevent discoloration or off-

odors due to the leaching of ClO_2 on/into the meat product. In addition, when trying to maximize the effectiveness of ClO_2 ice, increasing the ice melt by using warm (body temperature immediately after slaughter) meat will increase the ClO_2 released from the ice, which will offer more of an antimicrobial effect. Also, increasing the exposure time in an environmental temperature of 26°C could allow more ClO_2 to negatively affect the pathogen viability on the meat product. This research showed that the melted largely activated ClO_2 ice contained up to 3.6 $\log_{10}$ CFU/ml fewer microorganisms than the melted control ice. Future research could focus on implementing this new pathogen intervention strategy in food production systems from immediately after slaughter to time of purchase in the retail display case.

In a final thought, ClO_2 ice is not the cure-all to food safety in the meat industry. It is merely one step of a pathogen intervention strategy that minimizes the risk of a foodborne disease outbreak.