Post-Harvest Spray Treatments to Reduce *Salmonella* Contamination on Cantaloupe Surfaces

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ABSTRACT

Since the surfaces of cantaloupes are highly rough or irregular, *Salmonella* enteric and other bacteria can easily attach to these surfaces and are difficult to remove. Cetylpyridinium chloride (CPC) is the active ingredient of some antiseptic oral mouth rinses and has a broad antimicrobial spectrum with a rapid bactericidal effect on Gram-positive pathogens. Delmopinol hydrochloride (delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. The application of delmopinol or CPC to cantaloupe surfaces may be an alternative post harvest technique to reduce the frequency and level of *Salmonella* contamination.

Cantaloupe (Athena and Hale's Best Jumbo (HBJ) cultivars) rind plugs were inoculated with a broth culture of *Salmonella* Michigan. After 15 min, plugs were sprayed with 10 ml of a 1% delmopinol solution, or a CPC solution (0.5 or 1.0%) or distilled water (Control), and held at 37 ^{o}C for 1 hr or 24 hr. For additional samples, the chemical treatments were applied 15 min before pathogen inoculation. Melon plugs were submerged in Butterfield's Phosphate Buffer, shaken, sonicated and solutions were enumerated on Tryptic Soy Agar. The texture quality and color of additional melon samples were evaluated after delmopinol or CPC spray treatments and storage at 4 ^{o}C.

A 1.0% application of CPC reduced *Salmonella* levels up to 2.34 log CFU/ml (Athena) and 4.95 log CFU/ml (HBJ) in comparison to the control ($p<0.01$). A 1.0%

delmopinol treatment reduced *Salmonella* levels as much as 3.1 log CFU/ml in comparison to the control ($p<0.01$) on both cultivars. In general, the log recovery of *Salmonella* on cantaloupes treated with delmopinol or CPC solutions, after 1 hr storage, was significantly lower ($p<0.05$) than the recovery from control cantaloupes, but *Salmonella* recovery was not significantly different after 24 hr. No significant differences were observed in the texture and color of melons treated with delmopinol or CPC after 14 days. A surface spray application of delmopinol hydrochloride or cetylpyridinium chloride could be an alternative antimicrobial post-harvest treatment that could make cantaloupes surfaces more susceptible to sanitizers or enhance physical removal of bacteria.

Dedication

I dedicate this work to my wife, **Adelina Isabel Miranda**, her love, support and sacrifice has helped me achieve my goals.

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CHAPTER 1

INTRODUCTION

Salmonella infection was the most common food borne infection reported by FoodNet sites in 2010 resulting in the largest number of hospitalizations and deaths. The incidence of *Salmonella* infection in 2010 was not significantly different than during 1996–1998, but was significantly higher than during 2006–2008, resulting in an estimated $365 million in annual direct medical costs (USDA-ERS, 2010; Boriss 2012).

Salmonella enterica bacteria have been isolated from raw and fresh-cut cantaloupe in the U.S. and other countries. Furthermore, the consumption of cantaloupe and other fruits contaminated with this pathogen has caused individual cases and outbreaks of foodborne illness. A survey conducted by the United States Food and Drug Administration (USFDA), revealed a higher incidence of *Salmonella* contamination on both imported and domestic melons than other fruits and vegetables (CDC, 2006; CDC, 2011a; CDC, 2012). The last outbreak of salmonellosis associated with cantaloupes occurred in 2012 and resulted in a total of 261 persons infected with the outbreak strains of *Salmonella* Typhimurium and *Salmonella* Newport. Illnesses were reported from 24 states and the source of the outbreak was traced to Chamberlain Farms Produce, Inc. of Owensville, Indiana (CDC 2012b).

Salmonella is directly associated with the use of products of animal origin including, organic fertilizers and contaminated irrigation water. Reducing the level of bacterial contamination on the exterior of raw cantaloupe is difficult due to its highly irregular surface texture. Therefore, the use of appropriate post-harvest washing and

sanitizing procedures are key areas for control of *Salmonella* on fruit (Parnell et al., 2005). A (chlorinated) water rinse or dip may not remove all *Salmonella* and other microorganisms that can attach to, grow in crevices of, and build biofilms on the fruit surface. Produce growers and packers need additional options for reducing surface contamination by pathogenic microorganisms.

One of the most important scientific achievements of the past century has been humanity´s ability to control the detrimental activities of bacteria by the judicious use of antibacterial agents (Russell and Chopra, 1996). These agents can kill or inhibit growth of bacteria, or they may be able to prevent bacterial attachment to foods or food contact surfaces. Some of these chemicals, which have antimicrobial properties, could have applications for fresh produce such as cantaloupes. A direct spray application of these chemical compounds could dissolve the biofilm structure and expose hidden planktonic bacteria colonies, and reduce bacterial attachment. If the biofilm structures are weakened or fully destroyed, *Salmonella* and other microorganisms will be more susceptible to sanitizers.

Two chemical compounds used primarily in oral hygiene products- delmopinol hydrochloride (delmopinol) and cetylpyridinium chloride (CPC) were evaluated for their ability to reduce populations of *Salmonella* bacteria on two cantaloupe cultivars (Athena and Hale's Best Jumbo (HBJ)). These two cultivars differ greatly in their surface texture or striation and netting. The use of delmopinol on food products has not been permitted; however CPC is approved for use in poultry processing as an anti-microbial surface treatment (Burgemeister et al., 2001). While CPC has been studied as an

antimicrobial for some meat, poultry and seafood products, there is little information regarding the use of this compound to treat fruits and vegetables.

A spray application of delmopinol or CPC, rather than a dip, will minimize cross-contamination between fruit. Since each chemical has surfactant properties, they may inhibit the attachment of (loosely attached) *Salmonella* cells. A surface spray application of delmopinol or CPC may be an alternative antimicrobial post-harvest treatment that will interfere with bacterial attachment and biofilm development; and could make the cantaloupe surface more susceptible to sanitizers or enhance physical removal of bacteria.

The primary objective of the study is to evaluate the efficiency of a post-harvest treatment of delmopinol hydrochloride or cetylpyridinium chloride spray solutions to reduce *Salmonella* populations and the attachment of this pathogen on two different cultivars of cantaloupe ‘Athena’ and ‘Hale's Best Jumbo’. A second objective is to evaluate the effect of delmopinol and CPC on the color and texture of the treated cantaloupes.

CHAPTER 2

LITERATURE REVIEW

Cantaloupes: a fruit commodity

The United States is one of the world's leading consumers of cantaloupes. Cantaloupe (*Cucumis melo* group *reticulatus*) is among the fresh fruits that have been consumed in larger quantities in recent years. The annual US *per capita* consumption of cantaloupe increased from 5.8 lb in 1980 to 8.5 lb in 2010. And, the total consumption of cantaloupes was estimated at 26.1 pounds per person in 2010 in the U.S. (USDA,2003; Boriss, 2012). Cantaloupes consumption has remained high for a variety of reasons, including health consciousness of consumers, improved year-round availability, creative marketing and improved cultivars.

In 2010, the value of U.S. cantaloupe production continued to drop, falling to $314.4 million (Boriss 2012). In 2010, U.S. cantaloupe acreage remained at 74,730 acres but cantaloupes production declined to 18.8 million cwt. and domestically, cantaloupes acreage for the spring 2011 harvest was at 68,000 acres down 3 percent from the previous year.

The value of fresh cantaloupes imported into the United States, reached $478.2 million in 2010, while the volume decreased slightly to nearly 1.1 million metric tons. Today, the United States is a net importer (imports minus exports) of cantaloupes and the largest importer of cantaloupes and other cantaloupes worldwide. The majority of cantaloupes are imported from December through May; these imports generally originate in Latin American countries. Mexico is the largest supplier of cantaloupes in

2010 and Guatemala is the second supplier. Costa Rica and Honduras are also significant suppliers of cantaloupes (Boriss, 2012).

Commercial production of cantaloupe *(Cucumis melo* var. *reticulatus)*

Melons are members of the family curcurbitaceae. They are considered vegetables for the way they are consumed but botanical they are fruits. The curcurbitaceae, also known as the vine crops (Swiader and Ware, 2002). They largely originated in tropical Africa (Rubatzky and Yamaguchi, 1997). Since they are a warm-season crop and are very susceptible to cold injury, most of the commercial production in the USA is concentrated in southern and western states.

Muskmelons, known to consumers as cantaloupes, are a member of the reticulates group and were introduced to America in the early 1600s. Cantaloupes are a rough hard fruit having a characteristic meshwork netting on the rind, contain two blossom types: perfect (having both male and female parts) and male (staminate) flowers (Webster and Craig 1976, Swiader and Ware 2002). Usually the main stem produces 3 to 4 major branches of equal or longer length than the main stem. Additional laterals (branches) later arise from both the main stem and branches and can produce additional flushes of fruit if the vines remain healthy (Glimn-Lacy and Kaufman 2006).

Planting- Cantaloupes are planted by direct seeding and transplanting. The best range for soil temperatures for direct seeding is 77 to 90^{o}F, with an optimum of 90^{o}F. It is best to plant when the soil temperature is at least 78°F for germination. Seeds are placed about ½ to 1 inch deep and medium-textured soils will generally produce higher yields and better quality. In all cases, the soil must exhibit good internal and surface

drainage. The pH should be above 5.8 and preferably near 6.2. Rows should be raised 6 to 8 inches to facilitate soil drainage (Swiader and Ware 2002). Transplants should be grown in individual containers and planted directly into soil. Optimum greenhouse temperatures are 65 to 75^{o}F. After about three weeks, plants should not be past the three-leaf stage.

Fertilization - Irrigation prior to and after planting should be applied to ensure seed germination, emergence and stand establishment. Overhead irrigation is most commonly used; however, drip irrigation, with plastic mulch, is becoming more common and is highly desirable. Drip irrigation provides the plants with a more uniform application of water, placed it near the root zone and using less water. Drip irrigation also minimizes the amount of foliage and fruit disease compared with overhead irrigation. Furthermore, drip does not interfere with honeybees and subsequent pollination and fertilization (Swiader and Ware 2002). Row widths of 5 to 6 ft are desirable and in-row spacing should be 18 to 24 inches.

Plastic mulch should be used with trickle (drip) irrigation since it is very difficult to maintain proper soil moisture under the mulch using over-head irrigation. Fertilizer should also be applied through the drip tube. Again, the use of plastic mulch without irrigation is not recommended. Nitrogen and potassium fertilizer should be used with caution since there could be some danger of injury from high salt levels when placed in closed contact with seeds and roots (Swiader and Ware 2002). Cantaloupes require bees for pollination so toxic insecticides should be avoided.

Diseases- Fusarium wilt is a serious soil-borne disease of cantaloupes. Several blights, powdery and downy mildew, diseases as *Alternaria* and gummy stem blight and

insect like aphids also are serious. They usually attack the plants at fruit-sizing time and can be controlled with fungicide sprays. The term plasticulture refers to the practice of using plastic films as mulch in agricultural applications. Spray applications every 3, 5, or 7 days are used depending on insect pests and populations. Several insecticides will control these insects.

Harvest - Approximately 30 to 35 days are required from fruit pollination to harvest. Transplanted cantaloupes and those grown on plastic mulch will likely start 7 to 14 days earlier. Cantaloupes separate from the stem at maturity. When the stem separates completely (full slip) the fruit has achieved its maximum sugar content and if not consumed or cooled soon thereafter, the fruit will deteriorate and become unmarketable.

Cooling and packing - Cooling is an important part of handling cantaloupes after harvesting. In general, cantaloupes are cooled by forced air or hydro cooling and packed in cartons for long-distance shipping. At temperatures of 2.2°-5°C (36°-41°F), full slip cantaloupes can be held for about 14 days without significant loss in quality. Rapid pre-cooling soon after harvest is essential for optimal postharvest keeping quality. The pre-cooling endpoint is typically 10°C (50°F) but 4°C (39.2°F) is more desirable. Forced-air cooling is the most common practice but hydrocooling is also utilized (Crisosto 2010). Chilling injury typically occurs after storage temperatures < 2°C (35.6°F) for several days. Sensitivity to chilling injury decreases as melon maturity and ripeness increases. Symptoms of chilling injury include pitting or sunken areas, failure to ripen, off-flavors and increased surface decay.

Cantaloupe cultivars

There is a misunderstanding of the words “Cultivar” and “Variety” for most almost everyone outside of the science world. Varieties often occur in nature.
In fact cultivar means "cultivated variety." Therefore, a cultivar was selected and maintained by humans. Some cultivars originate as sports or mutations on plants. Other cultivars could be hybrids of two inbred lines. Propagation by seed usually produces something different than the parent plant (Haynes 2008, Merriam-Webster)

Cucumis melo var. reticulatus: **Athena cultivar -** Athena is a main season cantaloupes eastern type in a class of its own. It is an F-1 hybrid. It is a vigorous plant with coarse netting and thick salmon colored flesh, with a germination time of 10-20 days and a maturity of 75 days (total of 90 days). Athena has consistently delivered at the top for fresh color, flavor, aroma, firmness, and shelf life after harvest. Fruit average 5-6 lbs, High resistance, powdery mildew (Smart Garden 2013, Syngenta).

Cucumis melo var. reticulatus: **Hale's Best Jumbo cultivar -** Hale's Best Jumbo as well as `Athena´ is part of the cucumis genus. It is an heirloom with more than 100 years of been cultivated. Hale's Best Jumbo grows as an annual and this cantaloupe has a firm but thin rind (skin) that is slightly ridged. The fruits are large, ribbed, and heavily netted, with orange flesh. These cantaloupes can grow to 5-6 pounds and have a small seed cavity.

Hales Best dating back to 1920, Hales Best is a great early non-hybrid. The 'Hale's Best Jumbo' Cantaloupe introduced to the public over 80 years ago, it was originally discovered in a Japanese market gardener’s garden near Brawley, CA in 1923 (CSU 2000, Burpee 2013).

Foodborne illness linked to cantaloupe

Cantaloupes are considered to be a relatively high risk for foodborne illness. According to the Food and Drug Administration, cantaloupes were linked to 15 of 84 outbreaks involving fresh produce that FDA investigated between 1996 and 2008. Cantaloupe was implicated in 12 of those 15 outbreaks. (USFDA 2002, CDC 2012). Both domestic and imported samples of fresh produce have yielded evidence of microbiological contamination in testing by regulatory agencies. In 1999, the FDA surveyed eight different imported produce items and found that cantaloupe was the third most commonly contaminated item, with 7.3% of the sample cantaloupes yielding *Salmonella* or *Shigella* spp. (USFDA 2003). In a similar survey of domestic produce in 2000, cantaloupe was the second most commonly contaminated item since 3.0% of cantaloupe fruit tested yielded the microbial pathogens *Salmonella* or *Shigella* (FDA 2003).

Salmonella is estimated to cause more than 1.2 million illnesses each year in the United States, with more than 23,000 hospitalizations and 450 deaths (Scallan et al., 2011b). In the last few years, several *Salmonella* illness outbreaks associated with cantaloupes have been reported in the United States from imported and domestic production (CDC 2006). Recently, some large outbreaks of *Salmonella* Poona infections were associated with consuming cantaloupes, highlighting the need for enhancing cantaloupe safety and resulting in importation restrictions for implicated producers (USFDA 2010, USFDA 2003b).

A recent *Salmonella* outbreak from imported cantaloupe was traced back to a single harvested farm in Guatemala in 2010. In this outbreak, a strain of *Salmonella*

Panama infected 20 people in 10 states (CDC 2011b). And, in 2012, a total of 261 persons from 24 states were infected with the outbreak strains of *Salmonella* Typhimurium (228 persons) and *Salmonella* Newport (33 persons), and 94 ill persons were hospitalized and three deaths were reported. The source of the illnesses was traced to Chamberlain Farms Produce, Inc. of Owensville, Indiana (CDC 2012).

These outbreaks are evidence of the imperative need for a new alternative and additional technique to assure safety on whole and fresh cantaloupes. Furthermore, these illness outbreaks also confirm the importance of using a sanitizing agent in the wash water and areas where the cantaloupes are stored and displayed for sale. Eliminating pathogenic microorganisms, preventing their adherence to produce surfaces and preventing cross-contamination are important efforts for reducing foodborne illness caused by cantaloupe and other fruit.

Salmonella **characteristics**

The genus *Salmonella* was named in 1900 after a USDA bacteriologist, Dr. Salmon, who first described a member of the group, *Salmonella* choleraesuis, which he thought caused hog cholera. *Salmonella* is a gram-negative, rod-shaped bacillus that can cause diarrheal illness in humans. They are microscopic living creatures that pass from the feces of people or animals to other people or other animals (Doyle et al., 1997). The genus *Salmonella* is divided into two species, *enterica* and *bongori*. The species *Salmonella enterica* is further subdivided into six subspecies that are designated by taxonomic names and sometimes abbreviated by Roman numerals. The subspecies are further divided into more than 2500 serovars based on flagellar,

carbohydrate and lipopolysaccharide (LPS) structure (Coburn et al., 2006, Doyle et al., 1997). *Salmonella bongori* was originally designated *S. enterica* subspecies V; it has since been determined to be a separate species of *Salmonella*. However, for simplicity and convenience, these strains are still sometimes referred to as "subspecies V" (Doyle et al., 1997, Ray and Bhunia 2008).

Salmonella bacteria are estimated to be the leading cause of bacterial foodborne illness (Scallan, et al., 2011a). To reduce salmonellosis, a comprehensive farm-to-table approach to food safety is necessary. Two serotypes, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common in the United States, and approximately a million infections are caused by *Salmonella* serovars each year and they account for half of all human infections and 1.3 billion infections worldwide (Scallan et al., 2011a, 2011b). *Salmonella* can cause serious and sometimes fatal infections in young children, delicate or elderly people, and others with weakened immune systems. Healthy persons infected with *Salmonella* often experience fever, diarrhea (which may be bloody), nausea, vomiting and abdominal pain. In rare circumstances, infection with *Salmonella* can result in the organism getting into the bloodstream and producing more severe illnesses such as arterial infections (i.e., infected aneurysms), endocarditis (an infection of the inner lining of your heart) and arthritis (Doyle et al., 1997). Strains that cause no symptoms in animals can make people sick, and vice versa. If present in food, it does not usually affect the taste, smell, or appearance of the food. The bacteria live in the intestinal tracts of infected animals and humans.

Contamination of cantaloupe by *Salmonella*

When a fruit such as cantaloupe is subject to handling by different people, this fruit can become contaminated with whatever contaminant might be on their hands, and thereby accumulate human waste, chemical contaminants, and pathogenic microorganisms on its surface. These contaminants can cross-contaminate other produce or food contact surfaces. The physical characteristic of cantaloupes and the manner in which they are grown make it exceedingly difficult to prevent contamination and also to remove attached bacteria one in place (Sapers et al., 2009)

The outer surface of a cantaloupe has a variety of textures to which a bacterium may bind. The fruit epidermal cell surface is ruptured by a meshwork of raised tissues. The cell has a hydrophobic suberized wall (waxy waterproof substance) to reduce water loss and protect against pathogen ingress (Ukuku, 2006). The topography of cantaloupes plays a major role in the removal of microorganism. Washing cantaloupes with water does not have any significant effect at removing bacterial pathogens, and only those that are loosely attached may be removed if not located in areas that are out of reach of the water (Parnell et al., 2005).

Ukuku and Fett (2002) previously concluded that bacterial cell surface charge and hydrophobicity appear to be highly correlated with the strength of attachment of bacteria to the cantaloupes surface. Mesocarp tissues (the middle layer of pericarp, as the fleshy part of certain fruits) of fruits are particularly subject to contamination when rind surface integrity is compromised by disease, bruising, cutting, or peeling (Richards and Beuchat, 2005).

Another concern with the survival of *Salmonella* attached to cantaloupes surfaces is the ability of fresh cut cantaloupes to support the growth of *Salmonella*. Cutting though the rind of a cantaloupe harboring *Salmonella*, may result in contamination of fresh cut pieces with the pathogen (Ukuku and Samper 2001). The flesh of the cantaloupes is capable of supporting the growth of pathogens due to the mild acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99) (Bhagwa 2006). Once introduced on the surface of the cantaloupes, cells of *Salmonella* are almost impossible to remove completely, regardless of the sanitizer used or exposure time.

Control of *Salmonella* on cantaloupe

Antimicrobial chemicals and antimicrobial processes are used throughout the food industry to reduce and prevent microbial contamination. Some antimicrobial chemicals (biocides) are intended to kill or inactivate microorganisms, while others may be intended to perform as surfactants which can interfere with the mechanism of biofilm adherence to a food or food contact surface. Reducing the strength of attachment of bacteria on a surface can make these organisms more susceptible to sanitizers or physical removal. Some sanitizers evaluated such as hydrogen peroxide, sodium hypochlorite (chlorine), and ethanol are not effective in totally in eliminating pathogens, in part because organic materials in cantaloupes tissues neutralize the bactericidal activity (Beuchat 1997, Park and Beuchat 1999).

Recent control studies in laboratories with inoculated apples and cantaloupes have shown that 5% hydrogen peroxide solution can achieve log units reduction of 3 or higher when applied by full immersion of the commodity in the solution with vigorous

agitation and at a temperature of 50–60^{o}C for apples and 70–80^{o}C to cantaloupes (Sapers et al., 2001), However, it could be very difficult to apply in an industrial process.

Chlorine based disinfectants, applied when produce are packed, are widely used to control microorganisms. When applied properly, chlorine products are effective. However, hazardous disinfection breakdown products can be formed, and chlorine disinfectants have high oxidant activity that can affect produce quality and pose a risk to food handlers, which has lead to the search for new disinfection alternatives (Chaidez et al., 2007).

Fan et al., (2009) found that application of chlorine and other disinfectants such as acidified calcium sulfate (ACS), acidified sodium chlorite (ASC), and peroxyacetic acid (PAA) had a limited effect on the population of *Salmonella*, achieving no more than a 1.5-log reduction of the pathogen inoculated on the surface of the whole cantaloupes. Some researchers indicate that cantaloupes are especially difficult to sanitize (Alvarado-Casillas et al., 2007; Ukuku and Sapers 2001).

Cationic surfactants

According to McDonnell & Russell (1999), surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds. Of these, the cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants (Frier 1971).

QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization, and are membrane active agents (Hugo and Frier 1969). Salton (1968) proposed the following sequence of events with microorganisms exposed to cationic agents: (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) leakage of intracellular low-molecular-weight material; (iv) degradation of proteins and nucleic acids; and (v) cell wall lysis caused by autolytic enzymes.

Cetylpyridinium chloride (CPC) - CPC is a cationic, quaternary ammonium compound, and is highly effective for microbial destruction (Breen et al., 1997, Kim et al., 1996). In Gram-negative bacteria, such as *Salmonella* spp., the outer membrane contributes an extra barrier forms to slow or stop the entry of some antimicrobial agents, as a result, this makes the Gram-negative microorganism generally more difficult to destroy than Gram-positive microorganisms (Talaro and Talaro 1993). CPC can penetrate the bacterial cell wall, and react with the cytoplasmic membrane inducing cell wall lysis caused by autolytic enzymes (McDonnell & Russell 1999). CPC is a cell membrane active agent and is known to lower cellular surface tension, disrupt the bacterial cell membrane, and cause loss of selective permeability of the bacterial cell membrane. It has a broad antimicrobial spectrum with a rapid bactericidal effect on

gram-positive pathogens and a fungicidal effect on yeasts, in particular. There are gaps in its effectiveness against gram-negative pathogens and mycobacteria (Pitten 2001).

The antimicrobial effects of CPC are dependent on CPC binding to bacterial cells (Caputo *et al.,* 1975) and bactericidal activity in the presence of serum proteins and at different pH and temperatures (Quisno and Foter 1946). The toxic effects of CPC on bacteria are caused by the CPC adsorbing and penetrating into the cell wall and the cell membrane causing cell components to leak, which eventually leads to cell death (Cutter et al., 2000; Scheie 1989). CPC has been showed to interact strongly with negative charged surfaces and it antibacterial activity is related to its hydrophobicity (Kourai et al., 1986, Maeda et al., 1996). Electron microcopy studies showed that CPC damages the bacteria membrane and produces leakage of cellular material (Farber et al., 1989).

CPC has the molecular formula $C_{21}H_{38}NCl$ and at its pure form is in a solid state at room temperature. It has a melting point of 77°C when anhydrous or 80–83°C in its monohydrate form and is combustible. It is insoluble in acetone, acetic acid, or ethanol. It has a pyridine-like odor and concentrated solutions are destructive to mucous membranes. In its purest form, CPC is a fine white powder without taste or odor, which can be lethal if inhaled or ingested. CPC is an amphiphilic (possessing both hydrophilic (water-loving) and lipophilic (fat-loving) properties) quaternary compound with a long history of safe and effective use when incorporated into oral hygiene products (Haps *et al.,* 2008).

This molecule's positive charge facilitates binding to negatively charged bacterial surfaces (Lim and Mustapha 2007) and, consequently, its antimicrobial activity (Pitten and Kramer 2001). Investigations demonstrate the significant antimicrobial effects of

CPC on planktonic bacteria (Haps *et al.,* 2008) that include reducing microbial adhesion to surfaces (Xiong *et al.,* 1998).

QACs such as CPC are considered low level biocides. They are able to promote their own entry by displacing divalent metal cations in the outer membrane. The degree of damage to bacterial membrane is time and concentration dependent (Kim and Slavik 1996).

CPC has been approved to treat the surface of raw poultry carcasses prior to immersion in a water bath chiller in the United States (USFDA 2003a, USFDA 2004). Kim and Slavik (1996) demonstrated a 1.7 log reduction of *Salmonella* Typhimurium after immersion of poultry in 0.1% CPC and other study at 30 second spray with 0.1% CPC in chicken skin reduce up to 2.5 log (Wang et al., 1997). Other investigations demonstrated 0.4% CPC for 3 min exhibited a 4.9 log reduction and very effective in 0.8% in 10 min of *Salmonella* (Breen et al., 1997). Cutter et al., (2000) confirmed that CPC not only reduced *Salmonella* Typhimurium on poultry but also prevented cross-contamination.

CPC is the active ingredient of some antiseptic oral mouth rinses commonly used around the world due to its broad antimicrobial spectrum and relative safety for ingestion. The ability of cetylpyridinium chloride to inhibit plaque and thereby reduce gingivitis has been established. A recent meta-analysis from a systematic review supported the plaque and gingivitis inhibiting effect of CPC containing mouth rinses (Haps et al., 2008). In comparison to chlorhexidine, CPC has a lower residual effect, and as a result, a lesser effect against plaque and gingivitis (Pitten *and* Kramer 2001*)*.

Recent research indicates that CPC diffuses into oral biofilms irrespective of the thickness of extracellular components and appears to bind irreversibly (Sandt *et al*., 2007). Sreenivasan (2013) suggested that CPC inhibits insoluble glucan synthesis. Other researchers reported that the interaction of CPC with bacteria occurs by the disruption of membrane function, leakage of cytoplasmic material, and ultimately the collapse of the intra-cellular equilibrium (Pitten 2001, Scheie 1989).

According to Russell (1998, 2000), acquired resistance to biocide, involving outer cell changes, may occur as result of mutation or adaptation, plasmid-mediated changes in the outer membrane of Gram-negative bacteria that can reduce sensitivity of QAC compounds (Roussow and Rowbury 1989) and the antibacterial agent may cause extensive cytoplasmic membrane damage, this will not necessary result in cell lysis. (Russell 1998).

For this research, cetylpyridinium chloride (CPC) solutions were formulated as the commercially available Cecure® product that consists of cetylpyridinium chloride, as the active ingredient, and food-grade propylene glycol in a 1:1.5 ratio. Cecure® is a registered trademark of Safe Foods Corporation (North Little Rock, AR). This mixture is also approved for food uses in other countries, including Canada, Mexico, Panama, Costa Rica, Colombia, Russia, South Africa, Saudi Arabia, and Jordan (Safe Foods Corp., 2013). Concentrated solutions of Cecure® can be diluted with potable water to reach a concentration, not to exceed 1.0% CPC (10 mg/ml). These solutions have a neutral pH at ambient temperature. Propylene glycol is used in the formulation since it is considered generally recognized as safe (GRAS) by the U.S. Food and Drug Administration, and it is used as a humectants by the European Union (E1520), solvent,

and preservative in food and for tobacco products. In addition, propylene glycol is an excellent solvent for many organic compounds and is completely water-soluble.

CPC solutions are generally diluted to a concentration of 1.0% or less for food applications. The toxicity of the chemical to humans is considered by regulatory agencies in USA and the European Union for permitted applications to poultry, beef, and other foods in other countries around the world. For humans, direct ingestion of 1-3 grams of CPC is considered a fatal dose (Arena & Drew 1986). The available data indicate that CPC, tested as a working diluted solution in Cecure®, is not mutagenic in bacteria and not clastogenic (giving rise to or inducing disruption or breakages of chromosomes) in cultured mammalian cells. The development of enzymatic resistance to biocides and/ therapeutic antimicrobials as a result of exposure to CPC is highly unlikely. The mixture should be diluted to a $\leq$1% concentration of the active ingredient in potable tap water for use as a decontaminant treatment. Based on the available evidence, there is no concern for genotoxicity (which may lead to cancer) of CPC. Taking into account the estimated margins of safety and the conservative exposure estimates used to assess CPC exposure from consumption of poultry carcasses, there are no safety concerns for humans from the proposed use of Cecure® (EFSA 2012).

Delmopinol hydrochloride (delmopinol) – A recent entry into the oral mouth rinse market is a product formulated with delmopinol hydrochloride (delmopinol) as the active ingredient. Delmopinol has a chemical structure of 4-(2-Hydroxyethyl)-3-(4-propyl-heptyl)-morpholine hydrochloride. It is an antiseptic and oral hygiene compound and a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis.

Delmopinol inhibits bacterial adhesion to tooth and mucosal surfaces, and also inhibits cohesion between the bacterial cells themselves. In a range of studies, delmopinol hydrochloride has proven effective in reducing plaque and gingivitis (Lang et al., 1998, Addy et al., 2007).

Since the mode of action of delmopinol is to prevent bacterial attachment, this chemical has been classified as a medical device in the USA and it has been approved only to be used in oral hygiene products by the U.S. Food and Drug Administration, however it is approved as an antiseptic in Europe by the European Union legislation (Zee et al., 1997, USDA 2005a, 2005b).

Delmopinol works by disrupting the existing plaque matrix by reducing the viscosity of glucans and loosening the cohesive properties of plaque, making it easier to remove mechanically (Klinge et al., 1996; Rundegren and Arnebrant 1992; Rundegren et al., 1992). This chemical can prevent bacteria from synthesizing the sticky glucan polysaccharide compounds that cause the adhesion to tooth and gum surfaces, and to the other bacterial cells nearby, and disrupt existing dental plaque biofilm colonies Steinberg et al., (1992) reported that delmopinol may interfere with bacterial glucosyltranferase mediating glucan synthesis, which may play a role in bacterial colonization and in the formation of plaque matrix. Slow glucans formation of salivary pellicle on clean surfaces, is the priming step required for bacterial attachment to the teeth and gingival. Delmopinol also reduces the adherence of pioneer bacteria to salivary pellicle on tooth and gingival surfaces and reduces adherence of colonizing bacteria to the plaque matrix (Vassilakos et al., 1993, Steinberg et al., 1992). Short-term tests with delmopinol demonstrated little or no change in the salivary bacterial

counts but significant decreases in the surface area covered with bacterial deposits (Sjodin et al., 2011, Hancock and Newell 2000). Theoretically, delmopinol could dissolve or prevent formation of the complex structure of polysaccharide materials, expose hidden bacteria colonies, kill them and cover the surface with an invisible film that can last several hours, repelling or reducing bacterial attachment (Zee et al., 1997, Yeung et al., 1995, Hase et al., 1998).

As a result of this buildup, a microbial film is established. Not only do these microbial films provide protection to the microorganisms, they also provide the microorganisms with a source of food and nutrients, which in return allows the microorganisms within these microbial films to act synergistically as they are permitted to grow (Stier 2005). Delmopinol has been reported to be effective against both rapid and slow plaque (biofilms) formation (USFDA 2005a, USFDA 2005b), and to dissolve formed plaque in the absence of mechanical plaque control (Eley 1999, Brandon 2011, Hancock and Newell 2000). Dental clinical studies have been demonstrated that delmopinol reduced theses microbial films formation approximately 22% on treated, non-living objects (USFDA 2005a, FDA 2005b). Simonsson et al., (1991) and Rugrentet et al., (1992) suggested that delmopinol has a small bactericidal effect but the exact mode of action is not yet known. However Zee (1997) and Burgemeister (2001) research on planktonic and attached cells, showed a marked decrease in vitality following exposure to 0.2% delmopinol hydrochloride. They suggested that delmopinol does not just posses a bactericidal effect, but also an anti-aggregating effect. When there are existing plaque colonies, the cohesive forces between the bacteria are reduced by delmopinol, which makes removal by mechanical means much easier.

Biocides

The term ‘biocide’ is increasingly being used to describe compounds with antiseptic, disinfectant or, sometimes, preservative activity. A compound might be used in only one such capacity or possess two or even all of these properties (Russell 1997). Examples, of biocides that are used in dental care or oral hygiene products include triclosan, thymol and chlorhexidine.

A biocide, an antiseptic applied to living tissues, is an active chemical molecule agent that is capable of controlling the growth or destroying living organisms usually in a selective way. These are commonly used in the fields of medicine, agriculture and forestry (Russell and Chopra 1996; Russell 1998, 2000).

Most biocides are bactericidal because of their effects are on the cytoplasmic membrane of the bacterial cells. In contrast to the action of antibiotics, there are not specific receptor molecules to assist biocide penetration into a bacteria cell (Russell et al., 1998; 2000). Gram negative cells offer a supplementary barrier, the lipopolysaccharide layer, to biocide penetration which gram positive cells do not possess (Denyer 1995; Salton 1968). Biocides that interact strongly with the cell surface can reduce the charge and even, in some case, reverse it.

There is thus a loss of structural organization and integrity of the cytoplasmic membrane in bacteria, together with other damaging effects to the bacterial cell (Denyer 1995). The initial reaction between an antibacterial agent and a bacterial cell involves binding to the cell surface. Changes to outer layers may then occur to allow agents to penetrate the cell to reach their primary site of action and the cytoplasmic membrane or within the cytoplasm. The effect of the primary target site may lead to additional,

secondary, changes elsewhere in the organism. Such secondary alteration may also contribute to the bactericidal activity of the biocide (Russell and Chopra 1996).

Triclosan is an antimicrobial agent, which has been employed for a variety of purposes for more than 20 years. It is used clinically and in oral hygiene products, and is incorporated into many types of cosmetic formulations (Suller 2000).
In recent years, there has been renewed interest in the antibacterial properties of triclosan susceptible organisms that the growth-inhibitory activities of the phenyl ether resulted from blocking lipid synthesis by specifically inhibiting an NADH-dependent enoyl-acyl carrier protein (ACP) reductase, or Fab I (McMurry 1998). As with other biocide agents, triclosan possesses more than one type of action, and it is possible to delineate its growth-inhibitory and lethal effects.

Triclosan has a broad range of activity that encompasses many, but not all, types of Gram-positive and Gram-negative non-sporulating bacteria, and some fungi. Triclosan is bacteriostatic (an agent that stops bacteria from reproducing, while not necessarily harming them otherwise) at low concentrations but at higher levels it can be bactericidal (Suller 2000). Triclosan shows significant activity against some mycobacteria. Its growth-inhibitory properties result from an inhibition of enoyl reductase (it is a key enzyme of the type II fatty acid synthesis (FAS) system), Fab I. Membrane-destabilizing effects are likely to be responsible for bacterial inactivation by higher concentrations.

Thymol has strong antimicrobial attributes when used alone or with other biocides such as carvacrol (the essential oil of *Origanum vulgare* [oregano]). In

addition, naturally-occurring biocide agents such as thymol can reduce bacterial resistance to common drugs such as penicillin (Palaniappan and Holley 2010). Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties (Ündeğer et al., 2009). Clinical trials have demonstrated plaque (biofilm) reductions of 13.8% to 56.3%, and gingivitis reductions of 14% to 35.9% when using thymol essential oils (Sharma et al., 2004, Charles et al., 2004).

Chlorhexidine has been used as a medical and surgical disinfectant since the 1940s. In 1970, it was found to be effective for use within the oral cavity (Löe and Schiott 1970). Chlorhexidine is a chemical antiseptic that is effective on Gram-positive bacteria, but less effective with some Gram-negative bacteria.

Chlorhexidine binds via adsorption to the many surfaces within the oral cavity, as well as the pellicle and saliva. Based on the concentration of chlorhexidine, the bactericidal or bacteriostatic effects will compromise bacteria attaching to the oral surfaces and may be more effective as a plaque preventive agent, rather than a plaque removal agent (Jenkins et al., 1988). The mechanism of action being membrane disruption, not ATPase inactivation as previously thought (Ray et al., 1991).

While chlorhexidine is an extremely effective in a mouth rinse, it has some side effects. Teeth, dental restorations, and the dorsum of the tongue are affected by chlorhexidine gluconate staining and some dental patients experience taste alterations and nausea (Addy and Moran 2008).

CHAPTER 3

Delmopinol Hydrochloride Spray Reduces *Salmonella* on Cantaloupe Surfaces

ABSTRACT:

Cantaloupes become contaminated at centralized packaging facilities and distribution services during post-harvest operations and they are vulnerable to microbial cross contamination from contaminated water tanks, grading/sorting equipment, transport vehicles, and workers. Appropriate post-harvest washing and sanitizing procedures can help control *Salmonella* and other pathogens on cantaloupe or other melons. Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach to these surfaces and become difficult to remove.

Delmopinol hydrochloride (delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. The application of delmopinol to cantaloupe may be an alternative post harvest technique to reduce the frequency and level of *Salmonella* contamination.

Cantaloupe 'Athena' and 'Hale's Best Jumbo' (HBJ) rind plugs (2.5 cm. dia.) were inoculated with a broth culture of *Salmonella* Michigan (approx. 1.0×10^9 CFU/ml). After 15 min, rind plugs were sprayed with 10 ml of a delmopinol hydrochloride spray solution (0% or 1.0%) and held at 35 ^{o}C for 1hr and 24 hr. Cantaloupes rind plugs were diluted with Butterfield's Phosphate Buffer, shaken, and sonicated and solutions were enumerated on 50 ppm nalidixic acid-tryptic soy agar. The texture quality and color of additional cantaloupes were evaluated after 1% delmopinol spray treatments over 14 days storage at 4^{o}C.

A 1.0% (vol/vol) application of delmopinol after 1h at 35^{o}C reduced *Salmonella* concentration by ~3.1 log CFU/ml for both `HBJ´ skin rind plugs and `Athena´ stem scar rind plugs in comparison to the field control ($p<0.05$). No differences were observed in the texture and color (L*, a*, and b* values) of 1% delmopinol treated cantaloupes as compared to control. Storage of cantaloupes treated with 1.0% delmopinol hydrochloride solution for 1 hr had a greater effect on reducing concentration of *Salmonella* compared to 24 hr treatment. A surface spray application of 1% delmopinol hydrochloride on cantaloupes could be an alternative antimicrobial post-harvest treatment that could make surface bacteria more susceptible to sanitizers or physical removal.

INTRODUCTION:

As production and consumption of fresh fruits, including melons, and vegetables has increased in the United States (USFDA 2001, Pollack, 2001), so has the importance of the microbiological safety of these products. Scientists are looking for new methods that increase the safety of produce while keeping the sensory qualities consumers expect in their fruits and vegetables. In the last few years, foodborne illness resulting from contamination of these raw agricultural commodities, particularly melons, has become an increasing concern (USFDA 2003, CDC 2011, 2012). Several food safety programs, such as Good Agricultural Practices (GAPs), have been implemented to reduce outbreaks in areas from particular production fields. It is important to recall that GAPs are guidelines and not “mandatory” regulations in the United States of America or any company/country that wants to export its commodities to the USA (USFDA 1998).

After harvest, melons, including cantaloupe, honeydew, and watermelon, are susceptible to microbial contamination from mechanical damage and equipment, transport, grading/sorting, cleaning, packing and cooling, and during distribution or by the final consumer. *Salmonella* spp. are directly associated with the use of products of animal origin including organic fertilizers and contaminated irrigation water. Direct field packing greatly reduces the cross contamination potential, but it is not recommended in areas of high rainfalls; so centralized packaging facilities are another option. Centralized packaging facilities are vulnerable to rapid cross contamination from shared or poorly

cleaned water tanks, multiple melons harvested from different fields, and the possibility of fruit damage due to the additional manipulation of product.

Salmonella are extraordinary organisms that attach to rough surfaces and build biofilm complexes making them hard to remove using just chlorine and tap water (Ukuku and Fett, 2004, Donlan 2002). Parnell, et al. (2005) determined the effects of sanitizer and hot water treatments on microbial populations on cantaloupe surfaces and determined whether prior decontamination of melons by sanitizer treatment affects vulnerability to recontamination by *Salmonella.* The pathogen was reduced on the rind of cantaloupe by 1.8 log CFU/melon after soaking for 60 s in 200 ppm total chlorine, which was significantly better than the 0.7 log CFU/melon achieved when soaking in water. For both water and chlorine treatments, scrubbing with a vegetable brush was shown to be significantly (0.9 log CFU/cantaloupe) more effective than soaking alone. When honeydew melons were soaked or scrubbed in water, reductions of 2.8 log CFU/melon or 4.6 log CFU/melon (four of five samples), respectively, were observed. However, when water treatments were used, the presence of *Salmonella*-positive samples, at adjacent and remote sites, indicated that bacteria were spread from the inoculated site on the rind to uninoculated sites either through the rinse water (40–70 CFU/ml of *Salmonella*) or scrub brush (400–500 CFU/brush). When 200 ppm total chlorine was used, *Salmonella* could not be detected in the water or on the scrub brush (Parnell, et al., 2005).

Nevertheless, some chemicals approved and used for other food processes, food products, or oral hygiene products and which have antimicrobial and anti-biofilm properties (USFDA 2005a, 2005b) could have new applications for fresh agriculture

commodities that have not been investigated. Delmopinol hydrochloride ((3-(4-propylheptyl)-4-morpholinethanol)) is an antiseptic and oral hygiene compound that may be a new alternative. This chemical was approved, as a medical device, by the U.S. Food and Drug Administration in 2005 for use in oral hygiene products. The reason it is classified as a medical device is because its effectiveness is due to interference with dental plaque and biofilm formation and adherence of oral bacteria to teeth. This approval was based on clinical studies which showed that an oral rinse with 0.2% delmopinol hydrochloride decreases gingivitis up to 60% compared to no treatment when used as instructed with recommended brushing and flossing. Delmopinol HCl used as a direct spray application on foods or food contact surfaces could reduce *Salmonella* contamination. This chemical could be especially useful on surfaces with a highly irregular texture, such as the netted surface of cantaloupe, where a biofilm may be difficult to disrupt or remove. Short-term tests with delmopinol have demonstrated little or no change in salivary bacterial counts, but significant decreases in the surface area covered with bacterial deposits (Sjodin, et al., 2011, Hancock and Newell, 2000).

Theoretically, delmopinol could enable removal of exposed or hidden bacterial colonies, and cover a treated surface for several hours, repelling or reducing bacterial attachment (Zee et al.1997, Yeung et al. 1995, Hase et al., 2005).

Previous researchers have documented the inability of a variety of sanitizers and other treatments to completely remove and/or inactivate *Salmonella* inoculated onto cantaloupes (Sapers, 2001, Ukuku, 2001). However, most of the research done so far have been aimed to replace treatments previously implemented (Alvarado-Casillas et al. 2007; Ukuku 2006; Ukuku and Fett, 2004) and not as additional steps or treatments for

an extra food safety protocol. An additional step or post-harvest technique can be available to cantaloupe packers and distributors to reduce of the possibility of cross-contamination by *Salmonella* and other pathogens.

The objectives of the study are to evaluate the efficiency of microbial reductions of *Salmonella*, by a post-harvest treatment with delmopinol HCl, on the complex netted surface of two cantaloupes ‘Athena’ and ‘Hale's Best Jumbo’ (HBJ). Additionally, this study evaluated the color and firmness of cantaloupe during refrigerated storage for up to 14 days at 4^{o}C after a post-harvest treatment with 1% delmopinol hydrochloride.

MATERIALS AND METHODS

Nalidixic acid stock solution:

Sodium hydroxide solution (0.1 N NaOH) was prepared using 4 grams of NaOH pellets (Certified ACS, Beat UN182, Fisher Chemicals, Fisher scientific) in 1 liter of distilled water, then allow it to rest for 1 hour, then 0.5 grams of Nalidixic Acid (1-Ethyl-1,4-Dihydro-7-methyl-1,8-naphthyridin-4-on-3-carboxylic acid, 99.5%) powder (Acros Organics, 99.5%, Lot A0272062) was dissolved in NaOH solution; and mixed on a rotated magnetic plate at slow speed. Nalidixic Acid Solution (Nal stock) was stored in a crystal sterilized container, sealed, wrapped in aluminum foil and stored at 2 – 4^{o}C for maximum of 60 days.

TSA / Nalidixic acid plates:

Twenty grams of Difco™ Tryptic Soy Agar (TSA); (Becton–Dickinson and Company, lot 2058864, REF 236950) was diluted in 500 ml of distilled water, heated, dissolved and autoclaved at 121^{o}C x 15 min and cooled. Then, 5 ml of 50 ppm Nal stock was added and stirred for 10 minutes. Agar was poured into sterile petri dishes which were stored at room temperature to be used the next day.

Preparation of inocula:

Salmonella Michigan, isolated from a cantaloupe illness outbreak, was obtained from Dr. Larry Beuchat at University of Georgia. A culture was made nalidixic acid resistant by consecutive transfers every 24 hrs of isolated colonies from Tryptic Soy

Agar with increasing concentrations of nalidixic acid until colonies were resistant at a level of 50 ppm.

Colonies were added to a Tryptic Soy Broth (TSB) tubes (Becton–Dickinson and Company, lot 0363328, REF 211825) and placed at 24 hrs in an incubator at 35 +/-2C. Positives TSB grow colonies were transferred to a small vial and storage for further use.

Bacterial cultures were kept frozen in 80:20 glycerol solutions at –75 ^{o}C. Prior to each experiment, a culture vial was removed from frozen storage and defrosted slowly by hand. A 0.1 ml aliquot of bacterial culture were added to 9.9 ml of TSB and incubated for 24 hrs at 35 +/-2C.

A sample randomly picked from each group was evaluated to check for viability in the presence of 50 ppm nalidixic acid. For each sample culture of *Salmonella* Michigan, 100 µl were plated on 40 ppm TSANal Plates, 50 ppm TSANal Plates and 60 ppm TSANal Plates. Only colonies that grew on 50 ppm TSANal plates were used in subsequent experiments. *Salmonella* identification was confirmed with a biochemical test kit (API 20 E, identification system for Enterobacteriaceae; BioMérieux, bioMérieux, INC, Durham, NC). Only positive broth cultures were used.

Cantaloupe preparation:

'Athena' and 'Hale's Best Jumbo' cantaloupe were chosen because their surfaces are covered by a well-developed, firm, deeply striated and heavy netted skin and are more resistant to powdery mildew. The Athena´ cultivar is the most predominate commercial cantaloupe in the Eastern United States and ´Hales Best Jumbo´ is a heirloom melon that has been planted and sold in the Eastern U.S. for more than 100 years.

Planting and harvesting: Cantaloupes were transplanted and direct seeded at the Virginia Tech College of Agriculture and Life Sciences farm facility (Kentland Farm) in the summers of 2011 and 2012. First, seeds were planted at the greenhouse facility in 72 cell plug trays to obtain small melons transplants. These were transplanted in early June into black plastic mulch after the last frost. A second planting was done by direct seeding through holes into plastic mulch, to harvest the cantaloupes in sequential stages. Irrigation and fertilization was done using drip irrigation tubes under the plastic mulch. Plants were tended twice per week for weed removal, fruit rotation, and to confirm healthy growth. Insecticides were used only (under the Horticulture Department supervision) as a last resort and weeds were removed by hand. Cantaloupes were harvested when the stem part of the fruits was one-third or one-half off (slip stage), indicating that the fruits were ripe.

Transportation and storage: Undamaged cantaloupes were placed in a cleaned and sanitized plastic reusable box and transported to the Food Science and Technology building at Virginia Tech. Cantaloupes were sorted by size, cultivars, maturity and cleanness. Over-ripe, small and damaged cantaloupes were discarded, only whole good ones that did not show physical or insect damage or broken skins were used. Melons were transferred carefully to a clean water tank and debris was removed by hand and using a soft hair brush. Melons were rinsed using clean tap water and allowed to dry at room temperature (20 – 25 ^{o}C) for 30 minutes. Cleaned and sorted

melons were placed in dark plastic boxes and stored at 4 ^{o}C for a maximum of 7 days in a controlled walk-in refrigerator.

Rind plugs samples:

Cantaloupes were transferred to a biological safety cabinet at room temperature (20 ^{o}C) for 2 hr maximum before being sampled and treated. Cantaloupe rind plugs were collected (2.5 cm. diam., 2.5 cm. height, weight approx.10.0 grams) using a sanitized sterile cork bored plunger and the flesh adhering to the plug was trimmed off using a sterilized stainless steel single use scalpel. Rind plugs were inserted into a sterile sample container where 9.0 ml of Butterfield's Phosphate Buffer (3M, St. Paul, MN) was carefully added at the bottom of the container to prevent the sample from drying out and to preserve humidity.

Skin (SKN) samples were chosen that were well netted, thick, coarse, and corky, and stood out in bold relief over some part of the surface, the skin color (ground color) between the netting had changed from green to yellowish-buff, yellowish-gray, or pale yellow. Stem scar (SCR) samples were chosen that had a layer of cells around the stem that softens, yellowish cast rind, a smooth symmetrical, shallow base dish-shaped scar at the point of where the stem was attached (Appendix G). For each trial (3), 18 melons were used to obtain 40 skin rind samples and 40 melons were used to obtain stem scar rings.

Delmopinol hydrochloride treatments:

Preparation of delmopinol hydrochloride solutions: Delmopinol hydrochloride powder, (Sinclair IS Pharma, London, United Kingdom) was mixed with distilled water to create a 0.5% and 1.0% solution. Solutions were stored in clear airtight glass containers at room temperature, away from sunlight until further use, storage for a maximum of 60 days. Distilled water was used as a control (0% delmopinol). Two treatment applications were performed, where *Salmonella* "BAC" were applied first and then a spray "CHM" treatment (BAC/CHM), and also where the spray treatment was applied first, followed by the Salmonella (CHM/BAC).

Bacteria – Chemical spray application (BAC/CHM): Rind plugs were inoculated with 100 µL of a broth culture of *Salmonella* Michigan (approx. 1.0×10^9 CFU/ml inoculated amount) using a sterile syringe. This broth culture of *Salmonella* was placed drop by drop and spread evenly on the surface of the rind plugs. Then the melon rind plugs were left to stand for 1 h or 24 hrs, respectively, in an incubator at 35 +/-2 ^{o}C. Plugs were sprayed; using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the surface of the rind plugs samples with 10 ml (3 pump sprays) of a Delmopinol solution (0% or 1.0%) and left undisturbed for 15 min in a biosafety cabinet before microbiological analysis. Ten melon rind samples (3 sample treatment + 1 control) were enumerated after 1hr storage and 10 melon rind samples (3 samples treatment + 1 control) were enumerated after 24 hrs storage for each of three replications per trial.

Chemical - Bacteria application (CHM/BAC): Rind plugs were sprayed using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the samples with 10 ml (3 pump sprays) of a delmopinol solution (0% or 1.0%) in a biosafety cabinet. After 15 min, rind plugs were inoculated with 100 µL of an broth culture of *Salmonella* Michigan (approx. 1.0 x 10^9 CFU/ml inoculated amount) using a sterile syringe. The broth culture of *Salmonella* was placed drop by drop and spread evenly on the surface of the rind plugs. Cantaloupe rind plugs were left to stand for 1h or 24 hrs in an incubator at 35 +/-2C. Ten melon rind samples were enumerated after 1hr and 10 melon rind samples were enumerated after 24 hrs for each of three replications per trial.

Microbiological Analysis:

***Salmonella* recovery (Step 1, simple dilution):** Cantaloupe plugs separately were submerged in 90.0 ml of Butterfield's Phosphate Buffer. Bottles were shaken for 20 sec by hand and decimal dilutions were plated on TSA-Nal using an automated spiral plater (Autoplate 4000® spiral plater; Spiral Biotech, Norwood, MA).

***Salmonella* recovery (Step 2, dilution and sonication):** The plugs diluted in Step 1 above were transferred and placed in a new cup with fresh Butterfield's Phosphate Buffer (99 ml) and sonicated at 75 joules (15 watts for 5 seconds) in 3 intervals (1:1:1) using a CPX 130 ultrasonic processor (Cole Palmer Instruments,130 watts, frequency 20 khz). The ultrasonic probe had a 6 mm (1⁄4") titanium and length of 113 mm (Cole Parmer Instruments, model CV18, series # 2011026727).

Enumeration of samples: Dilutions were plated on TSA-Nal using an automated spiral plater (Autoplate 4000® spiral plater; Spiral Biotech, Norwood, MA). Plates were held at 35 +/- 2C for 24 hrs. Colonies were enumerated using a ProtoCOL® automated colony counter (Microbiology International, Frederick, MD). All samples were plated in duplicate and the experiment was replicated three times. The recovered cell concentrations for each sample enumerated with and without sonication were summed together prior to additional calculations of mean recovery and statistical significance.

Color analysis:

Fifteen whole cantaloupes (´Athena´) were sprayed using a bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the cantaloupe with 40 ml (5 spray pumps) of a 0%, 0.5% or 1.0% delmopinol hydrochloride spray solution and stored at 4 ^{o}C for 1, 2, 5, 7 and 14 days. Color measurements were recorded, for three replicate experiments, using a portable Chromameter (Minolta CR-300, Japan). For each sample, three readings were interpreted using the Hunter CIE L*a*b* (CIELAB) scale, where L* indicates the level of lightness and darkness, the a* value indicates the degree of redness and greenness, and the b* value indicates yellowness and blueness. A combination of these values were reported as Δ E which represents an overall color change. The instrument was standardized using black and white tiles previous to each reading, per the procedure described by the manufacturer of the Chromameter.

Texture analysis:

Fifteen whole cantaloupes (`Athena´) were sprayed using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees with 40 ml (5 spray pumps) of 0%, 0.5% or 1.0% delmopinol hydrochloride spray solution and stored at 4 ^{o}C for 1, 2, 5, 7 and 14 days. These cantaloupes were not additionally tested for color or microbial recovery. The firmness of the cantaloupes was analyzed using a TA-XT Plus, series 10545, texture analyzer (Texture Technology, New York,) with a model TA-23 plunger (½" diameter, ¼ R end, 3" tall). The auto trigger was used with 5 grams force and a 2.0 mm/sec test distance penetration speed. Readings were collected in triplicates.

Statistical analysis:

Three replicate experiments were conducted and two samples (skin rind plugs or stem scar rind plugs) of each treatment were analyzed for *Salmonella* Michigan at each sampling time. Data were analyzed by randomized complete block factorial design using general linear model (GLM) procedure of Statistical Analysis Software (Version 9.13, SAS Institute, Cary, NC). Significant differences ($p \leq 0.05$) in microbial recovery due to delmopinol hydrochloride treatment, storage time (1 h, 24 hrs) and order of application (CHM/BAC) or BAC/CHM) were determined using Tukey´s multiple range test.

RESULTS AND DISCUSSION:

In this study, 0% (control), 0.5% and 1.0% delmopinol hydrochloride direct spray solutions were evaluated for reduction of *Salmonella* Michigan on skin rind plug (SKN) and stem scar rind plug (SCR) of two cantaloupe cultivars- Athena and Hale's Best Jumbo (HBJ).

Athena´s cultivar:

Population reductions of *Salmonella* on stem scar rind plug (SCR) was approximately 3.1 Log CFU/ml, greater than the Control group, when 1% delmopinol (DEL) was applied either 1 hr before or after the *Salmonella* (DEL/BAC or BAC/DEL) (Table 1, Figure 1). *Salmonella* was reduced between 1.1 and 1.46 log CFU/ml on skin rind plugs (SKN). For both skin rind plugs (SKN) and stem rind plugs (SCR), *Salmonella* populations were significantly lower ($p<0.05$) after 1 hr with each delmopinol treatment. *Salmonella* reduction (from control) after 24 hr storage of skin rind plug (SKN) and stem scar rind plugs (SCR) was <1 Log CFU/ml (Table 1).

Hale's Best Jumbo (HBJ)´s cultivar:

Population reductions of the *Salmonella* on skin rind plugs (SKN) (3.16 Log CFU/ml) was significantly greater ($p<0.05$) when delmopinol was applied 1 hr after the bacteria (BAC/DEL) or 1 hr before the bacteria (reduction of 1.89 Log CFU/ml greater than the Control) (Table 2, Figure 1). Population reductions of the *Salmonella* on stem scar plugs (SCR) (1.98 Log CFU/ml) was higher when 1% delmopinol was applied 1 hr

before the bacteria (DEL/BAC). *Salmonella* reduction (from control) after 24 hr storage of skin rind plug (SKN) and stem scar rind plug (SCR) was <1 Log CFU/ml (Table 2).

1% delmopinol application on 'Athena' vs 'Hale's Best Jumbo (HBJ)':

Both Athena and HBJ cultivars have well netted, thick, coarse and corky skin, where pathogenic bacteria could hide and attach to many places on the surface. While *Salmonella* populations were reduced by the treatments, some organisms remained on cantaloupe sample surfaces. After 1 h, the application of 1% delmopinol, applied before or after *Salmonella* (DEL/BAC or BAC/DEL) on Athena stem scar rind plugs (SCR) resulted in similar a log reduction (>3.1 log CFU/ml) compared to the lesser effect on skin rind plugs (SKN). This 1% delmopinol spray application on stem scar rind plugs (SCR) was more effective on 'Athena' (3.08 to 3.14 log CFU/ml) than 'HBJ' where the log reduction was between 1.29 to 1.98 log CFU/ml. This difference could be associated because 'Athena' stem scars (SCR) are smaller, less soft and have less susceptibility to fracture than 'HBJ' stem scars. Also, bacteria may not have had sufficient time to internalize into the cantaloupes or have direct contact with 1% delmopinol. On the other hand treatment applications where *Salmonella* was applied first and then followed by a 1% delmopinol spray treatment (BAC/DEL), 1% delmopinol was significantly more effective compared to when the spray treatment was applied first, followed by the *Salmonella* (DEL/BAC). A 1% delmopinol application on 'HBJ' skin rind plugs (SKN), after 1h, was more effective than on stem scar rind plugs (SCR) of `HBJ´ and stem scar rind plugs (SCR) of 'Athena'.

No significant difference in *Salmonella* recovery was observed on either 'Athena' nor 'HBJ' cantaloupe after 24 hr storage on both stem scar rind plugs (SCR) and skin rind plugs (SKN). Storage of cantaloupes treated with 1.0% delmopinol solution for 1 hr had a greater effect on reducing *Salmonella* compared to 24 hr treatment for both Athena and HBJ cultivars, suggesting a rapid and short time bactericidal effect on bacteria cells.

After 7 days storage, the hardness of skin samples of 1% delmopinol treated cantaloupes, was not significantly different than control (DI water sprayed) samples. In color measurement, no differences were observed between 1% delmopinol treated cantaloupes and control cantaloupes after 14 days (Table 3).

This research suggests that a direct spray application with 1.0% delmopinol hydrochloride on the stem scar or on the skin could reduce *Salmonella* cells on cantaloupes by more than 3 log CFU/ml (Figure 1 and Table 3) and has no visible effect on color and texture changes.

Simonsson et al. (1991) and Rugrentet et al. (1992) suggested a small bactericidal effect of delmopinol HCl but the exact mode of action is not yet known. However Zee (1997) and Burgemeister (2001) research on planktonic and attached cells also showed a marked decrease in vitality followed by exposure to 0.2% delmopinol hydrochloride. They suggested that delmopinol does not just possess a bactericidal effect, but also possesses an anti-aggregating effect rather than an anti-adhesive effect on the pioneer bacteria. When there are existing plaque colonies, the cohesive forces between the bacteria are reduced by delmopinol, which makes removal by mechanical means much easier.

Experiments conducted to assess the relative strength of attachment of *Salmonella* on cantaloupe rinds demonstrated an increasing strength of attachment from days 0–7 during storage (Ukuku and Fett 2002). Annous et al., (2005), found that extracellular polymeric substance formation (biofilm) occurred rapidly following introduction of cells (2 h at 20 ^{o}C) onto the cantaloupe rind. Ukuku and Sapers (2001) speculated that increased contact time allowed for strong microbial attachment to the cantaloupe surface and the formation of a bacterial extracellular polymeric substance prior to sanitation. They found that *Salmonella* enterica sv. Michigan produces large amounts of extracellular polymeric substance following their introduction onto the cantaloupe rind.

This research illustrates that a direct spray application with 1.0% delmopinol hydrochloride could enhance the reduction of inoculated *Salmonella* on cantaloupe surfaces and make the bacteria more susceptible to sanitizers or physical removal. This new approach of using an oral hygiene chemical, incorporated as an additional action to the regular cleaning and sanitizing program for netted surfaced fruits such as cantaloupes, could be an option to reduce human pathogens like *Salmonella.*

Since, this pathogen is the predominant microorganism responsible for national and international outbreaks associated with consumption of cantaloupe (Richards and Beuchat, 2005), a new sanitizing option with great lethality is needed for cantaloupe and cantaloupe contact surfaces. Furthermore, any new antimicrobial chemical used for this purpose should have no residual effects and not affecting the visual appeal and texture qualities of the products. Post-harvest food industrial applications of novel antimicrobial and surfactant chemicals such as delmopinol hydrochloride could be beneficial for

reducing pathogenic bacteria, such as those found on cantaloupe and other raw produce.

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TABLES:

Table 1: Log CFU/ml recovery of *Salmonella* from stem scar rind plugs (SCR) and skin rind plugs (SKN) of 'Athena' after 1hr and 24 hrs incubation periods at 35 ^{o}C and 1% delmopinol spray solution applied.

Delmopinol hydrochloride (delmopinol)		Stem scar plugs (SCR)				Skin plugs (SKN)			
		1 hr		24 hrs		1 hr		24 hrs	
Athenas	Treatment	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD
	CONTROL	**8.12**^{a}	1.35	**9.58**^{a}	0.15	**7.51**^{a}	0.59	**9.85**^{a}	0.16
	BAC / 1% DEL	**4.98**^{b}	1.17	**9.45**^{a}	0.14	**6.05**^{b}	0.40	**8.93**^{b}	0.78
	1% DEL /BAC	**5.04**^{b}	0.97	**9.02**^{a}	0.50	**6.41**^{b}	0.48	**8.24**^{b}	0.20

Column means with the same letter are not significantly different.

Table 2: Log CFU/ml recovery of *Salmonella* from stem scar rind plugs (SCR) and skin rind plugs (SKN) of 'Hales Best Jumbo (HBJ)' after 1hr and 24 hrs incubation periods at 35^{o}C and 1% delmopinol spray solution applied.

Delmopinol hydrochloride (delmopinol)		Stem scar plugs (SCR)				Skin plugs (SKN)			
		1 hr		24 hrs		1 hr		24 hrs	
Hale's Best J.	Treatment	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD
	CONTROL	**7.34**^{a}	1.15	**8.71**^{a}	0.78	**7.44**^{a}	0.48	**9.33**^{a}	0.17
	BAC / 1% DEL	**6.05**^{a}	0.70	**7.95**^{a}	0.90	**4.28**^{b}	0.21	**8.54**^{b}	0.07
	1% DEL /BAC	**5.36**^{a}	1.14	**8.30**^{a}	0.43	**5.55**^{b}	1.13	**8.73**^{b}	0.27

Column means with the same letter are not significantly different.

Table 3: Color measurements after spray application of 1% delmopinol HCl on cantaloupe ('Athena') and 14 days storage at 4^{o}C.

Non Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	71.14	1.68	77.52	2.98	71.35	2.34	70.67	0.75	72.13	2.50
a mean	-0.91	0.20	0.29	0.97	-1.75	0.51	-0.98	1.24	-1.00	0.80
b mean	19.63	0.23	3.23	0.83	18.60	0.69	19.82	1.25	19.53	0.45
		Δ E	17.64	Δ E	1.34	Δ E	0.51	Δ E	1.00	

0.5% Delmopinol Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	72.74	0.97	78.32	0.53	73.30	0.30	73.90	1.12	73.41	1.59
a mean	-0.80	0.46	0.74	0.32	-1.21	0.20	-0.57	0.20	-0.92	1.51
b mean	20.98	0.11	4.56	2.07	20.35	0.66	20.61	0.41	21.46	1.01
		Δ E	17.40	Δ E	0.94	Δ E	1.24	Δ E	0.83	

1.0 % Delmopinol Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	70.67	0.69	74.95	2.10	70.23	1.50	71.17	1.59	70.96	2.13
a mean	-0.50	0.31	1.00	0.84	-0.53	0.43	-0.43	0.73	-0.53	0.81
b mean	19.59	0.42	4.17	0.92	19.99	0.57	20.89	0.45	20.37	0.61
		Δ E	16.07	Δ E	0.59	Δ E	1.39	Δ E	0.83	

L = 0 yields black and L = 100 indicates diffuse white; spectacular white
a = negative values indicate green while positive values indicate magenta
b =negative values indicate blue and positive values indicate yellow
where ΔE= Total color difference

FIGURES

Figure 1: Log CFU/ml reduction of *Salmonella* from stem scar rind plugs (SCR) and skin rind plugs (SKN) from 'Athena' (A) and 'Hale Best Jumbo (HBJ)' after 1hr incubation periods at 35 ^{o}C and 1% delmopinol (Del) spray solution applied

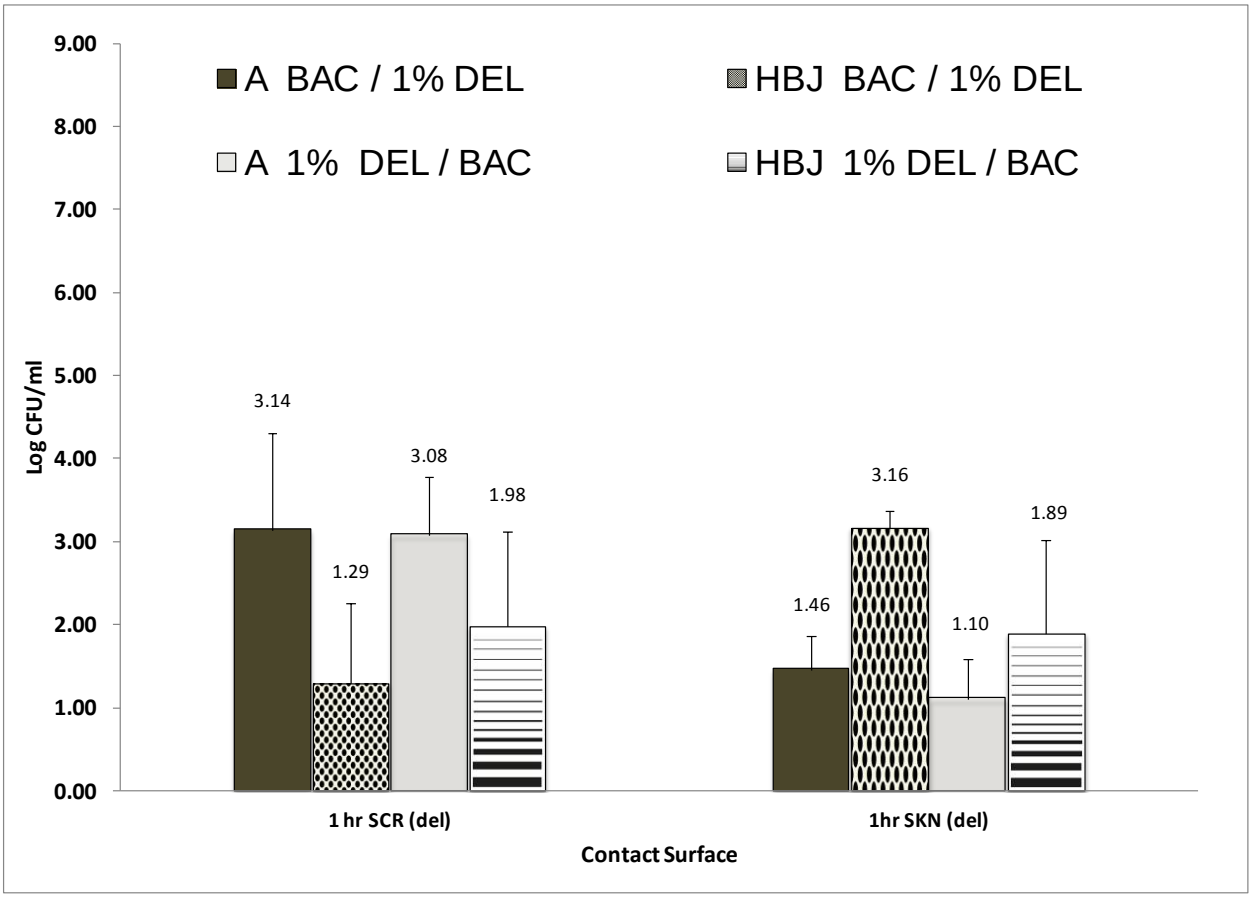


Figure 2: Skin hardness test (force (g) applied) on whole cantaloupes ('Athena') after 1% delmopinol spray solution applications and 1, 2, 5, 7 and 14 days storage at 4 ^{o}C.

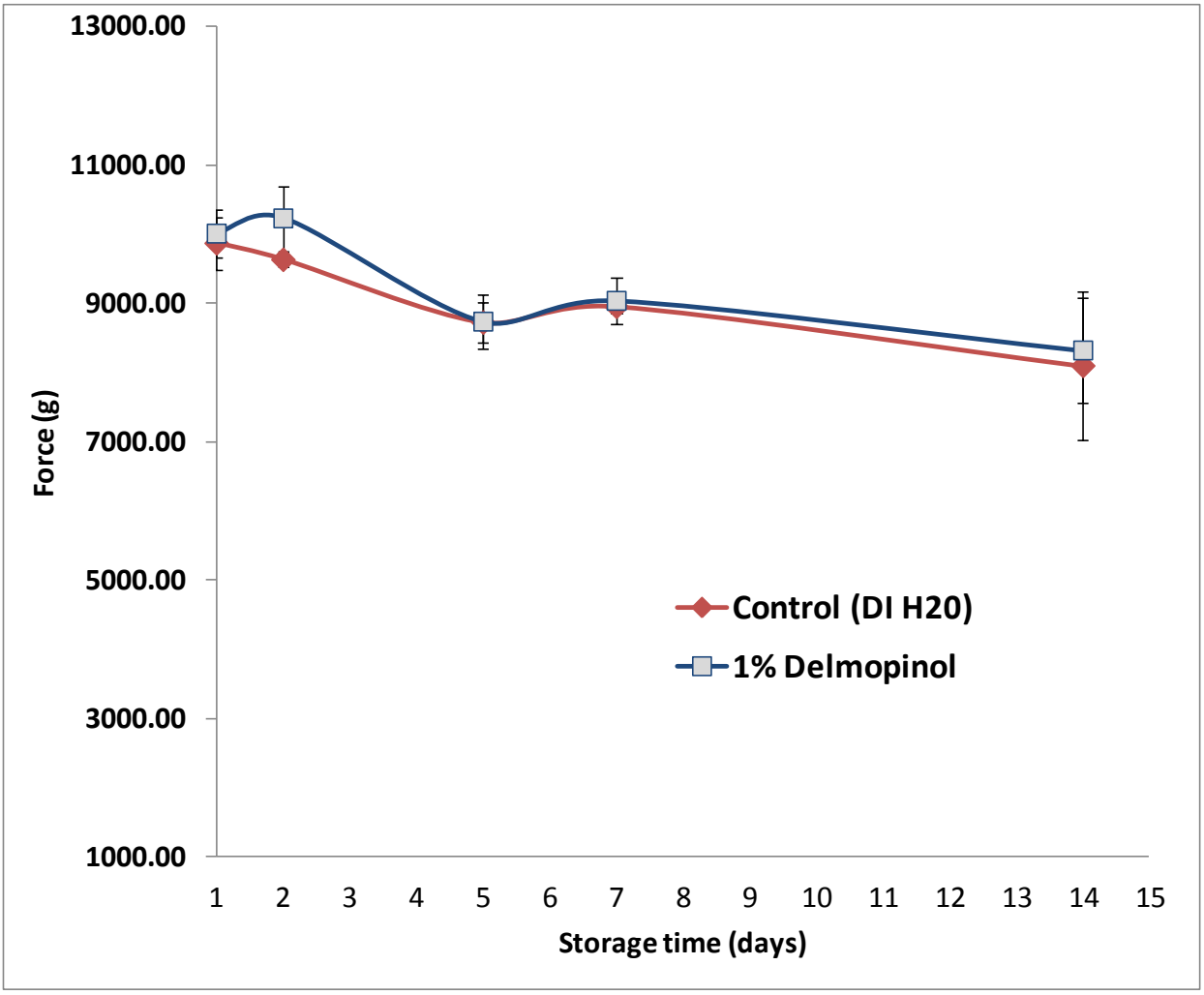
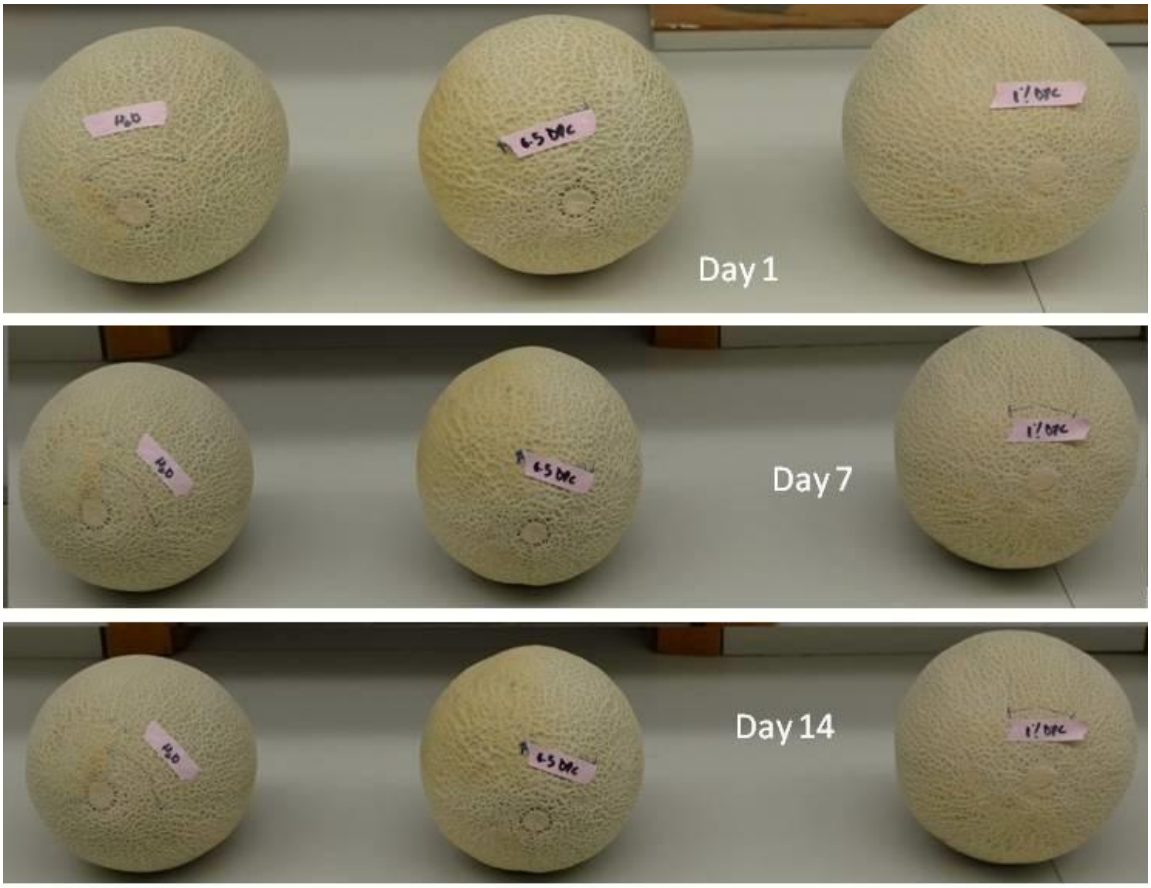


Figure 3: ‘Athena´s cantaloupe after 1% delmopinol spray application and 1, 7 or 14 days storage at 4^{o}C



CHAPTER 4

Cetylpyridinium Chloride Direct Spray Treatments Reduce *Salmonella* on Cantaloupe Rough Surfaces

ABSTRACT

Because cantaloupes are grown at ground level, their outer skins can be contaminated with pathogenic and spoilage bacteria during production from irrigation water and manure fertilizers and, during food processing by contaminated equipment and food handlers. Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach to these surfaces and become difficult to remove. Appropriate post-harvest washing and sanitizing procedures are needed that can help control *Salmonella* and other pathogens on melons especially on cantaloupes and their nested surface.

Cetylpyridinium chloride (CPC) is the active ingredient of some antiseptic oral mouth rinses, and has a broad antimicrobial spectrum with a rapid bactericidal effect on Gram-positive pathogens. The spray application of CPC solutions to cantaloupe may reduce the level of *Salmonella* surface contamination. In this study, cantaloupe (Athena and Hale's Best Jumbo cultivars) rind plugs (25 mm. diam.) were inoculated with a broth culture of *Salmonella* Michigan (~ 9 log CFU/ml). After 15 min, plugs were sprayed with 10 ml of a CPC solution (0, 0.2, 0.5 or 1.0%) and held at 37 ^{o}C for 1hr or 24 hrs. Cantalopus rind plugs were diluted with Butterfield's Phosphate Buffer, shaken and sonicated, and solutions were enumerated on 50 ppm nalidixic acid - tryptic soy agar.

Texture, quality and color of additional cantaloupes samples were evaluated after 0% and 1% CPC spray treatments over 14 days storage at 4^{o}C.

A 0.5% or 1.0% (vol/vol) application of CPC after *Salmonella* was applied reduced *Salmonella* levels between 2.34 log CFU/ml and 5.16 log CFU/ml in comparison to the control ($p<0.01$). No differences were observed in the firmness and color of 1% CPC treated cantaloupes. *Salmonella* concentration levels on cantaloupes, treated with 1.0% CPC, were lower after 1hr storage as compared to 24 hr. And, *Salmonella* on 'Athena' surface was more susceptible to CPC spray solution treatments than *Salmonella* on 'Hale's Best Jumbo'. A direct surface spray application of cetylpyridinium chloride may be an alternative antimicrobial post-harvest treatment to reduce pathogen contamination of cantaloupe melons.

INTRODUCTION

There has been an increase in cases of foodborne illness caused by *Salmonella* and other pathogenic bacteria associated with consumption of fruits and vegetable from both domestic and imported sources as well in the last two decades (USFDA 2001a; USFDA 2003; Bowen et al., 2006; CDC 2013). In the last few years, foodborne illness resulting from contamination of these raw agricultural products, particularly cantaloupe, has become an increasing concern (USFDA, 1998). Most of the outbreaks have been linked to poor or inappropriate cleaning and sanitation at the packing houses. Because cantaloupes are grown at ground level, their outer skins can be contaminated with pathogenic and spoilage bacteria during production from irrigation water and manure fertilizers, and during food processing by contaminated equipment and food handlers (Bowen et al., 2006; Mahmoud et al., 2012).

Produce packing houses utilize water dunk tanks to clean, sort and disinfect these cantaloupes to eliminate debris, soils and bacteria attached to the products. Chlorine and its derivatives are the most widely used disinfectants to sanitize cantaloupes. Fan et al. (2009) found that the application of chlorine and other disinfectants such as acidified calcium sulfate (ACS), acidified sodium chlorite (ASC), and peroxyacetic acid (PAA) had a limited effect on the population of *Salmonella*, achieving no more than a 1.5 log reduction of the pathogen from the surface of whole cantaloupes. There are disadvantages to using chlorine and its derivatives. For example, they are affected by organic matter; they are corrosive at high concentrations; they are not stable in diluted solutions and concentrates, and they cannot be stored for

a long time without losing their antimicrobial activity. These drawbacks have led to the search for new disinfection alternatives.

One of these alternatives includes quaternary ammonium compounds (QAC´s) which are widely used as disinfectants and antiseptics. QACs are more expensive than chlorine and its derivatives, but they have numerous qualities that make them an attractive alternative for washing fruits and vegetables. QACs are less affected by organic matter; are not corrosive except at high concentrations; they are stable even in diluted solutions and concentrates, and can be stored for a long time without losing their antimicrobial activity (Chaidez et al., 2007). According to Frier (1991), QACs are the most useful antiseptics and disinfectants. They are sometimes known as cationic detergents. QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization (McDonnell and Russell, 1999)

Cetylpyridinium chloride (CPC) is a quaternary ammonium molecule that is effective at concentrations of 0.5% for reducing cross-contamination in poultry washes giving reductions of up to 2.5 log in *Salmonella* Typhimurium levels (Breen et al., 1997; Kim and Slavick 1996). CPC has been approved to treat the surface of raw poultry carcasses prior to immersion in a chiller in the USA (USFDA 2003b; USFDA 2004). CPC is commonly used as an active ingredient in mouthwash and toothpaste around the world and it is generally recognized as a safe bactericide.

Salmonella has been the predominant serotype responsible for national and international outbreaks associated with consumption of cantaloupe (Richards and

Beuchat, 2004, 2005). In past years, *Salmonella* spp. has been implicated in outbreaks of foodborne illness linked to the consumption of fresh fruits, most especially, cantaloupe melons. Because *Salmonella* can attach to rough surfaces and build biofilm complexes, these organisms can be hard to remove using just chlorine and tap water. A direct spray CPC application could reduce hard to reach bacteria colonies between the netted surfaces of the cantaloupes. The use of CPC by cantaloupe packers could be an alternative post harvest technique for to reduce of possibility of *Salmonella* cross contamination at the packaging step.

The objectives of this study are to evaluate the efficiency of any microbial reductions in the level of *Salmonella* by direct spray application of CPC on the surface of two cantaloupe cultivars (Athena and Hale's Best Jumbo (HBJ)). Additionally, this study evaluated the color and texture of cantaloupe during refrigerated storage after a post-harvest treatment with a cetylpyridinium chloride spray solution.

MATERIALS AND METHODS

Nalidixic acid stock solution:

Sodium hydroxide solution (0.1 N NaOH) was prepared using 4 grams of NaOH pellets (Certified ACS, Beat UN182, Fisher Chemicals, Fisher scientific) in 1 liter of distilled water, then allow it to rest for 1 hour, then 0.5 grams of Nalidixic Acid (1-Ethyl-1,4-Dihydro-7-methyl-1,8-naphthyridin-4-on-3-carboxylic acid, 99.5%) powder (Acros Organics, 99.5%, Lot A0272062) was dissolved in NaOH solution; and mixed on a rotated magnetic plate at slow speed. Nalidixic Acid Solution (Nal stock) was stored in a crystal sterilized container, sealed, wrapped in aluminum foil and stored at 2 – 4^{o}C for maximum of 60 days.

TSA / Nalidixic acid plates:

Twenty grams of Difco™ Tryptic Soy Agar (TSA); (Becton–Dickinson and Company, lot 2058864, REF 236950) was diluted in 500 ml of distilled water, heated, dissolved and autoclaved at 121^{o}C x 15 min and cooled. Then, 5 ml of 50 ppm Nal stock was added and stirred for 10 minutes. Agar was poured into sterile petri dishes which were stored at room temperature to be used the next day.

Preparation of inocula:

Salmonella Michigan, isolated from a cantaloupe illness outbreak, was obtained from Dr. Larry Beuchat at University of Georgia. A culture was made nalidixic acid resistant by consecutive transfers every 24 hrs of isolated colonies from Tryptic Soy

Agar with increasing concentrations of nalidixic acid until colonies were resistant at a level of 50 ppm.

Colonies were added to a Tryptic Soy Broth (TSB) tubes (Becton–Dickinson and Company, lot 0363328, REF 211825) and placed at 24 hrs in an incubator at 35 +/-2C. Positives TSB grow colonies were transferred to a small vial and storage for further use.

Bacterial cultures were kept frozen in 80:20 glycerol solutions at –75 ^{o}C. Prior to each experiment, a culture vial was removed from frozen storage and defrosted slowly by hand. A 0.1 ml aliquot of bacterial culture were added to 9.9 ml of TSB and incubated for 24 hrs at 35 +/-2C.

A sample randomly picked from each group was evaluated to check for viability in the presence of 50 ppm nalidixic acid. For each sample culture of *Salmonella* Michigan, 100 µl were plated on 40 ppm TSANal Plates, 50 ppm TSANal Plates and 60 ppm TSANal Plates. Only colonies that grew on 50 ppm TSANal plates were used in subsequent experiments. *Salmonella* identification was confirmed with a biochemical test kit (API 20 E, identification system for Enterobacteriaceae; BioMérieux, bioMérieux, INC, Durham, NC). Only positive broth cultures were used.

Cantaloupe preparation:

'Athena' and 'Hale's Best Jumbo' cantaloupe were chosen because their surfaces are covered by a well-developed, firm, deeply striated and heavy netted skin and are more resistant to powdery mildew. The Athena´ cultivar is the most predominate commercial cantaloupe in the Eastern United States and ´Hales Best Jumbo´ is a heirloom melon that has been planted and sold in the Eastern U.S. for more than 100 years.

Planting and harvesting: Cantaloupes were transplanted and direct seeded at the Virginia Tech College of Agriculture and Life Sciences farm facility (Kentland Farm) in the summers of 2011 and 2012. First, seeds were planted at the greenhouse facility in 72 cell plug trays to obtain small melons transplants. These were transplanted in early June into black plastic mulch after the last frost. A second planting was done by direct seeding through holes into plastic mulch, to harvest the cantaloupes in sequential stages. Irrigation and fertilization was done using drip irrigation tubes under the plastic mulch. Plants were tended twice per week for weed removal, fruit rotation, and to confirm healthy growth. Insecticides were used only (under the Horticulture Department supervision) as a last resort and weeds were removed by hand. Cantaloupes were harvested when the stem part of the fruits was one-third or one-half off (slip stage), indicating that the fruits were ripe.

Transportation and storage: Undamaged cantaloupes were placed in a cleaned and sanitized plastic reusable box and transported to the Food Science and Technology building at Virginia Tech. Cantaloupes were sorted by size, cultivars, maturity and cleanness. Over-ripe, small and damaged cantaloupes were discarded, only whole good ones that did not show physical or insect damage or broken skin were used. Melons were transferred carefully to a clean water tank and debris was removed by hand and using a soft hair brush. Melons were rinsed using clean tap water and allowed to dry at room temperature (20 – 25^{o}C) for 30 minutes. Cleaned and sorted melons were placed in dark plastic boxes and stored at 4^{o}C for a maximum of 7 days in a controlled walk-in refrigerator.

Rind plugs samples:

Cantaloupes were transferred to a biological safety cabinet at room temperature (20 ^{o}C) for 2 hr maximum before being sampled and treated. Cantaloupe rind plugs were collected (2.5 cm. diam., 2.5 cm. height, weight approx.10.0 grams) using a sanitized sterile cork bored plunger and the flesh adhering to the plug was trimmed off using a sterilized stainless steel single use scalpel. Rind plugs were inserted into a sterile sample container where 9.0 ml of Butterfield's Phosphate Buffer (3M, St. Paul, MN) was carefully added at the bottom of the container to prevent the sample from drying out and to preserve humidity.

Skin (SKN) samples were chosen that were well netted, thick, coarse, and corky, and stood out in bold relief over some part of the surface, the skin color (ground color) between the netting had changed from green to yellowish-buff, yellowish-gray, or pale yellow. Stem scar (SCR) samples were chosen that had a layer of cells around the stem that softens, yellowish cast rind, a smooth symmetrical, shallow base dish-shaped scar at the point of where the stem was attached (Appendix G). For each trial (3), 18 melons were used to obtain 40 skin rind samples and 40 melons were used to obtain stem scar rings.

Cetylpyridinium chloride solution treatments:

Preparation of Cetylpyridinium chloride (CPC) solutions: Cetylpyridinium Chloride (CPC) (Sigma-Aldrich, Lot# 100M0211V, C0732-100G) was diluted in distilled water to concentrations of 0.2%, 0.5% and 1.0% (w/v). Propylene glycol (PG) (≥99.5%, SAFC,

Sigma-Aldrich, Lot # MKBB3091V, W294004-1KG-K) was added to each solution in a (1.5:1) ratio. Solutions were stored in clear airtight glass containers at room temperature, away from sunlight, until further use. Distilled water was used as a control (0% CPC). Two treatment applications were performed, where bacteria "BAC" were applied first and then a spray "CHM" treatment (BAC/CHM), and also where the spray treatment was applied first, followed by the bacteria (CHM/BAC).

Bacteria – Chemical spray application (BAC/CHM): Rind plugs were inoculated with 100 µL of a broth culture of *Salmonella* Michigan (approx. 1.0×10^9 CFU/ml inoculated amount) using a sterile syringe. This broth culture of *Salmonella* was placed drop by drop and spread evenly on the surface of the cantaloupes rind plugs. Then the melon rind plugs were left to stand for 1 h or 24 hrs, respectively, in an incubator at 35 +/-2 ^{o}C. Plugs were sprayed; using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the surface of the rind plugs samples with 10 ml (3 pump sprays) of a cetylpyridinium chloride (0%, 0.5% or 1.0%) solution and left undisturbed for 15 min in a biosafety cabinet before microbiological analysis. Ten cantaloupes rind samples (3 sample treatment + 1 control) were enumerated after 1hr storage and 10 melon rind samples (3 samples treatment + 1 control) were enumerated after 24 hrs storage for each of three replications per trial.

Chemical - Bacteria application (CHM/BAC): Rind plugs were sprayed using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the samples with 10 ml (3 pump sprays) of a cetylpyridinium chloride (0%,

0.5% or 1.0%) solution in a biosafety cabinet. After 15 min, rind plugs were inoculated with 100 µL of a broth culture of *Salmonella* Michigan (approx. 1.0 x 10^9 CFU/ml inoculated amount) using a sterile syringe. The broth culture of *Salmonella* was placed drop by drop and spread evenly on the surface of the cantaloupes rind plugs. Cantaloupe rind plugs were left to stand for 1h or 24 hrs in an incubator at 35 +/-2C. Ten melon rind samples were enumerated after 1hr and 10 melon rind samples were enumerated after 24 hrs for each of three replications per trial.

Microbiological analysis:

***Salmonella* recovery (Step 1, simple dilution):** Cantaloupe plugs separately were submerged in 90.0 ml of Butterfield's Phosphate Buffer. Bottles were shaken for 20 sec by hand and decimal dilutions were plated on TSA-Nal using an automated spiral plater (Autoplate 4000® spiral plater; Spiral Biotech, Norwood, MA).

***Salmonella* recovery (Step 2, dilution and sonication):** The plugs diluted in Step 1 above were transferred and placed in a new cup with fresh Butterfield's Phosphate Buffer (99 ml) and sonicated at 75 joules (15 watts for 5 seconds) in 3 intervals (1:1:1) using a CPX 130 ultrasonic processor (Cole Palmer Instruments,130 watts, frequency 20 khz). The ultrasonic probe had a 6 mm (1⁄4") titanium and length of 113 mm (Cole Parmer Instruments, model CV18, series # 2011026727).

Enumeration of samples: Dilutions were plated on TSA-NaI using an automated spiral plater (Autoplate 4000® spiral plater; Spiral Biotech, Norwood, MA). Plates were held at 35 +/- 2C for 24 hrs. Colonies were enumerated using a ProtoCOL® automated colony counter (Microbiology International, Frederick, MD). All samples were plated in duplicate and the experiment was replicated three times. The recovered cell concentrations for each sample enumerated with and without sonication were summed together prior to additional calculations of mean recovery and statistical significance.

Color analysis:

Fifteen whole cantaloupes ('Athena') were sprayed using a bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees to the cantaloupe with 40 ml (5 spray pumps) of a 0%, 5.0% or 1.0% CPC spray solution and stored at 4 ^{o}C for 1, 2, 5, 7 and 14 days. Color measurements were recorded, for three replicate experiments, using a portable Chromameter (Minolta CR-300, Japan). For each sample, three readings were interpreted using the Hunter CIE L*a*b* (CIELAB) scale, where L* indicates the level of lightness and darkness, the a* value indicates the degree of redness and greenness, and the b* value indicates yellowness and blueness. A combination of these values were reported as Δ E which represents an overall color change. The instrument was standardized using black and white tiles previous to each reading, per the procedure described by the manufacturer of the Chromameter.

Texture analysis:

Fifteen whole cantaloupes ('Athena') were sprayed using a commercial bottle atomizer with self adjusted spray nozzle, spraying at an angle of 45 degrees with 40 ml (5 spray pumps) of 0%, 0.5% or 1.0% CPC spray solution and stored at 4 ^{o}C for 1, 2, 5, 7 and 14 days. These cantaloupes were not additionally tested for color or microbial recovery. The firmness of the cantaloupes was analyzed using a TA-XT Plus, series 10545, texture analyzer (Texture Technology, New York,) with a model TA-23 plunger (½" diameter, ¼ R end, 3" tall). The auto trigger was used with 5 grams force and a 2.0 mm/sec test distance penetration speed. Readings were collected in triplicates.

Statistical analysis:

Three replicate experiments were conducted and two samples (skin rind plugs or stem scar rind plugs) of each treatment were analyzed for *Salmonella* Michigan at each sampling time. Data were analyzed by randomized complete block factorial design using general linear model (GLM) procedure of Statistical Analysis Software (Version 9.13, SAS Institute, Cary, NC). Significant differences ($p \leq 0.05$) in microbial recovery due to delmopinol hydrochloride treatment, storage time (1 h, 24 hrs) and order of application (CHM/BAC) or BAC/CHM) were determined using Tukey´s multiple range test.

RESULTS AND DISCUSSION

In this study, 0% (control), 0.5% and 1.0% CPC direct spray treatment solutions were evaluated for reduction of *Salmonella* Michigan on skin rind plugs (SKN) and stem scar rind plugs (SCR) of cantaloupe plugs from Athena and Hale's Best Jumbo (HBJ).

Athena´s cultivar:

Population reductions of *Salmonella* on stem scar plugs (SCR) was approximately 2.0 or 3.1 log CFU/ml when 1% CPC was applied either 1 hr before or after the bacteria, respectively. *Salmonella* was reduced between 1.84 and 2.34 log CFU/ml on skin plugs (SKN) when 1% CPC was applied. For both (SKN) and (SCR), *Salmonella* populations were significantly lower ($p<0.05$) after 1 hr with each CPC treatment (Table 1, Figure 1). *Salmonella* reduction (from control) after 24 hr storage on skin rind plugs (SKN) ranged from 0.74 to 1.93 log CFU/ml, while the difference in reduction, with no significant differences from control, was less than 0.5 log CFU/ml for stem scar plugs (SCR).

Hale's Best Jumbo (HBJ)´s cultivar:

Population reductions of the *Salmonella* on skin rind plugs (SKN) (4.95 log CFU/ml greater than control) was significantly greater ($p<0.05$) when 1.0% CPC was applied 1 hr before the bacteria (CHM/BAC). Additionally, when 1.0% CPC solution was applied after *Salmonella*, the reduction of *Salmonella* on skin rind (SKN) was 3.63 log CFU/ml greater than control. Population reductions of the *Salmonella* on stem scar plugs (SCR) (3.56 log CFU/ml) was also highest when 1% CPC was applied 1 hr before the bacteria (CHM/BAC). *Salmonella* reduction (from control) after 24 hr storage of skin rind (SKN) and stem scar rind (SCR) was <1.2 log CFU/ml, with no significant differences from control, for all combinations of CPC concentration and order of application (Table 2, Figure 1).

Athena and HBJ cultivars:

The log reduction (CFU/ml) of Salmonella with 1% CPC spray solution after 1h (BAC/CHM) on stem scar rind (SCR) of 'Athena' was significant greater ($p<0.05$) than with the 1h (BAC/CHM) treatment on stem scar rind (SCR) on 'HBJ'. Conversely, the log reduction (CFU/ml) with 1% CPC solution after 1h (CHM/BAC) on stem scar rind (SCR) HBJ was higher, but not statistically significant, than (CHM/BAC) on stem scar rind (SCR) of Athena's. For both cultivars, storage of cantaloupes treated with 1.0% CPC solution for 1 hr had a greater effect on reducing *Salmonella* compared to 24 hr treatment. For some test combinations, the log reduction of *Salmonella* was significantly higher when 1.0% CPC, rather than 0.5% CPC, was applied. In some tests, the log reduction with 0.5% CPC was slightly significant higher when compared to 1.0% CPC. This reinforces the argument that the effects of biocides on bacterial (and, other types of microbial) cells should be examined over a wide range of concentrations (Russell and McDonnell, 2000).

High recovery amounts for the CHM/BAC (1% CPC spray first and *Salmonella* inoculation second) treatment on 'Athena', demonstrate the significant antimicrobial effects of CPC on bacteria cells in a short period (1 hr). The antimicrobial effects of CPC are dependent on binding to bacterial cells (Caputo *et al.*, 1975) and bactericidal activity in the presence of serum proteins and at different pH and temperature (Quisno and Foter, 1946). On the other hand, after 24 hour storage, bacterial cells had time to adapt to the environment and population growth increased.

Texture and color

No significant differences were observed in the hardness of 1.0% CPC treated cantaloupes at 7 and 14 days compared to control. At day 14, a similar level of force was required to penetrate the skin of the control and CPC sprayed melons (Figure 2).

On day zero, the experiment control melons samples were slightly darker than those sprayed with the 0.5% and 1% CPC solution, but no major differences were observed in the color of 1.0% CPC solution treated cantaloupes throughout all storage days. In addition, the sensory (color and texture) quality of cantaloupes at the end of refrigerated storage did not suffer any major change based on the visual appearance of the outside of intact cantaloupes and their degree of deterioration.

Cetylpyridinium chloride (CPC) is a quaternary ammonium molecule that is highly effective for microbial destruction (Breen et al., 1997, Kim and Slavik, 1996). CPC is considered a low-level biocide. QACs are able to promote their own entry by displacing divalent metal cations in the outer membrane. CPC is bactericidal because of its effects on the cytoplasmic membrane of the bacterial cells; there are not specific receptor molecules to assist biocide penetration (Russell and Chopra 1996, Russell, 1998).

Previous studies with tomato and cantaloupes inoculated with human pathogens has revealed that when the time interval between inoculation and washing with sanitizer agent increased from one hour to several days, the efficacy of sanitizer treatment in reducing pathogen populations decreased (Ukuku and Sapers, 2001; Sapers and Jones, 2006).

In this study we demonstrate that a spray application of CPC solution can reduce *Salmonella* Michigan between 2.34 to 4.95 log CFU/ml. We can compare this result to

the previous work done by Hong et al., (2001), where experimental laboratory controlled research on vegetables treated with 0.1 and 0.5% CPC reduced *Salmonella* Typhimurium by 2.37 and 3.15 log CFU/g. Araya et al., (2008) found that the use of a 5 sec dip in 0.5% CPC significantly improved the microbial shelf life of cantaloupes and spanish melons when applied either directly to field harvested melons or after the current commercial processing and washing procedures allowing for a 99% reduction in aerobic plate count.

According to Fletcher (1996), bacterial adhesion occurs in three steps: reversible absorption, primary adhesion, and colonization. The initial reaction between an antibacterial agent and a bacterial cell involves binding to the cell surface. Changes to outer layers may then occur to allow agents to penetrate the cell to reach their primary site of action and the cytoplasmic membrane or within the cytoplasm. The toxicology effects of CPC on bacteria are caused by the CPC absorbing onto the cell well and the cell membrane (Cutter et al., 2000). The degree of damage to bacterial mebrane is time and concentration dependent (Kim and Slavik 1996). The effect on the primary target site may lead to additional, secondary, changes elsewhere in the organism. Such secondary alteration may also contribute to the bactericidal activity of the CPC (Russell and Chopra 1996).

CPC has been reported to be bactericidal to Gram-positive bacteria but relatively ineffective against some Gram-negative bacteria (Baker et al., 1941). The results from these trials suggest that the use of a 1.0% CPC treatment as a direct spray solution on cantaloupes could significantly improve the overall microbial safety of fresh cantaloupe, by inhibiting *Salmonella*, a gram-negative pathogen. The effect of CPC treatments on

the reduction of attachment bacteria to cantaloupes rough surface varied depending of the time and type of netted surface.

In conclusion, the data shows that a CPC spray solution is highly effective for microbial reduction when it was applied after *Salmonella* application for both ‘Athena’ and ‘HBJ’. There is an urgent need to investigate more fully the nature of the inhibitory and lethal effects of cetylpyridinium chloride on a range of microorganisms and the mechanisms of inhibition and inactivation of Gram-negative bacteria, such as *Salmonella*, on fruits and vegetables.

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TABLES

Table 1: Log CFU/ml recovery from stem scar plugs (SCR) and skin (SKN) plugs of 'Athena' after 1hr or 24 hrs incubation periods and CPC spray solution applied.

Cetylpyridinium chloride (CPC)		Stem scar plugs (SCR)				Skin plugs (SKN)			
		1 hr		24 hrs		1 hr		24 hrs	
Athenas	Treatment	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD
	CONTROL	**7.79**[a]	0.78	**9.58**[a]	0.15	**7.51**[a]	0.59	**9.84**[a]	0.16
	BAC / 0.5% CHM	**6.20**[ab]	0.72	**9.40**[a]	0.50	**6.24**[ab]	0.09	**8.13**[a]	0.49
	0.5% CHM / BAC	**4.89**[b]	1.10	**9.57**[a]	0.12	**7.25**[a]	0.38	**9.10**[a]	0.83
	BAC / 1.0 % CHM	**4.72**[b]	1.22	**9.15**[a]	0.93	**5.17**[b]	0.83	**7.91**[a]	0.38
	1.0 % CHM / BAC	**5.79**[ab]	0.80	**9.15**[a]	0.24	**5.67**[ab]	1.22	**8.29**[a]	1.59

Column means with the same letter are not significantly different.

Table 2: Log CFU/ml recovery from stem scar plugs (SCR) and skin (SKN) plugs of 'HBJ' after 1hr or 24 hrs incubation periods and CPC spray solution applied.

Cetylpyridinium chloride (CPC)		Stem scar plugs (SCR)				Skin plugs (SKN)			
		1 hr		24 hrs		1 hr		24 hrs	
Hale's Best J.	Treatment	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD	Log CFU/ml	SD
	CONTROL	**7.17**^{a}	0.88	**8.72**^{a}	0.78	**7.43**^{a}	0.48	**9.17**^{a}	0.53
	BAC / 0.5% CHM	**5.53**^{ab}	1.24	**7.52**^{a}	1.63	**3.72**^{bc}	0.08	**8.76**^{a}	0.77
	0.5% CHM / BAC	**4.70**^{b}	0.37	**8.32**^{a}	0.40	**4.38**^{b}	0.43	**8.50**^{a}	0.22
	BAC / 1.0 % CHM	**6.00**^{ab}	0.22	**8.72**^{a}	0.19	**3.80**^{bc}	0.69	**8.06**^{a}	0.62
	1.0 % CHM / BAC	**3.61a**^{b}	0.38	**7.94**^{a}	0.32	**2.48**^{c}	0.98	**8.68**^{a}	0.09

Column means with the same letter are not significantly different.

Table 3: Color analysis of CPC spray application on whole cantaloupes 'Athena', stored for 14 days at 4 ^{o}C.

Non Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	46.99	0.67	45.94	1.17	44.72	1.70	43.11	2.49	43.11	4.24
a mean	49.34	0.98	46.63	2.09	48.08	0.67	46.75	1.54	47.50	1.00
b mean	52.00	0.69	50.65	0.70	50.29	0.90	48.53	2.80	49.02	2.98
			ΔE 3.21		ΔE 2.71		ΔE 4.46		ΔE 3.98	

0.5% CPC Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	43.13	2.62	44.23	2.11	43.26	3.86	42.40	0.89	41.49	1.52
a mean	46.75	0.86	46.73	3.56	46.09	1.07	48.55	1.57	45.86	1.18
b mean	49.59	1.03	51.55	1.40	48.52	2.85	49.14	1.01	47.54	1.14
			ΔE 2.25		ΔE 3.10		ΔE 2.64		ΔE 3.53	

1.0 % CPC Treatment										
	Day 1	SD	Day 2	SD	Day 5	SD	Day 7	SD	Day 14	SD
L mean	45.16	0.52	42.20	1.25	43.65	1.30	45.07	0.73	43.19	0.85
a mean	44.58	1.68	47.33	1.42	47.31	0.95	47.79	1.24	47.29	1.46
b mean	49.55	0.83	48.35	1.21	49.64	0.36	51.94	1.08	49.43	1.67
			ΔE 4.21		ΔE 1.99		ΔE 2.35		ΔE 3.23	

L = 0 yields black and L = 100 indicates diffuse white; spectacular white
a = negative values indicate green while positive values indicate magenta
b =negative values indicate blue and positive values indicate yellow
where ΔE= Total color difference

FIGURES

Figure 1: Log CFU/ml reduction of Salmonella from stem scar plugs (SCR) and skin plugs (SKN) from 'Athena' (A) and 'Hale best Jumbo. (HBJ)' after 1hr incubation periods and CPC spray solution applied.

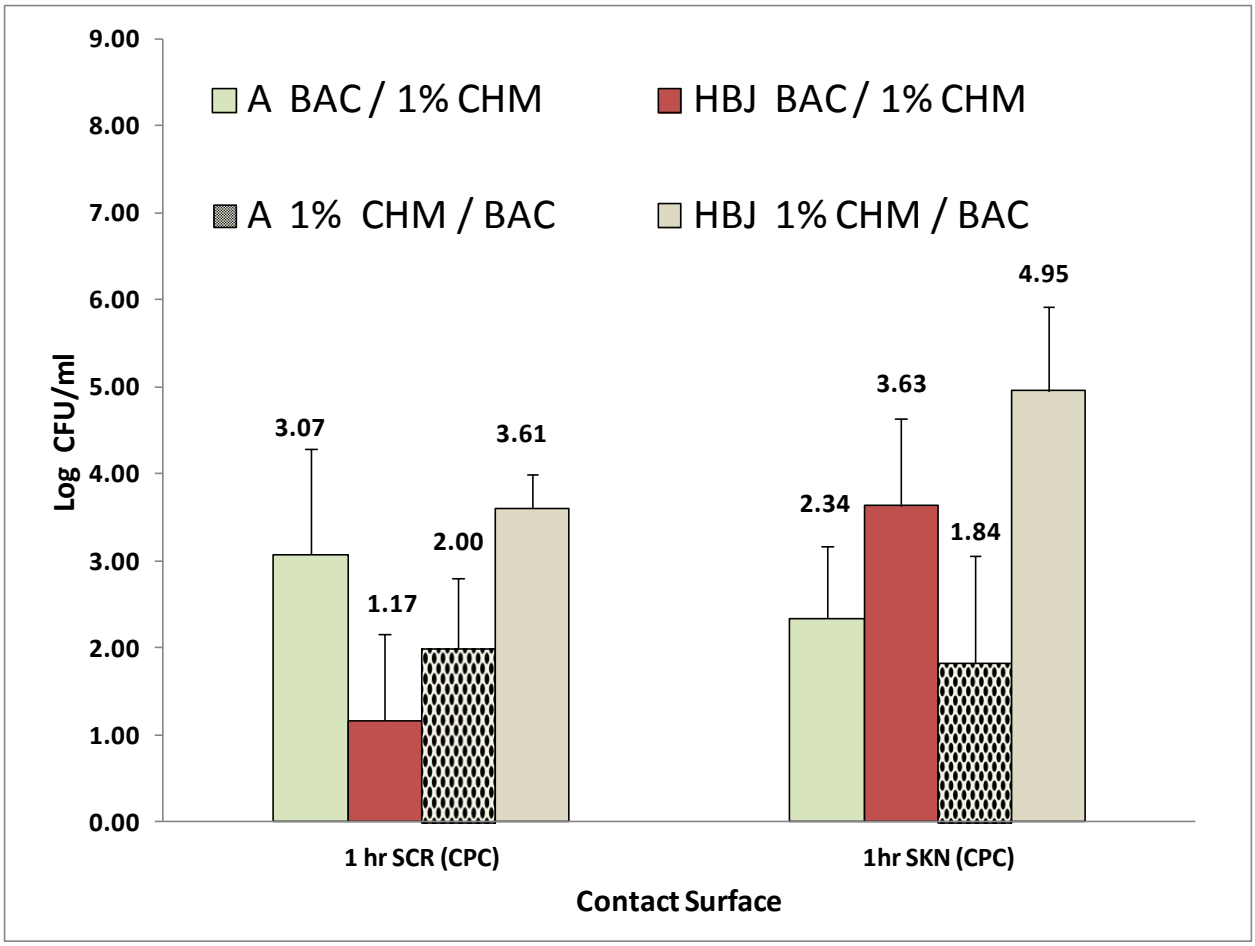
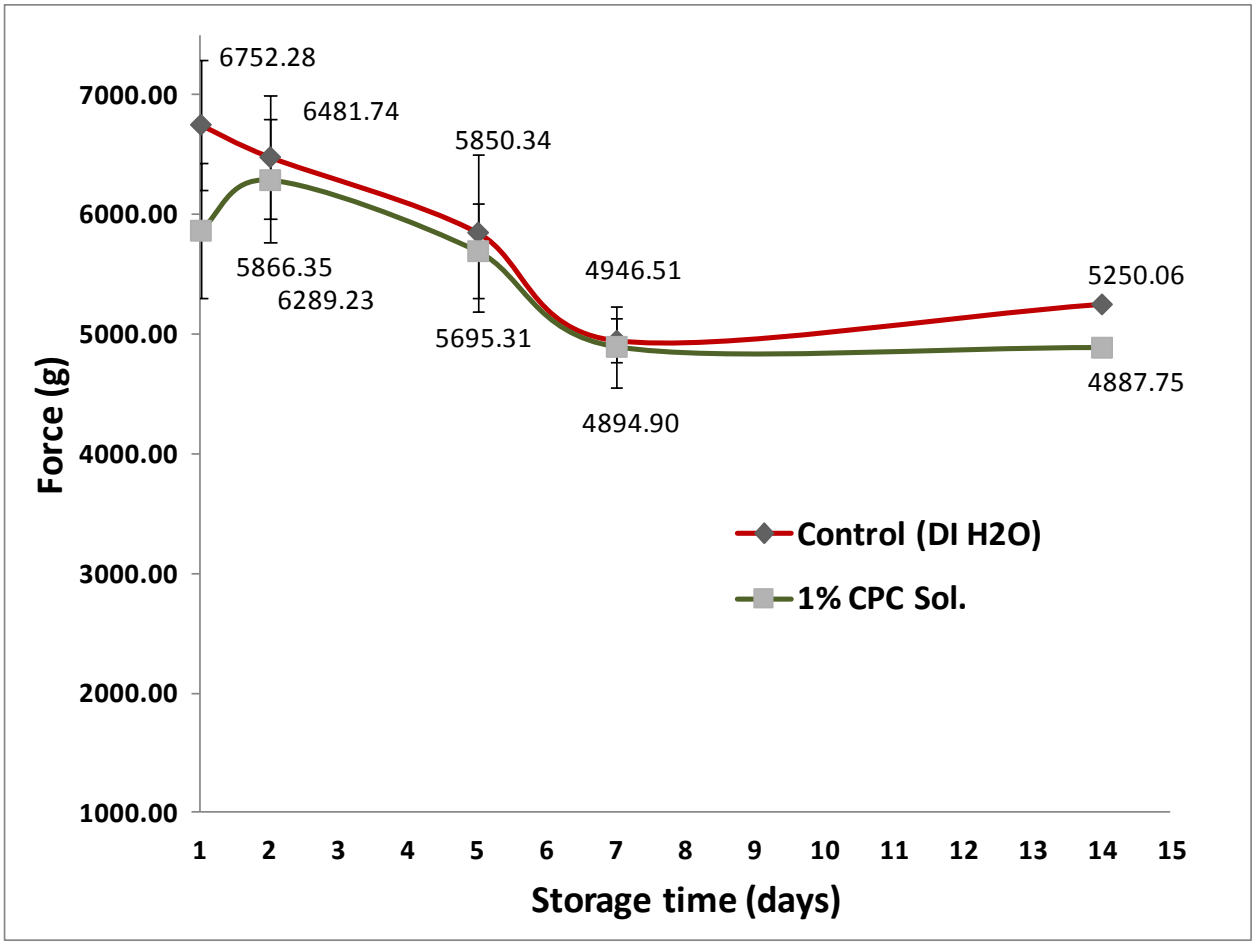


Figure 2 : Skin hardness test (force (g) applied) on whole cantaloupes ('Athena') after 0% (control) and 1.0% CPC spray solution applications and 1, 2, 5, 7 and 14 days storage at 4^{o}C.



CHAPTER 5

SUMMARY

The response of microorganisms to washing and sanitizing treatments will depend in part on the condition of contamination that affects attachment and survival on product surfaces (Sapers 2001). Conventional washing and sanitizing agents typically achieve 1-2 log units reduction in microbial populations under laboratory conditions, while reductions can be substantially smaller with commercials produce washing systems. Such reductions are not sufficient to assure microbiological safety. Among the factors limiting efficacy of conventional washing and sanitizing treatments are bacterial attachment to inaccessible sites, formation of resistant biofilms, and internalization of microorganisms within commodities (Sapers 2001).

Cationic surfactants including delmopinol and an antiseptic such as cetylpyridinium chloride (CPC) that interact strongly with cell surfaces could reduce the charge of the cell wall and, in some cases, reverse it and destroy the cell. The bactericidal activity of the biocide depends markedly on several factors, the most important are: time of exposure, concentration, temperature, pH, the presence of organic matter and the type of microorganism (Russell 1996). New washing procedures using cationic and antiseptic agents of greater lethality are needed to reduce and kill microorganisms on produce that survive conventional methods.

Salmonella cells that come in contact with the surface of cantaloupe melons in the field could easily become embedded within the fissures in the cuticle, protected from environmental stress and can survive through harvest and transport periods, as

evidenced by the large number of positive samples found during surveys of cantaloupe (USFDA 2001, 2003). Soil and soil amendments such as improperly composted manure, contaminated irrigation water, wild and domestic animals, and farm workers are potential vehicles of contamination of preharvest melons (Geldreich and Bordner 1971).

We have demonstrated that spray treatment with 1% CPC solution can reduce populations of *Salmonella* from netted surface of cantaloupes more than 3 log CFU/ml after 1hr at 35 ^{o}C on both cultivars 'Athena' and 'HBJ'. And, when 1% CPC spray solution was sprayed on cantaloupes before or after bacteria (BAC/CHM or CHM/BAC) a high reduction was observed for both cultivars and for both skin rind plugs (SKN) and stem scar rind plugs (SCR).

The results from these trials suggest that the use of a 1.0% CPC solution as direct spray solution on cantaloupes could significantly improve the overall microbial safety of fresh cantaloupe, by inhibition of human pathogens such as *Salmonella*. The effect of CPC treatments on the reduction of bacteria cells to cantaloupes rough surface varied depending on the time and type of netted surface.

Higher recovery (lower cell reduction) where spray "CHM" treatments were applied first and then bacteria "BAC" treatment (CHM/BAC) with 1% CPC was more effective compared to where the bacteria were inoculated, followed by the spray treatment (BAC/CHM). The antimicrobial effects of CPC are dependent on CPC binding to bacterial cells (Caputo et al*.,* 1975) and bactericidal activity in the presence of serum proteins and at different pH and temperature (Quisno and Foter 1946).

On the other hand, this study reports that when a direct spray of 1% delmopinol HCl was applied; there was an equivalent effect on either ‘Athena’ or ‘HBJ’ cantaloupes for skin (SKN) or stem scar (SCR) samples, thereby reducing *Salmonella* cells approximately 3.1 log CFU/ml after 1 hr. This demonstrates that 1% delmopinol hydrochloride could possess a light bactericidal and an anti-aggregate effect; this can make surface bacteria more susceptible to sanitizers or physical removal.

For 1% delmopinol hydrochloride and 1% CPC solution on stem (SCR) samples, we observed significance *Salmonella* reduction on ‘Athena’, but on skin samples a higher reduction was measured on ‘HBJ’ (Appendix H). No significant difference effect was observed at 24 hrs for ‘Athena’ or ‘HBJ' on skin (SKN) and stem scar (SCR) after 1% CPC solution nor 1% Delmopinol HCl application. No color or texture change was observed after 7 or 14 days of storage at 4 ^{o}C for either 1% CPC solution nor 1% Delmopinol HCl on ‘Athena’ or ‘HBJ’ cantaloupe.

Even though the bactericidal activity observed in the present study by CPC solution and delmopinol, at 1% concentrations, was efficient, a greater understanding of these oral hygiene chemicals on gram negative bacterial cells is clearly needed. Also, for other oral antiseptic (biocides) with antibacterial properties including compounds such as triclosan, thymol and **c**hlorhexidine there is an urgent need to investigate possible mechanisms of inhibition and inactivation of Gram-negative bacteria on organic surfaces such as fruits and vegetables. Further research could focus on optimizing chemical concentrations for lethality, exposure times, and possible combination of two or more of these compounds for treatment of fruits and vegetables to enhance food safety.

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APPENDICES

Appendix A: *Salmonella* Michigan identification and confirmation test.

Salmonella Michigan identification and confirmation test.

To identify and confirm the use of *Salmonella* Michigan, the API Kit, API 20 E, identification System (bioMérieux, Inc., Durham, NC) was used. API 20 E is a standardized identification system which uses 21 miniaturized biochemical tests to differentiate Enterobacteriaceae and other non-fastidious, Gram negative rods

Appendix B: Determination of Cetylpyridinium chloride (CPC) concentration.

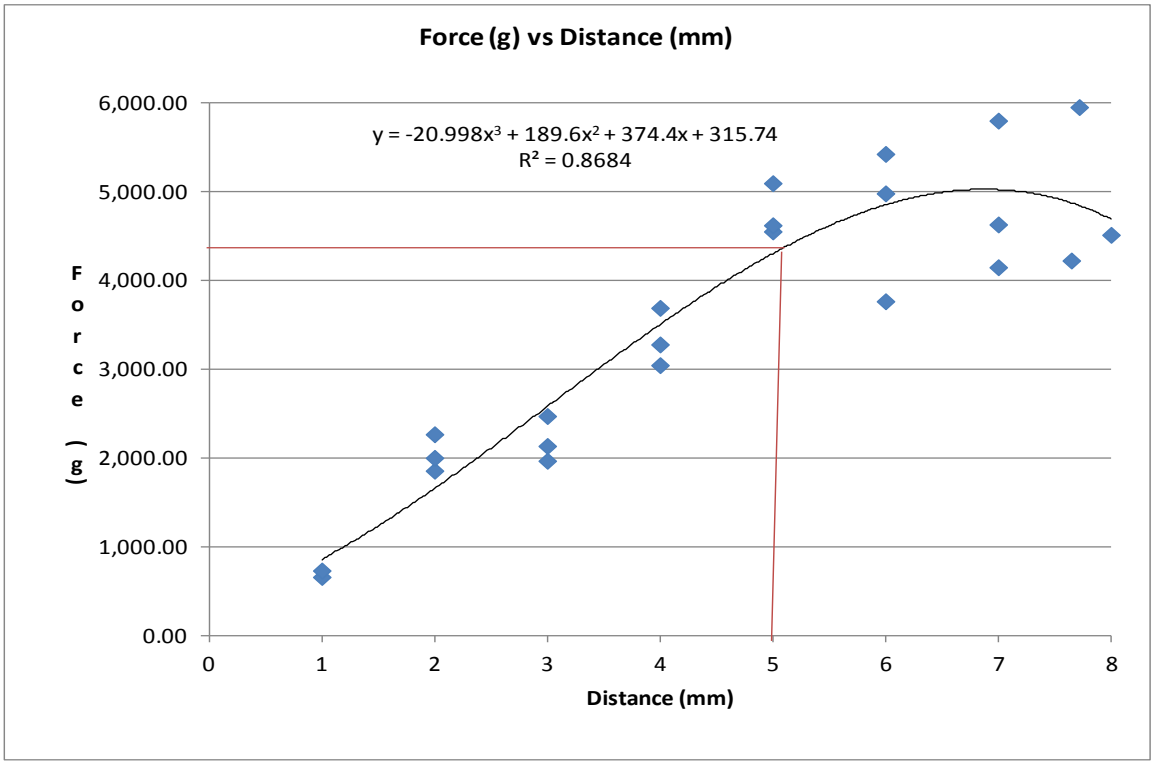
Determination of Cetylpyridinium chloride (CPC) concentration.
Cetylpyridinium chloride (CPC) concentrations were confirmed using a LaMotte direct reading titration test kit, 1649, Chestertown, Maryland, provided by Safe Foods Corporation, North Little Rock, AR.

Appendix C: Calculation of maximum penetration (Distance – Force) used for texture measurement for whole cantaloupe.

Batch	Distance	Force	Ave	Difference increase	Area F-T 1:2	Area F-T 2:3	Total area
	mm	g			g.sec	g.sec	
Control							
Control	1.00	661.63	1028.60		150.271	43.851	194.122
Control	1.00	733.95		1015.37	170.778	48.427	219.206
Control	2.00	1858.63	2043.97		865.122	166.433	1031.555
Control	2.00	2003.70			828.130	184.907	1013.036
Control	2.00	2269.59		150.29	947.349	220.076	1167.424
Control	3.00	2137.68	2194.26		1379.363	216.709	1596.072
Control	3.00	2474.58			1637.440	266.834	1904.274
Control	3.00	1970.53		1146.72	1422.859	197.390	1620.249
Control	4.00	3048.12	3340.98		2913.467	367.232	3280.699
Control	4.00	3281.39			3157.545	376.180	3533.725
Control	4.00	3693.44		1146.72	3558.580	436.356	3994.937
Control	**5.00**	4554.84	**4759.36**		5625.597	621.670	6247.268
Control	**5.00**	5098.69			6170.875	683.735	6854.610
Control	**5.00**	4624.55		**1418.38**	5497.066	599.255	6096.321
Control	6.00	5428.42	4726.88		7959.062	793.557	8752.620
Control	6.00	4983.96			7429.384	701.675	8131.059
Control	6.00	3768.26		-32.48	5599.502	530.116	6129.618
Control	7.00	5803.27	4862.85		10924.381	883.302	11807.682
Control	7.00	4633.68			9392.882	637.805	10030.687
Control	7.00	4151.60		135.97	6594.356	576.525	7170.881
Control	8.00	4516.34	4899.82		10284.203	629.037	10913.240
Control	7.72	5955.96			12734.193	1751.312	14485.505
Control	7.65	4227.18		36.97	9652.935	1291.658	10944.592

Calculation of maximum penetration (Distance – Force) used for texture measurement for whole cantaloupe. Pressure was applied for each mm of displacement until the skin Broke. (5.00 mm x g of pressure)

Appendix D: Calculation of maximum force applied to whole cantaloupe skin.



Calculation of maximum force applied to whole cantaloupe skin.
The figure shows the exact area where skin tension are disrupted and consequently the skin brake. Force applied after 5 mm of depth give scattered data.

Appendix E: Microbial recovery spreadsheet data sample for 0.0% CPC (control), 0.5% CPC, 1% CPC, 1% delmopinol treatments.

For: **Athenas** Part: Time: **24 Hrs** type **SAN/BAC**
ST1, ST2,ST3

Treatment	Sample #	CFU/ml	CFU/ml	AVG (CFU/ml)	Log CFU/ml	AVG (CFU/ml) Total	Log CFU/ml
Control	1	3.01E+09	2.32E+09	2.67E+09	9.43		
Control	2	2.92E+09	1.52E+09	2.22E+09	9.35		
Control	3	2.92E+09	1.52E+09	2.22E+09	9.35	2.37E+09	9.37
Control sonicated	1	1.67E+09	1.59E+09	1.63E+09	9.21		
Control sonicated	2	1.48E+09	1.64E+09	1.56E+09	9.19		
Control sonicated	3	1.48E+09	1.64E+09	1.56E+09	9.19	1.58E+09	9.20
Control + Control sonicated	1	4.68E+09	3.91E+09	4.3E+09	9.63		
Control + Control sonicated	2	4.40E+09	3.16E+09	3.78E+09	9.58		
Control + Control sonicated	3	4.40E+09	3.16E+09	3.78E+09	9.58	3.95E+09	**9.60**
0.5% CPC	1	2.12E+08	9.52E+08	5.82E+08	8.76		
0.5% CPC	2	1.40E+09	2.26E+09	1.83E+09	9.26		
0.5% CPC	3	3.59E+09	2.79E+09	3.19E+09	9.50	1.87E+09	9.27
0.5% CPC sonicated	1	3.65E+09	2.79E+09	3.22E+09	9.51		
0.5% CPC sonicated	2	1.52E+09	3.10E+08	9.15E+08	8.96		
0.5% CPC sonicated	3	1.72E+09	1.56E+09	1.64E+09	9.21	1.93E+09	9.28
0.5% CPC + 0.5% CPC sonicated	1	3.86E+09	3.74E+09	3.8E+09	9.58		
0.5% CPC + 0.5% CPC sonicated	2	2.92E+09	2.57E+09	2.75E+09	9.44		
0.5% CPC + 0.5% CPC sonicated	3	5.31E+09	4.35E+09	4.83E+09	9.68	3.79E+09	**9.58**
1% CPC	1	4.60E+08	3.06E+08	3.83E+08	8.58		
1% CPC	2	2.80E+08	1.40E+09	8.40E+08	8.92		
1% CPC	3	1.93E+09	2.19E+09	2.06E+09	9.31	1.09E+09	9.04
1% CPC sonicated	1	1.44E+09	1.14E+08	7.77E+08	8.89		
1% CPC sonicated	2	1.10E+08	1.20E+08	1.15E+08	8.06		
1% CPC sonicated	3	2.10E+08	2.10E+07	1.16E+08	8.06	3.36E+08	8.53
1% CPC + 1% CPC sonicated	1	1.90E+09	4.20E+08	1.16E+09	9.06		
1% CPC + 1% CPC sonicated	2	3.90E+08	1.52E+09	9.55E+08	8.98		
1% CPC + 1% CPC sonicated	3	2.14E+09	2.21E+09	2.18E+09	9.34	1.43E+09	**9.16**
1% Delmopinol	1	7.14E+08	2.24E+08	4.69E+08	8.67		
1% Delmopinol	2	3.12E+08	2.59E+08	2.86E+08	8.46		
1% Delmopinol	3	3.20E+08	2.70E+08	2.95E+08	8.47	3.50E+08	8.54
1% Delmopinol sonicated	1	5.47E+08	3.65E+08	4.56E+08	8.66		
1% Delmopinol sonicated	2	1.44E+08	2.60E+09	1.37E+09	9.14		
1% Delmopinol sonicated	3	4.00E+08	1.10E+08	2.55E+08	8.41	6.94E+08	8.84
1% Del.+ 1% Del. sonicated	1	1.26E+09	5.89E+08	9.25E+08	8.97		
1% Del.+ 1% Del. sonicated	2	4.56E+08	2.86E+09	1.66E+09	9.22		
1% Del.+ 1% Del. sonicated	3	7.20E+08	3.80E+08	5.5E+08	8.74	1.04E+09	9.02

Microbial recovery spreadsheet data sample for 0.0% CPC (control), 0.5% CPC, 1% CPC, 1% delmopinol treatments. Data includes enumerated rinses without sonication, sonicated rinses only and the sum of recoveries before sonication and with sonication.

Appendix F: Cantaloupe pictures

Figure F1: *Cucumis melo* var*. reticulatus:* Athena Cultivar

Figure F2: *Cucumis melo* var. *reticulatus:* Hales Best Jumbo Cultivar

Figure F3: Stem scar rind plugs sample

Figure F4: Skin rind plug samples (Athena).

Appendix G: Preliminary test to compare and choose percentage of CPC to use for the experimental design.

Means comparisons for all pairs using Tukey-Kramer HSD

Analysis of variance

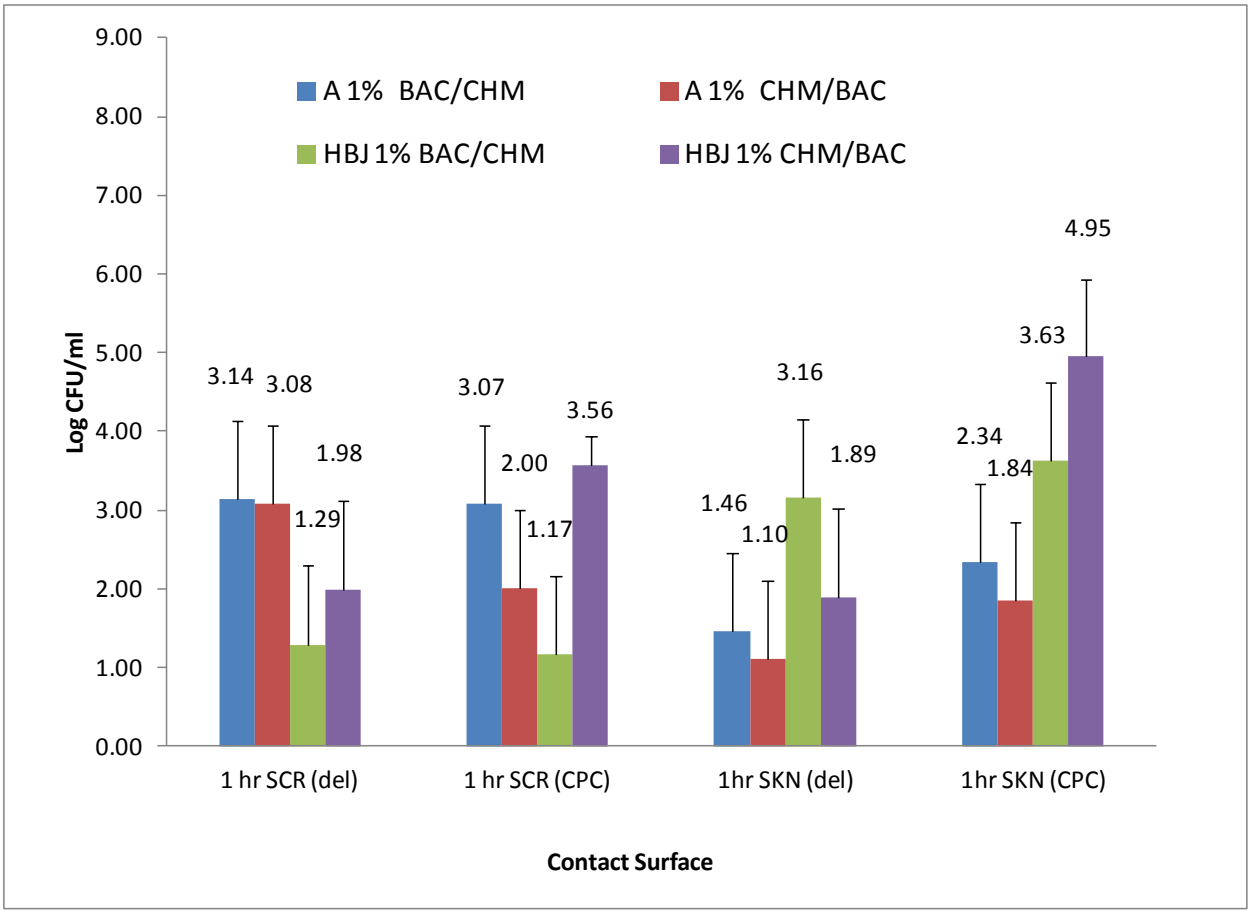
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	2.4833376	0.827779	7.5437	0.01
Error	8	0.8778446	0.109731		
C. Total	11	3.3611822			

Level			Mean
Control	**A**		9.3541881
0.2% CPC	A	B	8.5992646
0.5% CPC		**B**	8.2963987
1.0 % CPC		**B**	8.1910699

Levels not connected by same letter are significantly different.

Preliminary test to compare and choose percentage of CPC to use for the experimental design. 0.5 % and 1% CPC were used for the experiment deign because there was no a significantly different using 0.2% CPC, on preliminary data.

Appendix H: Maximum log CFU/ml reduction of *Salmonella* from stem scar plugs (SCR) and skin plugs (SKN) from 'Athena' (A) and 'Hale Best Jumbo (HBJ)' after 1hr incubation periods and 1% delmopinol HCl or 1% CPC spray solution applied, high reduction observed on both cultivars



Maximum log CFU/ml reduction of *Salmonella* from stem scar plugs (SCR) and skin plugs (SKN) from 'Athena' (A) and 'Hale Best Jumbo (HBJ)' after 1hr incubation periods and 1% delmopinol HCl or 1% CPC spray solution applied, high reduction observed on both cultivars