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Antimicrobial activity of CPC and ASC against foodborne pathogens and the physiological effect on fresh-cut cantaloupe cubes at refrigerator temperatures

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ANTIMICROBIAL ACTIVITY OF CPC AND ASC AGAINST FOODBORNE PATHOGENS AND THE PHYSIOLOGICAL EFFECT ON FRESH-CUT CANTALOUPE CUBES AT REFRIGERATOR TEMPERATURES

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Dedicated to
My Grandparents,
Mrs. Marie K. Nipper and the late Weldon A. Nipper
And
The late Mr. and Mrs. William L. Dumas

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ABSTRACT

In the last few decades, fresh-cut fruit products have gained popularity with consumers. They require little to no further processing prior to consumption. Fresh-cut products also make fruits and vegetables more conveniently available to consumers year-round. There are limitations, however, to fresh-cut fruit and vegetables in the marketplace. These products are very perishable and could become contaminated with foodborne pathogens. Most fresh-cut fruits and vegetables will only last a short period of time at refrigerator temperatures. There are many possible routes of bacterial contamination in the fresh-cut produce industry. There is a need for antimicrobial compounds that can be applied to fruits and vegetables to help maintain the shelf-life of these products without greatly altering their sensory qualities. Cetylpyridinium chloride (CPC) and acidified sodium chlorite (ASC) have both been shown to have antimicrobial effects.

This study was conducted to determine if cetylpyridinium chloride and acidified sodium chlorite would be effective in reducing *E. coli* 0157:H7, *Salmonella* Montevideo, and *Shigella sonnei* on the surface of inoculated and stored fresh-cut cantaloupe cubes. The effect of these compounds on the physiological quality of fresh-cut cantaloupe cubes was also investigated. Results obtained from this study show that both compounds were effective in significantly reducing the three pathogens. Significant reductions were achieved during both a preliminary 24 hour storage study and a 12 day shelf life study for all three pathogens. The 1.00% CPC and 1000 ppm ASC concentrations were most effective at reducing the three pathogens. It was also determined that CPC and ASC did not greatly affect the physiological quality (°Brix, firmness, color) of fresh-cut cubes stored at refrigerator temperatures for up to fourteen days. Treatment of fresh-cut cantaloupe cubes with CPC caused significant interaction effects between treatment levels and sampling day on the recovery of several characteristic impact flavor and aroma

compounds (CIFAC). Concentrations of ASC caused significantly higher levels of certain volatiles to be recovered over the 14 day study.

CHAPTER 1

INTRODUCTION

Fresh produce is an important part of a well-balanced diet. Produce provides us with vitamins, minerals, fiber and antioxidants among other nutrients (Anonymous 2004). Fresh-cut produce is a recent and expanding part of the produce industry. According to the International Fresh-Cut Produce Association, fresh-cut produce is any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state (Anonymous 2006). These products are peeled, sliced, cut, washed, or diced prior to sale to provide the consumer with more convenience. This sector of the industry has grown due to popularity with both retail and foodservice markets. Annual sales for the fresh-cut produce industry are currently in the $10-12 billion range (Anonymous 2006). It is estimated that the fresh-cut produce industry will continue to grow at a rate of 10-15% a year for the next five years in the United States. Fresh-cuts have become popular because consumers desire foods that are ready to cook or eat. A number of factors play into the consumer acceptability of fresh-cut fruits. Three of the most important factors are appearance, texture and flavor. First time purchases are largely based on the appearance and freshness but subsequent purchases depend on the consumer being satisfied with the texture and flavor of the product (Beaulieu and Baldwin 2002).

The Centers for Disease Control and Prevention (CDC) estimate that 76 million people become sick, more than 325,000 people are hospitalized, and 5000 people die from foodborne illnesses each year in the United States (Beuchat and Ryu 1997). It is very likely; however, that most infections go undiagnosed and unreported (Tauxe 1997). Even though they provide convenience to the consumer, fresh-cut fruits also provide an opportunity for microbial contamination. It is possible, given enough time at an appropriate temperature, that there can be populations of bacteria measuring over 10^7 CFU/g on the surface of produce (Beuchat and others 2001). It is recommended that consumers buy undamaged and unbruised fruits. They should

wash fruits with cool tap water immediately before slicing, avoid using soaps or detergents, and scrub fruits with a clean produce brush (Barak and others 2003). Incidences of foodborne illness seem to increase during the summer. The reasons behind this are not fully understood but it may be due to the fact that fresh produce is consumed in higher amounts during warmer months (Beuchat and Ryu 1997). Contamination of produce can occur at many points along the production chain. This includes processes before, during and after harvest of fresh produce. Contamination can also occur after purchase due to improper handling or storage. Contamination can come from both internal and external sources. Some preharvest sources of contamination include soil, irrigation water, inadequately composted manure, insects, wild and domestic animals, flooding and human handling. The chance for post-harvest contamination of fresh-cut fruits is great because of increased handling. Some post-harvest sources of contamination include human handling, harvesting equipment, insects, wash and rinse water, ice, improper storage, cross-contamination with other foods, and improper handling after purchase.

Species of *Salmonella*, *Escherichia coli* and *Shigella* have all been implicated in foodborne disease outbreaks associated with fresh-cut produce. The FDA performed a survey of domestic fresh produce in 2000 (Anonymous 2003). Many different fruit and vegetable samples were analyzed for the presence of *Salmonella* and *E. coli* 0157:H7. Some of the samples, including cantaloupe, were also analyzed for *Shigella*. A total of 1028 samples were collected for the study. Of these samples, only eleven tested positive for the presence of pathogens. *E. coli* 0157:H7 was not found on any samples (Anonymous 2003). Cantaloupe had the highest number of positive results with five, testing positive for *Salmonella* four times and *Shigella* once. *Salmonella* and *E. coli* are especially of interest to the fresh-cut produce industry. Several serovars of *Salmonella* have caused large outbreaks in association with the consumption of fresh-

cut cantaloupe (Mohle-Boetani and others 1999; Anonymous 1991; CDC 2002). There has been one outbreak associated with cantaloupe and *E. coli* 0157:H7 in August of 1993 (Del Rosario and Beuchat 1995).

Currently, the only compound approved for use as an antimicrobial agent on fresh-cut fruits and vegetables is sodium hypochlorite. Many other treatments have been tested on fresh-cut cantaloupe as well as other fruits and vegetables. Any treatment applied to fresh produce must be able to remove or inhibit any microorganisms present on the flesh. They must have as little effect on the sensorial and physiological attributes of the produce as possible. Treatments for fresh-cut fruits must be specialized because the physiology of fresh-cut fruits is very different from that of whole fruits. These treatments must help maintain the appearance of the product (Luna-Guzman and Barrett 2000). Cetylpyridinium chloride (CPC) and acidified sodium chlorite (ASC) have the potential to be effective antimicrobial agents on fresh-cut fruits. CPC is currently approved for use in raw poultry carcasses at levels not exceeding 0.3 grams per pound of carcass (FDA 2004). Acidified sodium chlorite has been approved for use on red meats, poultry, seafood and raw agricultural products at levels not to exceed 1200 ppm (FDA 2005).

The objective of this study was to determine if CPC and ASC would be effective as antimicrobial agents against *Salmonella* Montevideo, *Escherichia coli* 0157:H7 and *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes stored at refrigerated temperatures. This study also was undertaken to determine what effect these two compounds might have on the physiological properties of fresh-cut cantaloupe cubes stored over time.

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CHAPTER 2

LITERATURE REVIEW

Cantaloupe Melons (*Cucumis melo* L.)

Cantaloupe (*Cucumis melo* L.) is a tropical and subtropical fruit that is a member of the Cucurbitaceae family. There are approximately ninety genera and 750 species in this family (Whitaker and Davis 1962). This family includes produce such as watermelon (*Citrullus vulgaris*), squash (*Cucurbita pepo*), and cucumber (*Cucumis sativus*). The *Cucumis* L. genus consists of about thirty species. Cantaloupes are also known as muskmelons, nutmeg melons, winter melons, sweet melons, rock melons, and snap melons (Robinson and Decker-Walters 1997). The exact place of their origin is unknown but it is believed to be Africa. It is believed that these melons were brought to Cantaluppi, Italy from Africa and the Middle East. From there the melons were introduced to Europe and finally North America. The melons primarily sold in Europe are considered to be true cantaloupe melons from the *Cucumis melo* L. var. *cantalupensis* group. These melons are usually medium-sized fruits with netted, warty or scaly surfaces. The flesh of these melons is usually orange but sometimes green (Robinson and Decker-Walters 1997). The group of melons primarily grown and sold in the United States is *Cucumis melo* L. var. *reticulates*. These melons are usually larger with varied netting and more ribbing (Robinson and Decker-Walters 1997) than the *cantalupensis* group. Recently, the *Cucumis melo* L. *reticulates* and *cantalupensis* groups were combined into one group, *cantalupensis*, due to many similarities between the groups (Munger and Robinson 1991).

Melons, including cantaloupe, are one of the most widely produced fruits in the world. They rank behind only oranges, bananas, and grapes. In terms of cucurbits, melons are ranked third in production behind watermelon and cucumbers (Nayar and More 1998). Due to advances in agronomic practices, melons can be grown year round with suitable growing conditions. Cucurbits, such as cantaloupe melons, are not normally regarded as highly nutritious. However,

they do have many attributes that make them attractive to consumers. Like most cucurbits, the cantaloupe has very high moisture content. Typically, cantaloupes consist of approximately ninety-one percent moisture (Robinson and Decker-Walters 1997). They are low in fat and calories. Cantaloupes are high in carbohydrates, as are most fruits. The sugar content of cantaloupes is generally between eight and fourteen percent. One nutrient cantaloupes seem to have in abundance is vitamin A. There are approximately 3400 IU of vitamin A per 100 grams of cantaloupe (Robinson and Decker-Walters 1997). There are also low levels of tocopherols and tocotrienols present in cantaloupes (Chun and others 2006). The seeds can also be nutritious. They can be good sources of protein, calcium, phosphorus, iron, magnesium, arginine, methionine, aspartic acid, glutamic acid, thiamin and niacin (Robinson and Decker-Walters 1997).

Fresh-Cut Cantaloupes and Melons

Fruits and vegetables are an essential part of the human diet. They provide many important vitamins and minerals needed to sustain a healthy body. Consumption of fruits and vegetables has long been shown to help ward off illness and protect against certain diseases. Fresh-cut fruits have become increasingly more popular with consumers in the United States. Fresh-cut produce is defined as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state (Anonymous 2006b). Minimal processing may include processes such as dicing, trimming and low level irradiation (O'Connor-Shaw and others, 1994). The demand for fresh-cut fruits has risen dramatically in the last two decades. Sales of fresh-cut produce are between $10-12 billion dollars annually in the United States (Anonymous 2006b). Consumers want and need foods that are convenient and eaten quickly. Sales show that for fresh-cut salads consumers will pay for the convenience of

fresh-cut, if quality is perceived to be better than or equal to uncut product (Beaulieu and Lea 2003). Fresh-cut products are not only popular in retail markets but with the foodservice industry as well. These products are convenient because they have been thoroughly washed, cut and packaged before selling (Anonymous 2006). Probably the most important attribute of fresh-cut fruits is that they have a fresh and appealing appearance. This should mean that the product is relatively fresh in terms of days since processing (Beaulieu and Baldwin 2002). Initial purchases of fresh-cut produce are most often based on appearance of the product. Repeat purchases are normally driven by factors such as flavor and texture (Beaulieu and Baldwin 2002).

There are many processes that can affect internal quality factors. Quality can be affected both before and after processing. When fruits and vegetables are processed tissue disruption takes place causing vacuolar, cytoplasmic and nucleic enzymes and substrates to mix (Beaulieu 2005). Disruption leads to accelerated respiration of the plant tissue as well as physical decay (Bolin and Huxsoll 1989). It may also increase microbial spoilage through transfer of skin microflora to fruit flesh (O'Connor-Shaw and others 1994). One pre-harvest determinant of quality is the maturity of the commodity. In most climacteric muskmelons, an abscission layer where the stem attaches to the fruit begins to separate with ripening. The degree of separation is known as "slip". Partial to complete separation indicates full ripeness in melons. In the United States, the standard practice for commercial growers is to harvest melons at ¾ to full slip (Beaulieu and others 2004). Firm melons are desired for processing due to the fact that they hold up better when shipped and handled. Less mature fruit may also have a longer shelf-life than fully ripe melons. After harvesting, quality of fruit is mainly based on soluble solids content. This is often the only reliable, easily measured quality assessment for determining optimum melon harvest (Beaulieu and Lea 2003). Beaulieu and others (2003) showed that soluble solids

content may vary among commercial cultivars of cantaloupe melons as well as their parental lines. They also showed that soluble solids content may be affected by the growing season. Beaulieu (2005) showed that there were again differences in °Brix between commercial cultivars of cantaloupe.

Most consumers associate foodborne illness outbreaks with foods of animal origins. Fresh-cut produce has been implicated in several foodborne disease outbreaks in recent years. Outbreaks associated with fresh fruits and vegetables have nearly tripled since 1973 (Gagliardi and others 2003). The increase in outbreaks coincides with increased intake of fruits and vegetables (Osman and others 2006). Fruits, such as cantaloupe, provide an ideal environment for the growth and multiplication of foodborne pathogens. The flesh of most fruits is high in carbohydrates (Ray 2001) and the near neutral pH of most melons allows for pathogens to proliferate. The netting also gives the cantaloupe rind inherent surface roughness that favors microbial attachment (Ukuku 2006). There are many possible ways for fresh produce to be contaminated with foodborne pathogens. Contamination can occur at any time from the planting to the point of consumption. It is very possible for fruits and vegetables to be contaminated prior to harvest. The soil and water used during growing could be contaminated with human pathogens. Manure contaminated with pathogens such as *E. coli* 0157:H7 and *Salmonella* could be used as fertilizer in the fields. It is also possible for wild animals that serve as reservoirs for human pathogens to find access to growing fields. During and after harvest there is still a chance for contamination of whole and processed produce. Some of the pre-harvest sources can also cause contamination after harvest (Beuchat and Ryu 1997). In addition to these sources, harvesting equipment, wash and rinse water, human handling and processing equipment can all cause contamination. Contamination is also possible in retail markets, foodservice establishments

and the home. Several trade organizations have issued voluntary guidelines for several commodities, including melons, to help prevent contamination along the production chain. One document suggests that growers and processors train all employees to help avoid contamination through human handling (Anonymous 2005a). It also suggests monitoring of harvesting and processing equipment to ensure that cross contamination does not occur. Proper refrigeration of fresh-cut melons was also discussed. The guidelines suggest keeping fresh-cut melons between 0 and 5°C to help retard microbial growth (Anonymous 2005a). Bacterial populations on fresh produce can be as high as 10^7 colony forming units (CFU) per gram after harvesting, given enough time at the appropriate temperature (Beuchat and others 2001). Species of *Salmonella* and *Escherichia coli* are most often the causative agents in foodborne disease outbreaks associated with whole and fresh-cut produce. Other microorganisms have been implicated in foodborne disease outbreaks with fruits including species of *Shigella*.

Salmonella

Salmonella is a mesophilic Gram-negative rod. They are facultative anaerobes meaning they can survive with or without the presence of oxygen. There are over 2000 serovars of *Salmonella*. Most of the serovars are motile and are nonsporulating. *Salmonella* is normally found in the gastrointestinal tract of humans, animals, birds and insects. It has also been detected in soil, water and sewage contaminated with fecal matter (Ray 2001).

All serovars of *Salmonella* are considered to be pathogenic to humans. The illness caused by these bacteria is known as salmonellosis. It can be caused by the consumption of food containing viable cells of *Salmonella*. There are an estimated 2 to 4 million cases of salmonellosis that occur in the United States each year (Anonymous 1992b). Once ingested, the cells invade the mucosa of the small intestine, proliferate in the epithelial cells and produce a

toxin (Ray 2001). This toxin causes an inflammatory response in the intestine. Illness can be caused by as few as 15 to 20 cells (Anonymous 1992b). The amount of cells needed to cause illness largely depends on the age and health of the host. Salmonellosis can affect any age group but the elderly, infants and the immunocompromised are most often affected. Symptoms of salmonellosis can include abdominal cramps, diarrhea, nausea, vomiting, chills, fever, and prostration (Ray 2001). Depending on the person affected and the amount of cells ingested, symptoms usually appear within eight to 42 hours. They last for approximately two to three days. Some chronic symptoms could last for weeks however.

Foods of animal origin are most often implicated in outbreaks of salmonellosis. Various strains of *Salmonella* have been shown to cause illness when consumers eat contaminated raw meat, poultry, eggs, and dairy products. Fresh-cut fruits, such as cantaloupe, have been involved in several large outbreaks of salmonellosis in the last few years. Several large, multi-state outbreaks of salmonellosis involving cantaloupe have been reported since 1990. During June and July of 1991, *Salmonella* Poona was associated with more than 400 illnesses in 23 states and outbreaks in Canada (Anonymous 1991). Several laboratories were able to confirm *Salmonella* Poona as the causative agent. After recording food histories for both ill and control subjects, many recalled eating cantaloupe in fruit salad. One instance in New Jersey led to several guests at a party becoming ill after consuming fruit salad. The caterer had purchased cantaloupes from the same region the other contaminated cantaloupes had originated from. The cantaloupes were traced back to growers in several Texas counties. An outbreak in March 1997 was linked to *Salmonella* Saphra. This outbreak involved several persons who resided in twelve counties in California (Mohle-Boetani and others 1999). Food histories for ill subjects as well as case controls were again collected. Of the fifteen ill subjects who reported eating cantaloupe, 9 had

purchased cantaloupes from grocery stores, 3 ate cantaloupe at a restaurant and 3 others had both exposures (Mohle-Boetani and others 1999). Of those purchasing the cantaloupe in a grocery store, six subjects purchased the melon whole while five purchased pre-cut cantaloupe. Only one subject washed the whole cantaloupe prior to cutting. After further analysis of the food histories for the subjects, it was determined that all cantaloupes had come from the same shipper located in southern California. The source of these cantaloupes was from a packer in Mexico. No further cases were reported because imported cantaloupes were replaced by domestic cantaloupes (Mohle-Boetani and others 1999). A series of outbreaks occurred in the spring of the years 2000, 2001, and 2002. In the 2000 outbreak, 47 cases in six states were confirmed with *S*. Poona infections (CDC 2002). The median age of the infected patients was seven years old and most recalled consuming cantaloupe. For the 2001 outbreak, there was an initial cluster of infections in California. More cases were reported from five other states for a total of 50 infections. The age of the infected individuals was of interest in this outbreak. Nineteen children had a median age of three years while nine adults had a median age of 80 years. These ages constitute two at-risk groups for salmonellosis. Two older patients did in fact die due to *Salmonella* septicemia. Illness in this outbreak was only associated with eating cantaloupe (CDC 2002). There were a total of 58 cases in the 2002 outbreak. Ten states and four Canadian provinces reported cases of *S*. Poona infections. Seventy four percent of case patients in this outbreak recalled eating cantaloupe (CDC 2002).

The significance of the three outbreaks in 2000, 2001 and 2002 is the impact they had on the importation of cantaloupe. Investigations by the FDA found that the cantaloupes associated with these outbreaks had come from shippers and farms in Mexico. It was determined that these farms did not have proper controls in place to keep foodborne pathogens from contaminating

their product. Due to the 2001 and 2002 outbreaks, the shipper voluntarily recalled all cantaloupes that had been imported to the United States. It is important to note that *S*. Poona is a rare serotype. Regardless of this fact, four of the six outbreaks associated with cantaloupe have involved *S*. Poona. These four outbreaks were traced back to Mexican farmers and shippers so it is thought there may be a unique natural reservoir in that environment for *S*. Poona (CDC 2002). In 2002, the FDA issued an import alert to detain all cantaloupes imported to the United States from Mexico.

Escherichia coli

Species of *Escherichia coli* are straight, Gram-negative rods. They are mesophilic, facultatively anerobic, and can be motile or nonmotile. *E. coli* is normally found in the intestinal contents of humans, warm-blooded animals, and birds (Ray 2001). Not all strains of *E. coli* are pathogenic to humans. Nonpathogenic strains of *E. coli* are often used as indicators of sanitation. Some strains are pathogenic to humans and they are split into four groups based on their virulence mechanisms (Ray 2001). The groups include enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, and enterohemorrhagic *E. coli* (Ray 2001).

Enterohemorrhagic *E.coli* 0157:H7 is the strain of most importance with concern to foodborne illness. This strain was only recognized as a human pathogen in 1982 (Doyle and others 1997). This serotype is unique because it produces large amounts of one or more related toxins that cause severe damage to the lining of the intestine (Anonymous 1992a). The illness caused by *E. coli* 0157:H7 is known as hemorrhagic colitis. This illness is characterized by severe abdominal pain and diarrhea that begins as watery but becomes grossly bloody (Anonymous 1992a). Depending on the severity of the illness, vomiting and low-grade fever can also be symptoms. Hemorrhagic colitis usually lasts about eight days. As few as 10 cells of *E.*

coli 0157:H7 can cause hemorrhagic colitis. In certain groups, other complications arise due to *E. coli* 0157:H7 infection. Hemolytic uremic syndrome is an illness that can occur, especially in the very young. This illness causes kidney failure and hemolytic anemia (Anonymous 1992a). Elderly patients may develop fever and neurological symptoms in addition to hemolytic uremic syndrome.

Like *Salmonella*, outbreaks involving *E. coli* 0157:H7 usually involve foods of animal origin. *E. coli* 0157:H7 gained notoriety in the United States in early 1993. It was involved in a very large foodborne disease outbreak involving undercooked hamburger meat served at a fast food restaurant chain. More than 700 people were infected and four children died due to infection with *E. coli* 0157:H7. In recent years, outbreaks involving whole and fresh-cut produce have been noted. In August 1993, an outbreak of *E. coli* 0157:H7 infection involved the consumption of fresh-cut cantaloupe (Del Rosario and Beuchat 1996). Recently, a strain of *E. coli* 0157:H7 was implicated in a large multi-state outbreak associated with fresh spinach. Cases have been reported in 26 states as well as one Canadian province and nearly 200 people have become ill. Three deaths have been attributed to the consumption of the contaminated fresh spinach (Anonymous 2006a)

Shigella

Shigella is a nonmotile, Gram-negative rod. They are mesophilic and facultatively anaerobic. The optimal growth temperature is approximately 37°C. These bacteria are catalase positive and oxidase and lactose negative (Ray 2001). They can survive under a variety of stresses including refrigeration, freezing, increased salinity and low pH. *S. flexneri* has been shown to survive in the stationary phase at pH 2.5 for a few hours (Bagamboula and others 2002). *Shigella* is typically carried in the intestines of humans and primates. There are four

species of *Shigella*: *Shigella sonnei*, *Shigella dysenteriae*, *Shigella flexneri*, and *Shigella boydii*. Each species of *Shigella* has several serovars (Ray 2001). The most common cause of *Shigella* infection is transmission through the fecal-oral route. The illness caused by *Shigella* infection is known as shigellosis. This illness occurs when *Shigella* cells attach to, and penetrate, epithelial cells of the intestinal mucosa (Anonymous 1992c). The cells then multiply and spread causing tissue destruction. Tissue destruction likely occurs because the *Shigella* produces an enterotoxin. This toxin is known as a Shiga toxin (Ray 2001). The infective dose for shigellosis can be very small. As few as ten cells of *Shigella* are needed to cause illness. Once the cells are ingested, symptoms of shigellosis usually appear within twelve hours to seven days. In mild cases, symptoms normally last five to six days. In more severe cases, symptoms can linger for two to three weeks (Ray 2001). Even after symptoms have subsided a person may be a carrier of *Shigella* for months afterward. The symptoms of shigellosis include abdominal pain, diarrhea that is often bloody, mucus and pus in the stool, fever, chills, and headache (Ray 2001; Anonymous 1992c). Every age group is susceptible to shigellosis but children seem to be most at risk for the illness. *Shigella* is not usually associated with any one food in terms of illness. Outbreaks of shigellosis often occur due to improper food handling. Illness is usually spread by a person with poor hygiene and sanitation skills when preparing food.

Cetylpyridinium Chloride

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound that has been utilized by the dental industry for over fifty years. It can be found in mouthwashes and throat lozenges as well as hand sanitizing solutions. Quaternary ammonium compounds are detergents and sanitizers commonly used in the food industry due to their cleaning and germicidal properties (Ray 2001). They are formed when tertiary amines are reacted with alkyl halides or

benzyl chloride. The general structure of quaternary ammonium compounds is a central nitrogen atom surrounded by four alkyl groups. The cationic end of the molecule is hydrophobic while the anionic end is hydrophilic.

The general mode of action for quaternary ammonium compounds is through the denaturation of bacterial proteins and disruption of membrane functions. Quaternary ammonium compounds absorb into the bacterial cell surface, permeate and destroy the cell wall and cell membrane, and have a direct or indirect lethal effect on the cell (Cutter and others 2000). Quaternary ammonium compounds are generally more effective against Gram positive bacteria (Ray 2001). Cetylpyridinium chloride is a colorless compound that is freely soluble in water (Cutter and others 2000; Anonymous 2005). Cetylpyridinium chloride is only approved for use on raw poultry carcasses. The compound is applied as a fine mist spray at levels not to exceed 0.3 grams per pound of poultry carcass (FDA 2004). The compound must be sprayed at ambient temperature as part of an aqueous solution. This is done prior to chilling of the carcass. In order to be used, propylene glycol must be added to the solution. The propylene glycol must be at a concentration of 1.5 times higher than that of the cetylpyridinium chloride (FDA 2004). As noted above, it is approved for use in dentrifices. The FDA allows up to 0.1% CPC to be included in such products.

There have been several studies published documenting the experimental use of CPC in different food products; namely beef and poultry operations. It has also been tested in fresh cut lettuce and fresh-cut vegetables (Yang and others 2003; Wang and others 2001). A study by Lim and Mustapha (2004) showed that the efficacy of CPC to remove three different pathogens on fresh beef was largely dependent on the concentration of the compound during treatment. In this study, the researchers purchased whole beef round from a retail store to test the effect of CPC

against *L. monocytogenes*, *E. coli* 0157:H7 and *S. aureus*. The round was trimmed and then cut into cubes. These cubes were exposed to ultraviolet light to help minimize any natural microflora on the surface. After being exposed to the light, the cubes were dipped into a 10^5 to 10^6 CFU/g solution of each bacterium for 1 min and then allowed to air dry. The samples were then placed on absorbent pads that had been prepared with six different formulations of CPC, acidified sodium chlorite and potassium sorbate. The level of CPC in every formulation was 0.50%. The samples on the absorbent pads were then aseptically packaged and stored under conditions mimicking those in a retail grocery store. *E. coli* 0157:H7 was drastically reduced with CPC, ASC or a mixture of the two compounds. *E. coli* 0157:H7 was reduced by 2.78 Log by CPC while the mixture with ASC resulted in a 4.0 Log reduction compared to the controls. Cetylpyridinium chloride was found to be much more effective than the other two compounds on reducing levels of *L. monocytogenes* except on day two of the study. *S. aureus* was reduced by 4.01 Log during this storage study.

Acidified Sodium Chlorite

Acidified sodium chlorite (ASC) is formed by mixing a sodium chlorite solution with a generally recognized as safe organic acid (Inatsu and others 2005). Citric acid has been shown to work well with this compound. It has a broad spectrum of activity against bacteria, viruses, and fungi. This antimicrobial agent is currently approved by the FDA for use in beef, poultry, comminuted meat products, seafood and processed fruits and vegetables. It is approved at concentrations between 500 to 1200 parts per million (ppm) at a final pH of 2.5-2.9 when used for red meat, red meat parts, or organs (Bosilevac and others 2004). It can also be used in water or ice at concentrations of 40 to 50 ppm to rinse, wash, thaw, transport, or store seafood (Su and Morrissey 2003). The antibacterial activity of this compound is said to be the oxidative effect of

chlorous acids (Inatsu and others 2005). One drawback to using ASC in commercial settings is that at such low pH values it can be corrosive and might possibly damage processing equipment.

The effect of ASC on various food systems has been tested. It has been tested on beef and poultry carcasses, Chinese cabbage, and raw salmon just to name a few (Lim and Mustapha 2004; Bosilevac and others 2004; Kemp and others 2001; Oyarzabal and others 2004; Inatsu and others 2005; Su and Morrissey 2003). Inatsu and others (2005) studied the effect of ASC on *E. coli* 0157:H7 strains inoculated onto Chinese cabbage leaves. Four strains of *E. coli* 0157:H7 that were grown in tryptic soy broth and supplemented with rifampicin were used in the study. Supplementing with rifampicin and plating on media containing the antibiotic helped minimize the amount of natural flora that was allowed to grow. These strains were administered to cabbage leaves in a cocktail form. The final concentration of the cocktail was 7.1 Log CFU/ml. Four hundred grams of leaf pieces were used as one sample. This sample was dipped into 1200 ml of the inoculum and mixed gently for 10 min. For treatment, 100 g of inoculated leaves were mixed with 1000 ml of the wash solution. The researchers tested three different wash temperatures (4°C, 25°C and 50°C). After washing, 25 g of leaves was stomached with 225 ml of phosphate buffered saline for 90 seconds. Serial dilutions were then performed and surface plating took place on tryptic soy agar plates and sorbitol MacConkey agar supplemented with rifampicin. Acidified sodium chlorite showed nearly a 3.0 Log reduction of *E. coli* 0157:H7 on the washed cabbage leaves. In the Lim and Mustapha study (2004), acidified sodium chlorite was able to reduce levels of *E. coli* 0157:H7 by 4.62 Logs over a fourteen day period. When combined with CPC, ASC reduced *E. coli* 0157:H7 by 4.0 Logs. Acidified sodium chlorite was not as effective against *L. monocytogenes* but it did cause a 5.09 Log reduction in *S. aureus* for the length of the study.

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CHAPTER 3

EFFECT OF CETYLPYRIDINIUM CHLORIDE ON BACTERIAL SURVIVAL RATE AND PHYSIOLOGICAL QUALITY OF FRESH-CUT CANTALOUPE CUBES AT REFRIGERATOR TEMPERATURES

Introduction

Fresh-cut fruits are an expanding part of the fresh produce market. However, fresh-cut fruits could serve as vehicles of foodborne disease. They provide an environment that allows pathogens to survive and grow on the surface (Eswaranandam and others 2006). Minimal processing may transfer skin microflora to fruit flesh where microorganisms can grow rapidly upon exposure to nutrient laden juices (O'Connor-Shaw and others 1994). This can be an issue because produce is often consumed raw without any type of intervention that would reduce, control or eliminate pathogens (Anonymous 2004). The microstructure of the netting on the rind provides an atmosphere that favors bacterial attachment (Ukuku 2006). Bacteria on melon rinds can come from both pre- and postharvest sources. Preharvest sources can include feces, soil, water, wild and domestic animals and human handling. Postharvest sources may include feces, human handling, harvesting equipment, wash and rinse water, ice and improper storage (Beuchat and Ryu 1997). Species of *Salmonella* have been implicated several times in the last decade for causing illness after the consumption of cantaloupe (Anonymous 1991; CDC 2002; Mohle-Boetani and others 1999). There has been at least one outbreak pinpointing *E. coli* 0157:H7 as the causative agent of a cantaloupe associated foodborne illness (Del Rosario and Beuchat 1995). Many different treatments have been applied to whole and fresh-cut fruits to inhibit microbial growth on the surface of the fruit. These treatments include calcium lactate (Luna-Guzman and Barrett 2000), sodium hypochlorite (Barak and others 2003), organic acids (Materon 2003), chlorine dioxide (Rodgers and others 2004), hydrogen peroxide (Park and Beuchat 1999; Sapers and Sites 2003), hot water (Ukuku 2006; Solomon and others 2006) and electron beam irradiation (Palekar and others 1999).

Consumer acceptance of fresh-cut fruits is at first largely dependent on the appearance of the product. Repeat purchases depend on the overall flavor and texture of the product (Beaulieu and Baldwin 2002). Any compound applied to fresh-cut fruits, such as cantaloupe, must not affect the physiological and sensory properties to any great degree. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that has shown promise as an antimicrobial agent. It is a water-soluble, colorless compound (Cutter and others 2000) with little to no organoleptic effect on foods. The bactericidal activity of CPC may be linked to its ability to absorb negatively charged bacterial cell membrane phosphates (Wang and others 2001). This may cause the microorganism cell to become more permeable and allow the cell contents to leak out.

Cetylpyridinium chloride has been tested on products such as raw poultry, beef, shrimp and some fresh-cut vegetables (Yang and others 2003; Cutter and others 2000; Lim and Mustapha 2004; Wang and others 2001; Dupard and others 2006). Dupard and others (2006) investigated the effect of various CPC concentrations in the control of *Listeria monocytogenes* on raw and cooked, peeled and shell-on shrimp. Cetylpyridinium chloride treatments were performed both with and without a water treatment. They found that a 1.00% CPC treatment without a water treatment could reduce *Listeria monocytogenes* by about 2.5 Log CFU/g. When the 1.00% CPC treatment was followed by a water treatment, it could reduce *L*. monocytogenes on cooked shrimp by 7.0 Log CFU/g. A study by Yang and others (2003) concluded that CPC reduced *E. coli* 0157:H7 by 1.21 Log CFU/g and *S. typhimurium* by 0.96 Log CFU/g on fresh-cut lettuce. This study also discovered that the effectiveness of CPC is dependent on the species of bacteria being treated. Currently, CPC is approved for use on raw poultry carcasses. The level

used cannot exceed 0.3 grams per pound of poultry carcass (FDA 2004). The compound must be used as a fine mist spray at ambient temperature.

Experiments were designed to determine how effective CPC is at reducing counts of *E. coli* 0157:H7, *Salmonella* Montevideo, and *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes and to determine what effect CPC might have on the physiological attributes (°Brix, firmness, color, and volatile compounds) in stored (5° C) fresh-cut cantaloupe.

Materials and Methods

Culture Preparation

Escherichia coli 0157:H7 43889 (human isolate) was obtained from the American Type Culture Collection (ATCC; Manassas, VA). *Salmonella* Montevideo was obtained from the Centers for Disease Control and Prevention (CDC; Atlanta, GA). *Shigella sonnei* MO 4110 was obtained from Dr. Larry Beuchat at the University of Georgia. The cultures were maintained at a refrigerated temperature (5°C) on a Brain Heart Infusion (BHI) slant. One loopful (10 µl) of cells was transferred to 10 ml of BHI broth. The transferred cultures were incubated at 37°C for 24 h prior to use. Individual cultures were used at approximately 7.0 Log CFU/ml for all experiments.

Cetylpyridinium Chloride Preparation

Aqueous solutions of CPC were prepared using sterile distilled water. Depending on the experiment, the CPC was prepared at concentrations of 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00% (v/v basis). The solutions were made just prior to use and were used at ambient temperature (25°C).

24 Hour Microbial Enumeration

This study was performed to determine if CPC would be effective against the three pathogens selected. This preliminary study was designed to determine which concentrations

would be most effective. Cantaloupes (*Cucumis melo* L. *reticulates*) were purchased in retail markets the day before the experiment and kept at ambient temperature (25°C) until the experiment began. Prior to cutting, the melons were washed with distilled water and the outer surface was vigorously scrubbed. The cantaloupes were dipped in chlorinated distilled water (5700 mg/L available chlorine) and then washed again with distilled water. After washing, the cantaloupes were placed in a laminar flow hood for cutting. After drying for 5 min, cutting was done by hand using sanitized knives. The ends of the cantaloupe were removed first and knives were sanitized again. The outer surface of the cantaloupe was then removed. The fresh-cut cantaloupe cubes were prepared following a method described by Beaulieu and Lea (2003). Approximately 1 inch cubes were used for all analyses.

Cubes were placed on sanitized plastic trays covered with aluminum foil. Each tube of inoculum was vortexed to ensure proper mixing. The cubes were inoculated by pipetting 500 µl of the inoculum on one side of the cube. The cubes were then flipped onto the opposite side using sanitized forceps. Five hundred microliters of inoculum was pipetted onto the opposite side of the cube for a total of 1 ml of inoculum per cube. Cubes were allowed to air dry in the laminar flow hood for 1 h at ambient temperature to allow the bacteria to attach onto to the surface of the fresh-cut cantaloupe.

After bacterial attachment, cantaloupe samples were treated with 0.05, 0.10, 0.20, 0.40, 0.60, 0.80 or 1.00% (v/v basis) CPC. An untreated, inoculated control was also used. Fresh-cut cantaloupe samples were weighed to approximately 50 g. The samples were placed into Whirl-Pak bags and 50 ml of the appropriate CPC concentration was placed into the bag. Bags were then closed and the sample was gently shaken for 1 min. After 1 min, cubes were removed from

the bag using sanitized forceps and placed in a new Whirl-Pak bag. The samples were held at 5°C for 24 h prior to bacterial enumeration.

To determine *E. coli* 0157:H7, *S*. Montevideo and *S. sonnei* counts, 50 ml of PBS buffer was added to each Whirl-Pak bag containing the fresh-cut cantaloupe samples. The PBS buffer and cantaloupe were homogenized in a stomacher for 1 min at normal speed. Serial dilutions were prepared from the homogenate of each sample and 0.1 ml aliquots of each dilution were spread plated onto xylose lysine deoxycholate (XLD) agar for *S*. Montevideo and *S. sonnei* and sorbitol MacConkey (SMAC) agar for *E. coli* 0157:H7. Counts on these plates were determined following incubation at 37°C for 24 h and Log CFU/g was determined.

Shelf Life Study: Microbial Enumeration

Cantaloupe (*Cucumis melo* L. *reticulates*) cube preparation and sample inoculation were the same as the previously mentioned experiment. After bacterial attachment, cantaloupe samples were treated with 0.60, 0.80, or 1.00% (v/v basis) CPC. An inoculated control as well as an inoculated control washed with sterile distilled water was also used in this experiment. Fresh-cut cantaloupe samples were weighed to approximately 50 g. These samples were placed in Whirl-Pak bags and 50 ml of the appropriate CPC concentration was placed into the bag. Bags were then closed and the sample was gently shaken for 1 min. After 1 min, the cubes were removed from the bag using sanitized forceps, placed in a new Whirl-Pak bag and stored at 5°C until needed. Bacterial counts were determined at 0, 2, 4, 8 and 12 days. Microbial enumeration was done following the procedure discussed in the 24 h microbial enumeration study.

Shelf Life Study: Physiological Quality

Two separate experiments were performed during the summers of 2005 and 2006. Cantaloupes (cv. ‘Athena’) for the 2005 experiment were grown using standard cultural practices

in Moultrie, GA. Melons were received two days before the experiment began and were held at 5°C. Fruit was first washed in an ice water bath then dipped in another ice water bath containing 5.7 mg/L available chlorine. The melons were then rinsed with distilled water. They were then peeled on a CP-44 Melon Peeler (Muro Corp., Tokyo, Japan). The ends were then cut off and the melons were sliced lengthwise. Cantaloupe (cv. 'Athena') for the 2006 experiment were grown using standard cultural practices in North Carolina. The melons were received the day of the study and kept at ambient temperature. They were washed in distilled water and a water bath at ambient temperature containing 5700 mg/L available chlorine. Melons were rinsed with distilled water prior to peeling. The ends of the melons were removed with sanitized knives. Sides of the melons were then removed and they were sliced lengthwise. Fresh-cut cubes were prepared from these slices according to Beaulieu and Lea (2003).

The CPC concentrations used for this experiment were 0.60, 0.80 and 1.00% along with a Millipore water control and control fresh-cut treatment. Physiological quality measurements were made on days 0, 4, 7, 11 and 14. Approximately 250 g of 1 inch cubes were placed in Juice Catcher containers. The containers were held at 5°C until needed.

Color was measured using a Hunter colorimeter (DP-9000, Reston, VA). Firmness was measured with a handheld McCormick FT327 with an 8.0 mm probe. These two measurements were performed on cube surfaces that were sliced cleanly with a knife. Percent soluble solids (°Brix) were measured with an Atago PR101 (Tokyo, Japan) after extracting juice by squeezing cubes with a gloved hand.

GC-MS Volatile Preparation

Volatile samples were prepared in quadruplicate, from 4 to 5 cubes (80 – 100 g) from each 250 g Juice Catcher container, as previously described (Beaulieu and Grimm, 2001).

Briefly, tissue was rapidly juiced (~15 s) into a slurry with a Braun MP80 Juicer (Gillette Company, Boston, Mass), a 3-mL slurry (without foam) was immediately pipetted into 10-mL glass vials containing 1.1 g NaCl, then benzothiophene internal standard (IS) was added. Vials were sealed with a steel screw top fitted with a Teflon/silicon septum, and placed on a Combi-Pal Autosampler (Leap Technologies, Carrboro, N.C.) cooling rack at 4 °C.

Headspace SPME GC-MS Analysis

Sample vials were equilibrated 10 min via oscillation in a 40 °C autosampler, then a 1-cm 50/30μm stable flex divinyl benzene/carboxen/polydimethyl siloxane (DVB/Car/PDMS) SPME fiber was inserted into the headspace for 12.5 min at 40 °C. Volatile compounds were analyzed at approximately the temperature of the human palate, where mastication occurs (~37 °C). Vials were continuously swirled during SPME adsorption with an agitation speed of 100 rpm. Fibers were desorbed at 250 °C for 1 min in the injection port of an HP6890/ 5973 GC-MS (Agilent Technologies Wilmington, DE; formerly Hewlett-Packard, Palo Alto, CA) with a DB-5 column (J & W Scientific, Folsom, CA), 30 m, 0.25 mm I.D., 0.5 μm film thickness. Fibers remained in a needle bake out oven (270 °C) for 1 min. The GC was equipped with a Micro Cryo-trap (Scientific Instrument Services, Ringoes, N.J.) and compounds were cryofocused at -60 °C using carbon dioxide during the 1 min desorption in the injection port. The injection port (270 °C) was operated in pulsed splitless mode and subjected to a pressure of 173 kPa of ultrahigh purity helium (99.9995%) for the first minute, then flow velocity was constant at 40 cm s^{-1} for the remainder of the GC run. The initial oven temperature was 50 °C, held 1 min, ramped 5 °C min^{-1} to 100 °C then 10 °C min^{-1} to 190 °C, ramped 30 °C min^{-1} to 250 °C, and held 1 min. The HP5973 quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV (electron volts), a source temperature of 200 °C, with a continuous scan from *m/z* (mass to

charge ratio) 33 to 300. Samples were only run in one year since the repeated experiments were lost after hurricane Katrina.

GC_MS Data Analysis

Data were collected with MSD ChemStation software (D.01.02.16) and searched against the NIST (v. 1.5) and Wiley (v. 7.0 NIST98) libraries (Palisade Corp., Newfield, NY). Compounds were preliminarily identified by library search, and then the identities of most were confirmed by comparison of their GC retention time (RT) with authentic compounds, or an in-house cantaloupe retention index (RI) (Beaulieu 2006a; Beaulieu and Grimm, 2001). The RT's from a series of straight-chain alkanes (C_7-C_{20}), produced on the aforementioned column, under identical conditions, was used to calculate RI's for all identified compounds. Since this is mainly a qualitative appraisal for 14 (ethyl 2-methyl propanoate; methyl 2-methylbutanoate; ethyl butanoate; ethyl 2-methylbutanoate; 3 and 2-methylbutyl acetate; ethyl methylthioacetate; ethyl hexanoate; hexyl acetate; eucalyptol; (*Z*)-6-nonenal; 3-(methylthio)-propyl acetate; (*Z*)-6-nonenol; and benzyl acetate) of the 26 characteristic impact flavor/aroma compounds (CIFAC) in muskmelons (Beaulieu, 2006b), selected ion data (integrated ion areas) per compound were normalized on the IS, and averaged. Peaks for 3-methylbutyl acetate (isoamyl acetate) and 2-methylbutyl acetate were combined since the 3-isomer was a minor shoulder peak of the 2-isomer. Standards were acquired from Aldrich (Milwaukee, WI), Fluka (Switzerland), Poly Science (Niles, IL) and Ultra Scientific (North Kingstown, RI).

Statistical Analysis

All microbial analyses were based on two separate experiments each with three determinations (n=6). Significant differences among the means of the two controls and three sanitation dip treatment concentrations within each sampling day were determined using the

Student's t test following one-way analysis of variance (ANOVA). Means of each individual treatment concentration as well as each control sample were analyzed over time using the Student's t test following a one-way analysis of variance (ANOVA) in JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA). The statistical difference was set at $p<0.05$. All experiments were duplicated with the exception of the volatile recovery. This experiment was not repeated due to samples being lost after hurricane Katrina.

For the GC-MS procedure, sixteen different variables were of interest (eleven single compounds and five combinations of the eleven single compounds). The objective of the experiment was to compare the effects of the three sanitation dip treatment concentrations to two different control treatments (fresh-cut and water). Measurements were performed on days 0, 4, 7, 11 and 14 of the experiment with two to four replicates used per day per treatment. Analysis of variance (ANOVA) was used to test for differences among treatment and experimental day means (main effects) as well as for significant interactions between the two main effects. . If treatment and/or day effects were significant, then Dunnett's test was used to make post hoc pair-wise comparisons between the means of the two control groups and the three treatment concentrations, and between day 0 and the other experimental days. In the case of the treatment comparisons, a further correction was made on the Dunnett's tests since two sets of control comparisons were made. If the interaction term was significant, then contrasts were built to test for significant differences between the two control and three treatment concentration means within each of the five experimental days using Bonferroni-corrected significance levels to account for the multiple comparisons being made. Results were considered significant at the nominal level of 0.05 unless otherwise noted. All analyses were performed with SAS® version 9.1.

Results

24 Hour Microbial Enumeration

The 0.80 and 1.00% caused over a 5-Log reduction of *E. coli* 0157:H7 counts. The 0.60% CPC caused a 3.45 Log reduction while the 0.40% treatment caused a 3.42 Log reduction in *E. coli* 0157:H7 counts (Figure 1). The 0.80 and 1.00% concentrations were not significantly different from each other but were statistically different from the other concentrations and the control. The 0.20% CPC treatment caused a 2.31 Log reduction while the 0.05 and 0.10% treatments caused over a 1.5 Log reduction in *E. coli* 0157:H7 counts after 24 h (Figure 1). The 0.05, 0.10, 0.20, 0.40, and 0.60% CPC treatments were not significantly different from each other but were significantly different from the control and two other treatments.

The 1.00% CPC concentration caused a 3.92 Log reduction while the 0.80% concentration caused nearly a 3.5 Log reduction of *S*. Montevideo counts (Figure 2). The 0.60% CPC concentration caused a 1.94 Log reduction on *S*. Montevideo counts. The 0.05, 0.10 and 0.20% CPC treatments caused over a 1.0 Log reduction while the 0.40% treatment caused over a 1.5 Log reduction of *S*. Montevideo counts after 24 h (Figure 2). The 0.80 and 1.00% CPC concentrations again were not statistically different from each other, but were significantly different from the other treatments and the control. All treatments were significantly different than the control.

The 0.80% concentration caused a 2.77 Log reduction while the 1.00% concentration caused a 2.69 Log reduction on *Shigella sonnei* counts (Figure 3). The 0.80 and 1.00% CPC concentrations were statistically different from the other concentrations and the control. The 0.10, 0.20, 0.40 and 0.60% CPC treatments all caused at least a 1.0 Log reduction of *Shigella sonnei* counts after 24 h (Figure 3). The 0.05% CPC treatment caused a 0.96 Log reduction in *S.*

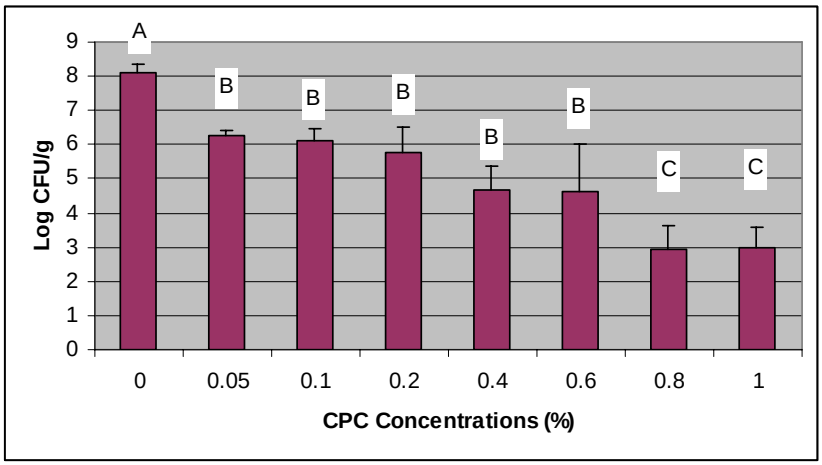

Figure 1. The effect of CPC against *E. coli* 0157:H7 on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

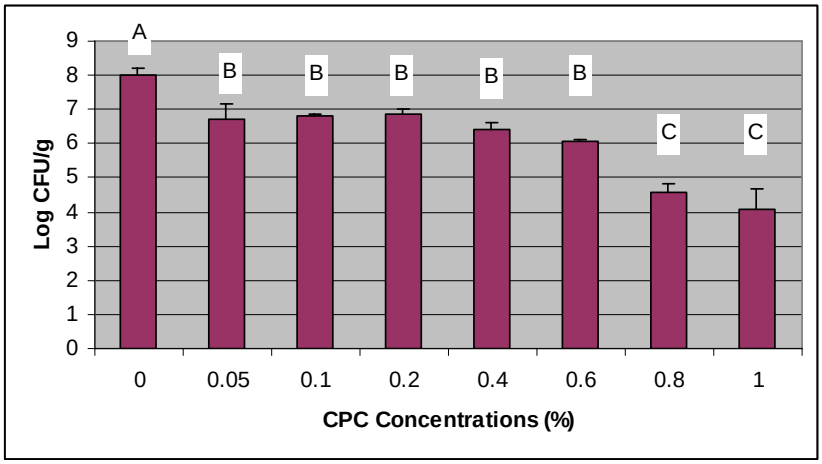

Figure 2. The effect of CPC against *Salmonella* Montevideo on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

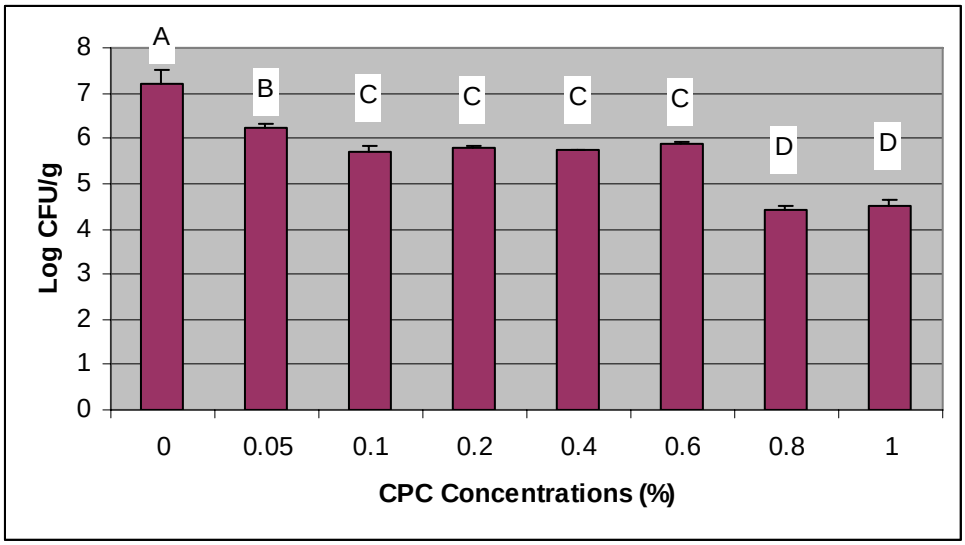

Figure 3. The effect of CPC against *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

sonnei counts. The 0.05% treatment was significantly different from the other treatments. The 0.10, 0.20, 0.40 and 0.60% treatments were not significantly different from each other but were significantly different from the control and other treatments.

Shelf Life Study: Microbial Enumeration

Cetylpyridinium chloride was less effective against *E. coli* 0157:H7 compared to *S.* Montevideo and *S. sonnei* over the 12 day period. On day 0, the 1.00% CPC concentration reduced *E. coli* 0157:H7 by 1.46 Log CFU/g. The 0.80% CPC treatment caused a 1.26 Log reduction while the 0.60% treatment caused a 1.22 Log reduction of *E. coli* 0157:H7 counts on day 0 (Table 1). The three CPC treatments were not significantly different from the water treated control and the untreated fresh-cut control on day 0. The 1.00% CPC treatment caused a 1.78 Log reduction on day 2 while the 0.80% CPC treatment caused a 1.87 Log reduction of *E. coli*

0157:H7 counts. These two concentrations were not significantly different from each other but were from the 0.60% treatment and the two controls. On day four, the 1.00% CPC concentration reduced *E. coli* 0157:H7 counts by 3.04 Log CFU/g. It was significantly different from the other two treatments as well as the two controls. On day 8, the CPC treatments reduced *E. coli* 0157:H7 counts by at least 1.5 Log CFU/g (Table 1). The three treatments were not significantly different from each other but were significantly different from the two controls. By day 12, the 1.00% concentration reduced *E. coli* 0157:H7 counts by 1.69 Logs (Table 1). It was significantly different from the other two CPC treatments as well as the water treatment and the control. The reduction of *E. coli* 0157:H7 counts were only significantly different for the fresh-cut control over time (Table 1).

The 1.00% concentration was successful at reducing *S.* Montevideo by nearly 2.0 Logs on day 0 (Table 1). The 0.80% concentration caused a 1.49 Log reduction while the 0.60% concentration reduced *S*. Montevideo by 1.22 Log CFU/g on day 0. The three CPC concentrations were significantly different from each other as well as the two controls. On day 2, the 1.00% CPC treatment caused a 2.58 Log reduction of *S*. Montevideo counts. The 0.80% concentration caused a 1.77 Log reduction of *S*. Montevideo and the 0.60% concentration caused nearly a 1.5 Log reduction on day 2. The 1.00% CPC concentration was significantly different from the two other CPC concentrations as well as the controls on day 2. The 0.80 and 1.00% CPC concentrations caused over a 1.5 Log reduction of *S*. Montevideo counts on day 4 (Table 1). These two concentrations were not significantly different from each other but were significantly different from the other 0.60% concentration and the two controls. The 1.00% concentration caused a 3.0 Log reduction of S. Montevideo counts on day 8 (Table 1). It was significantly different from the two controls on this day. By day 12, the 1.00% concentration reduced *S.*

Montevideo by 2.57 Log CFU/g. It was significantly different from the other two concentrations as well as the two controls on day 12. The levels of *S*. Montevideo over time were significantly different for the two controls and the 0.6 and 0.8% CPC treatments (Table 1).

Cetylpyridinium chloride was able to reduce *S. sonnei* by over 3 Log CFU/g on day 0 (Table 1). The 1.00% CPC treatment was not significantly different from the 0.80% concentration but was significantly different from the 0.60% treatment and the two controls. On day 2, the 1.00% CPC treatment caused a 2.78 Log reduction of *S. sonnei*. It was significantly different from the two other CPC concentrations as well as the two controls. The 0.80% concentration caused a 1.74 Log reduction while the 1.00% treatment caused a 1.86 Log reduction of *S. sonnei* on day four. The three concentrations were not significantly different from each other but were from the controls. The 1.00% CPC treatment caused over a 2.0 Log reduction of *S. sonnei* counts on day 8. By day 12, *S. sonnei* was reduced by 2.63 Log CFU/g (Table 1). On days 8 and 12, the 1.00% CPC treatment was not significantly different from the other two concentrations but was significantly different from the two controls. Over time, there were no significant differences of *S. sonnei* counts for the five samples (Table 1).

Shelf Life Study: Physiological Quality

On day zero, the untreated control had a °Brix reading of 8.29 while the water treated control had a reading of 8.27 (Table 2). The 0.8% CPC concentration had the lowest °Brix reading on day zero. The untreated control had the highest °Brix reading on day four of the study. The two highest CPC concentrations had °Brix reading of 7.89 and 8.16 for 1.0 and 0.8%, respectively, on day seven. The two control samples had the highest °Brix values on day eleven followed by the 1.0% CPC concentration. The two highest CPC concentrations had the highest °Brix readings on day fourteen of the study (Table 2). There was no significant difference

between the five treatments on any of the five sampling days of the study. The 1.0 and 0.6% CPC concentrations had the highest firmness readings on day zero (Table 3). On day zero, the firmness values were 18.12, 17.61, 19.77, 16.92 and 19.32 Newtons for the untreated control, water control, 1.0, 0.8, and 0.6% CPC concentrations, respectively. The highest CPC concentration was not significantly different from either control or the 0.6% CPC concentration on day zero. The lowest CPC concentration had the highest firmness readings on days four and seven. It was not, however, significantly different from the controls or other CPC treatments on either sampling day. The untreated fresh-cut control had the highest firmness reading on day eleven. The 1.0% CPC concentration had the lowest reading but was not significantly different from either control or the other two CPC concentrations. The 1.0% CPC concentration again had the lowest reading on day fourteen while the untreated control had the highest reading (Table 3). The five treatments were not significantly different from each other on day fourteen of the study. Over time, all five samples had significantly lower firmness values (Table 3).

The water control had the highest L* values (64.15) on day zero. The 0.8% CPC concentration had the lowest L* value on this sampling day. The untreated fresh-cut control as well as the 0.6 and 1.0% CPC concentrations were not significantly different from each other but were different from the water control and the 0.8% CPC concentration (Table 4). The untreated control had the highest L* value on day four. It was not significantly different from the water control. The 0.6 and 1.0% CPC samples were not significantly different from one another on day four but were different from the controls and the 0.8% CPC concentration. The untreated control also had the highest L* value on days seven, eleven and fourteen. On days seven and eleven, the three CPC treated samples were significantly different from the controls but not each other.

Table 1. The effect of CPC against *E. coli* 0157:H7, *Samonella* Montevideo, and *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes held at 5°C for 12 days.

Strain	Treatments	Days Storage (log CFU/g)[a]				
***E. coli* 0157:H7**		**0**	**2**	**4**	**8**	**12**
	Fresh-Cut Control	8.51±0.29 Aa	7.89±0.01 ABa	8.50±0.13 ABa	8.47±0.06 Aa	7.48±0.42 Ba
	Water Control	8.06±0.42 Aa	7.56±0.08 Ab	7.66±0.01 Ab	8.25±0.11 Aa	7.85±0.04 Aa
	0.6%	7.29±0.27 Aa	6.42±0.11 Ac	6.99±0.02 Ac	6.65±0.04 Ab	6.69±0.03 Ab
	0.8%	7.25±0.29 Aa	6.02±0.11 Ad	6.45±0.06 Ad	6.74±0.25 Ab	6.78±0.18 Ab
	1.0%	7.05±0.67 Aa	6.11±0.08 Ad	5.46±0.07 Ae	6.67±0.23 Ab	5.79±0.20 Ac
***S.* Montevideo**						
	Fresh-Cut Control	7.66±0.05 Aa	8.26±0.19 Aa	8.23±0.08 Aa	7.88±0.08 Aa	7.55±0.17 Ba
	Water Control	7.44±0.13 Aa	8.10±0.13 Aa	7.79±0.15 Ab	7.74±0.08 Aa	6.95±0.01 Bb
	0.6%	6.44±0.23 Ab	6.86±0.12 Ab	7.16±0.12 Ac	6.49±0.64 ABbc	5.96±0.10 Bc
	0.8%	6.17±0.14 Ac	6.49±0.23 ABb	6.49±0.04 ABd	5.74±0.04 ABbc	6.08±0.30 Bc
	1.0%	5.75±0.12 Ad	5.68±0.57 Ac	6.35±0.02 Ad	4.88±0.42 Ac	4.98±0.38 Bd
S. sonnei						
	Fresh-Cut Control	6.81±0.14 Aa	6.93±0.48 Aa	6.58±0.40 Aa	6.57±0.34 Aa	6.27±0.36 Aa
	Water Control	6.20±0.15 Aa	6.54±0.40 Aa	6.40±0.43 Aa	6.37±0.29 Aa	6.11±0.30 Aa
	0.6%	4.56±0.57 Ab	6.56±0.05 Aa	5.67±0.89 Aab	4.94±0.37 Ab	4.34±0.85 Ab
	0.8%	3.82±0.55 Ac	5.56±0.47 Ab	4.84±0.61 Ab	4.80±0.37 Ab	4.29±0.56 Ab
	1.0%	3.78±0.11 Ac	4.15±0.42 Ac	4.72±0.25 Ab	4.53±0.46 Ab	3.64±0.42 Ab

[a] All analyses were based on two separate experiments each with three determinations (n=6). Means for each sampling day (columns) followed by the same lower case letter were not significantly different (P > 0.05) from each other. Means (rows) followed by the same upper case letter were not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

By day fourteen, the highest and lowest CPC concentrations were not significantly different from the two controls (Table 4). When compared to day zero, all five samples had significantly lower L* values during the study (Table 4).

On the first four sampling days of the study, the untreated control had the highest a* value (Table 5). The untreated control was not significantly different from the 0.6 and 0.8% CPC concentrations on day zero. On day four, the a* values for the three CPC treatments were 9.07, 9.14 and 9.28 for the 0.6, 0.8 and 1.0% concentrations, respectively. The three highest a* values on day seven were for the untreated control, water control and the 1.0% CPC concentration. On day eleven, the three CPC treated samples were not significantly different from one another but were from the controls. The highest a* value on day fourteen was 10.46 for the water treated control. The lowest a* value was 8.70 for the 0.6% CPC sample. The 0.8 and 1.0% CPC samples were not significantly different from each other but were from the 0.6% CPC and the controls on day fourteen (Table 5).

The highest b* value on day zero was 34.50 for the untreated control. The water control had the lowest b* value on day zero. The highest CPC concentration was not significantly different from the untreated control or the other two CPC concentrations on day zero. The 1.0% CPC concentration had a b* value of 32.41 on day four. It was significantly different from the two controls and the 0.6% CPC sample. On days 7, 11 and 14 the untreated control had the highest b* value (Table 6). On day seven, the 1.0% CPC sample was not significantly different from the water control or the 0.6% CPC sample. The 1.0% CPC sample was not significantly different from the water control or the 0.6% CPC sample. The 1.0% sample was significantly

Table 2. The effect of CPC on the percent soluble solids (°Brix) value of fresh-cut cantaloupe cubes stored at 5°C for 14 days.

Concentration	Days Storage (°Brix)[a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	8.29±1.38 Aa	8.24±1.02 Aa	8.09±1.07 ABa	7.77±1.33 ABa	7.44±1.42 Ba
Water Control	8.27±1.57 Aa	8.08±0.99 Aa	7.89±0.88 Aa	7.70±0.71 ABa	7.18±1.09 Ba
0.6%	8.64±1.64 Aa	7.76±1.22 Ba	7.70±1.14 Ba	7.38±1.29 Ba	7.24±0.98 Ba
0.8%	8.05±1.43 ABa	8.09±0.96 ABa	8.16±1.08 Aa	7.38±1.09 Ca	7.52±1.04 BCa
1.0%	8.61±1.59 Aa	8.03±0.97 ABa	7.89±1.49 ABa	7.61±1.13 Ba	7.49±2.35 Ba

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 3. The effect of CPC on the firmness value of fresh-cut cantaloupe cubes stored at 5°C for 14 days.

Concentration	Days Storage (Newtons)[a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	18.12±6.10 Aab	16.28±4.85 ABa	15.20±5.15 ABa	14.90±6.99 ABa	14.05±6.19 Ba
Water Control	17.61±4.07 Aab	14.70±5.10 BCa	15.32±3.65 ABa	13.70±4.49 BCa	12.44±5.32 Ca
0.6%	19.34±4.25 Aab	17.48±5.85 ABa	15.45±4.23 BCa	13.50±4.53 Ca	15.03±6.38 BCa
0.8%	16.92±4.37 Ab	15.85±4.41 Aa	15.04±4.61 ABa	13.17±4.72 Ba	13.11±4.24 Ba
1.0%	19.77±5.12 Aa	15.44±4.82 Ba	15.15±4.33 Ba	13.01±6.39 BCa	12.17±4.81 Ca

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

different from the two controls on day eleven. It was not significantly different from the other two CPC concentrations. On day fourteen, the two controls were significantly different from each other and the three CPC treatments. The three CPC treatments were not significantly different from each other.

Eleven individual volatile compounds were recovered with consistency during the GC-MS procedure. These compounds include ethyl butanoate, ethyl 2-methyl butanoate, 3&2-methyl butyl acetate, ethyl (methylthio) acetate, ethyl hexanoate, hexyl acetate, eucalyptol, (*Z*)-6-nonenal, 3-(methylthio)-propyl acetate, benzyl acetate, and (*Z*)-6-nonenol. For statistical analysis, five combinations of the eleven compounds were created. These combinations were acetates plus sulfur compounds (acetate+(S)), acetates containing sulfur (sul-aces), acetates, and non-acetates. Treatment effects were significant for eight of the eleven single compounds (all but ethyl 2-methyl butanoate, 3 & 2-methyl butyl acetate, and eucalyptol) and three of the five combinations of compounds (all but the non-acetate and all compounds combinations).

Results of the two sets of pair-wise comparisons for the three CPC concentrations to the two control treatments for selected individual compounds and combinations may be found in Table 7. For four of the single compounds, hexyl acetate, 3-(methylthio)-propyl acetate, benzyl acetate, and (*Z*)-6-nonenol, and two of the combinations of compounds, all acetates including thio-compounds (acetate (+S)) and acetates not including thio compounds (acetates), all three of the CPC concentrations were significantly less than both the untreated fresh cut and water dip controls. For (*Z*)-6-nonenal, all three of the CPC concentrations were significantly less than the untreated fresh cut control, and while all three were also less than the water dip control, none of these differences reached statistical significance. Of further interest, in three of the single compounds, hexyl acetate, benzyl acetate, and Z-6-nonenol, the water dip control was

Table 4. The effect of CPC on L* value of fresh-cut cantaloupe cubes stored at 5°C for 11 days.

Concentration	Days Storage (L* value) [a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	62.70±4.81 ABa	62.11±3.55 Ba	63.22±3.31 ACa	64.59±3.08 Ca	63.91±2.44 CDa
Water Control	64.15±3.82 Ab	61.93±3.97 Ba	63.11±4.11 Ca	63.40±3.72 ACb	63.79±3.49 ACa
0.6%	62.20±3.87 ABa	60.31±5.27 Bb	61.54±4.59 Cb	60.18±5.56 Bc	63.26±2.38 Aab
0.8%	60.88±5.10 Ac	59.27±4.67 Bc	60.41±4.62 Ac	60.55±4.92 Ac	63.00±3.26 Cb
1.0%	62.17±4.61 Aa	60.62±3.99 Bb	60.93±5.17 Bbc	60.99±5.07 Bc	63.19±2.96 Aab

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different (P > 0.05) from each other. Means (rows) followed by the same upper case letter were not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 5. The effect of CPC on the a* value of fresh-cut cantaloupe cubes stored at 5°C for 11 days.

Concentration	Days Storage (a* value) [a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	10.30±1.76 Aa	9.75±1.44 Ba	9.47±1.33 Ba	9.15±1.40 Ca	10.29±0.87 Aa
Water Control	9.54±1.94 Ac	9.07±1.87 Bb	9.10±1.44 Bb	8.52±1.85 Cb	10.46±2.95 Da
0.6%	9.99±1.65 Aab	9.07±2.64 Bb	8.68±1.78 Bc	7.84±1.80 Cc	8.70±1.73 Bb
0.8%	10.14±1.88 Aa	9.14±4.77 BCab	8.73±1.87 Cc	7.83±1.53 Dc	9.47±1.72 Bc
1.0%	9.73±2.06 Abc	9.28±4.38 Bab	8.97±1.52 Bbc	8.11±1.73 Cc	9.43±1.38 ABc

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different (P = 0.05) from each other. Means (rows) followed by the same upper case letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 6. The effect of CPC on the b* value of fresh-cut cantaloupe cubes stored at 5°C for 11days.

Concentration	Days Storage (b* value)[a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	34.50±3.09 Aa	33.42±2.59 Ba	32.87±2.45 Ca	31.96±2.31 Da	31.95±2.35 Da
Water Control	32.61±2.64 Ab	31.56±2.25 Bb	31.44±2.32 Bb	30.39±2.23 Cb	29.85±2.45 Db
0.6%	33.65±2.18 Ac	31.47±3.18 Bb	30.71±3.21 Ccd	28.44±3.59 Dc	26.89±3.02 Ec
0.8%	33.54±2.52 Ac	31.88±2.48 Bbc	30.34±2.96 Cd	27.45±3.11 Dd	27.51±3.32 Dc
1.0%	34.04±2.70 Aac	32.41±3.01 Bc	30.92±3.22 Cbc	27.91±2.96 Dcd	27.25±2.68 Dc

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (vertical columns) followed by the same letter were not significantly different (P = 0.05) from each other. Means (horizontal rows) followed by the same letter were not significantly different (P = 0.05) from day zero. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

significantly less than the untreated fresh cut control (Table 7).

Experimental day effects were significant for all eleven of the single compounds and all five of the combinations of compounds. Results of the pair-wise comparisons for day 0 compared to days 4, 7, 11, and 14 of selected individual compounds and combinations may be found in Table 8. All CPC treatments and controls were combined to determine experimental day effects. For ethyl hexanoate, days 4, 7, 11, and 14 were significantly greater than day 0, while for (*Z*) 6-nonenal and (*Z*)-6-nonenol all days were significantly less than day 0. For hexyl acetate, days 7 and 11 were significantly greater than day 0 and day 14 was significantly less than day 0. For benzyl-acetate, days 4 and 7 were significantly less than day 0 while days 11 and 14 were significantly greater than day 0. For the acetate combination, days 4, 7 and 14 were significantly less than day 0. For the non-acetate combination, days 4, 7, and 11 were significantly greater than day 0.

Results of the two sets of pair-wise comparisons for the three CPC concentrations to the two control treatments for selected individual compounds and combinations may be found in Table 9. For ethyl 2-methyl butanoate and 3 & 2-methyl butyl acetate, none of the three CPC concentrations were significantly different from either the untreated fresh cut or water dip controls for any of the five experimental days (data not shown). There were no significant differences on day zero for hexyl acetate. However, for all four subsequent experimental days, all three CPC concentrations were significantly less than the untreated fresh cut control (Table 9). The comparisons to the water dip control revealed that on days 0 and 7 only the 1.00% CPC concentration was significantly less than the control. On days 4 and 14, both the 0.80 and 1.00% CPC concentrations were significantly less than the water dip control, while on day 11, all three CPC concentrations were significantly less than the control. For (Z) 6-nonenal, all three CPC

Table 7. Effect of treatment with CPC on the recovery of selected volatile compounds.

Compound	Treatment	Mean	p-value[a]	p-value[b]
Hexyl acetate	**Fresh-Cut Control**	3.93	NA	**0.0243**
	Water Control	3.60	**0.0246**	NA
	0.60%	2.09	**< 0.0001**	**< 0.0001**
	0.80%	2.09	**< 0.0001**	**< 0.0001**
	1.00%	1.70	**< 0.0001**	**< 0.0001**
(Z) 6-nonenal	**Fresh-Cut Control**	0.046	NA	0.0505
	Water Control	0.033	0.0511	NA
	0.60%	0.024	**0.0005**	0.3393
	0.80%	0.022	**0.0001**	0.1917
	1.00%	0.023	**0.0001**	0.1897
3-(methylthio)-propyl acetate	**Fresh-Cut Control**	0.084	NA	0.1107
	Water Control	0.094	0.1122	NA
	0.60%	0.052	**< 0.0001**	**< 0.0001**
	0.80%	0.060	**< 0.0001**	**< 0.0001**
	1.00%	0.065	**0.0009**	**< 0.0001**
Benzyl acetate	**Fresh-Cut Control**	1.06	NA	**0.0116**
	Water Control	0.92	**0.0117**	NA
	0.60%	0.59	**< 0.0001**	**< 0.0001**
	0.80%	0.64	**< 0.0001**	**< 0.0001**
	1.00%	0.60	**< 0.0001**	**< 0.0001**
Z-6-nonenol	**Fresh-Cut Control**	0.029	NA	**0.0002**
	Water Control	0.018	**0.0002**	NA
	0.60%	0.009	**< 0.0001**	**0.0103**
	0.80%	0.007	**< 0.0001**	**0.0004**
	1.00%	0.006	**< 0.0001**	**0.0001**
Acetate+S	**Fresh-Cut Control**	10.80	NA	0.7062
	Water Control	10.43	0.7123	NA
	0.60%	8.50	**<0.0001**	**<0.0001**
	0.80%	8.24	**<0.0001**	**<0.0001**
	1.00%	8.63	**<0.0001**	**<0.0001**
Acetate	**Fresh-Cut Control**	9.68	NA	0.5375
	Water Control	9.26	0.5438	NA
	0.60%	7.31	**<0.0001**	**<0.0001**
	0.80%	7.01	**<0.0001**	**<0.0001**
	1.00%	7.31	**<0.0001**	**<0.0001**

(table cont'd)

NA=not applicable
[a] Treatment compared to fresh-cut control
[b] Treatment compared to water treated control
Analysis of variance (ANOVA) was used to determine if effect of CPC treatment was different from that of the two control samples. If significance was found Dunnett's test (P=0.025) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (vertical columns) in bold font are significantly different from the control sample

concentrations were significantly less than both the untreated fresh cut and water dip controls for day 4 of the experiment. On day 7, all three CPC concentrations were again significantly less than the untreated fresh cut control, but the differences did not reach statistical significance for comparisons to the water dip control. No significant differences were seen for days 0, 11 and 14 for either of the control comparisons. For 3-(methylthio)-propyl acetate, no significant differences were seen for days 0, 4 and 7 for either of the control comparisons. However, on day 11, the 0.60% CPC concentration was significantly less than the untreated fresh cut control and both the 0.60 and 0.80% CPC concentrations were less than the water dip control. On day 14, all three CPC concentrations were significantly less than both the untreated fresh cut and water dip controls. For benzyl acetate, no significant differences were seen on day 0 for either control comparisons. On days 4 and 14, only the 1.00% CPC concentration was significantly less than the untreated fresh cut control. On day 7, both the 0.8 and 1.00% CPC concentrations were significantly less than the untreated fresh cut control, while on day 11, all three CPC concentrations were significantly less than the untreated fresh cut control. For the comparisons to the water dip control, on day 11 both the 0.60 and 0.80% CPC concentrations were significantly less, while on day 14, only the 1.00% concentration was significantly less than the water dip control. For Z-6-nonenol, all three CPC concentrations were significantly less than the untreated fresh cut control for experimental days 0, 4, and 7. No significant differences were seen in days 11 and 14 for comparisons to the untreated fresh cut control

Discussion

CPC has been shown to be an effective antimicrobial agent against a large number of bacteria. It has also been tested on a variety of food products such as fresh beef, poultry tissue, fresh-cut vegetables, and fresh-cut lettuce

Table 8. The effect of experimental day on the recovery of selected volatile compounds.

Compound	Day	Mean	p-value
Ethyl hexanoate	**0**	1.24	NA
	4	2.43	**< 0.0001**
	7	1.89	**< 0.0001**
	11	1.79	**< 0.0001**
	14	1.57	**0.0008**
Hexyl acetate	**0**	2.62	NA
	4	2.67	0.9708
	7	2.87	**0.1059**
	11	2.77	**0.4696**
	14	2.47	**0.5630**
(Z) 6-nonenal	**0**	0.083	NA
	4	0.042	**< 0.0001**
	7	0.021	**< 0.0001**
	11	0.000	**< 0.0001**
	14	0.001	**< 0.0001**
Z-6-nonenol	**0**	0.032	NA
	4	0.012	**< 0.0001**
	7	0.012	**< 0.0001**
	11	0.004	**< 0.0001**
	14	0.009	**< 0.0001**
Benzyl acetate	**0**	0.76	NA
	4	0.34	**< 0.0001**
	7	0.51	**< 0.0001**
	11	1.28	**< 0.0001**
	14	0.91	**0.0081**
Acetates	**0**	8.83	NA
	4	7.91	**0.0311**
	7	7.57	**0.0012**
	11	9.10	0.8476
	14	7.16	**<0.0001**
Non-acetates	**0**	4.40	NA
	4	6.91	**0.0003**
	7	6.00	**0.0303**
	11	6.49	**0.0027**
	14	5.17	0.5375

(table cont'd)

NA=not applicable
Analysis of variance (ANOVA) was used to determine if effect of experimental day was different from that of day zero. If significance was found Dunnett's test (P=0.05) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (columns) in bold font are significantly different from day zero.

Table 9. Interaction effects of treatment with CPC and experimental day on the recovery of selected volatile compounds.

Compound	Treatment	Day 0		Day 4		Day 7		Day 11		Day 14	
		p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]
Hexyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.1207	NA	0.1905	NA	0.0312	NA	0.2505	NA	0.1855	NA
	0.60%	0.6773	0.0500	**0.0009**	0.0401	**< 0.0001**	0.0027	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.0049
	0.80%	0.3999	0.0176	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.0086	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0010**
	1.00%	0.0479	**0.0005**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
(Z) 6-nonenal	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	**0.0006**	NA	0.1715	NA	0.0892	NA	0.9522	NA	0.9643	NA
	0.60%	0.0451	0.1391	**< 0.0001**	**0.0001**	**0.0008**	0.0888	0.9522	1.0000	0.9508	0.9873
	0.80%	0.1105	0.0590	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.0192	0.9522	1.0000	0.9830	0.9800
	1.00%	0.1516	0.0405	**< 0.0001**	**< 0.0001**	**0.0002**	0.0368	0.9522	1.0000	0.9879	0.9531
3-methylthio-propyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.6177	NA	0.7630	NA	0.1840	NA	0.2738	NA	0.0308	NA
	0.60%	0.7728	0.8331	0.0810	0.1476	0.7634	0.1040	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
	0.80%	0.2642	0.1074	0.0624	0.1119	0.2308	0.0124	0.0018	**< 0.0001**	**0.0002**	**< 0.0001**
	1.00%	0.1678	0.0614	0.2525	0.3983	0.1151	0.0042	0.0757	0.0046	**0.0001**	**< 0.0001**
Benzyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.3670	NA	0.2116	NA	0.0669	NA	0.0751	NA	0.9754	NA
	0.60%	0.0424	0.2541	0.0233	0.2992	0.0212	0.6279	**< 0.0001**	**< 0.0001**	0.0131	0.0186
	0.80%	0.3270	0.9374	0.0015	0.0381	**0.0006**	0.0920	**< 0.0001**	**< 0.0001**	0.0026	0.0047
	1.00%	0.0036	0.0407	**0.0005**	0.0232	**< 0.0001**	0.0085	**< 0.0001**	0.0252	**0.0007**	**0.0014**
Z-6-nonenol	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	**< 0.0001**	NA	0.3055	NA	0.0066	NA	0.4610	NA	0.2957	NA
	0.60%	**< 0.0001**	0.6681	**0.0002**	0.0051	**< 0.0001**	0.0202	0.2849	0.7384	0.0463	0.0049
	0.80%	**< 0.0001**	0.1833	**0.0003**	0.0059	**< 0.0001**	0.0083	0.1763	0.5358	0.0146	**0.0011**
	1.00%	**< 0.0001**	0.0126	**< 0.0001**	0.0021	**< 0.0001**	0.2390	0.1567	0.4941	0.0283	0.0024

(table cont'd)

NA=not applicable
[a] Treatment compared to fresh-cut control
[b] Treatment compared to water treated control
Analysis of variance (ANOVA) was used to determine if effect of CPC treatment and experimental day was different from that of the two control samples. If significance was found Dunnett's test (P=0.014) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (vertical columns) in bold font are significantly different from the control sample.

(Lim and Mustapha 2004; Breen and others 1997; Wang and others 2001; Yang and others 2003). To our knowledge this is the first time cetylpyridinium chloride has been tested on fruits and specifically, fresh-cut cantaloupe tissue. In the 24 h study, the mean Log CFU/g was significantly lower in CPC treated samples compared with the control sample. The 24 h study we conducted showed significant reductions for all treatments. A study by Dupard and others (2006) also found significant reductions after 24 h on cooked shrimp. The most effective concentrations for all three bacteria in this study were 0.80 and 1.00% CPC. This finding corroborates results reported by Lim and Mustapha (2004) that antimicrobial activity by CPC is dependent on the concentration used. A study of the bactericidal effect of CPC on fresh-cut vegetables by Wang and others (2001) also showed that efficacy is dependent on concentration. . The high initial reductions in our 24 h study did not carry over to the twelve day shelf life study but significant reductions were still recorded. *E. coli* 0157:H7 was reduced by nearly 1.5 Log CFU/g while *S.* Montevideo was reduced by 1.91 Log CFU/g. *S. sonnei* showed the highest reduction on day 0 with 3.03 Log CFU/g. By day 12 of our study, *E. coli* 0157:H7 counts were reduced by 1.69 Log CFU/g while *Salmonella* Montevideo and *Shigella sonnei* counts were reduced by over 2.5 Log CFU/g. The pathogens could possibly be secreting proteins to protect themselves from the refrigerated temperatures. This could keep the CPC from reaching the microbial cell. A water treated control sample was used during the 12 day shelf life study to mimic treatment washes. The water treated control was often not significantly different than the fresh-cut control for all three pathogens during the shelf life study. This is a positive finding because it indicates that a water wash alone is not effective at removing pathogens from the surface of fresh-cut cantaloupe.

Although many studies have investigated the antimicrobial effect of CPC, few have assessed the effects CPC on the organoleptic and physiological properties of different foods. Most studies have dealt with color changes while some have dealt with odor and firmness or texture of the treated product. Most of these studies have reported that treatment with CPC had little to no effect on the overall color of the treated food. There was fluctuation in the L* values for the five treatments during our study. They all decreased from day 0 to 4 then increased from day 4 to 7. All of the treatments except for the 0.60% CPC treatment increased again from day 7 to 11. On day 14, the water control and the three CPC concentrations all increased again. The L* value of the highest CPC concentration was not significantly different from the two controls on day 14. From day 0 to day 11 of our study, there was a general decrease in a* values for all five treatments. On day 14, all five treatments showed increases in a* values. The treatments of CPC seemed to have a greater impact on a* and b* values than it did on the L* values. By day 14, the highest CPC concentration had a significantly different a* value than the two controls. For the b* value, the 1.00% CPC concentration was significantly different from the untreated fresh-cut control on day 14. These differences could be due to the variability in fruit tested during this study. Yang and others (2003) tested the effect of three CPC concentrations on the color of fresh-cut lettuce. They found no significant difference in L*, hue and chroma values between the treated and untreated samples. A study by Lim and Mustapha (2004) showed that control samples of fresh beef inoculated with *E. coli* 0157:H7 showed the least brightness on day zero. The lightness of this sample was shown to be significantly different from samples treated with CPC. At the end of storage, the *E. coli* 0157:H7 control sample was shown to be significantly less bright than the treated samples. The treatments all had similar redness and yellowness values by the end of the study for *E. coli* 0157:H7 inoculated fresh beef surfaces (Lim and Mustapha

2004). Singh and others (2005) have also reported that a 1.00% CPC treatment did not affect the color of frankfurters stored for 42 days.

To our knowledge there are no published studies that discuss the effect CPC has on percent soluble solids (°Brix) and firmness in fruits and vegetables. The five treatments all showed decreased °Brix values over 14 days. Even so, the CPC treated samples were not significantly different from the controls within each sampling day of the study. In terms of firmness, there was a decline in values over the sampling period. The water control and the 0.60% CPC treatment showed fluctuations during the sampling period. This could be due to the variability of the fruit tested and not be caused by the treatment itself. Each sample had significantly lower firmness values when comparing day 0 to day 14 yet, firmness decline in stored fresh-cut cantaloupe is typical (Beaulieu and others 2004, Luna-Guzman and Barrett 2000).

The individual characteristic impact flavor and aroma compounds identified during our study can be grouped into two main categories: acetate and non-acetate esters. Treatment with the three different CPC concentrations on these compounds was studied. During our study, many of the acetate esters seemed to decrease while some of the non-acetate esters increased. Similar results have been reported in several studies. Beaulieu and others (2004) reported that for cantaloupe melons of varying maturities the amount of acetate esters decreased over time while non-acetate ester recovery increased with time. Beaulieu (2006b) showed that in two different varieties of cantaloupe (cv. ‘Sol Real’ and ‘Athena’) that again the concentration of acetate esters decreased over time while non-acetate esters increased. The 1.00% CPC treated samples had higher means than the control for 3&2-methylbutyl acetate (data not shown). The increases were not significant; however. This is a potentially positive result because 3-methylbutyl acetate is

responsible for banana, fragrant, fruit, pear and sweet flavor notes while 2-methylbutyl acetate is responsible for banana, candy, citrus, ether, floral, fresh, fruity, citrus, peanut and fresh notes. Treatment did seem to decrease the amount of some non-acetate esters. The recovery of (*Z*)-6-nonenol was decreased for cubes treated with CPC. This could affect the characteristic taste of the fresh-cut cube because this compound is responsible for cucumber, green, green melon, powerful, pumpkin, sweet and waxy flavor notes. There is currently no literature available to explain why CPC treated cubes had decreased levels of some non-acetate esters. One possibility could be the addition of propylene glycol to the concentrated CPC solution.

The recovery of hexyl acetate showed a transient increase then decrease over the storage period. Hexyl acetate was significantly different on days 7, 11 and 14 of the study. The recovery of (*Z*)-6-nonenal steadily decreased over time. The recovery of (*Z*)-6-nonenal, (*Z*)-6-nonenol and benzyl acetate was significantly different from day 0 on every subsequent day.

The interaction effect of treatment over time for the recovery of hexyl acetate was significantly different than that of the two controls on most days of the study. Every CPC concentration was significantly different from controls on day four for (Z) 6-nonenal. The effect of treatment over time was not significant for benzyl acetate or 3-methylthio-propyl acetate until nearly the end of the study. The interaction between experimental day and treatment was most often significant for the non-acetate esters on the first two or three days of the study while acetate esters showed significant interaction effects near the end of the study.

This study exhibited that CPC was able to significantly reduce levels of *E. coli* 0157:H7, *S*. Montevideo, and *S. sonnei* over a 12 day period. treatment with CPC did not significantly affect firmness, color or percent soluble solids of the fresh-cut cantaloupe cubes. The recovery of some characteristic impact flavor and aroma compounds was suppressed, however.

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CHAPTER 4

EFFECT OF ACIDIFIED SODIUM CHLORITE ON BACTERIAL SURVIVAL RATE AND PHYSIOLOGICAL QUALITY OF FRESH-CUT CANTALOUPE CUBES AT REFRIGERATOR TEMPERATURES

Introduction

Fruits and vegetables are an integral part of the human diet. They provide important vitamins and minerals in abundance without being high in calories or fat. Fresh-cut produce is a relatively new and growing sector of the fresh produce industry. These products provide consumers with added convenience. These products are convenient because they have been thoroughly washed, cut and packaged before selling (Anonymous 2006). They require no further processing before consumption. They are ideal for consumers seeking a quick and nutritious snack or meal.

Fresh-cut fruits can serve as a vehicle for foodborne disease outbreaks. Fresh-cut cantaloupe has been implicated in several foodborne disease outbreaks involving *Salmonella* (Mohle-Boetani and others 1999; Anonymous 1991; CDC 2002) and *E. coli* 0157:H7 (Del Rosario and Beuchat 1995). Their flesh can provide adequate growth conditions for an array of bacteria, viruses and fungi. Minimal processing may transfer skin microflora to fruit flesh where microorganisms can grow rapidly upon exposure to nutrient laden juices (O'Connor-Shaw and others 1994). Cantaloupe flesh can be contaminated at many different points along the production chain (Beuchat and Ryu 1997). Contamination can occur both pre- and postharvest. Rinses and dips containing antimicrobial compounds could help to reduce levels of microorganisms on the surface of fresh-cut fruits. A number of treatments have been previously studied on whole and fresh-cut fruits. These treatments include sodium hypochlorite (Barak and others 2003), organic acids (Materon 2003), chlorine dioxide (Rodgers and others 2004), hydrogen peroxide (Park and Beuchat 1999; Sapers and Sites 2003), hot water (Ukuku 2006; Solomon and others 2006), electron beam irradiation (Palekar and others 1999) and calcium lactate (Luna-Guzman and Barrett 2000).

One possible way to reduce foodborne disease outbreaks associated with fresh-cut cantaloupe is to treat the cut flesh with acidified sodium chlorite. Acidified sodium chlorite (ASC) is prepared by combining sodium chlorite with a generally recognized as safe (GRAS) acid (FDA 2005). This compound has been approved for use as an antimicrobial agent by the United States Food and Drug Administration. It has been approved for various applications on poultry, red meat, seafood, raw fruits and vegetables, and comminuted meat products. When used in the fresh produce industry, ASC can be used as a spray or dip. The compound can be applied to fresh produce at levels of 500 to 1200 parts per million (ppm) with a final pH value between 2.3 and 2.9 (FDA 2005). Certain processed fruits and vegetables can only be treated with ASC in the form of a dip. After treatment, fruits and vegetables must be washed with potable water and held for twenty four hours prior to consumption (FDA 2005).

The effect of ASC has been studied on raw salmon, ground beef, broiler carcasses, Chinese cabbage, fresh beef, almonds and tomatoes (Su and Morrissey 2003; Bosilevac and others 2004; Oyarzabal and others 2004; Inatsu and others 2005; Lim and Mustapha 2004; Pao and others 2006; Yuk and others 2005). Kemp and others (2000) found that ASC solutions of 500, 850 and 1200 ppm could significantly reduce populations of aerobes on broiler carcasses after only five seconds. Yuk and others (2005) investigated how effective ASC would be against *Salmonella* inoculated onto various surfaces on green, unwaxed tomatoes. They found that a 1200 ppm ASC solution significantly reduced levels of *Salmonella* on the surface of the tomatoes as well as in the stem scar and puncture wounds in the tomato. Inatsu and others (2005) showed that a water rinse only reduced levels of *E. coli* 0157:H7 inoculated onto the surface of cabbage leaves by about 1.0 Log CFU/g while a treatment with ASC reduced *E. coli* 0157:H7 by nearly 3.0 Log CFU/g.

The objective of this study was to determine if ASC was effective in reducing levels of *E. coli* 0157:H7, *Salmonella* Montevideo and *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes held at refrigerator temperatures for up to twelve days. Physiological parameters were also measured to determine the effect of ASC.

Materials and Methods

Culture Preparation

Preparation of *E. coli* 0157:H7 43889, *Salmonella* Montevideo, and *Shigella sonnei* MO 4110 cultures were detailed in chapter three.

Acidified Sodium Chlorite Preparation

Aqueous solutions of acidified sodium chlorite (Bio-Cide International, Redmond, WA) were prepared using sterile distilled water. An aqueous solution of sodium chlorite was combined with citric acid and allowed to sit for 10 min. Sterile distilled water was added to the mixture to make final concentrations of 500, 750 and 1000 ppm (pH 3.10) ASC. The solutions were made the day of the experiment and were used at ambient temperature (25°C).

24 Hour Microbial Enumeration

Cantaloupe (*Cucumis melo* L. *reticulates*) cube preparation and inoculation were previously described in chapter three.

After bacterial attachment, cantaloupe samples were treated with 500, 750, or 1000 ppm (v/v basis) ASC. An untreated, inoculated control was also used. Fresh-cut cantaloupe samples were weighed to approximately 50 g. These samples were placed into Whirl-Pak bags and 50 ml of the appropriate ASC concentration was placed into the bag. Bags were then closed and the sample was gently shaken for 1 min. After 1 min, cubes were removed from the bag using

sanitized forceps and placed in a new Whirl-Pak bag. The samples were held at 5°C for 24 h prior to bacterial enumeration.

To determine *E. coli* 0157:H7, *S*. Montevideo and *S. sonnei* counts, 50 ml of PBS buffer was added to each Whirl-Pak bag containing the fresh-cut cantaloupe samples. The PBS buffer and cantaloupe were homogenized in a stomacher for 1 min at normal speed. Serial dilutions were prepared from the homogenate of each sample and 0.1 ml aliquots of each dilution were spread plated onto xylose lysine deoxycholate (XLD) agar for *S*. Montevideo and *S. sonnei* and sorbitol MacConkey (SMAC) agar for *E. coli* 0157:H7. Plate counts were recorded following 24 h incubation at 37°C and Log CFU/g was determined.

Shelf Life Study: Microbial Enumeration

Cantaloupe cube preparation and sample inoculation were the same as the previously mentioned microbial enumeration experiments in chapter three. After bacterial attachment, cantaloupe samples were treated with 500, 750, or 1000 ppm (v/v basis) ASC. An inoculated control as well as an inoculated control washed with sterile distilled water was also used in this experiment. Treatment with ASC was identical to the procedure discussed in the previous experiment. The sample was then washed for 1 min with sterile distilled water in accordance with 21 CFR 173.325 and stored at 5°C until needed. Bacterial counts were determined at 0,2,4,8 and 12 days. Microbial enumeration was done following the procedure described in chapter three.

Shelf Life Study: Physiological Quality

Cantaloupe (*Cucumis melo* L. *reticulates*) cube preparation for all physiological quality measurements was previously discussed in chapter three. ASC treatment of cubes occurred after processing of the melons. The concentrations used for this experiment were 500, 750 or 1000

ppm ASC along with a Millipore water control and control fresh-cut treatment. All ASC solutions were made with Millipore water. Physiological quality measurements were made on days 0, 4, 7, 11 and 14 during the 2006 study. The previous year measurements were only made on days 0, 4, 7 and 11 due to a lack of tissue. Approximately 250 g of one inch cubes were placed in Juice Catcher containers. The containers were held at 5°C until sampled.

Color was measured using a Hunter colorimeter (DP-9000, Reston, VA). Firmness was measured with a handheld McCormick FT327 with an 8.0 mm probe. These two measurements were performed on cube surfaces that were sliced cleanly with a knife. Measurements were not made using the endocarp or rind side of cubes. Percent soluble solids were measured with an Atago PR101 (Tokyo, Japan) digital refractometer after expressing a portion of juice into the well of the device with a gloved hand.

GC-MS Volatile Preparation

Volatile samples were prepared in quadruplicate, from 4 to 5 cubes (80 – 100 g) from each 250 g Juice Catcher container, as previously described (Beaulieu and Grimm, 2001). Briefly, tissue was rapidly juiced (~15 s) into a slurry with a Braun MP80 Juicer (Gillette Company, Boston, Mass), a 3-mL slurry (without foam) was immediately pipetted into 10-mL glass vials containing 1.1 g NaCl, then benzothiophene internal standard (IS) was added. Vials were sealed with a steel screw top fitted with a Teflon/silicon septum, and placed on a Combi-Pal Autosampler (Leap Technologies, Carrboro, N.C.) cooling rack at 4 °C.

Headspace SPME GC-MS Analysis

Sample vials were equilibrated 10 min via oscillation in a 40 °C autosampler, then a 1-cm 50/30µm stable flex divinyl benzene/carboxen/polydimethyl siloxane (DVB/Car/PDMS) SPME fiber was inserted into the headspace for 12.5 min at 40 °C. Volatile compounds were analyzed

at approximately the temperature of the human palate, where mastication occurs (~37 °C). Vials were continuously swirled during SPME adsorption with an agitation speed of 100 rpm. Fibers were desorbed at 250 °C for 1 min in the injection port of an HP6890/ 5973 GC-MS (Agilent Technologies Wilmington, DE; formerly Hewlett-Packard, Palo Alto, CA) with a DB-5 column (J & W Scientific, Folsom, CA), 30 m, 0.25 mm I.D., 0.5 μm film thickness. Fibers remained in a needle bake out oven (270 °C) for 1 min. The GC was equipped with a Micro Cryo-trap (Scientific Instrument Services, Ringoes, N.J.) and compounds were cryofocused at -60 °C using carbon dioxide during the 1 min desorption in the injection port. The injection port (270 °C) was operated in pulsed splitless mode and subjected to a pressure of 173 kPa of ultrahigh purity helium (99.9995%) for the first minute, then flow velocity was constant at 40 cm s^{-1} for the remainder of the GC run. The initial oven temperature was 50 °C, held 1 min, ramped 5 °C min^{-1} to 100 °C then 10 °C min^{-1} to 190 °C, ramped 30 °C min^{-1} to 250 °C, and held 1 min. The HP5973 quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV (electron volts), a source temperature of 200 °C, with a continuous scan from *m/z* (mass to charge ratio) 33 to 300. Samples were only run in one year since the repeated experiments were lost after hurricane Katrina.

GC-MS Data Analysis

Data were collected with MSD ChemStation software (D.01.02.16) and searched against the NIST (v. 1.5) and Wiley (v. 7.0 NIST98) libraries (Palisade Corp., Newfield, NY). Compounds were preliminarily identified by library search, and then the identities of most were confirmed by comparison of their GC retention time (RT) with authentic compounds, or an in-house cantaloupe retention index (RI) (Beaulieu 2006a; Beaulieu and Grimm, 2001). The RT's from a series of straight-chain alkanes (C_7-C_{20}), produced on the aforementioned column, under

identical conditions, were used to calculate RI's for all identified compounds. Since this is mainly a qualitative appraisal for 14 (ethy 2-methyl propanoate; methyl 2-methylbutanoate; ethyl butanoate; ethyl 2-methylbutanoate; 3 and 2-methylbutyl acetate; ethyl methylthioacetate; ethyl hexanoate; hexyl acetate; eucalyptol; (*Z*)-6-nonenal; 3-(methylthio)-propyl acetate; (*Z*)-6-nonenol; and benzyl acetate) of the 26 characteristic impact flavor/aroma compounds (CIFAC) in muskmelons (Beaulieu, 2006b), selected ion data (integrated ion areas) per compound were normalized on the IS, and averaged. Peaks for 3-methylbutyl acetate (isoamyl acetate) and 2-methylbutyl acetate were combined since the 3-isomer was a minor shoulder peak of the 2-isomer. Standards were acquired from Aldrich (Milwaukee, WI), Fluka (Switzerland), Poly Science (Niles, IL) and Ultra Scientific (North Kingstown, RI).

Statistical Analysis

All microbial analyses were based on two separate experiments each with three determinations (n=6). Significant differences among the means of the two controls and three sanitation dip treatment concentrations within each sampling day were determined using the Student's t test following one-way analysis of variance (ANOVA). Means of each individual treatment concentration as well as each control sample were analyzed over time using the Student's t test following a one-way analysis of variance (ANOVA) in JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA). The statistical difference was set at $p>0.05$. All experiments were duplicated with the exception of the volatile recovery. This experiment was not repeated due to samples being lost after hurricane Katrina.

For the GC-MS procedure, sixteen different variables were of interest (eleven single compounds and five combinations of the eleven single compounds). The objective of the experiment was to compare the effects of the three sanitation dip treatment concentrations to two

different control treatments (fresh-cut and water). Measurements were performed on days 0, 4, 7, 11 and 14 of the experiment with two to four replicates used per day per treatment. Analysis of variance (ANOVA) was used to test for differences among treatment and experimental day means (main effects) as well as for significant interactions between the two main effects. . If treatment and/or day effects were significant, then Dunnett's test was used to make post hoc pair-wise comparisons between the means of the two control groups and the three treatment concentrations, and between day 0 and the other experimental days. In the case of the treatment comparisons, a further correction was made on the Dunnett's tests since two sets of control comparisons were made. If the interaction term was significant, then contrasts were built to test for significant differences between the two control and three treatment concentration means within each of the five experimental days using Bonferroni-corrected significance levels to account for the multiple comparisons being made. Results were considered significant at the nominal level of 0.05 unless otherwise noted. All analyses were performed with SAS® version 9.1.

Results

24 Hour Microbial Enumeration

The highest concentration (1000 ppm) of ASC resulted in a 3.66 Log reduction of *E. coli* 0157:H7 after 24 h (Figure 4). The 750 ppm ASC solution caused a 2.18 Log reduction while the 500 ppm concentration caused a 2.29 Log reduction in *E. coli* 0157:H7 counts. The 1000 ppm concentration was significantly different than the other two concentrations and the control. The other two concentrations were not significantly different from each other but were significantly lower than the fresh-cut control.

After 24 h, the 1000 ppm solution caused a 3.65 Log reduction in *Salmonella* Montevideo counts. The 750 ppm concentration caused a 3.21 Log reduction while the 500 ppm concentration caused nearly a 3.0 Log reduction (Figure 5) in *S*. Montevideo counts. The three concentrations were not significantly different from one another but were significantly different from the control.

The two highest concentrations of ASC caused over a 4.0 Log reduction of *Shigella sonnei* counts (Figure 6). The 500 ppm ASC concentration caused a 3.95 Log reduction of *Shigella sonnei* counts during this study. The three concentrations were not significantly different from one another but were significantly different from the control.

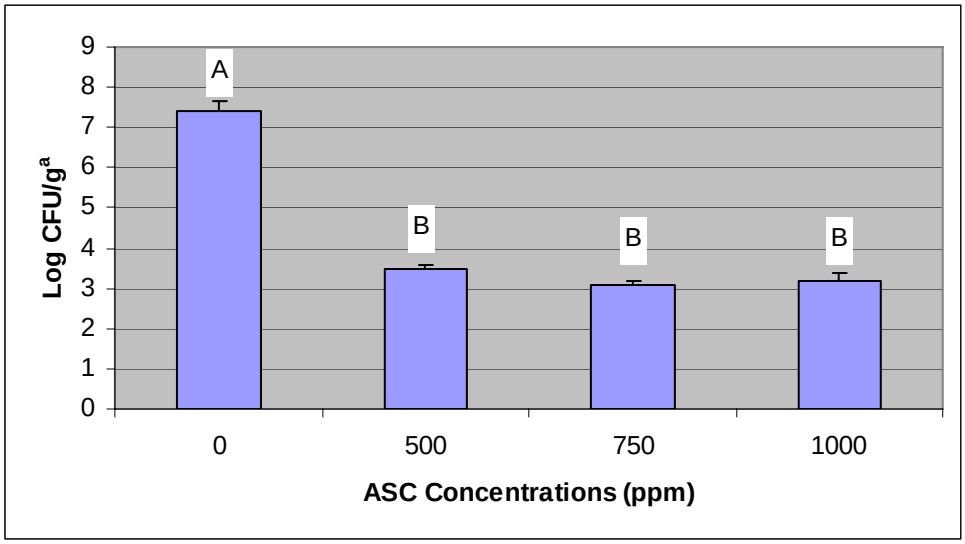

Figure 4. The effect of ASC against *E. coli* 0157:H7 on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

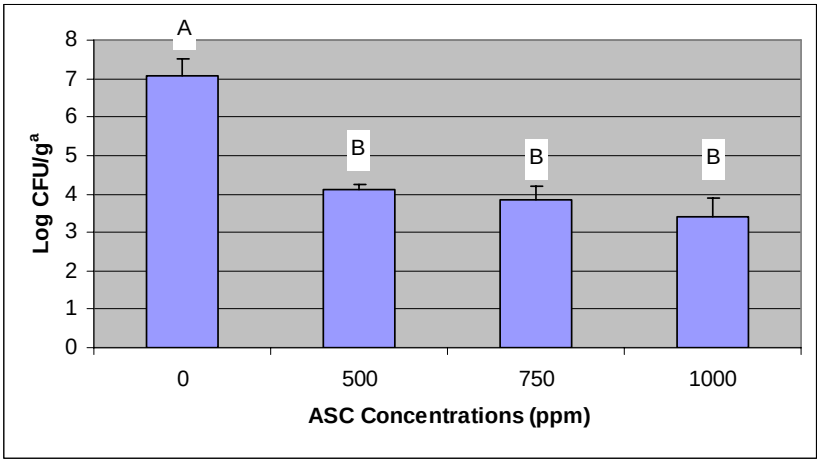

Figure 5. The effect of ASC against *Salmonella* Montevideo on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

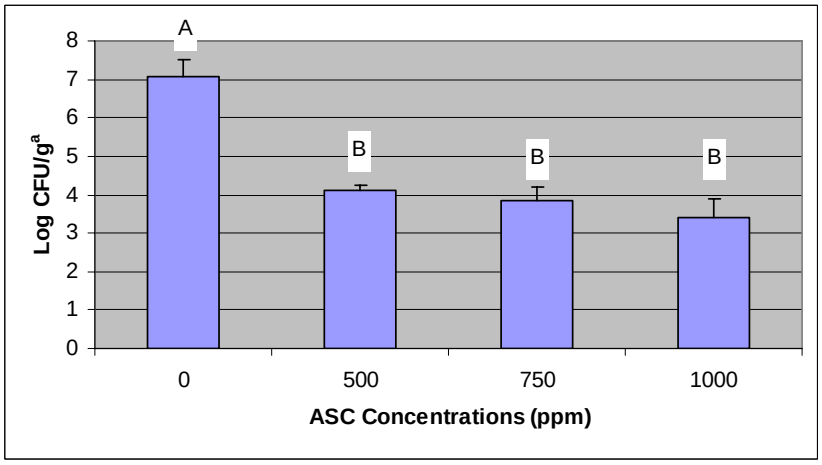

Figure 6. The effect of ASC against *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes held at 5°C for 24h.

[a]All analyses were based on two separate experiments with each mean ± standard deviation being average of two determinations. Means followed by the same letter were not significantly different (P = 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

The two highest concentrations of ASC caused over a 4.0 Log reduction of *Shigella sonnei* counts (Figure 6). The 500 ppm ASC concentration caused a 3.95 Log reduction of *Shigella sonnei* counts during this study. The three concentrations were not significantly different from one another but were significantly different from the control.

Shelf Life Study: Microbial Enumeration

On day 0, the 1000 ppm ASC concentration caused a 1.92 Log reduction of *E. coli* 0157:H7 (Table 10). On sample day 2 and 4, the highest concentration of ASC reduced *E. coli* 0157:H7 counts by 2.44 Log CFU/g. The 1000 ppm ASC treatment caused a 1.88 Log reduction of E. *coli* 0157:H7 counts on day 8 (Table 10). By day 12 of the study, the 1000 ppm ASC treatment caused a 1.67 Log reduction of *E. coli* 0157:H7. The 1000 ppm ASC treatment was significantly different from the other two ASC concentrations as well as the water treatment and untreated control on all five sampling days.

On day 0, *S*. Montevideo counts were reduced by 2.28 Log CFU/g when treated with the 1000 ppm ASC concentration (Table 10). On day 4, the 1000 ppm ASC treatment caused over a 2.5 Log reduction of *S*. Montevideo counts. By day 12, the 1000 ppm ASC concentration reduced *S*. Montevideo counts by 1.94 Log CFU/g. On day 0, the 500 and 750 ppm were statistically different from the untreated control and water treatment but not significantly different from each other. The 1000 ppm concentration was also significantly different from the control and water treatment as well as the other two ASC concentrations. By day 12, all three ASC concentrations were significantly different from each other as well as from the control and water treatment.

The 1000 ppm ASC treatment caused a 2.27 Log reduction of *S. sonnei* counts on day 0 (Table 10). *Shigella sonnei* was reduced by 2.82 Log CFU/g on day 4 by the 1000 ppm ASC

concentration. By day 12 of the study, the 1000 ppm treatment reduced counts of *S. sonnei* by 0.82 Log CFU/g. The 1000 ppm ASC concentration was significantly different from the two other concentrations as well as the controls for all five sampling days.

Shelf Life Study: Physiological Quality

On day zero, the untreated control had the highest °Brix reading (Table 11). The control had a reading of 8.81while the water treated sample had a reading of 8.09 °Brix. The °Brix readings on day zero for the ASC treated samples were 8.29, 7.79, and 7.82 for the 500, 750, and 1000 ppm concentrations, respectively. The untreated control was significantly different from the 750 and 1000 ppm ASC treatments on day zero. The control sample also had the highest °Brix reading on days four and seven of the sampling period. On day four, the control sample had 8.61 °Brix while the water treated sample had a reading of 8.56 °Brix. The 1000 ppm ASC treatment had a reading of 7.88 °Brix. The five treatments were not significantly different from one another on day four or seven of the study (Table 11). The control had a °Brix reading of 8.47 and the water treated sample had a reading of 8.25. The 500 ppm ASC sample had a 8.03 °Brix reading while both the 750 and 1000 ppm ASC sample had a reading of 7.91 °Brix. On day eleven of the study, the control (8.23 °Brix) was not significantly different from the 1000 ppm ASC (8.52 °Brix) treated sample. By day 14 of the study, there was a significant drop in °Brix values for the two controls (untreated and water) as well as three ASC concentrations. The ASC treated samples had higher °Brix values than those of the two controls (Table 11). The highest ASC concentration was not significantly different from the untreated control or the other two ASC concentrations by day fourteen.

reading (17.74 Newtons) followed by the untreated control and the 1000 ppm ASC

There was no significant difference in firmness between the two controls

Table 10. The effect of ASC against *E. coli* 0157:H7, *Salmonella* Montevideo, and *Shigella sonnei* on the surface of fresh-cut cantaloupe cubes held at 5°C for 12 days.

Strain	Treatments	Days Storage (Log CFU/g)[a]				
***E. coli* 0157:H7**		**0**	**2**	**4**	**8**	**12**
	Fresh-Cut Control	6.33±0.26 Aa	6.74±0.13 Aa	6.76±0.12 Aa	5.45±0.11 Ba	5.26±0.08 Ba
	Water Control	5.93±0.12 Ab	6.58±0.07 Bb	6.65±0.12 Ba	4.87±0.10 Cb	4.66±0.17 Db
	500 ppm	4.97±0.13 Ac	5.66±0.05 Bc	5.66±0.11 Bb	4.41±0.08 Cc	3.96±0.11 Dc
	750 ppm	4.69±0.06 Ad	5.39±0.17 Bd	4.56±0.14 Ac	4.30±0.09 Cc	3.77±0.22 Dd
	1000 ppm	4.41±0.07 Ae	4.30±0.06 Be	4.32±0.10 ABd	3.57±0.10 Cd	3.59±0.12 Ce
***S.* Montevideo**						
	Fresh-Cut Control	7.30±0.18 Aa	6.75±0.14 Ba	7.35±0.10 Aa	6.18±0.18 Ca	6.07±0.21 Ca
	Water Control	6.83±0.19 Ab	6.83±0.13 Aa	6.90±0.06 Ab	5.41±0.16 Bb	5.54±0.11 Bb
	500 ppm	5.62±0.04 Ac	5.35±0.05 Bb	5.54±0.04 Ac	4.65±0.16 Cc	4.79±0.05 Dc
	750 ppm	5.46±0.05 Ac	5.07±0.15 Bc	4.69±0.14 Cd	4.50±0.11 Dc	4.37±0.14 Dd
	1000 ppm	5.02±0.26 Ad	4.77±0.13 Bd	4.81±0.07 Be	4.22±0.12 Cd	4.13±0.16 Ce
S. sonnei						
	Fresh-Cut Control	5.82±0.08 Aa	6.22±0.26 Ba	6.38±0.15 BCa	6.59±0.08 Aa	6.94±0.08 Ca
	Water Control	5.24±0.14 Ab	5.65±0.13 Cb	5.41±0.14 Bb	5.82±0.28 Db	6.51±0.16 Eb
	500 ppm	4.68±0.16 Ac	4.78±0.02 Bc	4.82±0.05 Bc	5.41±0.09 Cc	6.28±0.07 Dc
	750 ppm	4.73±0.15 Ac	4.55±0.06 Bd	4.25±0.07 Cd	5.00±0.16 Dd	5.83±0.14 Ed
	1000 ppm	3.55±0.22 Ad	3.99±0.19 Be	3.56±0.26 Ae	4.39±0.25 Ce	6.12±0.13 De

[a] All analyses were based on two separate experiments each with three determinations (n=6). Means for each sampling day (columns) followed by the same lower case letter were not significantly different (P > 0.05) from each other. Means (rows) followed by the same upper case letter were not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

(untreated and water) and the three ASC concentrations on day zero. The water control had the highest firmness concentration. The firmness readings for the three ASC treated samples on day four were 12.40, 11.95 and 12.76 Newtons for 500, 750 and 1000 ppm ASC, respectively. The three ASC concentrations were significantly lower from one another or the water control on day four (Table 12). On day seven, the highest ASC concentration was not significantly different from the controls and the other two ASC concentrations. The three ASC concentrations were not significantly different from each other on day 11. The 750 and 1000 ppm concentrations were also not significantly different from the two controls on day 11. Cubes receiving 1000 ppm ASC had a firmness reading of 9.03 Newtons while the untreated control had a firmness reading of 10.43 Newtons on day 14. These two samples were not significantly different from each other

There was a general trend of increased L* color value for all five samples. The untreated control increased slightly from 62.39 to 65.06 over the four sampling days. The L* value for the water treated sample decreased slightly from day zero to day four. It then increased on days seven and 11. All three ASC treated samples showed increased L* values on days 0, 4 and 7. They then decreased on day 11 of the study. By day 11, the untreated control was significantly different from the other four samples (Table 13).

There were fluctuations in the a* value for all five samples during the study (Table 14). All samples decreased from day zero to day four then increased from day four to day seven. From day seven to day eleven, the a* values decreased again for all five samples. By day 11, the highest ASC concentration was significantly lower than the other two ASC concentrations and the two controls.

The untreated control displayed a gradual decrease in b* value over the eleven day sampling period (Table 15). From day zero to day 11, the b* values for the control decreased

Table 11. The effect of ASC on the percent soluble solids (°Brix) value of fresh-cut cantaloupe cubes stored at 5°C for 14 days.

Concentration	Days Storage (°Brix)[a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	8.81±1.41 Aa	8.61±1.49 Aa	8.47±1.49 Aa	8.23±1.71 Aa	6.46±0.41 Bab
Water Control	8.09±1.73 Aab	8.56±1.55 Aa	8.25±1.19 Aa	7.98±1.06 Aab	6.35±0.48 Bb
500 ppm	8.29±1.59 Aab	7.97±1.53 Aa	8.03±1.48 Aa	7.82±1.15 Aab	6.52±0.39 Bab
750 ppm	7.79±0.77 Ab	7.97±1.47 Aa	7.91±1.13 Aa	7.41±1.17 Ab	6.60±0.60 Bab
1000 ppm	7.82±1.38 Ab	7.88±1.19 Aa	7.91±0.61 ABa	8.52±1.29 Ba	6.78±0.62 Ca

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 12. The effect of ASC on the firmness value of fresh-cut cantaloupe cubes stored at 5°C for 14 days.

Concentration	Days Storage (Newtons)[a]				
	0	**4**	**7**	**11**	**14**
Fresh-Cut Control	17.62±5.82 Aa	14.75±3.17 Ba	13.49±3.25 BCab	12.35±4.34 CDa	10.43±3.06 Da
Water Control	17.74±4.57 Aa	13.61±4.21 Bab	14.50±3.18 Ba	12.84±3.80 Ba	8.34±2.38 Cb
500 ppm	16.88±4.50 Aa	12.40±2.98 Bb	11.98±2.51 BCbc	10.26±2.77 Cb	7.86±1.54 Db
750 ppm	15.55±2.39 Aa	11.95±2.05 Bb	11.46±2.40 Bc	11.05±3.26 Bab	7.92±2.56 Cb
1000 ppm	17.10±4.10 Aa	12.76±3.32 Bb	12.96±3.53 Babc	11.06±2.87 BCab	9.03±2.93 Cab

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

from 34.06 to 32.48. There was a general decrease in b* value for the water treated sample. It increased slightly from day four to day seven, however. The three ASC concentrations all decreased from day zero to day eleven. By day eleven, all five samples were significantly different from each other.

Eleven individual volatile compounds were recovered with consistency during the GC-MS procedure. These compounds include ethyl butanoate, ethyl 2-methyl butanoate, 3&2-methyl butyl acetate, ethyl (methylthio) acetate, ethyl hexanoate, hexyl acetate, eucalyptol, (*Z*)-6-nonenal, 3-(methylthio)-propyl acetate, benzyl acetate, and (*Z*)-6-nonenol. For statistical analysis, five combinations of the eleven compounds were created. These combinations were acetates plus sulfur compounds (acetate+(S)), acetates containing sulfur (sul-aces), acetates, and non-acetates. Treatment effects were significant for all eleven of the single compounds and all five of the combinations of compounds. Results of the two sets of pair-wise comparisons for the three ASC concentrations to the two control treatments for selected individual compounds and combinations may be found in Table 16. For ethyl (methylthio) acetate, both the ASC 750 and 1000 ppm concentrations were significantly less than the untreated fresh cut control while all three concentrations were significantly less than the water dip control. For ethyl hexanoate, only the ASC 1000 ppm concentration was significantly greater than the untreated fresh cut control. For (*Z*)-6-nonenal, the ASC 500 and 1000 ppm concentrations were significantly different from (less than) the untreated fresh cut control. For 3-(methylthio)-propyl acetate, the ASC 750 and 1000 ppm concentrations were significantly less than the water dip control. No significant differences were seen in the untreated fresh cut control comparisons for this compound. For benzyl acetate, only the ASC 500 ppm concentration was significantly different (less than) the untreated fresh cut control. No significant differences were seen in the water dip control

Table 13. The effect of ASC on L* value of fresh-cut cantaloupe cubes stored at 5°C for 11 days.

Concentration	Days Storage (L* values)[a]			
	0	**4**	**7**	**11**
Fresh-Cut Control	62.39±4.84 Aa	63.13±3.55 Ba	64.71±2.81 Ca	65.06±2.96 Ca
Water Control	63.44±3.74 ABb	63.10±3.12 Aa	63.79±3.33 BCb	64.15±3.58 Cb
500 ppm	62.08±5.29 Aac	64.37±3.04 Bb	64.48±3.09 Bab	63.92±3.50 Bb
750 ppm	61.13±5.70 Ac	64.10±3.39 Bb	64.71±3.43 Ba	63.71±5.58 Bb
1000 ppm	62.23±5.56 Aa	64.06±4.46 Bb	64.46±5.01 Bab	63.76±4.19 Bb

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 14. The effect of ASC on the a* value of fresh-cut cantaloupe cubes stored at 5°C for 11 days.

Concentration	Days Storage (a* values)[a]			
	0	**4**	**7**	**11**
Fresh-Cut Control	10.01±3.88 ABa	9.88±1.31 Ba	10.36±1.79 Aa	10.23±1.45 ABa
Water Control	9.63±2.87 Aab	9.54±1.27 Ab	10.27±3.16 Ba	9.78±1.96 Ab
500 ppm	9.34±2.31 Aab	9.27±1.42 Ab	9.52±4.30 Ab	9.02±1.73 Ac
750 ppm	9.38±4.75 Aab	8.92±1.65 Ac	9.39±2.34 Ab	8.98±1.71 Ac
1000 ppm	9.08±2.92 ABb	8.72±2.06 BCc	9.20±2.59 Ab	8.48±1.76 Cd

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

Table 15. The effect of ASC on the b* value of fresh-cut cantaloupe cubes stored at 5°C for 11 days.

Concentration	Days Storage (b* values)[a]			
	0	**4**	**7**	**11**
Fresh-Cut Control	34.06±3.15 Aa	33.64±2.42 ABa	33.41±2.20 Ba	32.48±2.33 Ca
Water Control	32.79±2.94 Ab	32.16±2.14 Bb	32.18±3.22 Bb	31.33±2.29 Cb
500 ppm	31.56±3.35 Ac	30.78±2.41 Bc	30.66±3.02 Bc	29.94±2.57 Cc
750 ppm	31.01±3.84 Acd	30.18±2.77 Bd	29.77±3.07 Bd	28.86±3.30 Cd
1000 ppm	30.60±4.22 Ad	29.23±2.81 Be	28.59±2.53 Ce	27.59±2.62 De

[a] All analyses were based on two separate experiments with each mean ± standard deviation being average of three determinations. Means for each sampling day (columns) followed by the same lower case letter were not significantly different ($P > 0.05$) from each other. Means (rows) followed by the same upper case letter were not significantly different ($P > 0.05$) from each other. Statistical comparisons of all pairs were analyzed using Student's t test following one-way analysis of variance (ANOVA) of JMP-IN (version 4.0.03, SAS Inst. Inc., Cary, North Carolina, USA).

comparisons for benzyl acetate, ethyl hexanoate, (*Z*)-6-nonenal and (*Z*)-6-nonenol. For (*Z*)-6-nonenol, all three ASC concentrations were significantly less than the untreated fresh cut control. Of further interest, in the single compound Z-6-nonenol, the water dip control was significantly less than the untreated fresh cut control.

Experimental day effects were significant for all eleven of the single compounds and all five of the combinations of compounds. All days were compared individually to day 0 of the experiment. Results of the pair-wise comparisons for day 0 compared to days 4, 7, 11, and 14 for selected individual compounds and combinations may be found in Table 17. For ethyl butanoate, day 4 was significantly greater than day 0, while day 14 was significantly less than day 0. For 3 & 2-methyl butyl acetate, both days 7 and 14 were significantly less than day 0. For ethyl (methylthio) acetate, days 4 and 7 were significantly greater than day 0, while day 11 was significantly less than day 0. For eucalyptol, day 4 was significantly greater than day 0, while day 11 was significantly less than day 0. For (*Z*) 6-nonenal and (*Z*)-6-nonenol, days 4, 7, 11, and 14 were all significantly less than day 0. For the acetate combination, only day 11 was significantly different from (greater than) day 0. For the sul-aces combination, both days 4 and 7 were significantly greater than day 0.

Interaction effects were significant for eight of the eleven single compounds (all but ethyl butanoate, ethyl 2-methyl butanoate, and hexyl acetate) and only one of the five combinations of compounds (sul-aces). Results of the two sets of pair-wise comparisons for the three ASC concentrations to the control treatments for selected individual compounds and combinations may be found in Table 18. For 3 & 2-methyl butyl acetate, all three of the ASC concentrations were significantly greater than the untreated fresh cut control on days 0, 4 and 11 of the experiment. On day 7 none of the concentrations were significantly different from the untreated

fresh cut control, while on day 14, only the ASC 500 and 1000 ppm concentrations were significantly greater than control. For the water dip control comparisons, on day 0 only the ASC 1000 ppm concentration was significantly greater than control; on day 4 all three ASC concentrations were significantly greater than control, on days 7 and 14 only the ASC 500 and 1000 ppm concentrations were significantly greater than control; and on day 11, only the ASC 500 and 750 concentrations were significantly greater than control. For eucalyptol, the ASC 500 and 1000 ppm concentrations were significantly greater than the untreated fresh cut control for experimental days 0 and 4. None of the comparisons were significant for any of the subsequent experimental days. For (*Z*) 6-nonenal, only the ASC 1000 ppm concentration was significantly less than the untreated fresh cut control on day 0 while all three concentrations were significantly less than control on day 4. None of the comparisons were significant for experimental days 7, 11 and 14. For the water dip control comparisons, only day 4 revealed significant differences, and only for the ASC concentrations of 500 and 1000 ppm which were significantly less than control. For 3-(methylthio)-propyl acetate, all three ASC concentrations were significantly less than the untreated fresh cut control for day 0; whereas, on day 14, all three concentrations were significantly greater than control. For the water dip control comparisons, both the ASC 750 and 1000 ppm concentrations were significantly less than control on day 0; whereas on day 11, only the ASC 1000 ppm concentration was significantly less than control. For benzyl acetate, no significant differences were seen on days 0 and 4 for either control comparisons. On day 7, no significant comparisons were seen for the untreated fresh cut control, while both the ASC 750 and 1000 ppm concentrations were significantly different from (greater than) the water dip control. On day 11, both the ASC 500 and 1000 ppm concentrations were significantly different from (less than) both the untreated fresh cut and water dip controls; whereas, on day 14, the ASC

750 and 1000 ppm concentrations were significantly different from (greater than) the untreated fresh cut control, but differences were not significant for comparisons to the water dip control.

Discussion

Acidified sodium chlorite has been shown to be an effective antimicrobial against a broad spectrum of bacteria. There were significant reductions after 24 h for all three pathogens used in our study. The highest ASC concentration was consistent in producing large reductions for all three pathogens. The reductions found during our shelf life study assessing antimicrobial activity were not as high as the 24 h results but, were still considered significant. The bacteria could possibly secrete proteins to protect themselves from refrigerated temperatures which might affect how much of the sanitation treatment actually comes in contact with the bacterial cell. The water treated controls normally did not show reductions over 1.0 Log CFU/g during the shelf life study. Similar results were reported by Su and Morrissey (2003) on raw salmon inoculated with *L. monocytogenes*. Lower reductions during the shelf life study could be due to the addition of a water rinse after treatment. The water rinse was used after ASC treatment to comply with current legal standards. Treatment of raw agricultural products with ASC must be followed by a potable water rinse as well as a 24 hour holding period prior to consumption or the product must be cooked. This rinse possibly removed some ASC from the surface of the cantaloupe cube causing the microbial reduction to be lower than that of the preliminary 24 hour study. There was fluctuation in the percent soluble solids (°Brix) of the five treatments during the study. During the first 7 days in storage, fresh-cut controls had higher Brix compared with all treatments. This is expected since rinsing can osmotically reduce sugar concentration from the tissue yet, as catabolism of sugars proceeded, general declines were not observed across treatments by day 11 through 14.

Table 16. The effect of treatment with ASC on the recovery of selective volatile compounds.

Compound	Treatment	Mean	p-value[a]	p-value[b]
Ethyl butanoate	**Fresh-Cut Control**	2.89	NA	0.4121
	Water Control	2.63	0.4074	NA
	500 ppm	3.39	0.0280	**0.0003**
	750 ppm	3.16	0.4043	**0.0171**
	1000 ppm	3.68	**0.0002**	**< 0.0001**
3&2-methyl butyl acetate	**Fresh-Cut Control**	4.69	NA	0.9983
	Water Control	4.74	0.9984	NA
	500 ppm	6.78	**< 0.0001**	**< 0.0001**
	750 ppm	6.12	**< 0.0001**	**< 0.0001**
	1000 ppm	6.92	**< 0.0001**	**< 0.0001**
Ethyl (methylthio) acetate	**Fresh-Cut Control**	1.03	NA	0.7628
	Water Control	1.08	0.7685	NA
	500 ppm	0.94	0.1276	**0.0143**
	750 ppm	0.68	**< 0.0001**	**< 0.0001**
	1000 ppm	0.75	**< 0.0001**	**< 0.0001**
Eucalyptol	**Fresh-Cut Control**	0.10	NA	0.9977
	Water Control	0.10	0.9980	NA
	500 ppm	0.14	**< 0.0001**	**< 0.0001**
	750 ppm	0.10	0.9259	0.9875
	1000 ppm	0.14	**< 0.0001**	**< 0.0001**
Z-6-nonenol	**Fresh-Cut Control**	0.029	NA	**0.0002**
	Water Control	0.018	**0.0002**	NA
	500 ppm	0.014	**< 0.0001**	0.4377
	750 ppm	0.017	**< 0.0001**	0.9809
	1000 ppm	0.018	**0.0002**	0.9999
Sul-Aces	**Fresh-Cut Control**	1.12	NA	0.6276
	Water Control	1.17	0.6341	NA
	500 ppm	1.03	0.2096	**0.0164**
	750 ppm	0.75	**<0.0001**	**<0.0001**
	1000 ppm	0.82	**<0.0001**	**<0.0001**
Acetates	**Fresh-Cut Control**	9.68	NA	0.6626
	Water Control	9.26	0.6690	NA
	500 ppm	11.78	**<0.0001**	**<0.0001**
	750 ppm	11.03	**0.0036**	**0.0001**
	1000 ppm	13.12	**<0.0001**	**<0.0001**

(table cont'd)

NA=not applicable
[a] Treatment compared to fresh-cut control
[b] Treatment compared to water treated control

Analysis of variance (ANOVA) was used to determine if effect of CPC treatment was different from that of the two control samples. If significance was found Dunnett's test (P=0.025) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (vertical columns) in bold font are significantly different from the control sample.

Table 17. The effect of experimental day on the recovery of selected volatile compounds.

Compound	Day	Mean	p-value
Ethyl (methylthio) acetate	**0**	0.77	NA
	4	1.36	< **0.0001**
	7	1.03	< **0.0001**
	11	0.61	**0.0040**
	14	0.70	0.3473
Ethyl hexanoate	**0**	1.49	NA
	4	2.68	< **0.0001**
	7	1.96	< **0.0001**
	11	1.82	**0.004**
	14	1.56	0.8866
(Z) 6-nonenal	**0**	0.079	NA
	4	0.048	< **0.0001**
	7	0.041	< **0.0001**
	11	0.002	< **0.0001**
	14	0.001	< **0.0001**
3-(methylthio)-propyl acetate	**0**	0.031	NA
	4	0.065	< **0.0001**
	7	0.083	< **0.0001**
	11	0.093	< **0.0001**
	14	0.144	< **0.0001**
Z-6-nonenol	**0**	0.036	NA
	4	0.021	< **0.0001**
	7	0.019	< **0.0001**
	11	0.007	< **0.0001**
	14	0.013	< **0.0001**
Benzyl acetate	**0**	0.85	NA
	4	0.54	**<0.0001**
	7	0.86	0.9988
	11	1.38	**<0.0001**
	14	1.33	**<0.0001**
Non-acetate	**0**	5.06	NA
	4	8.29	**<0.0001**
	7	6.62	**0.0383**
	11	7.04	**0.0055**
	14	5.64	0.7440

(table cont'd)

NA=not applicable
Analysis of variance (ANOVA) was used to determine if effect of experimental day was different from that of day zero. If significance was found Dunnett's test (P=0.05) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (vertical columns) in bold font are significantly different from day zero.

Table 18. Interaction effects of treatment with ASC and experimental day on the recovery of selected volatile compounds.

Compound	Treatment	Day 0		Day 4		Day 7		Day 11		Day 14	
		p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]	p-value[a]	p-value[b]
3&2-methyl butyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.1239	NA	0.8537	NA	0.1597	NA	0.5054	NA	0.9247	NA
	500 ppm	**< 0.0001**	0.0017	**0.0002**	**< 0.0001**	0.0200	**0.0003**	**< 0.0001**	**< 0.0001**	**0.0002**	**0.0005**
	750 ppm	**0.0004**	0.0379	**< 0.0001**	**< 0.0001**	0.7748	0.0914	**< 0.0001**	**0.0004**	0.1033	0.1091
	1000 ppm	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.0089	**< 0.0001**	**0.0004**	0.0038	**0.0001**	**0.0003**
Eucalyptol	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.5308	NA	0.9418	NA	0.3029	NA	0.2008	NA	0.3903	NA
	500 ppm	**0.0003**	0.0027	**< 0.0001**	**< 0.0001**	0.0231	0.0011	0.3942	0.0343	0.0100	0.2044
	750 ppm	0.8175	0.6918	0.0437	0.0516	0.0365	0.2817	0.2330	0.9304	0.6888	0.2365
	1000 ppm	**0.0004**	0.0033	**< 0.0001**	**< 0.0001**	0.0102	**0.0004**	0.3781	0.0320	0.0314	0.3615
(Z) 6-nonenal	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	**0.0006**	NA	0.1715	NA	0.0892	NA	0.9522	NA	0.9643	NA
	500 ppm	0.4151	0.0080	**< 0.0001**	**0.0001**	0.0172	0.4841	0.9838	0.9361	0.9803	0.9825
	750 ppm	0.0564	0.1150	**< 0.0001**	0.0021	0.8146	0.1418	0.9223	0.8749	0.9675	0.9944
	1000 ppm	**< 0.0001**	0.3341	**< 0.0001**	**0.0002**	0.2094	0.0035	0.5351	0.4965	0.9950	0.9597
3-(methylthio) -propyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.6177	NA	0.7630	NA	0.1840	NA	0.2738	NA	0.0308	NA
	500 ppm	**0.0004**	0.0019	0.8896	0.6598	0.0056	0.1395	0.9598	0.2963	**< 0.0001**	0.0684
	750 ppm	**< 0.0001**	**0.0004**	0.1734	0.2880	0.8047	0.1158	0.0403	0.0019	**0.0012**	0.3795
	1000 ppm	**< 0.0001**	**< 0.0001**	0.1163	0.2032	0.8894	0.2336	0.0238	**0.0010**	**< 0.0001**	0.1228
Benzyl acetate	**Fresh-Cut Control**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Water Control	0.3670	NA	0.2116	NA	0.0669	NA	0.0751	NA	0.9754	NA
	500 ppm	0.5540	0.7555	0.0097	0.1717	0.7725	0.0345	**< 0.0001**	**< 0.0001**	0.5058	0.5583
	750 ppm	0.0979	0.4472	0.7210	0.1092	0.1303	**0.0010**	0.0030	0.2190	**0.0008**	0.0020
	1000 ppm	0.8561	0.4706	0.3265	0.0269	0.0843	**0.0005**	**< 0.0001**	**0.0005**	**0.0008**	0.0020

(table cont'd)

NA=not applicable
[a] Treatment compared to fresh-cut control
[b] Treatment compared to water treated control
Analysis of variance (ANOVA) was used to determine if effect of CPC treatment and experimental day was different from that of the two control samples. If significance was found Dunnett's test (P=0.014) was used for post hoc pair-wise comparisons between the means of the two control samples and three treatment concentrations. P values (vertical columns) in bold font are significantly different from the control sample.

Overall, the five samples (two controls and three ASC treatments) showed decreases in soluble solids content during the course of the study. These results are similar to those found by Beaulieu (2005) when comparing soluble solids content of various cultivars. The untreated control sample decreased through storage while the water control increased from day 0 to 4 then decreased on each subsequent day. The 1000 ppm concentration actually increased from day 0 to 11 then decreased from day 11 to 14. We were only able to record °Brix readings on day 14 during the 2006 experiment due to lack of tissue the previous year. This may account for the large decrease experienced by all the treatments on day 14. Individual fluctuations in °Brix may be due to variations in melons sampled.

Firmness generally decreased during fresh-cut storage. There was a large drop in firmness from day 0 to 4 for all five treatments. The untreated fresh-cut control then dropped on each subsequent sampling day. On day seven, the water control and the 1000 ppm both increased while the two lower ASC concentrations decreased. On days 11 and 14, all five treatments showed decreased firmness readings. The fluctuations seen in the water control and the 1000 ppm concentration could again be due to variations in melons sampled. Regardless, firmness decline in stored fresh-cut cantaloupe is typical (Beaulieu and others 2004, Luna-Guzman and Barrett 2000). Several studies have shown that firmness will generally decrease over time after processing. This may be due to increased ethylene production or enzymatic activity.

Our results for color showed a general trend of increased L* value in all samples. The untreated fresh-cut control increased slightly on each sampling day while the water treated control decreased from days 0 to 4 and then increased on each subsequent sampling day. The three ASC treated samples showed increased L* values between days 0 and 7 then decreased on day 11. There were fluctuations in a* values for all five treatments. The values decreased from

day 0 to 4 then increased from day 4 to 7. They again decreased from day 7 to 11. The b* values for all five treatments decreased over the sampling period. The fluctuations seen in individual treatments could be due in part to variations within the melons used for the experiments.

Treatment of fresh-cut cantaloupe cubes appeared to markedly elevate recovery levels for several CIFACs. The recovery of all acetate volatiles was increased while the sulfur containing acetate volatiles were decreased. The recovery of volatile compounds fluctuated over the 14 day sampling period. Most of the compounds showed transient increases before decreasing. The 1000 ppm ASC concentration had the highest recovery for the majority of compounds investigated.

It has been shown in several studies that acetate esters in muskmelons generally decrease while non-acetate esters increase. This general trend was also seen in our study but treatment with the three concentrations of ASC actually markedly increased the recovery of some acetate esters. All three concentrations of ASC significantly increased the levels of 3&2-methylbutyl acetate recovered during our study. This is a potentially positive result because these two volatile compounds are responsible for several sweet and fruity flavor notes.

Levels of hexyl acetate increased over the 14 day physiological study. These increases were determined to be significantly greater than the control. Hexyl acetate is normally associated with flavor notes such as apple, cherry, floral, pear, and pine. The levels of some non-acetate esters actually slightly decreased over time instead of increasing as previously reported (Beaulieu and others 2004; Beaulieu 2006a. Decreased levels of non-acetate esters can lead to the loss of several characteristic flavor notes in fresh-cut cantaloupe. Interaction effects showed that the three concentrations of ASC had significant interaction on the recovery of 3&2-methyl butyl acetate every day of the study. Interaction effects for most non-acetate esters were significant

during the beginning of the study but usually not at the end. Most acetate esters had significant interaction effects during the middle to end of the study.

Treatment with ASC significantly reduced the counts of three pathogens on the surface of fresh-cut cantaloupe. There was a general decrease over a 14 day sampling period in color, firmness and soluble solids of cantaloupe cubes when treated with ASC. These differences in most cases were not significantly different though. Recovery of some important volatile compounds was actually increased after treatment with ASC.

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CHAPTER 5

CONCLUSION

Currently, CPC is only approved for use in raw poultry carcasses at a level not to exceed 0.3 grams per pound. This study has shown that CPC shows strong potential as an antimicrobial agent on fresh-cut cantaloupe cubes. Levels of *E. coli* 0157:H7, *Salmonella* Montevideo and *Shigella sonnei* were significantly reduced on the surface of fresh-cut cantaloupe cubes over the 12 day sampling period. They were also significantly reduced after a 24 h refrigeration period. Treatment with CPC also did not significantly affect the soluble solids, firmness or color of the fresh-cut cantaloupe cubes. Treatment of cantaloupe cubes with ASC followed by a water rinse was also effective at reducing levels of *E. coli* 0157:H7, *Salmonella* Montevideo and *Shigella sonnei*. These pathogens were significantly reduced over the12 day shelf life study. There was a general decrease over a 14 day sampling period in color, firmness and soluble solids of cantaloupe cubes when treated with ASC. These differences in most cases were not significantly different though. CIFAC recovery was suppressed by the treatment of cubes with CPC while treatment with ASC often caused volatile levels to be elevated. More work is needed with a sensory panel to determine how acceptable these compounds would be when used on fresh-cut cantaloupe cubes.

VITA

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