Allyl isothiocyanate reduces *Salmonella enterica* Michigan and *Listeria monocytogenes* on the surface of whole cantaloupe (*Cucumis melo* L.).

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ABSTRACT

Since 2006 there have been four *Salmonella enterica* and one *Listeria monocytogenes* foodborne outbreaks linked to whole cantaloupe fruit. No post-harvest intervention to reduce potential contamination on cantaloupe currently exists. The complex surface topography of netted cantaloupes aids bacterial attachment. This research evaluates the use of allyl isothiocyanate (AITC; a natural antimicrobial) to reduce populations of *S. enterica* Michigan and *L. monocytogenes* on the surface of cantaloupe. Fifty µl of *S.* Michigan or *L. monocytogenes* was inoculated onto whole 'Athena' or 'Hales Best Jumbo' ('HBJ') cantaloupe fruit in 22 mm diameter circles and allowed to dry for 90 min. resulting in 6.60 log CFU/g. Cantaloupe received either AITC liquid or vapor, sterile deionized water, 200 ppm sodium hypochlorite per circle, or no treatment. All cantaloupes were stored in separate sealed glass desiccators for 1 or 24 h at 25°C or 35°C. To enumerate the bacteria following treatment, 22 mm sections of the rind were removed, homogenized and plated onto appropriate agar. Headspace analysis using Gas Chromatography-Mass Spectrometry (GC-MS) quantified the concentration of each AITC vapor treatment. The texture quality of the pericarp tissue of whole cantaloupes was evaluated after 24 h treatments, followed by two weeks of storage at 4°C.

The concentration of vapor ranged from 3.4 to 19.6 µl AITC/L inside the desiccators. The liquid treatment reduced ($P < 0.05$) *S.* Michigan populations on 'Athena' (3 log CFU/g) and *L. monocytogenes* on 'HBJ' (2.6 log CFU/g). The longer exposure time to the AITC vapor (24 h versus 1 h) resulted in a greater reduction of both *S.* Michigan and *L. monocytogenes* on 'Athena' and treatments at 35°C reduced microbial populations up to 4.5 times greater ($P < 0.05$). The highest vapor concentration reduced ($P < 0.05$) both pathogens at least 3.0 log CFU/g on 'Athena' at 25°C. Generally, bacterial pathogens from the surface of 'Athena' cantaloupe were reduced more than pathogens inoculated on the surface of 'HBJ.' The application of AITC liquid or vapor is a natural alternative post-harvest treatment to 200 ppm free chlorine to reduce the level of bacterial contamination on cantaloupe surfaces for certified organic production.

Dedication

This dissertation is dedicated to all those I have lost.

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CHAPTER 1

Introduction

Fresh fruits and vegetables add a wide range of nutrients into a daily diet. Since the first Dietary Guidelines for Americans was released in 1980, Americans have been encouraged to maintain a healthy weight by including exercise into their daily routine as well as limiting the amount of sugar, sodium, and saturated fat in their diet. The U.S. Department of Agriculture (USDA) dietary guidelines recommend the consumption of 2.5 to 6.5 cups of fresh produce daily for a healthy lifestyle (Department of Health and Human Services, 2010). This is one factor that has led to an increase in sales of fresh produce, both whole and cut. Melons, like cantaloupe, are sold to consumers as whole fruit, pre-cut slices or cubes, or in fruit salads with other fresh produce that are available year-round at grocery stores. Cantaloupes are a highly versatile agricultural product that can be consumed at breakfast, as a snack, at picnics, or as a dessert (Boriss et al., 2012). Due to their popularity and easy accessibility, there has been a recent focus on marketing them as whole melons, pre-cut products, convenience products in packages for individual servings, or in salad bars (FDA, 2013).

Cantaloupes, like other fresh fruits and vegetables, are at risk of becoming contaminated with foodborne pathogens. The introduction of foodborne pathogens can occur at several different points in the production chain. Bacteria can be present in the growing environment from the soil or water or can be introduced via infected animals or humans who come into contact with them. Contamination can also take place between the field and shipping in the packinghouse through unsanitized equipment, dirty floors, leaking roofs, or by workers. Within the last decade, there have been four multistate (including one international) outbreaks of

Salmonellosis linked to cantaloupe consumption, and one in 2011 linked to cantaloupe contaminated with *Listeria monocytogenes*, the first ever associated with the pathogen (Marler, 2012). As demonstrated by these and several other cases of salmonellosis associated with cantaloupes over the past two decades, new or improved methods of sanitizing to eliminate or reduce the amount of pathogenic microorganisms present on the surface or after cutting is needed.

The surface of many cultivars of cantaloupe contains netting which provides a hospitable porous environment for bacteria to attach and thrive (Ukuku and Fett, 2006). This morphological characteristic makes them susceptible to bacterial attachment and provides a good medium for pathogen growth (FDA, 2013). When the fruit is cut or if the surface is punctured, bacteria can enter and multiply rapidly because of the abundant nutrient source provided by the flesh and juice and its favorable pH of 6.1 to 6.6 (Parnell et al., 2003; Ukuku and Sapers, 2001). Since they are a low acid food, their flesh is a natural breeding ground for bacteria if they can get past the outer rind (FDA, 2013). Additionally, fresh-cut cantaloupe is considered a potentially hazardous food and therefore should be stored at less than 5°C to reduce the food safety risk (FDA, 2008). However, from a horticultural standpoint cantaloupe fruits are also chilling sensitive. Storage at temperatures less than 13°C causes chilling injury because cell membranes leak resulting in off flavors and a loss of fruit quality. Therefore, storing whole melons at less than 5°C is not a routine practice in distribution channels because of the decrease in product quality that may occur. Only cut fruit are routinely refrigerated during retail sale.

Current commercial sanitizers used to treat cantaloupes include chlorine and hydrogen peroxide (H_2O_2), but they only may reduce the total bacterial population by 1 to 3 logs (Adams et al., 1989; Beuchat et al., 1998; Ukuku et al., 2001; Wei et al., 1995). Natural antimicrobials have

become a ‘green alternative’ for preventing pathogenic and spoilage organisms in and on foods. The essential oils of natural antimicrobials have been traditionally tested and utilized in the food processing industry in their liquid forms. The majority of natural antimicrobials are secondary metabolites that are produced as plant defense mechanisms in response to predation by microorganisms, insects, and herbivores (Cowan, 1999). One potential antimicrobial, allyl isothiocyanate (AITC), an extract obtained from brown mustard seeds, has antimicrobial activity in the liquid phase. However, the vapor phase of essential oils can possess greater antimicrobial activity because of their ability to reach locations, such as cracks and stem ends, where pathogens may reside and can evade aqueous sanitizers (Obaidat and Frank, 2009b). More specifically, the vapor state of some volatile oils, like AITC, is more effective and stable than their liquid counterparts (Sekiyama et al., 1996). There is some research on AITC vapor and its antimicrobial activity when applied to fresh produce, but it is limited to only a handful of commodities (alfalfa, lettuce, spinach, and tomatoes) (Obaidat and Frank, 2009a; Obaidat and Frank 2009b; Weissinger et al., 2001). A recent study revealed the ability of a coating containing chitosan, nisin, and liquid allyl isothiocyanate to reduce the population of *Salmonella* on cantaloupe rind, but the vapor form has not been investigated (Chen et al., 2012).

The primary objective of this study is to evaluate the effect of AITC in liquid and vapor phase to reduce foodborne pathogenic bacteria (*Salmonella enterica* serovar Michigan and *Listeria monocytogenes*) on the surface of two cantaloupe cultivars: Athena and Hales Best Jumbo. The corky netted surface of cantaloupe rind supports the formation of biofilms and thus makes it more difficult for liquid sanitizers to remove them efficiently. AITC vapor applied as an additional processing step after the conventional washing procedure at the packinghouse may provide an extra level of safety. Vapor treatment could also be utilized prior to packing for those

cantaloupe fruit that do not undergo a washing step, especially because they do not currently receive any sanitizing step. The comparative evaluation of the effect of AITC in liquid and gaseous phase against pathogenic microorganisms on the surface of whole cantaloupe is the first step in demonstrating the potential use of AITC vapor to reduce or eliminate foodborne pathogens in the melon industry. In the future, AITC vapor could allow growers and packers to more effectively sanitize their cantaloupes with a natural antimicrobial desired by consumers instead of using chemical sanitizers. AITC may have applications for sanitizing other types of vegetables as well if it is effective on cantaloupe.

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CHAPTER 2

Review of the Literature

Outbreaks associated with cantaloupe

The first multistate outbreak of salmonellosis associated with cantaloupe was reported in 1990. The implicated strain was *Salmonella enterica* serovar Chester, which affected 245 people (two deaths) in 30 states (Mead et al., 1999; Ries et al., 1990). The second case involved more than 400 laboratory confirmed *S. enterica* Poona infections in 23 states and Canada in 1991 (CDC, 1991). *S. enterica* Poona was also the verified culprit in several multistate outbreaks associated with consumption of cantaloupes in 2000, 2001, and 2002 (CDC, 2002). Additionally, S. *enterica* Oranienburg was culture confirmed in a 1998 outbreak in 10 U.S. states and one Canadian province involving 41 cases that were traced back to a fruit salad that contained cantaloupe and honeydew melon (CDC, 2007).

Microbiological surveys of domestic cantaloupes revealed that 4 (2.4%) of 164 samples tested positive for *Salmonella* (U.S. Department of Health and Human Services, 2003), while another reported *Salmonella* spp. on 0.5% of domestic cantaloupes and 0.3% on imported cantaloupes from Mexico (Castillo et al., 2004). In a third study, 3 (3.3%) of 90 cantaloupe samples were contaminated with *S. enterica* Montevideo (Johnston et al., 2005). While the melon rind is not commonly consumed, as few as 150 microorganisms per cm^2 on the rind of cantaloupe can contaminate the interior flesh after cutting (Suslow and Cantwell, 2001). Data from Ukuku and Sapers (2001), suggests that an outbreak of disease might result from eating contaminated fresh-cut melon prepared from improperly washed or surface-sanitized cantaloupe rind stored at 4 or 20°C for more than 3 d.

In the past 6 years, there have been 3 multistate outbreaks of *S. enterica* subspecies associated with cantaloupes. The first of these outbreaks involved *S. enterica* Litchfield in 2008 from cantaloupes imported from Honduras (CDC, 2008). Fifty one individuals became ill in 16 states (CDC, 2008). In 2011, an outbreak strain of *S. enterica* Panama was traced back to imported cantaloupes from a single farm in Guatemala (CDC, 2011c). Twenty people fell ill in 10 states, including 3 who were hospitalized (CDC, 2011c). The most recent multistate outbreak occurred in 2012 when a total of 261 persons were infected with *S. enterica* Typhimurium (228 persons) and *S. enterica* Newport (33 persons) from 24 states (CDC, 2012a). Ninety four of those individuals were hospitalized and 3 people died in Kentucky (CDC, 2012a). The source of the outbreak was traced back to cantaloupe from Chamberlain Farms Produce, Inc. in Owensville, Indiana (CDC, 2012a). The initial contamination was reported to have likely occurred in the production field and was then most likely spread by operations and practices in the packinghouse (FDA, 2013b). Both food and non-food contact surfaces in the packinghouse were constructed of materials that could not be easily cleaned or sanitized, including carpet and wood (FDA, 2013b). Additionally, water was allowed to pool on the floor near equipment, and there was evidence of birds roosting in the buildings rafters (FDA, 2013b). Bird droppings were seen on equipment and the floor, which were directly above food contact surfaces (FDA, 2013b). An environmental assessment inspection report by the U.S. Food and Drug Administration indicated that records were not available to demonstrate whether Chamberlain Farms had monitored the pH of the water for washing the cantaloupes (FDA, 2013b). The report also noted that there was an accumulation of debris including trash, wood, food pieces, standing water, mud, and dirt observed beneath the conveyer belt and that the cantaloupes were not pre-cooled

after packing before shipment to retailers, which may have facilitated *Salmonella* survival and growth on the rind (FDA, 2013b).

The first and only *Listeria monocytogenes* related cantaloupe outbreak to date occurred in September 2011 and was traced back to Jensen Farms in Colorado (CDC, 2012b). The total number of outbreak-associated illnesses was 147 persons in 28 states (CDC, 2012b). There were 33 deaths as a result of listeriosis and 1 miscarriage (CDC, 2012b). Ten other deaths may have been attributed to *L. monocytogenes* among persons who had been infected with an outbreak-associated subtype (CDC, 2012b). This was the third deadliest foodborne disease outbreak in U.S. history (Flynn, 2012).

The Food and Drug Administration, along with Colorado state officials, conducted an inspection at Jensen Farms. Their findings indicated several factors that potentially contributed to the introduction, growth, and spread of *L. monocytogenes*. There was a low level of *L. monocytogenes* in the growing field and incoming cantaloupe may have contributed to the introduction and spread of the pathogen into the packing facility (CDC, 2011d). A truck that was used to transport culled cantaloupe to a cattle operation was parked adjacent to the packing facility and it may have introduced *L. monocytogenes* to the facility (CDC, 2011d). Neither the packing facility floor nor the packing equipment were easily cleanable or sanitized (CDC, 2011d). The washing and drying equipment used for cantaloupe packing had previously been used for postharvest handling of another raw agricultural commodity, which could have led to cross-contamination (CDC, 2011d). Lastly, there was no pre-cooling step to remove field heat before the cantaloupes were transferred to cold storage (CDC, 2011d).

***Salmonella*: Basic characteristics**

Each year in the U.S. *Salmonella* is estimated to cause more than 1.2 million illnesses, with more than 23,000 hospitalizations and 450 deaths (Scallan et al., 2011). *Salmonella* spp. are facultatively anaerobic Gram-negative rod-shaped bacteria belonging to the family *Enterobacteriaceae* (D'Aoust and Maurer, 2007; Montville and Matthews, 2005). Members of the genus are motile by peritrichous flagella; however, there are nonflagellated variants such as *Salmonella enterica* serovar Pullorum and *Salmonella enterica* serovar Gallinarum, and nonmotile strains resulting from dysfunctional flagella also exist (D'Aoust and Maurer, 2007; Montville and Matthews, 2005). *Salmonella* are oxidase negative and catalase positive, grow on citrate as a sole carbon source, generally produce hydrogen sulfide, decarboxylate lysine and ornithine, but do not hydrolyze urea (D'Aoust and Maurer, 2007). Salmonellae are chemo-organoleptic, with the ability to metabolize nutrients by both respiratory and fermentative pathways (D'Aoust and Maurer, 2007).

The genus *Salmonella* consists of 2 species that both contain multiple serovars: *S. enterica* and *S. bongori* (Brenner et al., 2000; Popoff et al., 2004). *S. enterica* subspecies are differentiated on the basis of their biochemical properties and genomic sequences (D'Aoust and Maurer, 2007). The biochemical identification is generally coupled with serological confirmation based on the agglutination of bacterial surface antigens with *Salmonella*-specific antibodies (D'Aoust and Maurer, 2007). These antigens include somatic (O), lipopolysaccharides (LPS) on the external surface of the outer membrane, flagellin (H) antigens associated with the peritrichous flagella, and the capsular (Vi) antigen, which only occurs in serovars Typhi, Paratypchi C, and Dublin (Le Minor, 1981). Meanwhile, capsular (K) antigens that are commonly encountered in members of the *Enterobacteriaceae* are limited to the Vi antigen in the *Salmonella* genus (D'Aoust and Maurer, 2007).

Salmonellae have the ability to readily adapt to extreme environmental conditions, ranging from elevated temperatures (≤ 54°C) to refrigeration temperatures (2 to 4°C) (D'Aoust et al., 1975). They are mesophilic, with optimum growth temperature between 35 and 37°C, but generally have a growth range of 5 to 46°C (Ray and Bhunia, 2008). They can proliferate at a pH range from 4.5 to 9.5, with an optimum pH for growth of 6.5 to 7.5 (D'Aoust and Maurer, 2007; Montville and Matthews, 2005). *Salmonella* spp. can live in foods with water activities as low as 0.93 (D'Aoust, 1989). Even though *Salmonella* is generally inhibited in the presence of 3 to 4% NaCl, bacterial salt tolerance increases as temperature increases in the range of 10 to 30°C (D'Aoust and Maurer, 2007; Montville and Matthews, 2005). Several studies have revealed the increased ability of *Salmonella* spp. to grow under acidic (pH, ≤ 5.0) conditions or in environments of high salinity (≥ 2% NaCl) with increasing temperature (D'Aoust, 1989; Ferreira et al., 1987; Montville and Matthews, 2005; Thomas et al., 1992).

Salmonella spp. are found widespread in the natural environment in both the gastrointestinal tracts of animals, but also in soil, water, and sewage contaminated with fecal matter (Andrews and Baumler, 2005; D'Aoust, 1989; Ray and Bhunia, 2008). Fruits and vegetables can become contaminated with *Salmonella* by the fertilization of crops with untreated sludge or sewage effluents, irrigation water from contaminated sources, contaminated equipment, or poor worker sanitation. Since the 1950s, foodborne salmonellosis has been recognized as the major cause of all foodborne diseases by pathogenic bacteria and viruses, both in number of incidents (sporadic and outbreaks), as well as number of cases (Ray and Bhunia, 2008). There are an estimated 1.4 million cases of salmonellosis each year in the U.S. and over 2,500 serotypes of *Salmonella* have been identified (Brenner et al., 2000; Mead et al., 1999; Popoff et al., 2000).

Salmonella enterica subspecies *enterica* includes most serotypes that are frequently associated with foodborne salmonellosis (Ray and Bhunia, 2008). A dose of 10^1 to 10^5 cells must be consumed in order to initiate infection (Ray and Bhunia, 2008). Foodborne salmonellosis is characterized by gastrointestinal disorder in the form of nonbloody diarrhea and abdominal cramps (Ray and Bhunia, 2008). Other symptoms include nausea, vomiting, chills, fever, and prostration (Ray and Bhunia, 2008). The illness is usually self-limiting and symptoms appear within 8 to 42 h, generally in 24 to 36 h, and last for about 2 to 3 d, but in certain individuals they can last longer (Ray and Bhunia, 2008). Salmonellosis can be fatal, especially for sick individuals, infants, and the elderly (Andrews and Baumler, 2005; D'Aoust, 1989). An infected individual can be a carrier for several months after recovering (Ray and Bhunia, 2008). Newborns, infants, the elderly, and immunocompromised individuals are more susceptible to *Salmonella* infections than healthy adults (D'Aoust, 1989; Montville and Matthews, 2005). The infectious dose for humans can be as few as 15 to 20 cells, depending on the age and health of the host and the specific strain (FDA, 2013a).

***Listeria monocytogenes*: Basic characteristics**

Listeria monocytogenes is 1 of 6 species in the genus *Listeria.* There are 13 serotypes of *L. monocytogenes*, which can cause disease, but more than 90% of human isolates belong to 3 serotypes: 1/2a, 1/2b, and 4b (Salova et al., 2005; Seeliger and Hohne, 1979). It is a Gram-positive, psychrotrophic, facultative anaerobic, nonsporulating, motile, small rod that displays tumbling motility facilitated by the presence of peritrichous flagella (Ray and Bhunia, 2008). However, motility is temperature dependent, and flagellar expression is at its maximum when at a temperature of 20 to 30°C (Ray and Bhunia, 2008). *L. monocytogenes* is hemolytic and produces β-hemolysis on sheep or horse blood agar plates (Ray and Bhunia, 2008).

L. monocytogenes has an optimum growth temperature range of 30 to 37°C and can initiate growth in a temperature range of 0 to 45°C, but growth occurs more slowly at lower temperatures (Montville and Matthews, 2005; Ray and Bhunia, 2008; Swaminathan et al., 2007). At pH values below 4.3, cells may survive, but they do not grow (Montville and Matthews 2005). It grows optimally at water activities > 0.97, with 0.93 the minimum water activity for growth (Swaminathan et al., 2007; Montville and Matthews, 2005). Some strains may grow below a water activity of 0.90 and the bacterium may be capable of surviving long periods at water activities as low as 0.83 (Shahamat et al., 1980; Swaminathan, 2007). *L. monocytogenes* is able to grow in the presence of 10 to 12% sodium hypochlorite (NaClO) and can grow to high populations in salt concentrations of 6.5% (Swaminathan et al., 2007; Montville and Matthews, 2005).

L. monocytogenes can enter into food processing facilities through soil on workers' shoes and clothing, on transport equipment, raw plant tissue, and even healthy human carriers (Swaminathan et al., 2007). It is most often detected in moist areas such as condensed and stagnant water, floors, floor drains, residues, and processing equipment (Cox et al., 1989). The bacterium can attach itself to many types of surfaces, including stainless steel, glass and rubber (Jeong and Frank, 1994). Due to its tendency to adhere to food contact surfaces and form biofilms, it is very difficult to eliminate from food processing facilities (Gravani, 1999). Post-processing contamination is the most likely source of contamination of processed foods by *L. monocytogenes* (Swaminathan et al., 2007).

Certain ready-to-eat foods, such as unpasteurized milk and products prepared from unpasteurized milk, soft unfermented cheeses, uncooked frankfurters, certain delicatessen meats and poultry products, and some seafoods, are high-risk vehicles for transmitting listeriosis for

susceptible populations (Swaminathan et al., 2007). The first foodborne outbreak of listeriosis occurred in 1981 in Nova Scotia, Canada and was traced to contaminated coleslaw (Schlech et al., 1983). Since then listeriosis has emerged as a major foodborne disease (Swaminathan et al., 2007). Human disease caused by *L. monocytogenes* occurs mostly in pregnant women, neonates, immunocompromised adults, and the elderly; however, it can also occur in persons with no predisposing underlying health conditions (Swaminathan et al., 2007). In nonpregnant adults, no symptoms may develop or they may show a very mild enteric from of the disease (Ray and Bhunia, 2008). In adults it can cause septicemia, meningitis, and meningoencephalitis, with a mortality rate of 20 to 30% (Swaminathan et al., 2007). Pregnant women, particularly in the third trimester, may experience mild flu-like symptoms (fever and/or myalgias with or without diarrhea), but listeriosis has severe consequences for the fetus, leading to stillbirth or abortion (Swaminathan et al., 2007). Neonates who are less than 7 d old, can experience sepsis and pneumonia, while neonates older than 7 d experience meningitis and sepsis (Swaminthan et al., 2007). *L. monocytogenes* is highly fatal (30 to 40%) to fetuses, newborns, infants, the elderly, pregnant women, and immunocompromised people (Ray and Bhunia, 2008).

The infectious dose of *L. monocytogenes* depends on many factors, including the immunological status of the host (Swaminathan et al., 2007). It varies between individuals, with immunosuppressed persons being most susceptible (Ray and Bhunia, 2008). On the basis of the epidemiological data, the infectious dose has been estimated to be in the range of 100 to 1,000 cells in an immunocompromised host (Ray and Bhunia, 2008). In immunocompromised individuals, pregnant women, unborn fetuses, infants, and elderly people, *L. monocytogenes* causes an invasive systemic disease, which has a 2 to 3 week incubation period before bacteremia (septicemia) resulting in fever and headache, meningitis, encephalitis, endocarditis,

liver abscess, and other symptoms begin (Ray and Bhunia, 2008). Listeriosis is less severe in healthy individuals where it causes febrile gastroenteritis with a dose of 10^8 to 10^{10} cells (Ray and Bhunia, 2008). Symptoms appear within 1 to 7 d following ingestion and include mild flu-like symptoms with slight fever, abdominal cramps, and diarrhea (Ray and Bhunia, 2008). These symptoms subside in a few days, but the individual continues to shed the bacteria in their feces for some time (Marsden, 1994; Rocourt, 1994). As a result of its high fatality rate, the U.S. government has established a "zero tolerance" policy for *L. monocytogenes* in ready-to-eat products (Montville and Matthews, 2005; Ray and Bhunia, 2008).

Cantaloupes (*Cucumis melo* L.)

The word "melon" was first documented around the year 1395 and according to John Ayto's Dictionary of Word Origins it is derived from Melos (the Greek Cyclades Islands), which is best known for the Venus de Milo (National Garden Bureau, 2005). Today "melon" is used as a generic term for a range of round-shaped fruits, like cantaloupes. Cantaloupes (*Cucumis melo* L.) belong to the cucurbit family (Cucurbitaceae), commonly known as the gourd family, which also includes cucumbers, squash, zucchini, pumpkins, chayote, watermelon, and honeydew to name a few. Fellow members of their genera *Cucumis* include cucumbers, honeydew, and muskmelons. Besides cantaloupes, the species *Cucumis melo* can refer to the "winter melons" like 'Honeydew,' 'Casaba,' 'Persian,' 'Canary,' 'Crenshaw,' and 'Santa Claus.' However, *Cucumis melo* is composed of two groups of commercial importance in the U.S.: Inodorus and Reticulatus. The "winter melons" are classified in the Inodorus Group because at maturity they do not separate from the vine, they lack heavy netting, are generally less aromatic, and can have a longer shelf life than those of the Reticulatus Group (Welbaum, 2014). The winter melons ripen later, their leaves are light to medium green, their surface is usually smooth, and they

require a longer growing season at relatively higher temperatures under semi-arid conditions (Salunkhe and Kadam, 1998). Additionally, they have a shorter shelf life (Salunkhe and Kadam, 1998).

A third group is Cantalupensis, which are melons native to the Middle East that are warty, scaly, rough, and lack netting (Salunkhe and Kadam, 1998). The term "cantaloupe" refers more correctly to the Cantalupensis Group of *Cucumis melo*. Therefore, the term "muskmelon" is the preferred name of the members of the Reticulatus Group that contains both western and eastern shipping cultivars of muskmelon (Welbaum, 2014). Muskmelons are identifiable by their pronounced sutures and ribs, coarse and heavy netting, as well as having a relatively larger seed cavity, juicier flesh, spicier flavor, and stronger aroma (Salunkhe and Kadam, 1998; Welbaum, 2014). Nevertheless, since no true cantaloupes (Cantalupensis) are grown commercially in the U.S., the generic and widely accepted name of all netted, musk-scented melons has become "cantaloupe" (Miller, 2009; Welbaum, 2014). Hence, cantaloupe today simply refers to cultivars that are highly uniform in overall netting with relatively indistinct vein tracts (Salunkhe and Kadam, 1998). Botanically cantaloupes are a fruit but are also considered a vegetable based on how they are consumed.

Cantaloupe as a commodity

Cantaloupes can trace their origin as a cultivated crop back over 4,000 years ago to the Near East, from Turkey to China, including northwest India, Afghanistan, and Uzbekistan (Siddiq et al., 2012). The early colonists brought them to North America in the sixteenth century and the Native Americans around present day Montreal, Canada began cultivating them in 1535, while others near Philadelphia did the same around 1748 (Siddiq et al., 2012). Commercial production in the U.S. began after the 1870s in Maryland, Delaware, and New Jersey (Orzolek et

al., 1998; Siddiq et al., 2012). In the last available Food and Agriculture Organization of the United Nations' crop production and trade data report from 2012, the top ten melon producing countries in order were China, Iran, Turkey, Egypt, India, U.S., Spain, Morocco, Brazil, and Mexico (FAO, 2012). In 2012, China by itself produced nearly twice the amount of melons (17,500,000 metric tons) compared to the other nine countries combined (FAO, 2012).

Cantaloupes sold in the U.S. primarily come from the U.S., Central America, and Mexico (Parnell et al., 2013). A 2003 study indicated that California alone produced approximately 60% (1 million tons, or 907,000 metric tons, per year) of the total U.S. market (Parnell et al., 2003). Cantaloupes are harvested in the U.S. from May to November, with June to October as the peak harvest months, and are imported from Mexico, Costa Rica, Guatemala, Honduras and other countries from November to May (Parnel et al., 2003). In 2010, the value of U.S. cantaloupe production was $314.4 million, total acreage was 74,730 acres, and the per capita consumption was 8.5 lbs (Boriss et al., 2012). California was still the largest producer with 10.9 million cwt (or hundredweight is equivalent to 100 lbs), followed by Arizona with 4.9 million cwt, and Georgia with 1.5 million cwt (Boriss et al., 2012). Other cantaloupe producing states were Colorado, Indiana, Pennsylvania, South Carolina, and Texas (Boriss et al., 2012; NASS 2011). At the same time, in 2010 the U.S. exported more than 261,900 metric tons of cantaloupes (Boriss et al., 2012). The major importers were Canada, Mexico and Japan (Boriss et al., 2012). Overall, the U.S. is the largest importer of cantaloupes and other melons in the world (Boriss et al., 2012). The import value of fresh melons in 2010 was $478.2 million (Boriss et al., 2012).

Commercial production of cantaloupes

Cantaloupe plants are andromonoecious, meaning that they produce separate (male) and hermaphroditic (perfect) flowers (contain both male and female parts) on the same plant

(Salunkhe and Kadam, 1998). Male flowers appear first in clusters on the main and secondary branches (laterals), followed by the perfect flowers only on the laterals. Male flowers typically appear 7 to 10 d before perfect flowers. Both types of flowers are only open for 1 d in which fertilization must occur, or unpollinated flowers will rapidly senesce and abscise. If pollination successfully occurs, the ovary rapidly enlarges into a fruit (Salunkhe and Kadam, 1998; Welbaum, 2014). However, vines are only able to support the development of about 1 to 3 fruit at a time (Salunkhe and Kadam, 1998). Flowers developing after maximum fruit set will fail to develop due to the inability of the vine to sustain additional fruit (Welbaum, 2014). Nonetheless, cantaloupes set in cycles, so additional fruit can be set later after fruit are harvested. The most effective pollinators are bees. To develop a large and marketable fruit, several hundred grains of pollen must be deposited on the pistil of each fruiting flower (Welbaum, 2014). Ten to 15 bee visits must occur during the 1 day that the flower is open for pollination to be successful (Welbaum, 2014). For that reason, 1 or 2 strong colonies of bees per acre are needed to maintain that frequency of flower visitations (Welbaum, 2014). Pollen can be transferred from male stamens to female pistils by hand pollination, but this a very time consuming and unreliable method of fertilization.

While sandy loams are considered best for early crops and loams are good for high yield of fruit, sandy soils can also be treated with humus or compost (Salunkhe and Kadam, 1998). Clay soils are not recommended for growing cantaloupes because its poor aeration and restricted drainage will reduce root growth and the final fruit quality (Welbaum, 2014). Overall, medium-textured soils with high water-holding capacity and good internal drainage produce maximum yields (Welbaum, 2014). Cantaloupe is a warm-season, frost-intolerant crop that requires a hot and dry climate with an optimum temperature of 27 to 30°C (80.6 to 86°F). Planting should be

conducted after the threat of frost as past. Soils with high salinity should be avoided due to the sensitivity of cantaloupes to salt (Welbaum, 2014). The fruit are also sensitive to acidic conditions and soils with a pH of less than 6.0 may produce plants that are weak and do not properly mature the melons (Motes et al., 2013). Compared to other vegetable crops, fertilizer requirements for cantaloupes are moderate (Welbaum, 2014). The amount of nitrogen applied should be monitored because excessive quantities will cause plants to spend their energy on vegetative growth instead of on reproductive growth (Welbaum, 2014).

The first irrigation should be applied immediately after sowing the seeds or transplanting. Regular irrigation every 5 to 7 d is required prior to the fruit-setting stage (Salunkhe and Kadam, 1998). Irrigation should be stopped or limited during the ripening period. If it is not, the sugar content will be reduced, the stem-end may crack, and fruit rotting may occur (Motes et al., 2013). This is especially important because almost half of the final concentration of sugars is accumulated during the last week of fruit maturation (Hemphill, 2010). The last irrigation usually is applied 7 to 10 d before harvest (Welbaum, 2014). In the Eastern U.S., drip irrigation is commonly used because it provides the most uniform application of water, ensures that water goes directly to the root zone, and prevents water from becoming stagnant on the vine canopy surface, which could result in significant vine and fruit disease (Welbaum, 2014).

Female flower production is promoted by short day length (Salunkhe and Kadam, 1998). Cloudy weather and high atmospheric humidity adversely affect the texture, sugar formation, and flavor (Salunkhe and Kadam, 1998). To have proper ripening of the fruit and the production of a high sugar content, low humidity, high temperature, and lots of sunshine are required (Salunkhe and Kadam, 1998). Cantaloupes will take about 70 to 90 d after sowing or 30 to 35 d after fruit set to mature, depending upon the variety and temperature of the growing season (Salunkhe and

Kadam, 1998). Rather than harvesting by the size of the fruit, cantaloupe cultivars grown in the U.S. should be harvested by their maturity for best fruit quality. Ideally, commercial maturity is when the cantaloupe is at the firm-ripe stage, which correlates to ¾ to full-slip. This means that there is a clear abscission or separation of the fruit from the vine when light pressure is applied. Cantaloupes may differ in their external coloration at harvest. During growth and maturation their skin color usually transitions from gray to dull green (immature), deep uniform green (maturity), and light yellow (full-slip). Another indicator of commercial maturity is the presence of a raised corky netting on the fruit surface (Suslow et al., 2012). However, the amount and presence of netting varies among cultivars.

The level of sweetness is the most important factor controlling the quality of melons (Salunkhe and Kadam, 1998; Welbaum, 2014). Vegetative growth should be limited once fruit set has started because it draws sugars away from the developing fruit (Welbaum, 2014). Cantaloupes ripen after harvest; however, their sugar content is highest when plants are attached to the vine and does not increase after harvest (Parnell et al., 2003). The highest sugar content is produced during warm sunny days and cool clear nights when the plants are healthy and not under water stress (Welbaum, 2014). The sugar content of expressed juice can be measured by a hand refractometer.

In the U.S. there are 4 Federal Grade Standards for cantaloupes: Fancy, No. 1, Commercial, and No. 2. These grades are based mostly on external appearance and measured soluble solids. U.S. Fancy must be well-formed, well-netted, have "very good internal quality" and a minimum of 11% soluble solids. Meanwhile, U.S. No. 1 cantaloupes need to be well-formed, well-netted, have a "good internal quality" and a minimum of 9% soluble solids (Agriculture Marketing Service, 2008; Suslow et al., 2012). U.S. Commercial and U.S. No. 2

grades consist of cantaloupes, which must have a minimum of 9% soluble solids, are mature but they have no internal quality requirement (Agriculture Marketing Service, 2008). Although 9% soluble solids is the minimum industry standard, a good tasting cantaloupe contains 14% soluble solids (Welbaum, 2014). In addition to appearance (color) and soluble solids, quality of cantaloupes depend on 5 other characteristics: well-shaped almost spherical, smooth stem end, absence of defects (scars, punctures, bruises, or sunburn), firmness, and appropriate weight and size with an internal cavity without loose seeds or liquid accumulation (Suslow et al., 2012).

A good indication of a recently harvested melon is the presence of a "wet nose," or moist stem end (Welbaum, 2014). Melons are typically harvested early in the morning or at night when the temperatures are low to reduce the amount of field heat that must be removed (Welbaum, 2014). Field heat is the amount of sensible heat, which must be removed when cooling produce to the desired storage temperature (Chakraverty et al., 2003). It is a function of produce mass, specific heat, and the difference between the initial and desired transport temperatures (Chakraverty et al., 2003). After harvest, melons are forced-air cooled to remove field heat, which increases their shelf life (Orzolek et al., 1998; Welbaum, 2014). 10°C (50°F) is typically the pre-cooling endpoint, but 4°C (39.2°F) is more desirable (Suslow et al., 2012). Besides forced-air cooling, hydrocooling, using chilled water to cool perishable crops, is also utilized to remove field heat (Suslow et al., 2012; Thompson and Chen, 1989). Depending on where they are grown, melons may undergo a rinsing or dip tank procedure with chlorinated water to control bacterial populations on their surface. Then they are shipped at 10 to 12.8°C (50 to 55°F) (Salunkhe and Kadam, 1998; Welbaum, 2014).

Cantaloupe is a climacteric fruit, thus it emits the plant hormone ethylene during the ripening process and has an increased rate of respiration, which may be a problem during

distribution and short-term storage (Suslow et al., 2012). The recommended storage temperature for whole cantaloupes is 2 to 5°C and near 0°C for minimally processed cantaloupe pieces (Bett-Garber et al., 2011; Parnell et al., 2003). If storage temperatures are less than 2°C (35.6°F) for several days, chilling injury occurs which causes pitting or sunken areas on the surface, off-flavors, and increased surface decay (Suslow et al., 2012). The relative humidity should be 95 to 100% to prevent drying. Under these conditions, freshly harvested cantaloupe, picked at full slip, lasts about 5 to 15 d, depending on cultivar and growing conditions. After melons are purchased from a grocery store, they will keep in the refrigerator for approximately 5 d, depending on their initial degree of ripeness at purchase (Parnell et al., 2003).

Cantaloupe cultivars

All organisms are classified using a taxonomic ranking that groups individuals based on shared characteristics (from most general to specific): domain, kingdom, phylum, class, order, family, genus, and species. Using botanical nomenclature, "botanical variety" ranks right below that of species and is sometimes called subspecies. A cultivated variety, often termed cultivar, is maintained by man and does not persist in the wild. Cultivars differ from one another by at least one important trait such as rind thickness, days to maturity, disease resistance, etc. The terms "variety" and "cultivar" are often used interchangeably and incorrectly. Botanical varieties occur in nature as true breeding populations that continue to reproduce, each succeeding generation the same as their predecessors. Cultivars are created and maintained by humans as the result of genetic mutations, plant breedings, genetic engineering, cuttings, grafting, or tissue culture (Haynes, 2008). Those plants that have been produced by the combination of 2 genetically distinct parents are referred to as hybrids. F-1 hybrids are the first filial generation of offspring from distinctly parental types that are inbred for several generations so they are

homozygous. In other words, F-1 hybrids are the result of crossing 2 inbred lines with unique characteristics to produce offspring that express heterosis and possible new traits (Anonymous, 2014). Characteristics of F-1 hybrids include: high vigor, high yields, and uniformity (Anonymous, 2014).

Cantaloupes in the U.S. are often divided into 2 types: western and eastern types. Western shipping cultivars, as the name suggests, are grown in desert regions of the western U.S. and are adapted for long distance shipping. Western shipping cultivars average 3 to 4 lbs, have thick flesh with a dry seed cavity, little aroma, no solid netting, grow in desert regions where there are high temperatures and low humidity, and can travel long distances to be sold (Welbaum, 2014). Eastern cultivars are larger averaging between 5 to 7 lbs, each have a strong aroma, are generally sweeter, have coarse netting, softer flesh, large moist seed cavities, are ridged with prominent sutures, and are not able to travel long distances from harvest to market (NCFVSD, 2012; Welbaum 2014). Therefore, eastern cultivars are often sold in local markets close to where they are grown but may also be shipped from the south to the north early in the season. Eastern melons also are better adapted for growth under high humidity. Since eastern growers also want some of the same characteristics of western shipping melons, like hard rinds, better shelf life, and heavy netting to reduce abrasion, new cultivars developed for eastern production have incorporated many of the same fruit characteristics as western shipping melons, so the differences between the 2 types are not as great as they once were (Welbaum, 2014).

***Cucumis melo* L. 'Athena'**

Athena is not only the Greek goddess of wisdom, but is also a widely grown cantaloupe cultivar. 'Athena' was first introduced in 1995 and quickly gained popularity with eastern growers, shippers, and consumers because of its wide adaptability, high yields, good shipping

characteristics, uniformity, excellent flavor, and longer shelf life (NCFVSD, 2012). Its longer shelf life, up to 2 weeks under optimum conditions, allows it to be shipped and sold in more distant markets than older cultivars developed for production in the Eastern U.S. (NCFVSD, 2012). Within 2 years of its release, 'Athena' accounted for 80 to 90% of all cantaloupes grown in the Southeast and Midwest U.S. (NCFVSD, 2012). Although new cultivars have been released, 'Athena' remains the industry standard (Kelley and Bertrand, 2007). The cultivar Aphrodite has found some popularity, but many growers consider it too large for many markets (Kelley and Bertrand, 2007).

'Athena' is an early to midseason eastern cultivar with 80 d between seeding and harvesting with an elongated oval shape that ranges from 4 to 7 lbs (Hemphill, 2014; Schultheis, 1998). The skin is slightly sutured with coarse netting, the flesh is firm, thick, and yellow-orange in color, and its sugar content is high (Hemphill, 2014; National Garden Bureau, 2005). Also, it is less susceptible to cracking than many other eastern cultivars (Schultheis, 1998). It is an F-1 hybrid and has resistance to fusarium wilt and powdery mildew (Hemphill, 2014). The optimum storage conditions for 'Athena' are 1.7 to 7.2°C (35 to 45°F) and 95% relative humidity (NCFVSD, 2012).

***Cucumis melo* L. 'Hales Best Jumbo'**

'Hales Best Jumbo' ('HBJ') is an heirloom cultivar that was developed by a Japanese market gardener in Brawley, California and discovered in 1923 by I.D. Hale, who was a representative of the C.B. Weaver Co. of Chicago (1 of the largest cantaloupe distributors operating in the Imperial Valley) (Bonnie Plants, 2014; CooksInfo, 2009; LocalHarvest, 2012; Tapley, 1937; Victory Seed Company 2014; W. Atlee Burpee & Company, 2013). It is slightly more drought tolerant than most cultivars (Bonnie Plants, 2014). 'HBJ' is an oval melon with

deep green skin with golden netting that became widely popular due to its excellent flavor and good quality (W. Atlee Burpee & Company, 2013). The flesh is salmon colored and it has a sweet aroma (W. Atlee Burpee & Company, 2013). It is a heavily netted melon that takes 80 to 90 d to mature (Vista Horticultural Group, 2014; W. Atlee Burpee & Company, 2013). At maturity it ranges from 3 to 5 lbs (Bonnie Plants, 2014). However, it is not sold by grocery chains and is not shipped long distances because of its shorter shelf life compared to other cultivars like Athena (CooksInfo, 2009).

Cantaloupe harvests

In the U.S., primary processing of cantaloupes varies from state to state. For example, California-grown cantaloupes are field-packed in 40 lb (18 kg) cartons, the industry standard, without receiving aqueous processing procedures, while melons in Georgia are transported to a facility where they are washed and packed (Akins et al., 2008; Siddiq et al., 2012). Eastern types of cantaloupes are typically washed in large dump tanks containing 100 to 150 ppm chlorinated water at 45 to 50°C within a few hours of harvesting (Richards and Beuchat, 2004; Suslow, 1997). Prior to undergoing the washing step at the packinghouse, melons, like other fruits and vegetables, can contain populations of 10^4 to 10^6 microorganisms/g (Brackett, 1994), and may contain pathogens such as *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., and *Escherichia coli* O157:H7 (Brackett, 1992). A chlorinated washing step, containing up to 200 ppm chlorine, may only result in a 1 to 2 log unit reduction of the total population of microorganisms on the fruit (Adams et al., 1989; Beuchat et al., 1998; Wei et al., 1995). Concentrations as high as 1000 ppm chlorine or 5% hydrogen peroxide (H_2O_2) solution can reduce the native microflora on cantaloupes approximately 2 to 3 log CFU/cm^2 within 24 h of inoculation (Ukuku et al., 2001).

Bacteria have the ability to adhere to the surface of cantaloupes and form biofilms, which are communities of microorganisms immobilized and living on a solid surface exposed to air or liquid (White, 2007). Increased resistance of *S. enterica* to chlorine has been associated with the bacterial cell's ability to produce cellulose, which aids in biofilm formation (Solano et al., 2002). One study found that when *S. enterica* was left undisturbed on the surface of cantaloupe for more than 24 h, the efficacy of rinsing with chlorine and H_2O_2 decreased significantly (Ukuku and Sapers, 2001). This was hypothesized to occur because of the increased contact time between the bacterium and fruit surface allowing biofilms to form (Ukuku and Sapers, 2001).

Contamination of pathogens on the surface of cantaloupes

Produce that is grown on the ground, like cantaloupe, may become contaminated with foodborne pathogens present in the growing or packing environment. If present, foodborne pathogens can be introduced to the exterior surface during pre-harvest from unclean irrigation water, animal feces, or pathogens due to poor worker hygiene, which can include bacteria like *Salmonella* (CDC, 1991). Contamination can also occur either during harvesting or post-harvest, in a packing shed, particularly during the washing step (Gagliardi et al., 2003). Poor equipment and facility sanitation can transfer contamination to cantaloupes and other produce during processing and packaging. Furthermore, the meshwork of lenticellar netting on the surface of cantaloupes can increase the number of attachment sites available for *Salmonella* to adhere to during packing (Annous et al., 2005; Johnston et al., 2005). Not only does the netting on cantaloupe rinds favor microbial attachment, but the pH range of the internal fruit (6.1 to 6.6) is conducive to microbial growth (Johnston et al., 2005).

The meshwork of raised tissue on the epidermal cell surface of cantaloupe fruit consists of lenticels (pores for direct exchange of gases) and phellum (cork) cells. Both of these cells

contain hydrophobic suberized walls in order to reduce water loss and to protect against pathogen entry. A cuticle, containing waxes and cutin cover epidermal cells and are responsible for the hydrophobic nature of the cantaloupe rind (Webster and Craig, 1976; Ukuku and Fett, 2006). The attachment of bacterial pathogens to the surface of cantaloupes is influenced by both surface charge and hydrophobicity (Ukuku and Fett, 2002). Cantaloupe rinds are hydrophobic in nature and *Salmonella* binds stronger to its surface than *E. coli* O157:H7 and *L. monocytogenes* because *Salmonella* has the most hydrophobic surface of the three genera of bacteria (Ukuku and Fett, 2002). Internalization of pathogens into the fruit can also occur. For example, *S. enterica* Typhimurium has been shown to internalize into cantaloupes through the ground spot and, secondarily, through the stem scar (Sapers et al., 2009; Suslow, 2004). After post-harvest processing, *S. enterica* Typhimurium was found 5 mm under the rind (Sapers et al., 2009). This occurred because the ground spot is a thin and underdeveloped rind that is poorly netted and is thus more susceptible to bacterial or fungal growth (Sapers et al., 2009)

Salmonella is known to form lattice-like matrices on the netting of cantaloupe rind and this sheet-like material may provide a defense for the bacteria cells and aid in their resistance against aqueous sanitizers (Annous et al., 2004). Both *E. coli* and *Salmonella* are able to produce an extracellular carbohydrate polymer cellulose and curli (aggregative fimbriae), which are the principle components of the extracellular matrix proteins, and are believed to be the cause of biofilm formation (Brown et al., 2001; Zogaj et al., 2001). This matrix is also composed of extracellular polysaccharides (EPS) (Fett and Cooke, 2003). *Salmonella*'s production of cellulose and the presence of curli may be the reason for its strong attachment to cantaloupe rind (Ukuku and Fett, 2006). However, strains related to cantaloupe-implicated outbreaks in general have not been found to attach more strongly than those of non-cantaloupe *Salmonella* related

strains (Ukuku and Fett, 2006). Therefore, the ability of *Salmonella* to bind and proliferate on the rind of cantaloupe varies between individual strains. Moreover, EPS may protect bacterial cells within biofilms from desiccation and may provide resistance to antimicrobial compounds (Annous et al., 2005). In addition, the ability of cellulose and curli to form a strong bond to surfaces like cantaloupe rind may allow bacteria to develop resistance to removal by washing steps during handling and later by consumers before consumption (Ukuku and Fett, 2006). Annous et al. (2005) chose a storage temperature of 20°C and 10°C to simulate consumer temperature abuse to demonstrate biofilm formation by *Salmonella* spp. on the rind of cantaloupes. After approximately 2 h following inoculation at 20°C, a biofilm was already present on the melon rind (Annous et al., 2005).

Use of sanitizers

Chlorine has been used as a sanitizer in the food processing industry for several decades (Artés et al., 2007; Martín-Belloso and Soliva-Fortuny, 2011). Chlorine, as acidified sodium chlorite, is permitted at levels from 50 to 1500 ppm as an antimicrobial agent in water (Martín-Belloso and Soliva-Fortuny, 2011). However, it can react with organic materials, thus potentially producing harmful by-products, such as chloramines and trihalomethanes, to human health (Richardson et al., 1998). Chlorine also becomes inactivated and made inert in the presence of organic compounds (Zhang and Farber, 1996). Additionally, hypochlorous acid, which is formed when chlorine dissolves in water, is not able to reach microorganisms in the natural pores and crevices within the hydrophobic surface of cantaloupe and therefore contributes to the lack of effectiveness of using chlorine treatments to sanitize whole melons (Alvarado-Casillas et al., 2007).

In recent years H_2O_2 has been studied on whole produce. When whole cantaloupes, honeydew melons, and asparagus spears were treated with 1% H_2O_2, it was found to be less effective at reducing levels of *Salmonella* spp. and *E. coli* O157:H7 than hypochlorite, acidified sodium chlorite, and a peracetic acid-containing sanitizer (Martín-Belloso and Soliva-Fortuny, 2011; Park and Beuchat, 1999). Water can also be used to disinfect cantaloupes. Fouladkhah and Avens (2010) reported that a thermal water immersion (95°C) for 2 min in primary processing of fresh melons resulted in a 3 log CFU/cm^2 reduction of natural microflora surface contamination (Martín-Belloso and Soliva-Fortuny, 2011).

Natural antimicrobials

In response to the inefficiency and chemical origins of common food processing sanitizers, there has been a surge in the use of natural antimicrobials as part of 'green consumerism' (Tuley, 1996). Three groups of natural antimicrobials exist: bacteriocins (which are produced by microorganisms), plant derived compounds, and animal derived compounds. The majority of natural antimicrobials are secondary metabolites that are produced as plant defense mechanisms in response to predation by microorganisms, insects, and herbivores (Cowan, 1999). The antimicrobial activity found in plants, herbs, and spices may come from several major components. These include phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, organic acids, flavonoids, tannins, lectins, and isothiocyanates (Davidson, 2005).

Plant derived antimicrobials

Essential oils (EOs) are aromatic oily liquids, or volatile or ethereal oils, obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, or roots) by steam distillation, solvent extraction, or pressing (Burt, 2004; Davidson, 2005). Their composition

originating from a specific species of plant may differ between harvesting seasons and between geographical sources (Burt, 2004). The antimicrobial activity of EOs depends on several factors, including the amount used, genus, species, and strain of microorganism, and environmental factors such as pH, temperature, water activity, atmospheric composition, initial microbial load, and the presence of proteins, lipids, salts, and phenolic substances present in the food environment (Burt, 2004; Negi, 2012).

Additionally, the individual components of the chemical structures of EOs affect their particular mode of action and antibacterial activity (Dorman and Deans, 2000). Gram-positive bacteria have been found more susceptible to the effects of EOs (Dorman and Deans, 2000). On the other hand, it is believed that Gram-negative microorganisms are less susceptible to these antimicrobials since they have an outer membrane surrounding the cell wall, which limits diffusion of hydrophobic compounds through their lipopolysaccharide covering (Vaara, 1992). A considerable number of EOs and their components have Generally Recognized as Safe (GRAS) status and are approved food flavorings.

Antimicrobials in the vapor phase

In general, EO vapors may be more effective than their liquid phase because of the ability of lipophilic molecules in the aqueous state to form micelles and in turn suppress the attachment of the EOs to microorganisms. In comparison, the vapor phase allows free attachment (Inouye et al., 2003). The vapor state also offers an advantage since the components of the EO are dispersed; therefore, they are less likely to affect the organoleptic properties of the food the same way that EO liquids would (Goñi et al., 2009). Heating EOs can also increase the volatility of the oils (Su et al., 2007).

Although EOs have traditionally been used as antimicrobials in the liquid phase, they are generally more effective in the liquid and vapor phase *in vitro* than when tested in systems and subsequently higher concentrations are needed to obtain the same result (Laird and Phillips, 2011). The vapor phases of some antimicrobials have been shown to have greater antimicrobial activity when compared to the liquid phase. Chlorine dioxide gas has 2.5 times the oxidation capacity compared to chlorine liquid and is more effective at inactivating surface bacteria on fresh produce, with reductions between 3 to 4 log CFU/g from using concentrations of 3 to 10 ppm (Benarde et al., 1965; Han et al., 2001; Singh et al., 2002).

Other examples of effective sanitizers in their vapor forms include H_2O_2, ethanol, and ozone. H_2O_2 vapor significantly reduced microbial populations on whole cantaloupes, grapes, prunes, raisins, walnuts, and pistachios (Martín-Belloso and Soliva-Fortuny, 2011; Sapers and Simmons, 1998). Likewise, ethanol vapor effectively extended the shelf life of fresh produce, such as cut apples, broccoli florets, and eggplant, by inhibiting mold growth and retarding tissue senescence (Corcuff et al., 1996; Han et al., 2006; Hu et al., 2010). The shelf life of high-moisture bakery products is also extended by ethanol emitters in the form of sachets (Robertson, 2006). In addition, ethanol vapor generators are effective in controlling at least three species of spoilage yeast, 10 species of molds, and 15 species of bacteria, including *Salmonella* and *E. coli* spp. (Robertson, 2006). Meanwhile, controlled levels of ozone vapor have effectively reduced fungal growth and in turn increased the shelf life of grapes and apples (Selma et al., 2008). Bubbling ozone gas in water has also been shown to decrease the native microflora on postharvest shredded iceberg lettuce leaves by 1.5 to 1.9 log CFU/g in 3 min (Kim et al., 1999). While ozone gas may cause physiological injury to produce, which could cause a negative

impact on sensory qualities, 10,000 ppm ozone gas for 30 min did not affect the sensory qualities of fresh-cut cantaloupes after 8 d of storage in a previous study (Selma et al., 2008).

Allyl isothiocyanate

An example of a post-infectional plant antimicrobial compound is isothiocyanate from the family Brassicaeae, which includes broccoli, cabbage, cauliflower, horseradish, mustard, radish, rapeseed, turnip, and wasabi. The intact plant contains sulphur and nitrogen containing compounds known as glucosinolates. When plant tissues are disrupted by mechanical or physical damage from pathogens or animal attacks, the glucosinolates come into contact with a specific thioglucosidase, myrosinase (Enzyme Commission Number 3.2.1.147) which is located in a separate part of the plant in myrosin cells. If water is also present, the endogenous enzyme hydrolyzes the glucosinolates into 1 of 3 classes of aglycones: isothiocyanates, nitriles, or thiocyanates. These products are responsible for the associated spicy or pungent odors and flavors of Brassica vegetables (Bennett and Wallsgrove, 1994). AITC is one of four distinct aglycones produced from the enzymatic hydrolysis of sinigrin, a specific glucosinolate that is found in brown or oriental mustard (*Brassica juncea*) (Shofran et al., 1998) (Figure 1).

AITC has GRAS status and is known to have strong antimicrobial activity in both liquid and vapor forms against *E. coli* O157:H7, *S. enterica* Montevideo, *S. enterica* Typhimurium, *L. monocytogenes*, *E. coli* JCM-1649, *Vibrio parahaemolyticus*, and *Pseudomonas aeruginosa* (Isshiki et al., 1992; Lin et al., 2000b). In addition, it is exempted from the requirement for a residue tolerance in or on all raw agricultural commodities as approved by the Environmental Protection Agency (EPA) (Environmental Protection Agency, 1996). Unlike other essential oils, Gram-negative bacteria are more sensitive than Gram-positive bacteria to inhibition by AITC. For example, *L. monocytogenes* is more resistant to AITC than *E. coli* O157:H7 and *S. enterica*

Montevideo (Lin et al., 2000a). However, the antimicrobial mechanism(s) of AITC is not well understood (Lin et al, 2000a; Lin et al., 2000b).

AITC is composed of a polar isothiocyanate end and a nonpolar allyl side chain (Lin et al, 2000a; Lin et al., 2000b). Two studies have shown that AITC may modify protein structures at a concentration, which inhibits microbial growth (Kawakishi and Kaneko, 1985; Kawakishi and Kaneko, 1987). Another analysis illustrated how oxidized glutathione is cleaved by AITC at the disulfide bond (Kawashi and Kaneko, 1985; Lin et al., 2000b). Additionally, AITC like other isothiocyanates may inhibit cytochrome *c* oxidase in the electron transport chain and be an uncoupler of oxidative phosphorylation (Kojima and Ogawa, 1971).

Antimicrobial effect of liquid allyl isothiocyanate

AITC in the liquid phase against foodborne pathogens has predominately been tested *in vitro*. The inhibitory bactericidal effects of liquid AITC were found to be time-related for *E. coli* O157:H7, *S. enterica* Montevideo, and *L. monocytogenes* at the lag, early exponential, late exponential and stationary growth phases (Lin et al., 2000b). Additionally, at the early and late exponential growth stages, *E. coli* O157:H7 and *S. enterica* Montevideo were more susceptible to AITC (Lin et al., 2000b). Nonetheless, AITC liquid has been incorporated into coatings for foods. AITC liquid was tested in combination with the bacteriocin nisin in coatings containing chitosan (edible polymer), the deacetylated derivative of the by-product of seafood processing called chitin, and alone with chitosan. Chen et al. (2012) tested 6 different coatings: chitosan, chitosan and 10 µl/ml AITC, chitosan and 30 µl/ml AITC, chitosan with 30 µl/ml AITC and 25 mg/ml nisin, chitosan and 60 µl/ml AITC, and chitosan with 60 µl/ml AITC and 25 mg/ml nisin. These combinations were applied to whole cantaloupe to investigate their ability to inactivate *Salmonella* on the surface of the melon (Chen et al., 2012). The most successful grouping was

the chitosan-AITC-nisin coating, which resulted in more than a 5 log CFU/cm^2 reduction (Chen et al., 2012). Jin et al. (2013) also studied antimicrobial coatings containing polylactic acid (nonedible polymer) or chitosan containing nisin and AITC for decontaminating and preventing cross-contamination of shell eggs with *S. enterica* Enteritidis. Polylactic acid coatings containing nisin and 20 and 60 µl AITC reduced *Salmonella* by approximately 0.95 and 1.2 log CFU/cm^2, respectively (Jin et al., 2013). At the same time, chitosan coatings with nisin and 20 and 60 µl AITC reduced *Salmonella* populations by 1.1 and 1.7 log CFU/cm^2, respectively (Jin et al., 2013).

Antimicrobial effect of allyl isthiocyanate vapor

Some previous work indicates that AITC can be more effective as a vapor treatment. AITC vapor was discovered to be 500 to 1,000 times more effective as an antimicrobial agent than the same amount of AITC liquid in agar (Sekiyama et al., 1996). In another study, AITC vapor at a concentration of 1,000 µg/L of air yielded greater than or equal to a 5.65 log CFU/cm^2 reduction of *E. coli* O157:H7, *S. enterica* Typhimurium, and *L. monocytogenes* when grown on tryptic soy agar (TSA) disks at 35°C (Delaquis and Sholberg, 1997). There are some theories as to why AITC vapor causes greater antibacterial activity than the liquid form. For example, the liquid has low solubility in broth, there is the potential for possible degradation of AITC in aqueous solution, and the liquid would have limited contact with bacterial cells compared to the vapor (Lin et al., 2000b). All of these theories could be related to the high volatility of AITC.

Volatile AITC vapor has been previously tested as a preservative for raw beef, cured pork, sliced raw tuna, fresh chicken breast, cheese, egg sandwiches, noodles, pasta, rye bread, and pears (Isshiki et al., 1992; Mari et al., 2002; Nielsen and Rios, 2000; Shin et al., 2010). It has also been combined with modified atmosphere packaging (MAP) for some of those foods.

For example, after 21 d, 1.2 µg/h AITC in combination with MAP led to approximately a 2.1 log CFU/g reduction of *S. enterica* Typhimurium on fresh chicken breast compared to ambient air and a 1.3 log CFU/g reduction compared to MAP alone (Shin et al., 2010). In spite of this, there is not a considerable amount of research investigating the use of AITC vapor for reducing or inhibiting microbial growth on fresh produce.

AITC vapor has been applied to test the lethality of *Salmonella* on alfalfa seeds and sprouts, *Salmonella* and *E. coli* O157:H7 on sliced and whole tomatoes, and *E. coli* O157:H7 on the intact and damaged portions of lettuce and spinach leaves (Obaidat and Frank, 2009a; Obaidat and Frank 2009b; Weissinger et al., 2001). Volatile AITC successfully eliminated or reduced *Salmonella* populations on the surface of alfalfa sprouts; however, the sensory quality attributes were adversely affected (Weissinger et al., 2001). The lowest level of AITC (8.3 µl/L of air) reduced the population of *Salmonella* on sliced tomatoes by 3.5 log CFU/g at 10°C in 10 d and on whole tomatoes to the detection limit of < 2 log CFU/g at 25°C in 10 h (Obaidat and Frank, 2009b). In comparison, another study reported that AITC at 132 µl/L of air reduced *Salmonella enterica* Montevideo by 8 log CFU/g on whole tomato surface at 4°C after 2 d (Lin et al., 2000a). Meanwhile, AITC concentrations of 16.6 and 33.3 µl/L of air inactivated *E. coli* O157:H7 by more than 2 log CFU/g after 7 d storage at 4°C on tomato slices and a level of 33.3 µl/L of air caused a 4.4 log CFU/g decrease of *E. coli* O157:H7 after storage of whole tomatoes at 10°C for 10 d (Obaidat and Frank, 2009b). In addition, AITC at 16 µl/L of air reduced *E. coli* O157:H7 on undamaged lettuce by 3.66 log CFU/leaf after 4 d at 0°C and to below the detection limit for damaged lettuce at the same storage time and temperature (Obaidat and Frank, 2009a). However, in this study no significant difference was found in the inactivation of *E. coli* O157:H7 on lettuce and spinach after AITC treatment (Obaidat and Frank, 2009a). Furthermore, Yun et

al. (2013) evaluated the effect of gaseous AITC on cherry tomatoes inoculated with *S. enterica* Typhimurium and found the pathogen to be reduced by more than 5.52 log CFU/g after 18 h at 22°C. In spite of this, the cherry tomatoes became discolored, softened, and lost vitamin C and lycopene during 21 d of storage at 10°C with exposure to AITC vapor (Yun et al., 2013).

There are also several patents in the U.S. involving isothiocyanate (ISOTC) vapors. For example, Ohama and Kato (1993) developed a treatment procedure using ISOTC vapor generated from ISOTC liquid dissolved in an oily liquid (e.g., olive oil, sesame oil, safflower oil, soybean oil, corn oil, rape oil, castor oil, sunflower oil, peanut oil, etc.). The vapor is generated within a chamber, which is subsequently blown into a treatment chamber (Ohama and Kato, 1993). The concentration of ISTOTC vapors within the treatment chamber range from 5 to 2,000 ppm, preferably 10 to 500 ppm, and they are maintained for 1 min to 12 h (Ohama and Kato, 1993). Under these conditions, microorganisms are almost completely destroyed (Ohama and Kato, 1993). Another U.S. patent filed by Fujita et al. (1999) of The Green Cross Corporation and Rengo Co., LTD., of Osaka, Japan discusses a method for controlling the release speed of AITC in packaging. AITC is impregnated within a porous packaging substrate, which can be either a hydrophilic material, a hydrophobic material, or a mixture thereof, with preference given to a hydrophilic material (Fujita et al., 1999). According to the patent, the AITC vapor release speed can be controlled, which enables sustained release of vapors and persistent effects of antimicrobial action (Fujita et al., 1999). In addition, the antimicrobial action can be sustained for a long duration with a small amount of AITC (Fujita et al., 1999).

In contrast to research studying the effects and uses of AITC from brown mustard (*Brassica juncea*) seeds, more recently in July 2013, Ekanayke et al. received a U.S. patent as a continuation of an application filed in December 2009 looking into the preservation effect of 4-

hydroxybenzyl isothiocyanate from white mustard (*Sinapis alba*) seeds. 4-hydroxybenzyl isothiocyanate is a moisture-sensitive compound that begins to degrade (i.e. hydrolyze) within hours of being exposed to moisture (Ekanayke et al., 2013). However, Ekanakye et al. (2013) discovered a method to stabilize the moisture-sensitive ISOTC compounds and to add them to solid food products, which can then be stored at a reduced temperature (< 10°C) for at least 12 h.

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Figures

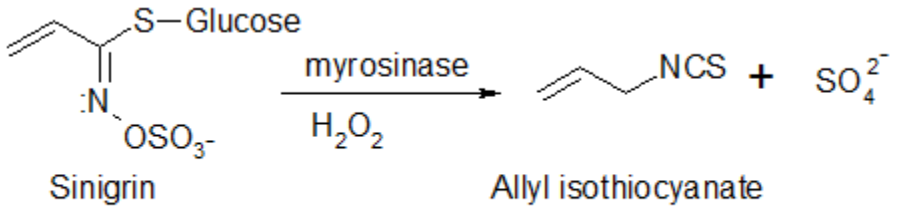

Figure 1. Hydrolysis of sinigrin (redrawn with permission from Bones and Rossiter, 1996).

CHAPTER 3

Liquid allyl isothiocyanate reduces populations of *Salmonella enterica* Michigan and *Listeria monocytogenes* on cantaloupe (*Cucumis melo* L.) surfaces*

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ABSTRACT

Cantaloupes (*Cucumis melo* L.) can become contaminated with foodborne pathogens in the field, during transportation, or at packinghouses. Current post-harvest sanitizer treatments of 200 ppm chlorine and 5% hydrogen peroxide may only result in a 1 to 3 log CFU/g reduction of bacteria. Allyl isothiocyanate (AITC; a natural antimicrobial) has Generally Recognized as Safe (GRAS) status. This study shows that application of AITC reduces *Salmonella enterica* Michigan and *Listeria monocytogenes* on cantaloupes surfaces. Fifty µl of *S.* Michigan or *L. monocytogenes* (10^8 CFU/ml) was inoculated into 22 mm diameter circles on the surface of two cantaloupe cultivars (Athena and Hales Best Jumbo (HBJ)) and allowed to dry for 90 min (resulting in approximately 6.80 log CFU/g). Circles then received 5 µl liquid AITC (95% purity), 25 µl 200 ppm sodium hypochlorite, 25 µl sterile deionized water, or no treatment. All cantaloupes were stored in sealed desiccators for 24 h at 25°C. Following treatment, the 22 mm diameter sections were removed, the rind was separated from the flesh, and 10 g of rind was homogenized with 90 ml 0.1% sterile peptone water. Homogenate was plated onto appropriate agar and incubated for 24 or 48 h. AITC significantly reduced ($P < 0.05$) *S.* Michigan (3.0 log CFU/g) on 'Athena' and *L. monocytogenes* (2.6 log CFU/g) on 'HBJ' compared to the controls. In most cases, there was greater reduction of *L. monocytogenes* by all treatments when compared to *S.* Michigan. A surface application of liquid AITC on cantaloupes is an alternative post-harvest sanitizer to current industrial treatments.

INTRODUCTION

Between 1998 and 2008, 46% of the more than 9 million foodborne illnesses each year, 38% of hospitalizations, and 23% of deaths in the U.S. were attributed to produce consumption (Painter et al., 2013). Raw fruits and vegetables can become contaminated with pathogenic bacteria directly or indirectly via soil, water, animals, insects, dirty equipment, or humans (Harris et al., 2003). Cantaloupes and other produce grown in direct contact with the soil are susceptible to contamination with human pathogens that may be present in the soil (Richards and Beuchat, 2005). They may also become contaminated during harvest, packing, and shipping. Mechanical and physical damage can allow pathogens to enter through punctures, cracks, or bruises and contaminate the mesocarp tissue or flesh of the cantaloupe (Richards and Beuchat, 2005). Although melons can have smooth or netted rind surfaces, more foodborne illness outbreaks have been linked to melons with netted rinds (FDA, 2009). Since cantaloupes do not receive a 'lethal' treatment to kill pathogens prior to consumption, pathogens introduced at any point of the production chain may be present when it is consumed (Abadias et al., 2011).

Numerous safe melon-handling practices have been adopted and implemented into the production chain beginning with growers and processors. Examples include Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs). In the U.S., primary processing of cantaloupes varies from state to state. California-grown cantaloupes are field-packed without receiving aqueous processing procedures, while melons in the Eastern U.S. are transported to a packing facility where they are more often washed and packed (Akins et al., 2008; Siddiq et al., 2012). The water used to wash cantaloupes is typically chlorinated (up to 200 ppm) to reduce microbial contamination in produce processing lines (Ukuku, 2006; Wei et al., 1995). Although it is widely used commercially, chlorine compounds can react with trace amounts of organic

material and be inactivated or form various carcinogenic organochlorine compounds and chlorine compounds on fruit (Richardson et al., 1998; Rodgers et al., 2004). Additionally, washing with chlorinated water achieves only a 1 to 2 log CFU/g reduction (Richards and Beuchat, 2004; Rodgers et al., 2004). Conventional washing technology was developed primarily to remove soil from produce and not to remove microorganisms from their surface (Sapers, 2001). Chlorine in wash water does not disinfect produce surfaces, as only low bacterial reductions are obtained to make a contaminated product safe to consume without additional treatment (FAO, 2009).

There is interest in using more natural plant antimicrobial compounds, possibly to replace chlorine (Kim et al., 2002). Allyl isothiocyanate (AITC), a naturally occurring flavor compound from the Brassicaceae family is known to have antimicrobial and antioxidant effects (Brown, 2009). It is listed as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (USFDA) and the use of AITC as a preservative is permitted in Japan, if the compound is extracted from natural sources (Code of Federal Regulations, 1999; Sekiyama et al., 1994).

AITC is effective against various foodborne pathogens such as *Escherichia coli* O157:H7, *L. monocytogenes*, *S. enterica* Typhimurium, *Bacillus cereus*, *Staphylococcus aureus*, and *Campylobacter jejuni*, *in vitro* or in meat products, including ground beef, fresh chicken, fermented sausage, and Westphalian ham (Olaimat and Holley, 2013). Little is known about the effect of AITC liquid in direct contact with pathogens present on food surfaces. Chen et al. (2012) tested the effect of a chitosan, nisin, and liquid AITC edible coating on cantaloupe rind previously inoculated with *Salmonella*. They discovered that the coating produced greater than a 5 log CFU/cm^2 reduction in *Salmonella* (Chen et al., 2012).

The purpose of this study is to evaluate whether AITC would be an effective post-harvest treatment for cantaloupe. The ability of aqueous AITC to reduce populations of *S. enterica* serovar Michigan and *L. monocytogenes* previously inoculated on the netted surface of two cantaloupe cultivars: Athena and Hales Best Jumbo (HBJ) was evaluated.

MATERIALS AND METHODS

Bacterial cultures and culture conditions:

Salmonella enterica serovar Michigan:

Salmonella enterica serovar Michigan (isolated from a cantaloupe associated outbreak) was obtained from Dr. Larry Beuchat at the University of Georgia. The culture was made nalidixic acid resistant by consecutively transferring a loopful (10 µl) from Tryptic Soy Broth (TSB, Bacto, Difco, Becton-Dickinson, Sparks, MD) to TSB containing increasing concentrations of nalidixic acid (99.5%, Acros Organics, Morris Plains, NJ) (TSBN) (5, 10, 25, 40, and 50 ppm) every 24 h until cultures were resistant at a level of 50 ppm (50 µg/ml). Simultaneously, a loopful (10 µl) of culture was t-streaked onto Tryptic Soy Agar (TSA, Difco, Becton-Dickinson, Sparks, MD) with increasing concentrations of nalidixic acid (TSAN) (5, 10, 25, 40, and 50 ppm) until colonies were resistant at a level of 50 ppm.

Nalidixic acid resistant colonies were preserved in TSBN containing 30% glycerol (99+%, pure, synthetic, Acros Organics Glycerol, Morris Plains, NJ) and stored at -80°C until use. Prior to each experiment, a vial (2 ml) of frozen culture was removed from storage and thawed at room temperature. Cells were activated by 3 successive 24 h transfers into TSB containing 50 ppm nalidixic acid (TSBN) and incubated at 37°C. Samples were then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Difco, Becton-Dickinson, Sparks, MD),

incubated at 37°C for 24 h, and a representative colony was confirmed using the *Salmonella* latex test (Oxoid Ltd.; Basingstoke, Hants, UK).

After confirmation, a single representative colony was inoculated into 10 ml of sterile TSBN and incubated for 24 h at 37°C. The overnight culture was centrifuged (Sorvall Legend RT+, Thermo Scientific, Waltham, MA) at 7000 x g for 10 min at 22°C, the pellet was suspended in 0.1% sterile peptone water, and washed twice more to yield a bacterial inoculum of approximately 10^8 CFU/ml.

***Listeria monocytogenes*:**

Listeria monocytogenes 0072 and *Listeria monocytogenes* 0089, both isolated from the 2011 cantaloupe outbreaks, were obtained from the U.S. Food and Drug Administration (USFDA). Cultures were made nalidixic acid resistant using methods described for *S. enterica* Michigan, except 0.6% yeast extract was added to all TSA and TSB (TSA+YE, TSB+YE).

Nalidixic acid resistant colonies were preserved in TSB+YE containing 30% glycerol and 50 ppm nalidixic acid and stored at -80°C until use. Prior to each experiment, a culture of each strain was removed from frozen storage and thawed at room temperature. Cells were activated by three successive 24 h transfers into TSB+YE containing 50 ppm nalidixic acid (TSBN+YE) and incubated at 37°C. Samples were then streaked onto Modified Oxford Agar (MOX, Becton-Dickinson, Sparks, MD), incubated at 37°C for 24 h, and a representative colony was selected for further confirmation. Cultures were confirmed using API *Listeria* strips (Biomerieux, Inc., Durham, NC).

After confirmation, a single representative colony from each culture was inoculated into separate 10 ml of sterile TSBN+YE and incubated for 24 h at 37°C. Equal volumes of the 2 *L. monocytogenes* overnight cultures were combined to create a 2-strain cocktail. The cocktail was

then centrifuged (Sorvall Legend RT+, Thermo Scientific, Waltham, MA) at 7000 x g for 10 min at 22°C, the pellet was suspended in 0.1% sterile peptone water, and was washed twice more to yield a bacterial inoculum of approximately 10^8 CFU/ml.

Cantaloupe preparation:

'Athena' and 'Hales Best Jumbo' ('HBJ') cantaloupe were chosen because of the structural differences of their surfaces. Athena is the predominant commercial cultivar in the Eastern U.S. and has slightly sutured skin with coarse netting. HBJ is an heirloom cultivar with thicker, denser netting.

Planting and harvesting:

'Athena' and 'HBJ' seeds were planted in cell plug trays and grown inside a Virginia Tech Department of Horticulture greenhouse. Cantaloupe plants with 3 to 5 true leaves were transplanted in early- to mid-June to the Virginia Tech Kentland Research Farm in the summers of 2012 and 2013. Some plants were established by direct seeding in order to have fruits maturing later in the season. Rows were set on 1.8 m centers with in row spacing of 0.9 m per hill. Two transplants or 4 to 5 seeds were placed into holes cut into black-plastic mulch laid on raised beds. At planting complete analysis fertilizer was banded below transplants or seeds in conjunction with soil testing results in both years. Plants were hand watered following planting. A drip irrigation system installed underneath the plastic cover provided approximately 2.5 cm of water per week throughout the season. Irrigation was terminated late in the season just prior to harvest. Cantaloupes were harvested from early September to early October at the ¾ slip stage of the stem and fruit.

Transportation and storage:

Immediately after harvest, undamaged whole cantaloupes were placed inside clean, sanitized, reusable plastic containers and transported to the Virginia Tech Food Science and Technology Building approximately 10 mi from the field site. In the building, cantaloupes were carefully transferred to a clean sink filled with tap water, lightly scrubbed with a soft hair brush, and rinsed under tap water for approximately 30 s to remove any soil and organic material. They were passively dried at room temperature (20 to 25°C) for at least 1 h or until any excess moisture evaporated. Fruits were then sorted by cultivar and size. Over-ripe, small, and damaged cantaloupes were discarded so only whole melons with no visual cracking, insect damage, or puncture wounds were used for treatments. Whole intact melons were placed in clean plastic containers and stored at 4°C inside a controlled walk-in refrigerator for a maximum of 10 d because of their shelf life.

Surface preparation:

Cantaloupes were transferred to room temperature the night before treatment for their temperature to adjust and surface condensation to dry before treatment. Cantaloupes were placed with the stem scar side facing down and approximately 45, 22 mm diameter circles were drawn on the top half of the cantaloupe spheres with a fine point, nontoxic, permanent marker (Sharpie®, Series No. 35000; Sanford, Bellwood, IL). Six cantaloupes of each cultivar were used per replication (3) per pathogen. In preliminary studies, liquid AITC ran outside of the 22 mm diameter circles due to the hydrophobicity of the cantaloupe surface. Therefore, caulk (Acrylic Latex Caulk Plus Silicone, Brilliant White DAP®, Alex Plus® Exceeds A STMC 834, Baltimore, MD) was applied around the pre-drawn circles of 2 cantaloupes of each cultivar per trial to keep the allyl isothiocyanate liquid within the designated area.

Surface inoculation of whole cantaloupes:

Cantaloupes were transferred to a biological safety cabinet at room temperature. For surface inoculation, 50 µl (4 to 6 drops) of prepared inoculum was spotted within each 22 mm diameter circle. Following inoculation, cantaloupes were left undisturbed in the biological safety cabinet for 90 min. An uninoculated cantaloupe from each cultivar was tested to ensure there was no background microflora that could grow on TSAN and TSAN+YE.

All desiccators were sanitized, rinsed with deionized water, and allowed to dry prior to treatments. Five of the 6 cantaloupes per cultivar received one of 5 treatments while in individual 12 L glass desiccators with the stem scar facing down. Three cantaloupes per cultivar were negative controls and received either no treatment, but 25 µl sterile deionized water (DI H_2O) was dispensed within each circle, or had caulk surrounding the 22 mm diameter circles without hydration (caulk control). One cantaloupe per cultivar received 25 µl 200 ppm sodium hypochlorite (NaClO) per circle (positive control) and one received 5 µl AITC (95% purity, Sigma Aldrich, Milwaukee, WI) applied inside each caulk-surrounded circle per cultivar. Besides the caulk control cantaloupes, the 5 µl AITC treated cantaloupes were the only others to have caulk surrounding the circles. As soon as each cantaloupe was treated, the desiccator lid was sealed. Humidity and temperature within the desiccators were monitored and maintained at 95.5 ± 2.5% RH and 25.2 ± 1.1°C throughout the 24 h treatment. The sixth cantaloupe of each cultivar was enumerated immediately to determine the initial population of pathogen on the cantaloupe surface. The 5 µl AITC volume was chosen because preliminary experiments showed that 10 and 15 µl AITC volumes per 22 mm diameter circle deteriorated the rind of the cantaloupe.

Microbiological counts:

At time 0 h for the initial count and 24 h for the treatments, cantaloupes were placed inside a biological safety cabinet and cantaloupe plugs from the inoculated surfaces were removed with a sanitized, sterile 22 mm cork borer (Humboldt Manufacturing Company, Elgin, IL). A sterile stainless steel spatula separated the rind from the cantaloupe flesh of each plug. Rind plugs were randomly selected and carefully weighed to obtain 10 g of rind per sample. Three 10 g samples of rind were used per cantaloupe per replication (n=9). Each 10 g sample was separately placed into a sterile stomacher bag (Whirl-Pak, Nasco, Ft. Atkinson, WI), diluted with 90 ml of 0.1% sterile peptone water, and pummeled in a stomacher (Easy Mix, AES Laboratories, Princeton, NJ) for 2 min. Homogenates were serially diluted into 0.1% sterile peptone blanks and pour plated with the appropriate agar in duplicate. Cantaloupe rinds inoculated with *S. enterica* Michigan were plated on TSAN and rinds inoculated with *L. monocytogenes* were plated on TSAN+YE. TSAN plates were incubated at 37°C for 24 h and TSAN+YE plates were incubated at 37°C for 48 h. Following incubation, the plates were counted. One typical colony was chosen from TSAN and TSAN+YE plates to confirm the pathogen. To confirm *Salmonella* spp., a single representative colony was selected for further confirmation using the *Salmonella* latex test. To confirm *L. monocytogenes*, a colony was chosen at random from TSAN+YE plates, streaked onto MOX, and incubated at 37°C for 48 h. A single representative colony was selected for further confirmation using API *Listeria* strips.

Statistical analysis:

Microbiological counts:

Each treatment was replicated 3 times with triplicate samples tested for each replication (n=9) per pathogen. Enumeration data were transformed into log values prior to statistical analysis. Data were analyzed by factorial analysis in a completely randomized design using the

general linear model (GLM) procedure of Statistical Analysis Software (Version 9.13, SAS Institute, Cary, NC). Significant differences ($P < 0.05$) in microbial recovery after liquid treatments for 24 h were determined using Tukey's Honestly Significant Difference to compare mean log reductions from treatments.

RESULTS AND DISCUSSION

The initial (0 h) population of *S. enterica* Michigan on 'Athena' was 6.36 ± 0.68 log CFU/g and 5.94 ± 1.06 log CFU/g on 'HBJ'. Meanwhile, the initial (0 h) population of *L. monocytogenes* on 'Athena was 7.08 ± 0.70 log CFU/g and 6.62 ± 0.54 log CFU/g on 'HBJ.' There was no growth of *S. enterica* Michigan or *L. monocytogenes* on the TSAN or TSAN+YE plates from the uninoculated cantaloupe controls.

Five µl AITC significantly reduced ($P < 0.05$) populations of *S. enterica* Michigan by 3.01 log CFU/g on the surface of 'Athena' (Figure 1). All other treatments, led to an increase of *S. enterica* Michigan on the surface of 'Athena' during 24 h at 25°C. Even though 5 µl AITC reduced the population of *S. enterica* Michigan by approximately 0.92 log CFU/g, it was not statistically significant from the other treatments against the bacteria on the surface of 'HBJ.' On the other hand, 5 µl AITC significantly reduced ($P < 0.05$) *L. monocytogenes* on 'HBJ' when compared to other treatments, with a reduction of 2.59 log CFU/g (Figure 2). Similar to the results on 'Athena,' the reduction in microbial populations by the AITC treatment was significant between pathogens on 'HBJ.' Five µl AITC applied to 'Athena' reduced the microbial load of *S. enterica* Michigan 3 times greater than on 'HBJ.' Compared to 'Athena,' the reduction of *L. monocytogenes* on 'HBJ' was significant. The AITC treatment applied to 'HBJ' decreased the population of *L. monocytogenes* by approximately 1.5 times greater than on 'Athena.'

The surface topography of Athena and Hales Best Jumbo (HBJ) cultivars both consist of a coarse skin with well-developed netting where pathogenic bacteria can attach between the netting and form biofilms, thus making it difficult to remove bacterial populations once they have attached. This study demonstrates that a 24 h treatment of 5 µl liquid AITC on the rind of cantaloupes reduces populations of *S. enterica* Michigan up to 3 log CFU/g on 'Athena' and of *L. monocytogenes* up to approximately 2.6 log CFU/g on 'HBJ,' respectively. Treatment with DI H_2O increased growth of both pathogens on both cultivars. The increase was significantly greater (more than double) for *S. enterica* Michigan on 'Athena' and 'HBJ' compared to *L. monocytogenes*. Treatment with 200 ppm NaClO increased the population of *S. enterica* Michigan by 0.50 log CFU/g, while decreasing *L. monocytogenes* by 0.36 log CFU/g on 'Athena'.

These results are in contrast to what Ukuku and Fett (2002) previously found. Since cantaloupe rinds are hydrophobic in nature and *Salmonella* has a more hydrophobic surface than *L. monocytogenes*, it would be expected that *Salmonella* would bind stronger to the surface of cantaloupes and thus be more difficult to reduce (Ukuku and Fett, 2002). This was true for all but two treatments in this study: 5 µl AITC against *S. enterica* Michigan on 'Athena' and 200 ppm NaClO against *S. enterica* Michigan on 'HBJ.' However, the difference between the results of 200 ppm NaClO on 'HBJ' for the two pathogens was not statistically significant.

Dorman and Deans (2000) reported that Gram-positive bacteria are more susceptible to the effects of essential oils and Vaara (1992) stated that Gram-negative bacteria are less susceptible to essential oils because they have an outer membrane surrounding the cell wall, which limits diffusion of hydrophobic compounds through their lipopolysaccharide covering. Nevertheless, unlike other essential oils, Gram-negative bacteria (like *S. enterica* Michigan) are

more sensitive than Gram-positive bacteria (like *L. monocytogenes*) to inhibition by AITC (Lin et al., 2000). The results from the 5 µl liquid AITC treatment on 'HBJ' are in agreement with Dorman and Deans (2000) and Vaara (1992), since *L. monocytogenes* had a greater reduction than *S. enterica* Michigan. However, the 5 µl liquid AITC treatment on 'Athena' concurs with Lin et al. (2000). The stronger attachment of the hydrophobic *S. enterica* Michigan to the surface of 'HBJ' could be due to the tighter netting on the epidermal cell surface of 'HBJ' versus 'Athena,' since both of these cells contain hydrophobic suberized walls in order to reduce water loss and to protect pathogens from entering and are responsible for the hydrophobic nature of cantaloupe rind (Webster and Craig, 1976; Ukuku and Fett, 2006).

This research demonstrates that a liquid treatment of AITC is as effective or more successful in reducing populations of *S. enterica* Michigan and *L. monocytogenes* than the commonly used 200 ppm chlorine treatment in packinghouse wash water, which may only result in 1 to 2 log CFU/g reduction, and 1000 ppm chlorine or 5% hydrogen peroxide solution, which achieves a 2 to 3 log CFU/cm^2 reduction (Adams et al., 1989; Beuchat et al., 1998; Ukuku et al., 2001; Wei et al., 1995). Although in spite of these conclusions, 5 µl liquid AITC still deteriorated the rind of both cultivars to an extent, which would not be desired by the consumer because it softened and discolored the cantaloupe rind, but less than it visually caused in preliminary experiments with 10 and 15 µl, respectively. Further research is needed to identify an acceptable concentration of liquid AITC which can be applied to the surface of cantaloupes without causing deterioration of the skin and netting, as well as, a more acceptable exposure time since 24 h is quite a long time for cantaloupes to undergo treatment in the fresh produce industry.

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Figures

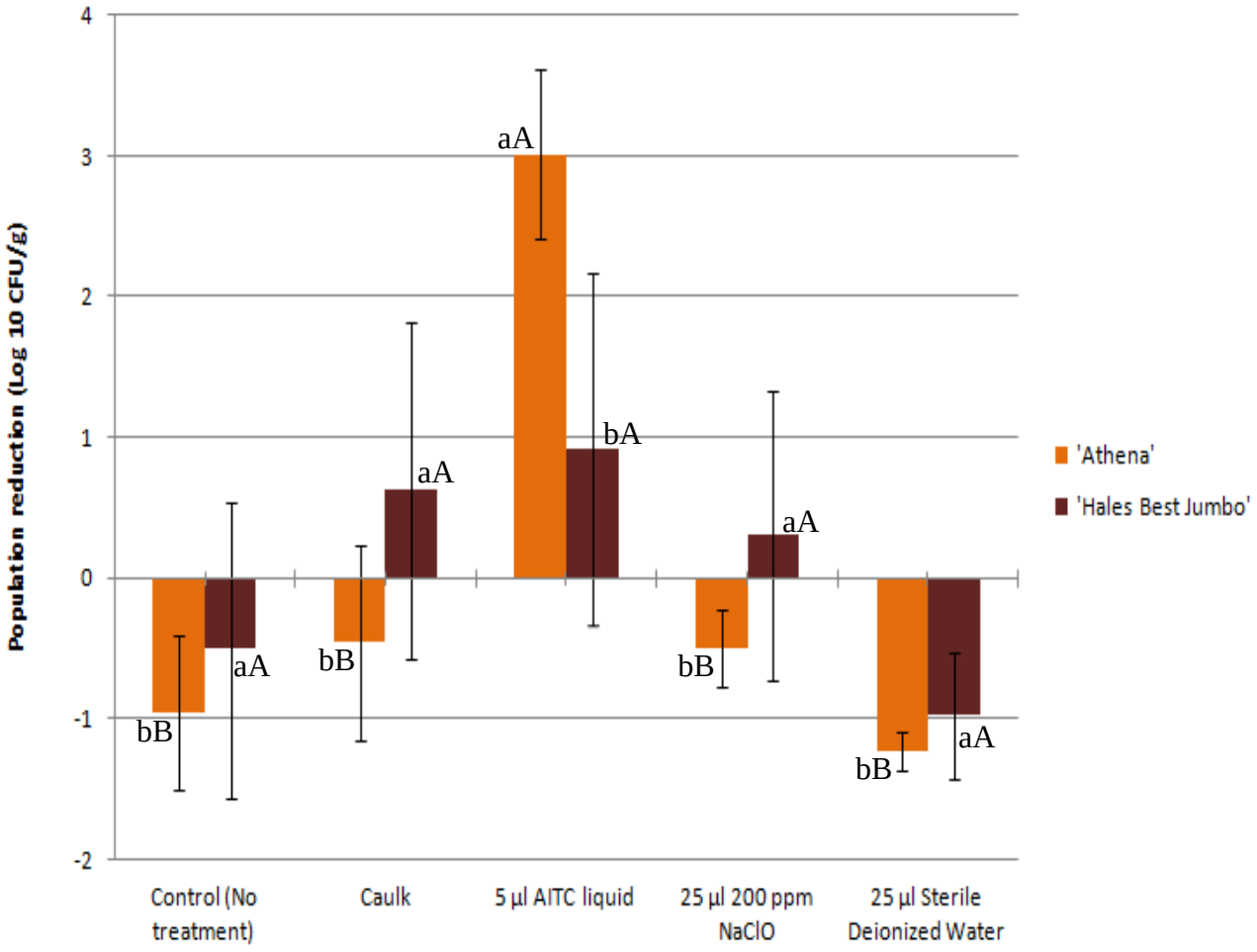

Figure 1. Population reduction (Log 10 CFU/g) of *S. enterica* Michigan on the surface of 'Athena' and 'Hales Best Jumbo' after 24 h treatments at 25°C. The bars represent the standard deviation of the mean of nine replications. Means with the same lower case letters for a treatment are not significantly different ($P < 0.05$) between cultivars and those with the same upper case letters are not significantly different ($P < 0.05$) between treatments per cultivar.

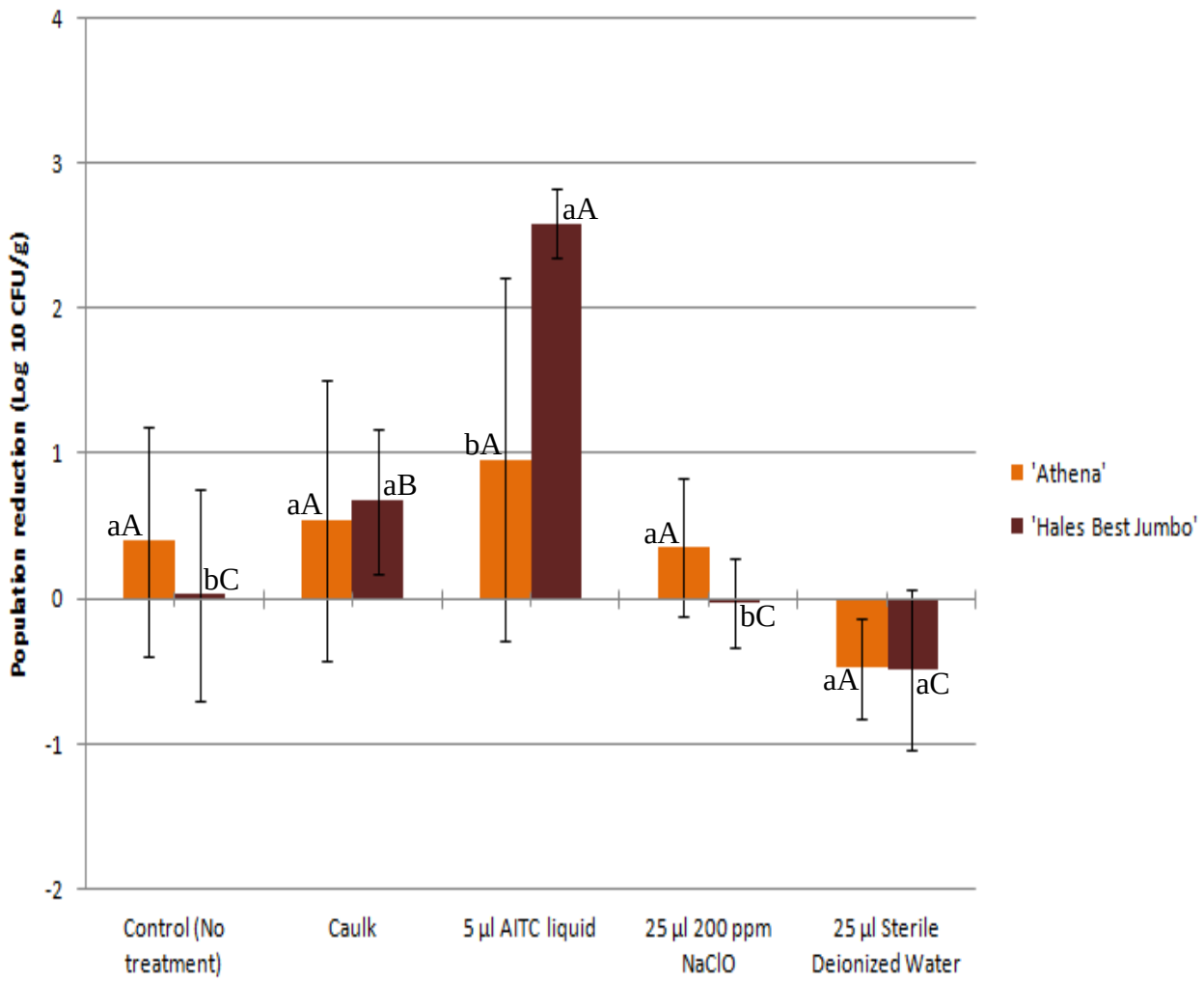

Figure 2. Population reduction (Log 10 CFU/g) of *L. monocytogenes* on the surface of 'Athena' and 'Hales Best Jumbo' after 24 h treatments at 25°C. The bars represent the standard deviation of the mean of nine replications. Means with the same lower case letters for a treatment are not significantly different ($P < 0.05$) between cultivars and those with the same upper case letters are not significantly different ($P < 0.05$) between treatments per cultivar.

CHAPTER 4

Allyl isothiocyanate vapor reduces populations of *Salmonella enterica* Michigan and *Listeria monocytogenes* on the surface of cantaloupe (*Cucumis melo* L.)*

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Key words: cantaloupe, allyl isothiocyanate, vapor, *S. enterica* Michigan, *L. monocytogenes*, temperature, time

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ABSTRACT

By the time cantaloupes (*Cucumis melo* L.) reach the packinghouse, they can contain 10^4 to 10^6 bacteria/g, including some foodborne pathogens. Many cultivars contain a corky netted fruit epidermis that supports the formation of biofilms, making it more difficult for sanitizers, like chlorine, to remove them efficiently. The application of sanitizers in the gaseous phase may be more effective. Allyl isothiocyanate (AITC), a plant derived compound, reduces microbial loads on various food products in both its liquid and vapor states. Due to its high volatility, AITC vaporizes faster at increased temperatures and may reduce more pathogens when applied in the vapor phase. This study evaluates AITC vapor for its ability to reduce foodborne pathogens on the surface of cantaloupe fruit. Fifty µl of *Salmonella enterica* Michigan or *Listeria monocytogenes* (10^8 CFU/ml) were inoculated onto Athena or Hales Best Jumbo (HBJ) cultivars in 22 mm diameter circles and allowed to dry for 90 min. resulting in approximately 6.35 log CFU/g. Each cantaloupe was placed inside a separate 12 L glass desiccator and exposed to AITC vapor or control (no treatment) at 25°C or 35°C for 1 or 24 h. Vapor was created by dispensing liquid (100 or 300 µl) onto Whatman No. 1 filter paper attached to the lid of the desiccator prior to sealing. Following treatment, the 22 mm sections were removed, homogenized and plated onto appropriate agar. AITC vapor concentrations were quantified by Gas Chromatography-Mass Spectrometry (GC-MS) headspace analysis. Rind hardness of 'Athena' was evaluated after treatments. Vapor concentrations produced ranged from 3.4 to 19.6 µl/L. The 300 µl treatment reduced ($P < 0.05$) both pathogens at least 3.0 log CFU/g on 'Athena' at 25°C. Generally, the longer exposure time to the AITC vapor (24 h versus 1 h) reduced both *S.* Michigan and *L. monocytogenes* on 'Athena' and 35°C reduced populations up to 4.5 times greater. Pathogens on

the surface of ‘Athena’ were reduced to lower numbers than those inoculated on the surface of ‘HBJ.’ AITC vapor shows promise as a way to reduce bacteria on fresh whole cantaloupe.

INTRODUCTION

From 2000 to 2011, there were over 1,100 documented foodborne illnesses associated with 24 outbreaks of melon consumption (Nguyen, 2012). Cantaloupes were either confirmed or suspected to be the melon associated with 12 of the 24 outbreaks (Nguyen, 2012). Foodborne pathogens such as *Escherichia coli* O157:H7, *Campylobacter*, *Shigella*, and Norovirus are all of concern in melon associated outbreaks; however, *Salmonella* is the most common pathogen responsible for these outbreaks (Nguyen, 2012). *Listeria monocytogenes* was responsible for the first multistate outbreak of listeriosis involving cantaloupes and became an additional pathogen of concern in 2011.

Cantaloupes are traditionally either field packed (Western U.S.) or subjected to a chlorinated water wash prior to packing (Eastern U.S.). However, use of 200 ppm chlorine may only result in a 1 to 2 log CFU/g reduction of bacteria (Adams et al., 1989; Beuchat et al., 1998; Wei et al., 1995). There is need for a more effective sanitation procedure to reduce the risk of microbial contamination. One challenge is that pathogens may become embedded in the corky netting of the epidermal tissue of cantaloupe fruit, avoiding sanitizing agents which penetrate poorly (Seo and Frank, 1999). The use of antimicrobial compounds in their vapor phase may enhance pathogen reduction because they may penetrate those protected locations more effectively. Gaseous antimicrobials tend to dissolve in wound sites on fruits and vegetables, and microbes in those areas are likely to be inactivated, thus making vapor application of antimicrobials attractive (Yun et al., 2013). For example, chlorine dioxide (ClO_2) gas has greater penetration ability on fresh producing leaving behind less residue than its liquid equivalent (Knapp and Battisti, 2001).

The industry is moving towards more natural plant antimicrobial compounds, possibly to replace chlorine (Kim et al., 2002). Few studies have looked into the antibacterial activities of essential oils (EOs) in the vapor phase (Yun et al., 2013). Furthermore, little research exists about how exposure to gaseous essential oils affects the quality of fresh fruits and vegetables, such as cantaloupes (Yun et al, 2013). The antimicrobial compound allyl isothiocyanate (AITC) in its gaseous state was the most effective among the isothiocyanates tested and could inhibit the growth of various microorganisms on the surface of agar (Ahn et al., 1999; Kim et al., 2002). Several studies have demonstrated the outstanding antimicrobial activity of AITC vapor (Isshiki et al., 1992; Nadarajah et al., 2005; Park et al., 2000; Park et al., 2012). AITC has also been incorporated into controlled release matrices, such as calcium alginate beads, cyclodextrin, maize, mesoporous silica, and *Saccharina japonica* (brown seaweed) powder to reduce foodborne bacteria in food (Paes et al., 2011; Li et al., 2007; Kim et al., 2008; Park and Pendleton, 2012; Siahaan et al., 2014). Shin et al. (2010) concluded that AITC vapor is a more effective antimicrobial agent than liquid AITC. Nevertheless, the use of AITC in food systems is limited because of its strong odor, which can significantly affect the taste of the food (Chacon et al., 2006; Delaquis and Mazza, 1995; Kim et al., 2002).

The goal of this research is to evaluate the effect of AITC vapor at different time (1 and 24 h) and temperature (25°C and 35°C) combinations on the survival of *S. enterica* Michigan and *L. monocytogenes* on the surface of 'Athena' cantaloupe fruit and at 24 h at 25°C on 'Hales Best Jumbo' ('HBJ'). This study also assesses the texture of 'Athena' during 2 weeks of refrigerated storage following post-harvest AITC vapor treatment.

MATERIALS AND METHODS

Bacterial cultures and culture conditions:

***Salmonella enterica* serovar Michigan:**

Salmonella enterica serovar Michigan (isolated from a cantaloupe associated outbreak) was obtained from Dr. Larry Beuchat at the University of Georgia. The culture was made nalidixic acid resistant by consecutively transferring a loopful (10 µl) from Tryptic Soy Broth (TSB, Bacto, Difco, Becton-Dickinson, Sparks, MD) to TSB containing increasing concentrations of nalidixic acid (99.5%, Acros Organics, Morris Plains, NJ) (TSBN) (5, 10, 25, 40, and 50 ppm) every 24 h until cultures were resistant at a level of 50 ppm (50 µg/ml). Simultaneously, a loopful (10 µl) of culture was t-streaked onto Tryptic Soy Agar (TSA, Difco, Becton-Dickinson, Sparks, MD) with increasing concentrations of nalidixic acid (TSAN) (5, 10, 25, 40, and 50 ppm) until colonies were resistant at a level of 50 ppm.

Nalidixic acid resistant colonies were preserved in TSBN containing 30% glycerol (99+%, pure, synthetic, Acros Organics Glycerol, Morris Plains, NJ) and stored at -80°C until use. Prior to each experiment, a vial (2 ml) of frozen culture was removed from storage and thawed at room temperature. Cells were activated by 3 successive 24 h transfers into TSB containing 50 ppm nalidixic acid (TSBN) and incubated at 37°C. Samples were then streaked onto Xylose Lysine Deoxycholate Agar (XLD, Difco, Becton-Dickinson, Sparks, MD), incubated at 37°C for 24 h, and a representative colony was confirmed using the *Salmonella* latex test (Oxoid Ltd.; Basingstoke, Hants, UK).

After confirmation, a single representative colony was inoculated into 10 ml of sterile TSBN and incubated for 24 h at 37°C. The overnight culture was centrifuged (Sorvall Legend RT+, Thermo Scientific, Waltham, MA) at 7000 x g for 10 min at 22°C, the pellet was suspended in 0.1% sterile peptone water, and was washed twice more to yield a bacterial inoculum of approximately 10^8 CFU/ml.

***Listeria monocytogenes*:**

Listeria monocytogenes 0072 and *L. monocytogenes* 0089, both isolated from the 2011 cantaloupe outbreaks, were obtained from the U.S. Food and Drug Administration (USFDA). Cultures were made nalidixic acid resistant using methods described for *S. enterica* Michigan, except 0.6% yeast extract was added to all TSA and TSB (TSA+YE, TSB+YE). Nalidixic acid resistant colonies were preserved in TSB+YE containing 30% glycerol and 50 ppm nalidixic acid and stored at -80°C until use. Prior to each experiment, a culture of each strain was removed from frozen storage and thawed at room temperature. Cells were activated by 3 successive 24 h transfers into TSB+YE containing 50 ppm nalidixic acid (TSBN+YE) and incubated at 37°C. Samples were then streaked onto Modified Oxford Agar (MOX, Becton-Dickinson, Sparks, MD), incubated at 37°C for 24 h, and a representative colony was selected for further confirmation. Cultures were confirmed using API *Listeria* strips (Biomerieux, Inc., Durham, NC).

After confirmation, a single representative colony from each culture was inoculated into separate 10 ml of sterile TSBN+YE and incubated for 24 h at 37°C. Equal volumes of the 2 *L. monocytogenes* overnight cultures were combined to create a 2-strain cocktail. The cocktail was then centrifuged (Sorvall Legend RT+, Thermo Scientific, Waltham, MA) at 7000 x g for 10 min at 22°C, the pellet was suspended in 0.1% sterile peptone water, and was washed twice more to yield a bacterial inoculum of approximately 10^8 CFU/ml.

Cantaloupe preparation:

Athena and Hales Best Jumbo (HBJ) cultivars of cantaloupe were chosen because of the structural differences of their fruit surfaces. Athena is the predominant commercial cultivar in

the Eastern U.S. and has slightly sutured fruit architecture with coarse netting. HBJ is an heirloom cultivar with thicker, denser netting.

Planting and harvesting:

'Athena' and 'HBJ' seeds were planted in cell plug trays and grown inside a Virginia Tech Department of Horticulture greenhouse. Cantaloupe plants with 3 to 5 true leaves were transplanted in early- to mid-June to the Virginia Tech Kentland Research Farm in the summers of 2012 and 2013. Some plants were established by direct seeding in order to have fruits maturing later in the season. Rows were set on 1.8 m centers with in row spacing of 0.9 m per hill. Two transplants or 4 to 5 seeds were placed into holes cut into black-plastic mulch laid on raised beds. At planting complete analysis fertilizer was banded below transplants or seeds in conjunction with soil testing results in both years. Plants were hand watered following planting. A drip irrigation system installed underneath the plastic cover provided approximately 2.5 cm of water per week throughout the season. Irrigation was terminated late in the season just prior to harvest. Cantaloupes were harvested from early September to early October at the ¾ slip stage of the stem and fruit.

Transportation and storage:

Immediately after harvest, undamaged whole cantaloupes were placed inside clean, sanitized, reusable plastic containers and transported to the Virginia Tech Food Science and Technology Building approximately 10 mi from the field site. In the building, cantaloupes were carefully transferred to a clean sink filled with tap water, lightly scrubbed with a soft hair brush, and rinsed under tap water for approximately 30 s to remove any soil and organic material. They were passively dried at room temperature (20 to 25°C) for at least 1 h or until any excess moisture evaporated. Fruits were then sorted by cultivar and size. Over-ripe, small, and

damaged cantaloupes were discarded so only whole melons with no visual cracking, insect damage, or puncture wounds were used for treatments. Whole intact melons were placed in clean plastic containers and stored at 4°C inside a controlled walk-in refrigerator for a maximum of 10 d because of their shelf life

Cantaloupes were transferred to room temperature the night before treatment for their temperature to adjust and surface condensation to dry before treatment. Cantaloupes were placed with the stem scar side facing down and approximately 45, 22 mm diameter circles were drawn on the top half of the cantaloupe spheres with a fine point, nontoxic, permanent marker (Sharpie®, Series No. 35000; Sanford, Bellwood, IL). Six fruits of each cultivar were used per replication (3) per pathogen.

Surface inoculation of whole cantaloupes:

Cantaloupes were transferred to a biological safety cabinet at room temperature. For surface inoculation, 50 µl (4 to 6 drops) of prepared inoculum was spotted within each 22 mm diameter circle. Following inoculation, cantaloupes were left undisturbed in the biological safety cabinet for 90 min. An uninoculated cantaloupe from each cultivar was tested to ensure there was no background microflora that could grow on TSAN and TSAN+YE.

Allyl isothiocyanate vapor treatments:

Preparation of storage containers (desiccators):

Glass desiccators with a volume of 12 L were sanitized, rinsed with deionized water, and allowed to dry prior to treatment. Depending on the liquid concentration to be applied, either 2 or 4 Whatman Qualitative Grade 1 Filter Papers measuring 5.5 cm in diameter (Whatman Inc., Piscataway, NJ) were attached to the inner lid of each glass desiccator with labeling tape (Fisher Scientific, Fairlawn, NJ).

Vapor application for 'Hales Best Jumbo':

After 90 min of drying, all but 1 of the 6 fruits was transferred into individual 12 L glass desiccators with the stem scar still facing down. Two cantaloupes served as negative controls and received either no treatment or 25 µl sterile deionized water (DI H_2O) dispensed within each circle. One cantaloupe received 25 µl 200 ppm sodium hypochlorite (NaClO) per circle. Two concentrations of allyl isothiocyanate (AITC) (95% purity, Sigma Aldrich, Milwaukee, WI) vapor treatments were tested on 2 cantaloupes. Either 100 µl liquid AITC (50 µl on 2 filter papers) or 300 µl liquid AITC (75 µl on 4 filter papers) was dispensed onto the filter paper attached to the lid of the glass desiccators. As soon as each cantaloupe received its treatment, the desiccator lid was sealed. Humidity and temperature within the desiccators were monitored and maintained at 95.5 ± 2.5% RH and 25.2 ± 1.1°C throughout the 24 h treatment. The sixth cantaloupe was enumerated immediately to determine the initial population of pathogen on the cantaloupe surface.

Vapor application for 'Athena':

Similar to the procedure for 'HBJ,' after 90 min of drying all but 1 of the 'Athena' cantaloupes was transferred into its own individual 12 L glass desiccator with the stem scar still facing down and received its respective treatment. However, 4 temperature-time storage conditions were evaluated for 'Athena': 1) 1 h at 25°C, 2) 1 h at an elevated temperature (35.1 ± 3.1°C), 3) 24 h at 25°C, or 4) 24 h at 35°C. Each storage temperature-time experiment included 1 cantaloupe that was used as a control that was inoculated and received no treatment and 1 that also was inoculated with no treatment that was enumerated at time 0 h to determine the initial population of pathogen on the 'Athena' surface. For the 24 h 25°C experiments, 1 cantaloupe received 25 µl 200 ppm NaClO per circle and another had 25 µl DI H_2O dispensed within each

circle. All 4 temperature-time storage conditions consisted of 2 concentrations of AITC vapor treatments that were tested against 2 cantaloupes as previously described for 'HBJ.'

Microbiological counts:

At time 0 h for the initial count and after 1 or 24 h for the treatments, cantaloupes were placed inside a biological safety cabinet and a sanitized, sterile 22 mm cork borer (Humboldt Manufacturing Company, Elgin, IL) was used to remove cantaloupe plugs from the inoculated surfaces. A sterile stainless steel spatula was used to separate the rind from the cantaloupe flesh of each plug. Rind plugs were randomly selected and carefully weighed to obtain 10 g of rind per sample. Three 10 g samples of rind were used per cantaloupe per replication (n=9). Each 10 g sample was separately placed into a sterile stomacher bag (Whirl-Pak, Nasco, Ft. Atkinson, WI), diluted with 90 ml of 0.1% sterile peptone water, and pummeled in a stomacher (Easy Mix, AES Laboratories, Princeton, NJ) for 2 min. Homogenates were serially diluted into 0.1% sterile peptone blanks and pour plated with the appropriate agar in duplicate. Cantaloupe rinds inoculated with *S. enterica* Michigan were plated on TSAN and rinds inoculated with *L. monocytogenes* were plated on TSAN+YE. TSAN plates were incubated at 37°C for 24 h and TSAN+YE plates were incubated at 37°C for 48 h. Following incubation, the plates were counted. One typical colony was chosen from TSAN plates and TSAN+YE plates to confirm the pathogen. To confirm *Salmonella* spp., a single representative colony was selected for further confirmation using the *Salmonella* latex test. To confirm *L. monocytogenes*, a colony was chosen at random from TSAN+YE plates, streaked onto MOX, and incubated at 37°C for 48 h. A single representative colony was selected for further confirmation using API *Listeria* strips.

Texture analysis:

Twenty one ‘Athena’ cantaloupes were tested for rind hardness. After the fruits were washed and dried, treatments were applied the same day as harvest. Glass desiccators with a volume of 12 L were sanitized, rinsed with deionized water, and dried prior to treatment.

Controls:

Nine ‘Athena’ fruit (3 per experiment) were 24 h controls and received no AITC application. Six cantaloupe fruits were separately placed in 12 L glass desiccators. Three were stored at 25°C and 3 were stored at 35°C. Three additional cantaloupes were not sealed in a desiccator and held at 25°C. Humidity and temperature within the desiccators were monitored and maintained throughout the 24 h treatments.

After 24 h, the melons were removed from their storage environment and their firmness was analyzed using a TA-XT Plus, Series 10545, texture analyzer (Texture Technology, New York, NY) with a model TA-23 plunger (½ inch diameter, ¼ inch R end, and 3 inches tall). The auto trigger applied 5 g of force and a 2.0 mm/sec test distance penetration speed. Following the skin hardness tests all fruit were placed in clean plastic containers and stored inside a controlled walk-in refrigerator set at 4°C and held for 14 d. Every 24 h the cantaloupes were removed and their firmness was analyzed again. All applied force measurements were tested on the surface of fruit with similar amounts of netting and all readings were collected in triplicate. Humidity and temperature were monitored and maintained at 81 ± 17% RH and 4 ± 4°C throughout the 2 week storage period inside the walk-in refrigerator.

Treatments:

The firmness of the cantaloupe rind in the remaining 12 ‘Athena’ fruits (3 per experiment) were evaluated for effects of 24 h AITC vapor treatments. Their firmness was measured using the TA-XT Plus, Series 10545, texture analyzer as previously described for the

controls. Then they were all were stored in their own 12 L glass desiccator that had been sanitized, rinsed with deionized water, and allowed to dry prior to treatment. Cantaloupes were exposed to 2 concentrations of AITC vapor as previously described for microbial studies. Six fruits a piece were exposed to both concentrations of AITC vapor, 3 of each were stored at 25°C and the other 3 were stored at 35°C. Humidity and temperature within the desiccators were monitored and maintained throughout the 24 h treatments.

After 24 h, the fruits were removed from their desiccators and their firmness was analyzed using the TA-XT Plus, Series 10545, texture analyzer as previously described. Following the skin hardness tests all cantaloupes were placed in clean plastic containers and stored inside a controlled walk-in refrigerator set at 4°C for 14 d. Every 24 h the cantaloupes were removed and their firmness was analyzed again. All applied force measurements were tested on the surface of fruit with similar amounts of netting and all readings were collected in triplicate. Humidity and temperature were monitored and kept at 81.5 ± 16.5% and 4 ± 4°C throughout the 2 week storage period inside the walk-in refrigerator.

Headspace analysis:

Headspace analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS) following vapor treatments to quantify the amount of liquid AITC that entered into the headspace as a vapor inside the desiccators. Vapor treatments were performed inside a 12 L glass desiccator as previously described, but cantaloupes were not present during analysis. To extract gas samples after the treatment period without opening the desiccator lid, a 1 cm diameter hole was cut out of the desiccator lid. Prior to sealing the lid to begin treatment, a piece of self-stick weathering strip tape (Frost King, Rubber Foam Weatherseal, Black R512H, ½”W, 5/16”T,

10 ft L, Mahwah, NJ) was placed over the hole to ensure no vapor could escape from the desiccator and to allow easy removal of gas samples with a gastight syringe following treatment.

Allyl isothiocyanate vapor procedure:

The 12 L glass desiccator was sanitized, rinsed with deionized water, and allowed to dry prior to treatment. Depending on the liquid concentration to be applied, either 2 or 4 Whatman Qualitative Grade 1 Filter Papers measuring 5.5 cm in diameter (Whatman Inc., Piscataway, NJ) were attached to the inner lid of the glass desiccator with labeling tape (Fisher Scientific, Fairlawn, NJ). Next, either 100 µl liquid AITC (50 µl on 2 filter papers) (95% purity, Sigma Aldrich, Milwaukee, WI) or 300 µl liquid AITC (75 µl on 4 filter papers) was dispensed onto the filter paper. Immediately after application of liquid AITC, the desiccator lid was sealed and the liquid evaporated during the treatment period. Depending upon the treatment, the desiccator was stored at 25°C for 1 or 24 h or at 35°C for 1 or 24 h. Humidity and temperature within the desiccator were monitored throughout the treatment periods. All treatments were performed in triplicate (n=3).

Direct injection Gas Chromatography-Mass Spectrometry (GC-MS):

After 1 or 24 h, a gastight syringe removed 25 µl from the headspace inside the desiccators, which was directly injected into a QP2010 Ultra (Shimadzu, Columbia, MD) gas chromatography equipped with a GCMS-QP2010 Ultra gas chromatograph-mass spectrometer. The injection port was set to 200.0°C and all injections were made in split mode with a split ratio of 1:10. Volatile compounds were separated using a nonpolar (SHRXI-5MS) 30 m x 0.25 mm I.D., 0.25 µm film thickness capillary column with helium as the carrier gas at a column flow rate of 0.68 mL/min and total flow rate of 8.0 mL/min (linear velocity 30.0 cm/sec). Pressure was fixed at 3.9 psi and the purge flow was 0.5 mL/min. The GC column oven temperature

program was set at 50.0°C, held for 5 min, and then increased to 250.0°C. GC-MS was performed to identify the presence and intensity of AITC vapor after 1 and 24 h treatments at 25°C and 35°C. AITC peaks were identified using standardized retention time (retention index values, RI) and the Wiley 2010 mass spectral library. The area under the AITC peaks was recorded and a standard was obtained to determine the concentration of AITC vapor for each treatment.

Standard of AITC vapor:

A 10,000 ppm (1%) AITC liquid solution was prepared in hexane and 1.0 µl was injected into the injection port of the GC-MS following the same protocol as described previously for the AITC vapor treatments. The area under the AITC peaks were averaged and used in calculations to quantify the AITC vapor inside the desiccator for each treatment (n=4). Final AITC concentrations were calculated assuming complete vaporization and a constant desiccator volume of 12 L.

Quantification of percent netting on cantaloupe surfaces:

Photographs were taken of both 'Athena' and 'HBJ' and saved as JPEG files. The images were viewed using Adobe Photoshop CS3. For both cultivars, the flattest part of the cantaloupes in the photographs were selected and the total number of pixels in the selected region was recorded. Then the netting was circled and the inverse was selected so that the surface of the fruit not containing netting became white. By selecting the inverse of the specific region, the number of pixels of netting was obtained and recorded. Lastly, to calculate the percent netting on the fruit surfaces, the total number of pixels was divided by the number of pixels of netting.

Statistical analysis:

Microbiological counts:

Each treatment was replicated 3 times with triplicate samples tested for each replication (n=9) per pathogen. Enumeration data were transformed into log values prior to statistical analysis. Data were analyzed by factorial analysis in a completely randomized design using the general linear model (GLM) procedure of Statistical Analysis Software (Version 9.13, SAS Institute, Cary, NC). Significant differences ($P < 0.05$) in microbial recovery due to treatment concentration (100 or 300 µl liquid AITC applied on filter paper), exposure time (1 or 24 h), and storage temperature (25°C or 35°C) were determined using Tukey's Honestly Significant Difference to compare mean log reductions from treatments.

Texture analysis:

Each treatment was repeated 3 times with triplicate measurements taken per replication (n=9). Data were analyzed by factorial analysis in a completely randomized design using the general linear model (GLM) procedure of Statistical Analysis Software (Version 9.13, SAS Institute, Cary, NC). Significant differences ($P < 0.05$) in applied force (kg) due to treatment concentration (100 or 300 µl liquid AITC applied on filter paper) and storage temperature (25°C or 35°C) were determined using Tukey's Honestly Significant Difference. The same procedure was used to analyze statistically significant differences ($P < 0.05$) in applied force (kg) due to treatment concentration (100 or 300 µl liquid AITC applied on filter paper) and storage temperature (25°C or 35°C).

RESULTS

Headspace analysis using GC-MS identified the area under the allyl isothiocyanate (AITC) peaks to range from 10^7 to 10^8 after 1 or 24 h at 25°C and 35°C) when 100 and 300 µl, respectively, were applied to filter paper inside a sealed desiccator. The average area under the

AITC peak obtained from the standard was $3.77 \times 10^8 \pm 1.52 \times 10^7$. From these values, the concentration of AITC vapor in the headspace of the desiccator for each treatment was determined. Concentrations ranged from 3.4 µl/L (for 100 µl liquid on filter paper for 24 h at 25°C) to 19.6 µl/L (for 300 µl liquid on filter paper for 1 h at 35°C) (Table 1).

The temperatures and relative humidity inside the desiccators containing the control cantaloupes for the texture analysis tests were 26.8 ± 1.0°C and 47.5 ± 4.5% RH (room temperature and low relative humidity), 26.8 ± 1.0°C and 94.5 ± 1.5% RH (room temperature and high relative humidity), and 34.2 ± 0.9°C and 94.5 ± 1.5% RH (elevated temperature and high relative humidity). Whereas the relative humidity inside the desiccators containing the cantaloupes that received the AITC vapor treatments was 95.0 ± 3.0% and the temperatures were 25.2 ± 0.6°C and 36.8 ± 1.0°C. There were no significant differences in the hardness of the 3 'Athena' controls for each specific day (Table 2). All cantaloupes regardless of treatment became softer over the 14 d storage period. Generally cantaloupe stored at 25°C and 47.5% RH became softer than cantaloupe stored at 35°C and 94.5% RH, but there were no significant differences. The least amount of difference in force (2.06 kg) between days 1 and 14 existed for the 35°C and 94.5% RH controls.

The greatest difference between the applied force on the cantaloupes treated with AITC occurred after treatment with 300 µl AITC at 35°C between days 0 and 14 (5.04 kg reduction) (Table 3). The second greatest difference in applied force between untreated cantaloupes after 2 weeks of refrigerated storage was 3.58 kg for the 100 µl AITC at 35°C treatment. Similarly, 300 µl AITC decreased the amount of force required (by 3.42 kg) to penetrate the rind compared to 3.31 kg needed by 100 µl AITC on filter paper between days 0 and 14. These results are identical to the order of decreasing force needed to be applied to cantaloupes after 24 h vapor

treatments on day 1. By day 11, the cantaloupes treated with 300 µl AITC at 35°C were significant from the other three treatments.

The applied force (2.43 kg) for the 25°C and 94.5% RH control was within the range of the forces applied (2.23 to 2.90 kg) to the cantaloupes treated with 100 µl and 300 µl AITC at 25°C after 24 h (day 1) and day 14 of storage (Figure 1). However, the amount of force (2.06 kg) for the 35°C and 94.5% RH control was slightly above that of the 100 µl and 300 µl AITC treatments at 35°C (1.54 and 1.92 kg). Hence, while AITC vapor exposure for 24 h did soften the rind of 'Athena' by decreasing the amount of force needed to penetrate the surface, after 14 d of refrigerated storage post-treatment, the difference in softness was minimal and therefore may not be due to the AITC vapor treatments. Nonetheless, the overall texture of cantaloupes after 2 weeks of storage following AITC vapor treatments required 3.31 to 5.04 kg less force than the untreated cantaloupes (Day 0) and the controls required 2.06 to 2.62 kg less applied force to pierce the surface than on Day 1.

Microbiological treatment effects after 1 and 24 h:

After 1 h the relative humidity in the desiccators was 86.5 ± 5.5% and after 24 h it was 95.5 ± 2.5% regardless of temperature. Initial (0 h) populations of *S. enterica* Michigan were 6.19 ± 0.96 log CFU/g and 6.87 ± 0.91 log CFU/g for *L. monocytogenes*. There was no growth of *S. enterica* Michigan or *L. monocytogenes* on the TSAN or TSAN+YE plates from the uninoculated cantaloupe controls.

Among the 1 h treatments against *S. enterica* Michigan, 300 µl AITC at 35°C significantly reduced ($P < 0.05$) the population by 3.68 log CFU/g (Figure 2). The greatest reduction of *S. enterica* Michigan after 24 h (3.92 log CFU/g) was caused by 300 µl AITC at 25°C, followed by 100 µl AITC at 35°C (3.37 log CFU/g), and then a 3.07 log CFU/g reduction

attributed to the 300 µl AITC at 35°C treatment. On 'Athena,' both 24 h vapor concentrations of AITC at 35°C significantly reduced ($P < 0.05$) the population of *L. monocytogenes* (3.64 and 3.86 log CFU/g, respectively). In response to the 1 h treatments on 'Athena,' the 300 µl AITC treatment at 35°C resulted in the greatest reduction of *L. monocytogenes* (1.75 log CFU/g). Exposure of cantaloupes to AITC vapor for 24 h significantly increased the reduction of both pathogens in all cases except for 100 µl at 35°C. The population reductions of *S. enterica* Michigan and *L. monocytogenes* were not significantly different ($P < 0.05$) after treatment with 100 µl AITC at 35°C for 24 h. All 1 h vapor treatments, except for 100 µl AITC at 25°C, led to a significant reduction ($P < 0.05$) of *S. enterica* Michigan compared to reductions of *L. monocytogenes*. Generally, the longer exposure time to the AITC vapor (24 h versus 1 h) resulted in greater reduction of both *S. enterica* Michigan and *L. monocytogenes* on 'Athena' and 35°C reduced microbial populations up to 4.5 times greater. The highest vapor concentration reduced ($P < 0.05$) *S. enterica* Michigan and *L. monocytogenes* at least 3.0 log CFU/g on 'Athena' at 25°C.

Treatment at elevated temperature:

After 1 h, 35°C caused 0.93 and 1.72 log CFU/g greater reductions of *S. enterica* Michigan compared to 25°C treatments. There was a slight increase in the reduction of *L. monocytogenes* (0.10 and 0.39 log CFU/g greater compared to 25°C) after both vapor concentrations at 35°C for 1 h, but the reductions were not statistically significant. AITC vapor significantly reduced ($P < 0.05$) the populations of *L. monocytogenes* (2.84 and 0.79 log CFU/g greater compared to 25°C) and the lower vapor concentration caused a significant reduction of *S. enterica* Michigan (1.46 log CFU/g compared to 25°C) after 24 h at 35°C. However, 24 h

treatment at 25°C with the higher concentration was 0.85 log CFU/g more effective in decreasing the population of *S. enterica* Michigan than at 35°C.

24 h treatments at 25°C compared to NaClO:

For 24 h 25°C treatments on 'HBJ,' initial (0 h) populations of *S. enterica* Michigan were 5.94 ± 1.06 log CFU/g and *L. monocytogenes* populations were 6.80 ± 0.72 log CFU/g. Initial (0 h) populations on 'Athena' were 7.08 ± 0.70 log CFU/g for *S. enterica* Michigan and 6.42 ± 0.74 log CFU/g for *L. monocytogenes*. There was no growth of *S. enterica* Michigan or *L. monocytogenes* on the TSAN or TSAN+YE plates from the uninoculated cantaloupe controls.

300 µl AITC significantly reduced ($P < 0.05$) populations of *S. enterica* Michigan (3.92 log CFU/g) on the surface of 'Athena' (Figure 3). 100 µl AITC exposed to *S. enterica* Michigan on 'Athena' was also significant ($P < 0.05$) from the controls, reducing the population by 1.91 log CFU/g. 300 µl AITC significantly reduced ($P < 0.05$) *L. monocytogenes* (3.07 log CFU/g) on the rind of 'Athena' (Figure 4). Simultaneously, on 'HBJ' 300 µl AITC significantly reduced ($P < 0.05$) *S. enterica* Michigan (1.67 log CFU/g) and 100 µl AITC decreased the population of *S. enterica* Michigan by 0.55 log CFU/g, which was significantly less than the control, but no more than the NaClO treatment. However, 100 µl AITC caused the greatest reduction of *L. monocytogenes* (1.36 log CFU/g) on 'HBJ.' The 300 µl AITC treatment only reduced the population of *L. monocytogenes* by 0.11 log CFU/g and was not significant ($P < 0.05$) from the untreated control nor the 200 ppm NaClO treatment on 'HBJ.' Furthermore, AITC vapor reduced the population of both foodborne pathogens on the surface of 'Athena' greater than compared to 'HBJ' for 3 of the 4 vapor concentrations at 25°C for 24 h. The reductions of *S. enterica* Michigan on 'Athena' were significantly greater (up to 2.25 log CFU/g) ($P < 0.05$) than

reductions on ‘HBJ’ for all treatments. Also, *L. monocytogenes* was significantly reduced ($P < 0.05$) (approximately 3 log CFU/g) by 300 µl AITC on ‘Athena’ compared to ‘HBJ.’

DISCUSSION

Strength analysis was performed on cantaloupe fruits to determine if storage for 24 h at 35°C would soften the surface more than a 24 h treatment at 25°C. The rinds of cantaloupes stored at 25°C at a lower relative humidity were softest after two weeks. This could be explained by the ideal relative humidity storage conditions for cantaloupes at 95 to 100% relative humidity to prevent the fruit from drying. Thus, it is possible that the drier air pulled moisture from the cantaloupes, which in turn led to the phellum (cork) cells being disrupted and altering the texture of the rind; therefore, making the rind softer. If cork cells are disrupted, it may allow easy access for pathogens to enter through the cantaloupe surface.

Although the AITC vapor treated ‘Athena’ required approximately 3.3 to 4.5 less force (kg) than the control melons after 14 d of storage, this was more than likely due to the quality of the individual cantaloupes. The day 0 (same day as harvested) ‘Athena’ required less initial force to penetrate the rind than controls required after 24 h of storage at their respective temperatures and relative humidity. This is attributed to the below average environmental conditions experienced in the growing season. The weather was cooler, there was more rain than normal and less sunshine, which led to an inferior cantaloupe crop in terms of yield and fruit quality. The first cycle of fruit set (crown fruit) was used for the controls for texture analysis, whereas the AITC treated ‘Athena’ were from later harvests. For that reason, a very low force (5.75 to 7.39 kg) was needed for the AITC treated ‘Athena’ on day 0 compared to the controls after 24 h storage (7.96 to 8.75 kg). When comparing the amount of force required after 24 h

storage, with and without AITC treatment, the forces between the control and treatments are similar.

Yun et al. (2013) treated cherry tomatoes inoculated with *S. enterica* Typhimurium with 10 µl AITC vapor in a 250 ml glass jar for 18 h at 22°C. It resulted in a reduction of *S. enterica* Typhimurium by 5.52 log CFU/g tissue (Yun et al., 2013). However, the use of AITC vapor induced discoloration, softening, and loss of vitamin C and lycopene during 21 d of storage at 10°C (Yun et al., 2013). The tomatoes and apples also became softer following 2 d of treatment with high levels of AITC (> 300 µl) in a study by Lin et al. (2000). These results are similar to the effect in softness of cantaloupes caused by AITC treatment and storage for 14 d at 4°C.

Effect of heat on vapor concentrations

Increased temperature corresponded to greater population reductions of *S. enterica* Michigan and *L. monocytogenes*. The only treatment in which this was not true was for the 300 µl AITC treatment at 35°C for 24 h against *S. enterica* Michigan. However, it still reduced the population of *S. enterica* Michigan by 3.07 log CFU/g. At the same time, the higher temperature led to higher concentrations of AITC in the headspace of the desiccators for all but one of the treatments (AITC vapor concentrations ranged from 3.4 to 19.6 µl/L of air). The vapor concentration of 100 µl AITC at 25°C was 17.0 µl/L, while its 35°C equivalent was 14.8 µl/L. This could be explained by the volatility of AITC and the variability of the dispersal of the liquid into the vapor phase, which can lead to significant error in the amount of vapor from liquid application on filter paper (Delaquis and Sholberg, 1997).

S. enterica Michigan was more susceptible to AITC than *L. monocytogenes* after all but the 24 h 35°C treatments. These results agree with Lin et al. (2000), who concluded that unlike other essential oils, Gram-negative bacteria (like *S. enterica* Michigan) are more sensitive than

Gram-positive bacteria (like *L. monocytogenes*) to inhibition by AITC. The lethal effects of gaseous AITC against *S. enterica* Michigan and *L. monocytogenes* correlated to the increase in temperature and concentration. This agrees with a previous study that found AITC vapor gradually increased its effectiveness towards *S. enterica* Typhimurium and *L. monocytogenes* as the range of temperatures and concentrations tested increased (Delaquis and Sholberg, 1997).

In addition, Delaquis and Sholberg (1997) observed the inactivation of *S. enterica* Typhimurium, *L. monocytogenes*, and *E. coli* after 48 h exposure to 1,500 µl/L at 40°C. Higher temperatures increase the volatility of AITC and in turn improve its diffusion into bacterial cells leading to bactericidal effects (Delaquis and Sholberg, 1997). Another report in the literature cited more than a 7 log reduction in population of *E. coli* O157:H7 at 37°C within 24 h using an agar disk assay exposed to 10 µl AITC (Park et al., 2000). In comparison the lethality of 10 µl AITC vapor was less at 20°C resulting in slightly greater than a 1 log reduction after 24 h (Park et al., 2000). Meanwhile, treatment with 10 µl of AITC for 5 h at 47°C caused a 6 log reduction of *E. coli* O157:H7 (Park et al., 2000).

Obaidat and Frank (2009a) determined that 4 µl AITC/L of air reduced the population of *E. coli* O157:H7 on lettuce at 0°C by 3.66 log CFU/leaf by the fourth day of treatment. Obaidat and Frank (2009b) also observed a reduction of *Salmonella* on whole tomatoes to the detection limit of < 2 log CFU/g at 4 and 10°C in 10 d and by 1.3 log CFU/g at 25°C in 10 h with 8.3 µl AITC/L of air. AITC vapor has been shown to reduce the population of *S. enterica* Montevideo on the surface of whole tomatoes by as much as 8 log CFU/g at 4°C after 2 d at a concentration of 132 µl/L of air (Lin et al., 2000). Also, in a study conducted by Obaidat and Frank (2009b), 33.3 µl AITC/L of air caused a 4.4 log CFU/g decrease of *E. coli* O157:H7 after storage of whole tomatoes at 10°C for 10 d. The high level of bacterial population reduction by these two studies

compared to this cantaloupe study is probably due to the surface topography difference of the fruit. The surface of tomatoes is much smoother and lacks the netting found on cantaloupe. In this study, 3 of the 4 high vapor concentrations (1 h at 35°C, 24 h at 25°C, and 24 h at 35°C) and the low vapor concentration at 35°C for 24 h reduced bacterial populations between 3.64 and 3.92 log CFU/g.

Comparison between vapor concentrations

The bactericidal activity of AITC was affected by the exposure time and concentration. AITC vapor caused a greater reduction of *S. enterica* Michigan than *L. monocytogenes* for 7 out of the 8 treatments, with 6 of them being statistically significant between the 2 pathogens. The higher vapor concentrations produced the greatest reduction (approximately 2 log CFU/g) for both *S. enterica* Michigan and *L. monocytogenes* after 24 h at 25°C. This is consistent with other studies in the literature that reported as the concentration increases so does the amount of reduction. Lin et al. (2000) previously detected correspondingly higher levels of bactericidal effects of AITC vapor against *S. enterica* Montevideo and *E. coli* O157:H7 on lettuce and tomatoes as the concentration increased from 0 to 400 µl. Additionally, reductions increased when exposure to AITC vapor was extended from 1 to 2 d (Lin et al., 2000).

Comparison between 'Athena' and 'Hales Best Jumbo'

AITC vapor was expected to reduce the microbial populations of *S. enterica* Michigan and *L. monocytogenes* greater on the surface of 'Athena' than 'HBJ' because of the difference in the amount and proximity of the netting on the 2 cultivars (Figures 5 and 6). The netting on 'Athena' covered approximately 52.3% of the total surface and 59.7% on 'HBJ,' respectively Results of these experiments revealed AITC vapor was able to reduce the population of both foodborne pathogens on the surface of 'Athena' greater than compared to 'HBJ' for 3 of the 4

vapor concentrations according to the 24 h 25°C results. 300 µl AITC produced nearly a 3 log greater reduction of *L. monocytogenes* and more than doubled the reduction of *S. enterica* Michigan on the surface of 'Athena' compared to on 'HBJ.' These results suggest that AITC vapor may not be able to reach bacteria attached within the crevices of thicker netting, like on the surface of 'HBJ,' as well as it can on a less netted exterior, like that of 'Athena.'

This research suggests that AITC in its gas phase can be an effective antimicrobial agent against *S. enterica* Michigan and *L. monocytogenes* on the surface of 2 cultivars of cantaloupe with varying degrees of netting. Increasing the temperature has a positive correlation on the amount of AITC that can enter into the headspace of the treatment chamber, which in turn increases the ability of the compound to reduce the overall population of pathogens present on the rind of cantaloupe.

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Tables

Table 1. Estimation of AITC vapor concentrations in the headspace of desiccators calculated from the standard at 25°C and 35°C for 1 and 24 h with 100 and 300 µl AITC liquid applied to filter paper (n=3).

	µl/L	
Treatment	**1 h**	**24 h**
100 µl AITC at 25°C	17.0	3.4
100 µl AITC at 35°C	14.8	10.3
300 µl AITC at 25°C	12.1	11.7
300 µl AITC at 35°C	19.6	19.4

Table 2. Rind hardness test (force (kg) applied to achieve penetration) on whole 'Athena' cantaloupe fruit after 24 h storage at 25°C & 47.5% RH, 25°C & 94.5% RH, and 35°C & 94.5% RH followed by storage at 4°C for 2 weeks (n=9).

	Force (kg) applied		
Day	**25°C & 47.5% RH**	**25°C & 94.5% RH**	**35°C & 94.5% RH**
1	8.12 ± 1.03	7.96 ± 1.19	8.75 ± 0.92
2	7.93 ± 1.25^{e}	7.73 ± 1.36^{d}	8.29 ± 1.08^{b}
3	7.58 ± 1.42^{de}	7.28 ± 1.51^{cd}	7.67 ± 0.97^{ab}
4	7.13 ± 1.48^{cde}	6.75 ± 1.25^{abcd}	7.46 ± 0.75^{ab}
5	7.11 ± 1.23^{cde}	6.92 ± 1.37^{bcd}	7.43 ± 1.19^{ab}
6	7.25 ± 1.31^{cde}	6.82 ± 1.00^{abcd}	7.08 ± 0.79^{ab}
7	6.67 ± 1.29^{abcd}	6.42 ± 0.98^{abcd}	7.12 ± 0.58^{ab}
8	6.83 ± 1.71^{bcde}	6.40 ± 0.83^{abc}	7.14 ± 0.51^{ab}
9	6.34 ± 0.77^{abc}	5.86 ± 1.83^{ab}	6.89 ± 0.58^{ab}
10	6.03 ± 1.53^{abc}	5.72 ± 1.30^{ab}	6.72 ± 0.44^{a}
11	5.48 ± 1.42^{a}	5.78 ± 1.30^{ab}	6.71 ± 1.03^{a}
12	5.80 ± 1.18^{ab}	5.63 ± 0.94^{ab}	6.83 ± 0.31^{a}
13	5.49 ± 1.67^{a}	5.55 ± 1.01^{a}	6.55 ± 0.45^{a}
14	5.50 ± 1.27^{a}	5.53 ± 0.96^{a}	6.69 ± 0.43^{a}

Mean values (mean ± S.D.) with the same letters within a column are not significantly different ($P < 0.05$) between days.

Table 3. Rind hardness test (force (kg) applied to achieve penetration) on whole 'Athena' cantaloupe fruit with AITC vapor treatments after 24 h storage at 25°C and 35°C with 95 ± 3% relative humidity followed by storage at 4°C for 2 weeks (n=9). Results vary dependent on the degree of ripeness and fruit size.

	Force (kg) applied			
	25°C		35°C	
Day	**100 µl AITC***	**300 µl AITC***	**100 µl AITC***	**300 µl AITC***
0	5.89 ± 0.67	5.75 ± 1.22	5.81 ± 0.83	7.39 ± 0.95
1	5.48 ± 0.96^{gD}	4.56 ± 0.50^{fC}	3.77 ± 0.36^{dB}	4.27 ± 0.23^{gA}
2	4.78 ± 0.81^{fgC}	4.14 ± 0.66^{efC}	3.32 ± 0.29^{cdB}	3.80 ± 0.30^{fgA}
3	4.52 ± 0.92^{efC}	3.89 ± 0.69^{defC}	3.06 ± 0.32^{bcdB}	3.52 ± 0.34^{efA}
4	4.45 ± 0.77^{efC}	3.86 ± 0.73^{deC}	2.82 ± 0.26^{abcB}	3.51 ± 0.21^{efA}
5	3.92 ± 0.95^{defC}	3.50 ± 0.85^{cdeC}	2.88 ± 0.20^{abcB}	3.15 ± 0.36^{cdeA}
6	3.87 ± 0.76^{cdeC}	3.25 ± 0.69^{bcdC}	2.73 ± 0.29^{abcB}	3.20 ± 0.30^{deA}
7	3.49 ± 0.87^{bcdC}	2.93 ± 0.65^{abcC}	2.54 ± 0.34^{abcB}	3.03 ± 0.16^{cdeA}
8	3.23 ± 0.70^{abcdC}	2.92 ± 0.73^{abcBC}	2.50 ± 0.18^{abB}	2.90 ± 0.34^{bcdA}
9	3.22 ± 0.68^{abcdC}	2.65 ± 0.56^{abBC}	2.51 ± 0.25^{abB}	2.79 ± 0.40^{abcdA}
10	3.03 ± 0.50^{abcC}	2.64 ± 0.56^{abBC}	2.38 ± 0.23^{abB}	2.70 ± 0.26^{abcdA}
11	2.84 ± 0.66^{abB}	2.41 ± 0.60^{aB}	2.28 ± 0.08^{abB}	2.64 ± 0.21^{abcA}
12	2.82 ± 0.57^{abB}	2.46 ± 0.70^{aB}	2.19 ± 0.16^{aB}	2.45 ± 0.36^{abA}
13	2.58 ± 0.64^{aB}	2.30 ± 0.45^{aB}	2.17 ± 0.11^{aB}	2.39 ± 0.20^{abA}
14	2.58 ± 0.68^{aB}	2.33 ± 0.49^{aB}	2.23 ± 0.36^{aB}	2.35 ± 0.24^{aA}

*****Quantity of AITC applied to filter paper for vapor treatment. See Table 3 for equivalent µl/L concentration for each temperature/time combination.

Mean values (mean ± S.D.) with the same letters within a column are not significantly different ($P < 0.05$) between days and the same upper case letters within a row are not significantly different ($P < 0.05$) between treatments.

Figures

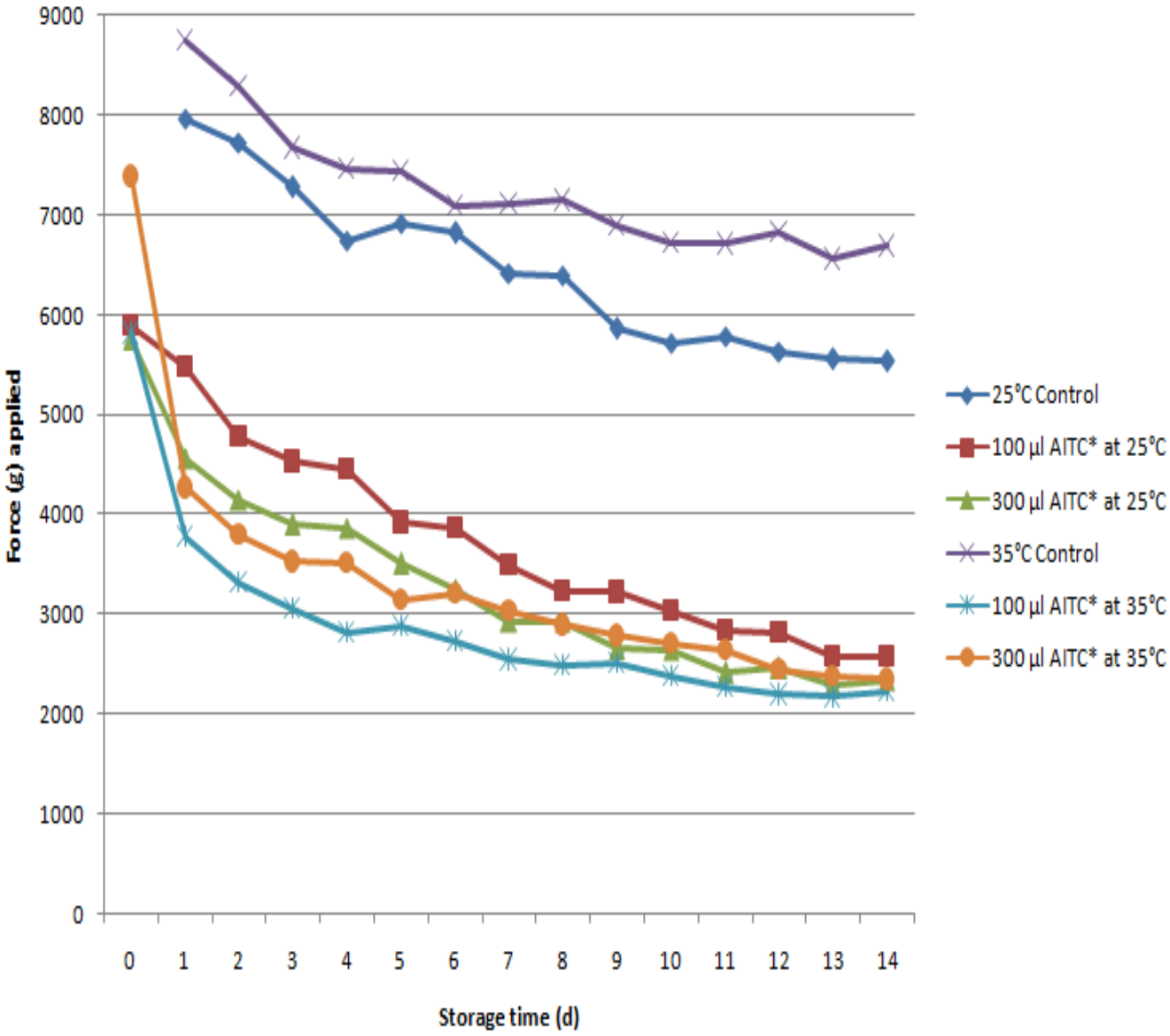

Figure 1. Rind hardness test (force (g) applied to achieve penetration) on whole 'Athena' cantaloupe fruit with AITC vapor treatments and controls (no treatment) after 24 h storage at 25°C and 35°C with 95 ± 3% relative humidity followed by storage at 4°C for 2 weeks (n=9). Results vary dependent on the degree of ripeness and fruit size.[1]

*****Quantity of AITC applied to filter paper for vapor treatment. See Table 3 for equivalent µl /L concentration for each temperature/time combination.

[1] No data was collected for the 25°C and 35°C Controls on Day 0.

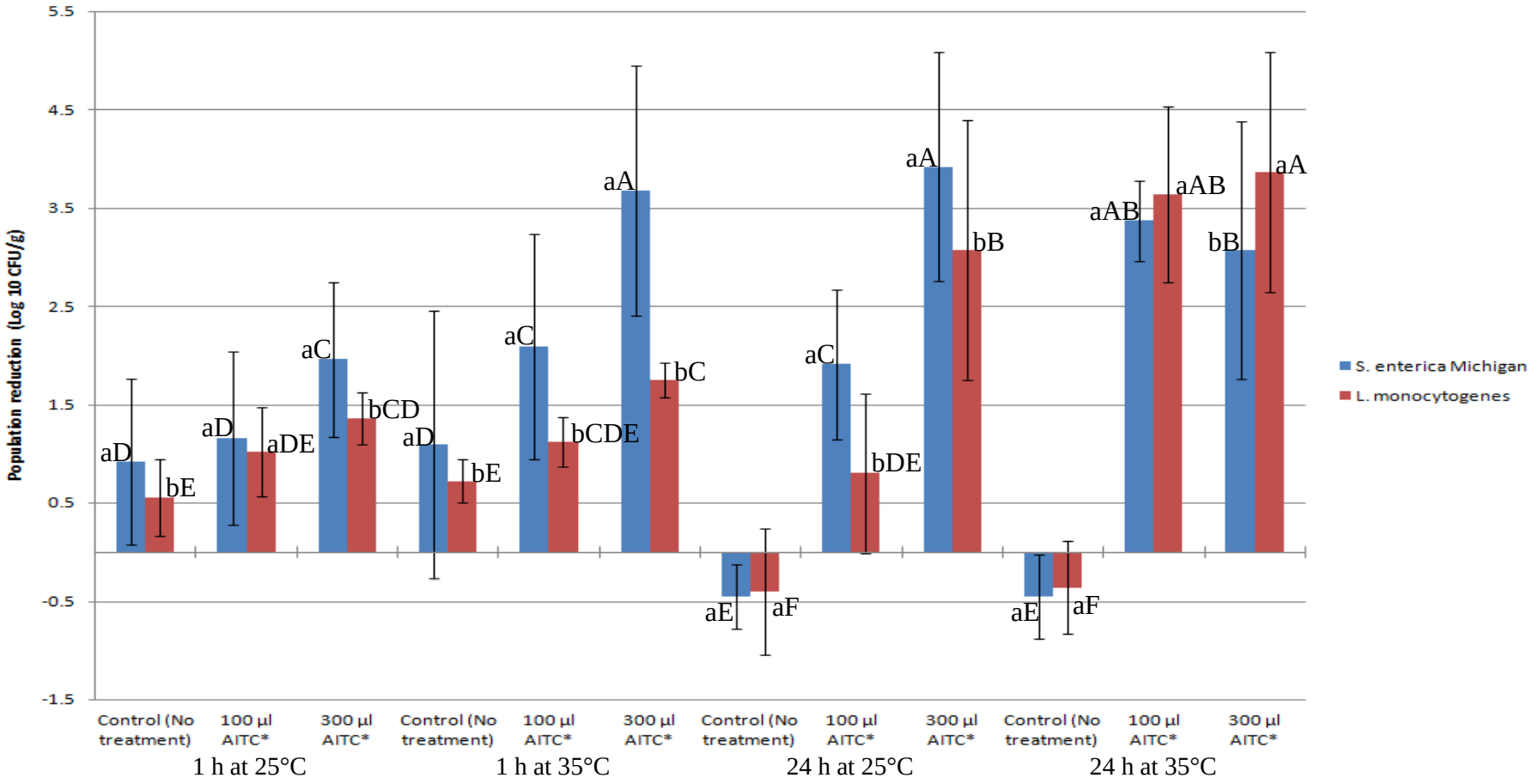

Figure 2. Population reduction (Log 10 CFU/g) of *S. enterica* Michigan and *L. monocytogenes* on 'Athena' surface after 1 or 24 h AITC vapor treatments at 25°C or 35°C. The bars represent the standard deviation of the mean of nine replications. Means with the same lower case letters for a treatment are not significantly different ($P < 0.05$) between pathogens and those with the same upper case letters are not significantly different ($P < 0.05$) between treatments per pathogen. *****Quantity of AITC applied to filter paper for vapor treatment. See Table 1 for equivalent concentration of µl AITC/L of air for each temperature/time combination.

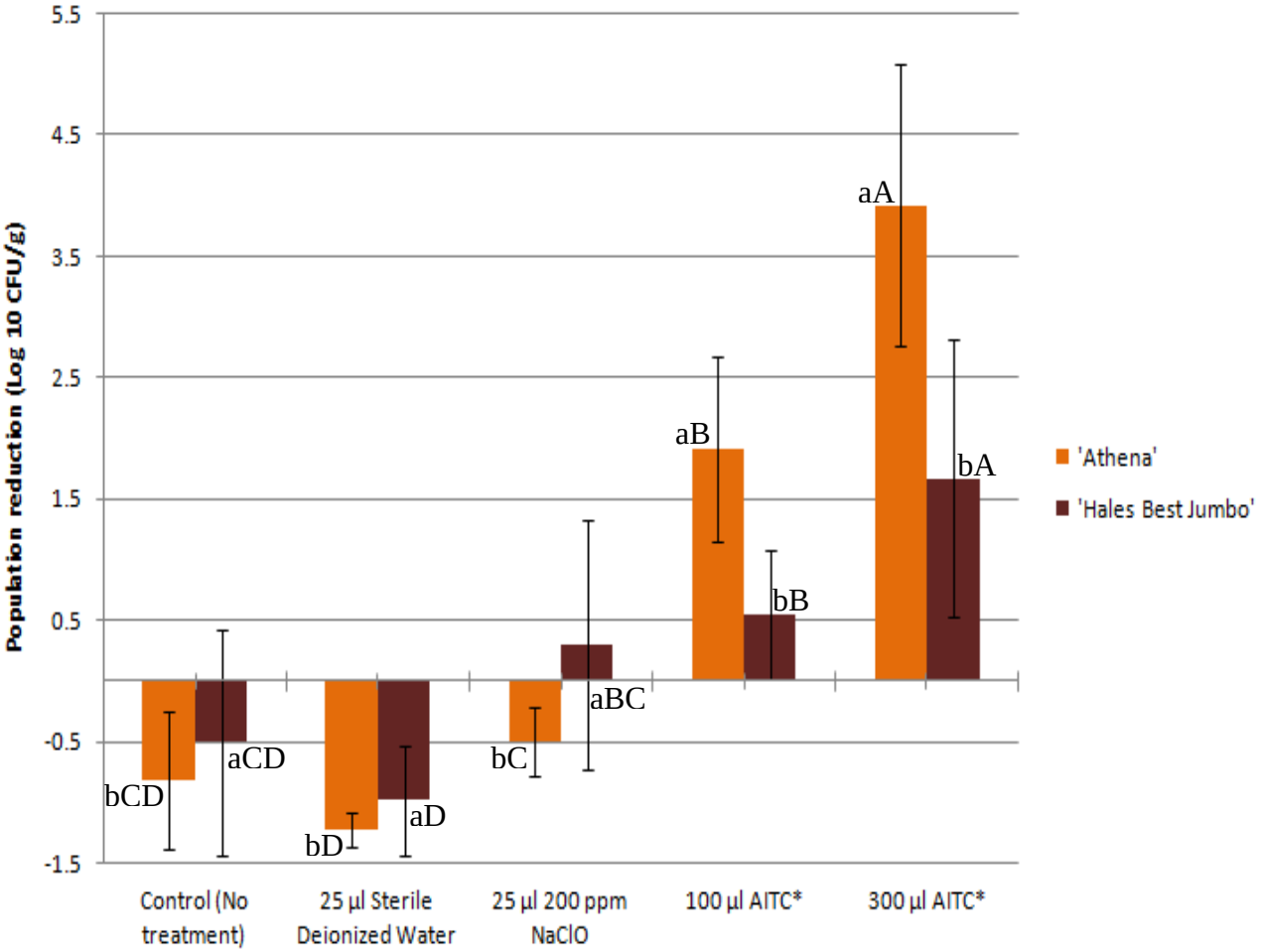

Figure 3. Population reduction (Log CFU/g) of *S. enterica* Michigan on the surface of 'Athena' and 'Hales Best Jumbo' after 24 h treatments at 25°C. The bars represent the standard deviation of the mean of nine replications. Means with the same lower case letters for a treatment are not significantly different ($P < 0.05$) between cultivars and those with the same upper case letters are not significantly different ($P < 0.05$) between treatments per cultivar. *****Quantity of AITC applied to filter paper for vapor treatment. See Table 1 for equivalent concentration of µl AITC/L of air for each temperature/time combination.

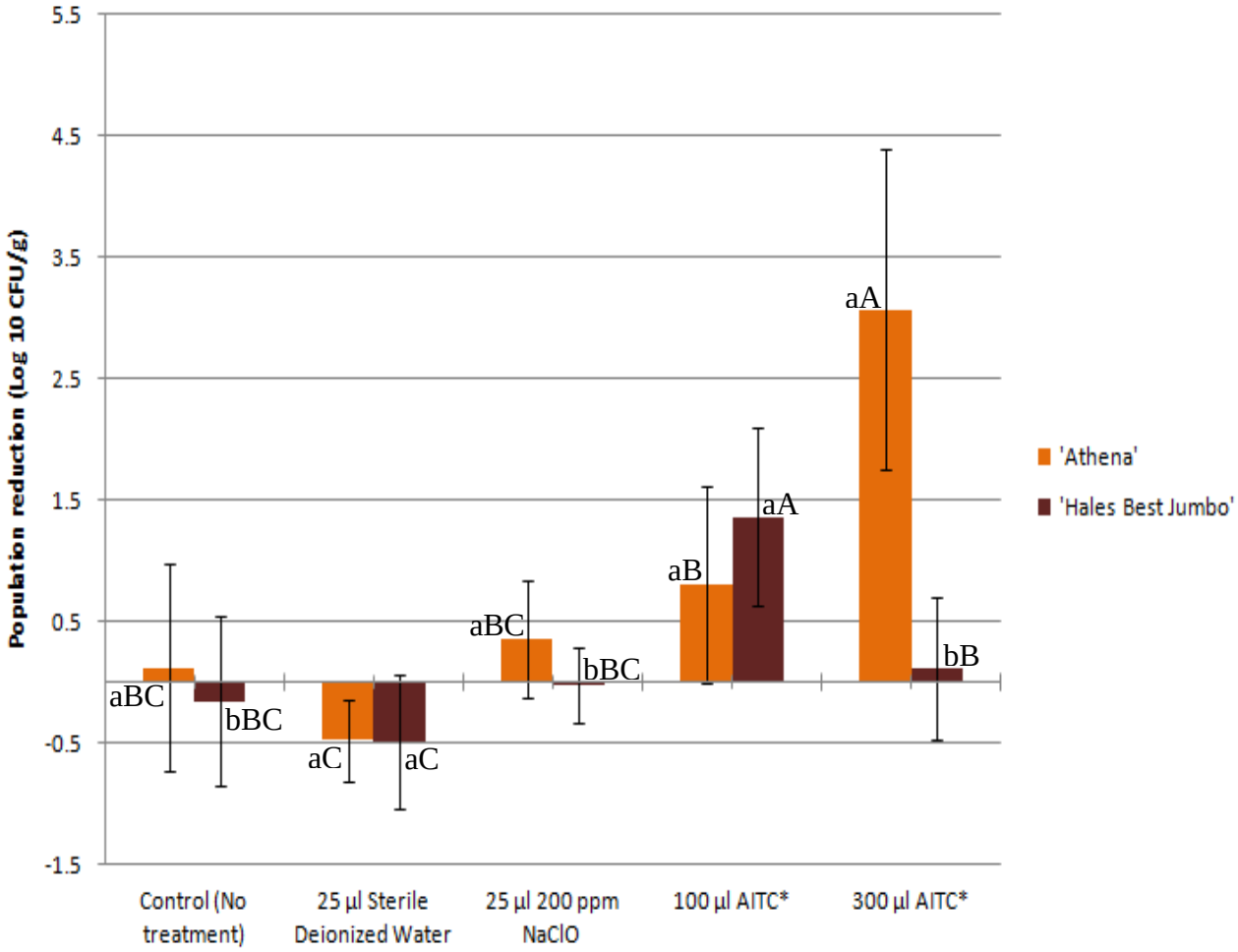

Figure 4. Population reduction (Log CFU/g) of *L. monocytogenes* on the surface of 'Athena' and 'Hales Best Jumbo' after 24 h treatments at 25°C. The bars represent the standard deviation of the mean of nine replications. Means with the same lower case letters for a treatment are not significantly different ($P < 0.05$) between cultivars and those with the same upper case letters are not significantly different ($P < 0.05$) between treatments per cultivar. *****Quantity of AITC applied to filter paper for vapor treatment. See Table 1 for equivalent concentration of µl AITC/L of air for each temperature/time combination.

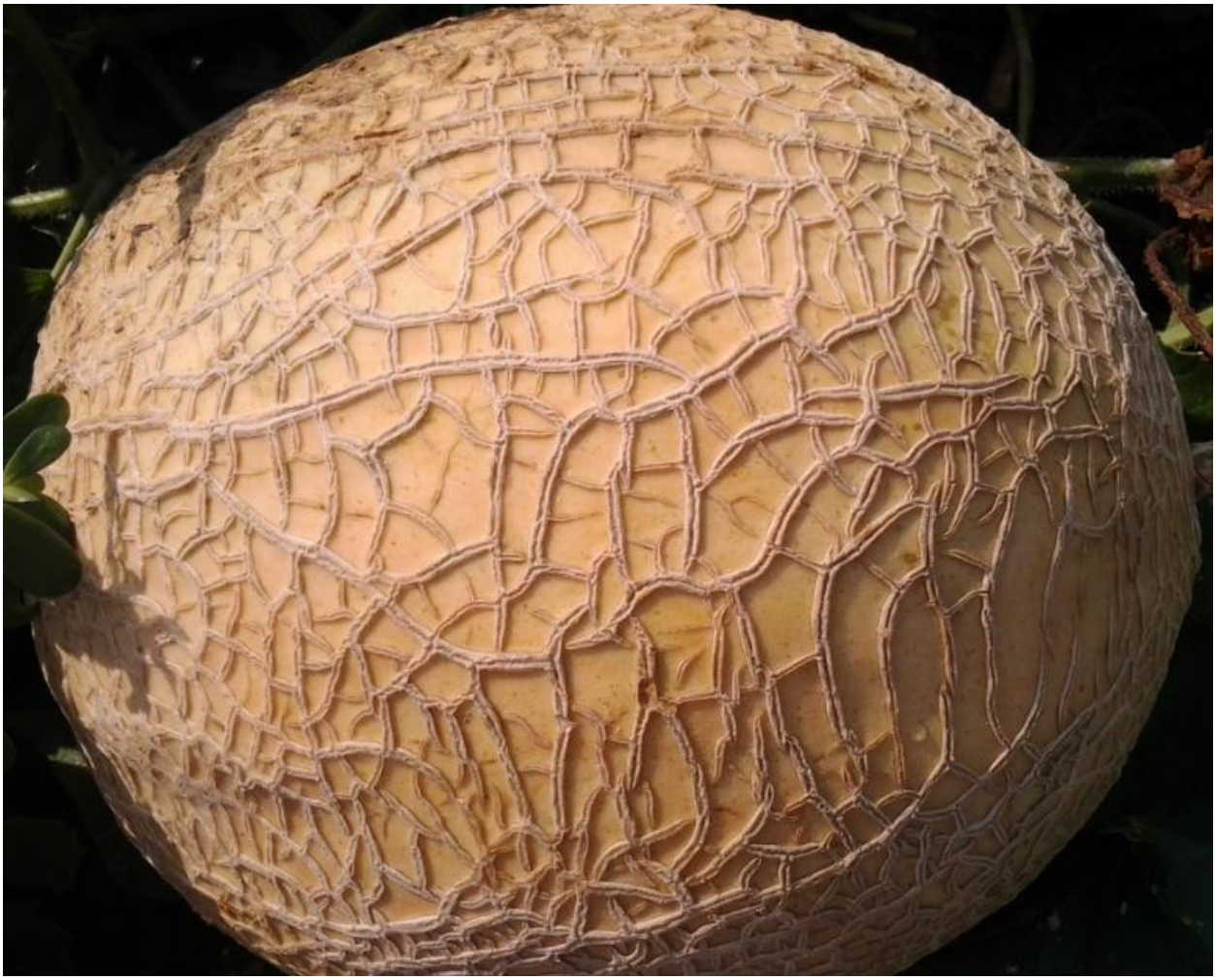

Figure 5. ***Cucumis melo L.*** **'Athena.'**

Figure 6. *Cucumis melo* L. 'Hales Best Jumbo.'

CHAPTER 5

Conclusions and Future Directions

The purpose of this research was to better understand the effects of allyl isothiocyanate (AITC) in liquid and vapor phase as an antimicrobial agent against foodborne pathogens on the surface of whole cantaloupes with varying amounts of netting. More specifically, how the amount of netting on the surface of cantaloupes affects the ability of the liquid and gas phases to destroy the pathogens present on the rind and if the time and temperature of vapor treatments has an effect on the reduction of the bacteria. In response to the 3 *Salmonella enterica* and 1 *Listeria monocytogenes* associated cantaloupe outbreaks in the past 6 years, this research was conducted to provide more information on the use of AITC as a post-harvest treatment for eastern cultivars, such as Athena, that are washed in water containing 200 ppm chlorine, sorted, and packed in packinghouses.

This study revealed that in general, AITC vapor treatments on 'Athena' for a longer exposure time (24 h versus 1 h) resulted in greater reduction of both *S. enterica* Michigan and *L. monocytogenes*, more than double the decrease in pathogen populations compared to 1 h treatments. Also, treatments at 35°C reduced populations of *S. enterica* Michigan and *L. monocytogenes* during AITC vapor treatments greater than reductions at 25°C. AITC gas treatments should be tested on a smooth surface melon, such as honeydew or a smooth cantaloupe cultivar, like Delicious 51, since AITC vapor resulted in a greater reduction of *S. enterica* Michigan and *L. monocytogenes* on the surface of a slightly sutured cultivar with coarse netting (Athena) than it did on a cultivar (Hales Best Jumbo (HBJ)) containing more netting. Although AITC has a strong mustard flavor that can be absorbed into foods, cantaloupes could be an acceptable product to apply a vapor treatment of AITC to compared to other fresh produce

commodities, because they contain an outer rind which is not consumed, unlike apples or pears. Thus, the rind of cantaloupe can prevent any pungent flavors of AITC from entering the edible tissue of the melon.

When comparing the results of the vapor and liquid treatments under the same experimental conditions (24 h exposure at 25°C), three out of four pathogen/cantaloupe cultivar combinations received the greatest reduction from the high concentration of AITC vapor. Meanwhile, liquid treatments of 5 µl per 22 mm diameter circle were more effective than the low concentration of AITC vapor. However, this was not true for *L. monocytogenes* on the surface of 'HBJ,' in which case the liquid treatment was the most effective. In conclusion, on 'Athena' 300 µl AITC for 24 h at 25°C reduced the population of *S. enterica* Michigan up to approximately 3.9 log CFU/g and 300 µl AITC for 24 h at 35°C decreased *L. monocytogenes* up to approximately 3.9 log CFU/g.

There are a number of opportunities for this research to be expanded upon in the future. Residual AITC on the surface after gas and liquid treatments should be studied, as well as the penetration of AITC vapor and liquid through the cantaloupe rind (depth of absorption through the edible tissue). Forced circulation of AITC vapor inside treatment chambers should also be tested to determine how increased circulation of AITC around cantaloupes effects reductions of microbial populations on the rind. Further studies could be done testing lower concentrations of AITC liquid for the same period of time (24 h) or 5 µl could be tested in shorter exposure/treatment periods. Lower concentrations of AITC could be tested to determine if they have the same antimicrobial effect, but without softening the rind. Additionally, cantaloupes could be rinsed after liquid AITC treatment and residual AITC could be measured. Furthermore, this research could also be expanded to include other cantaloupe associated foodborne

pathogens, like *Escherichia coli* O157:H7, *Campylobacter jejuni*, and other *Salmonella enterica* serovars.

Appendices

Appendix A. Images of 'Athena' after 24 h storage conditions.

24 h after 35°C storage **24 h after 4°C storage**

Figure A.1. Effect of 24 h storage temperature on 'Athena.'

Appendix B. Average percent of allyl isothiocyanate liquid evaporated from filter paper during vapor treatments.

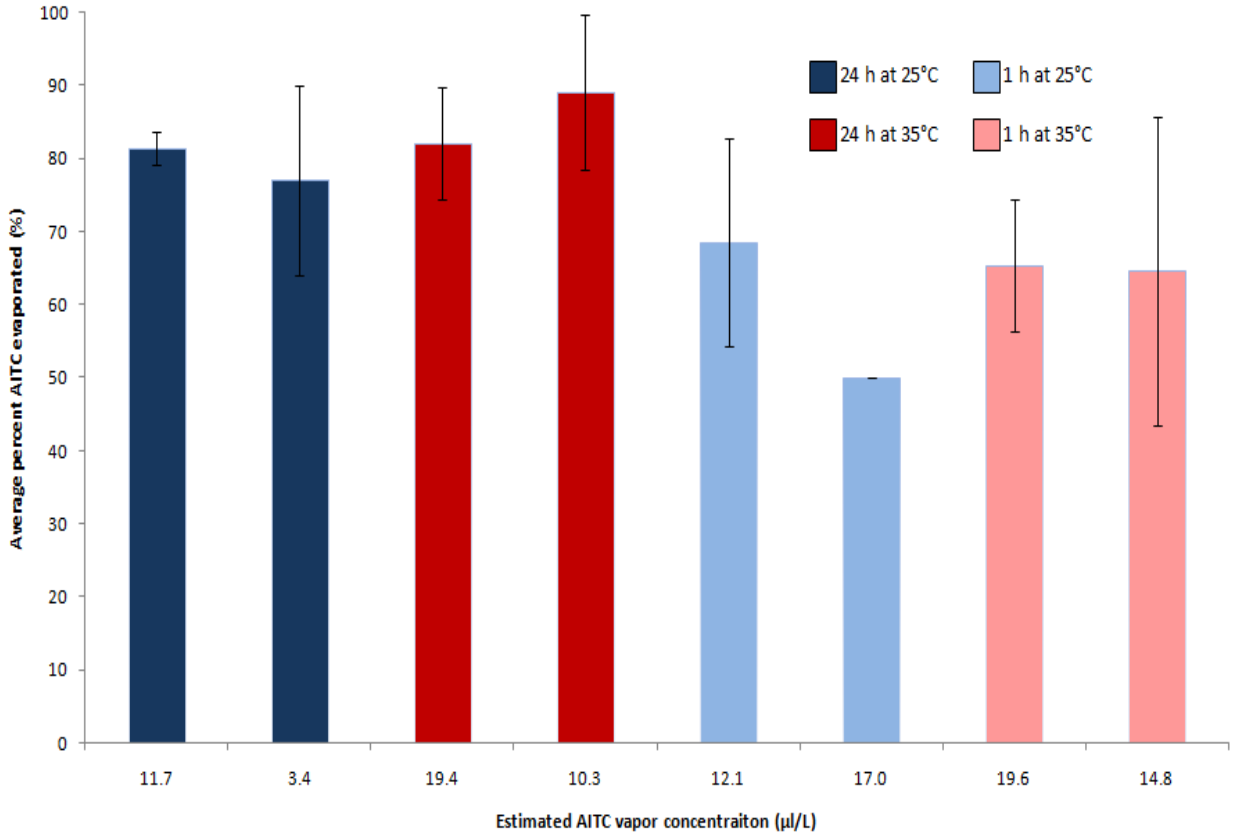

Figure B.1. Percent (mean ± S.D.) of AITC liquid evaporated from filter paper during vapor treatments in the absence of cantaloupe during 1 and 24 h storage at 25°C and 35°C (n=3).

Appendix C. Force (g) applied to achieve penetration for 'Athena' controls.

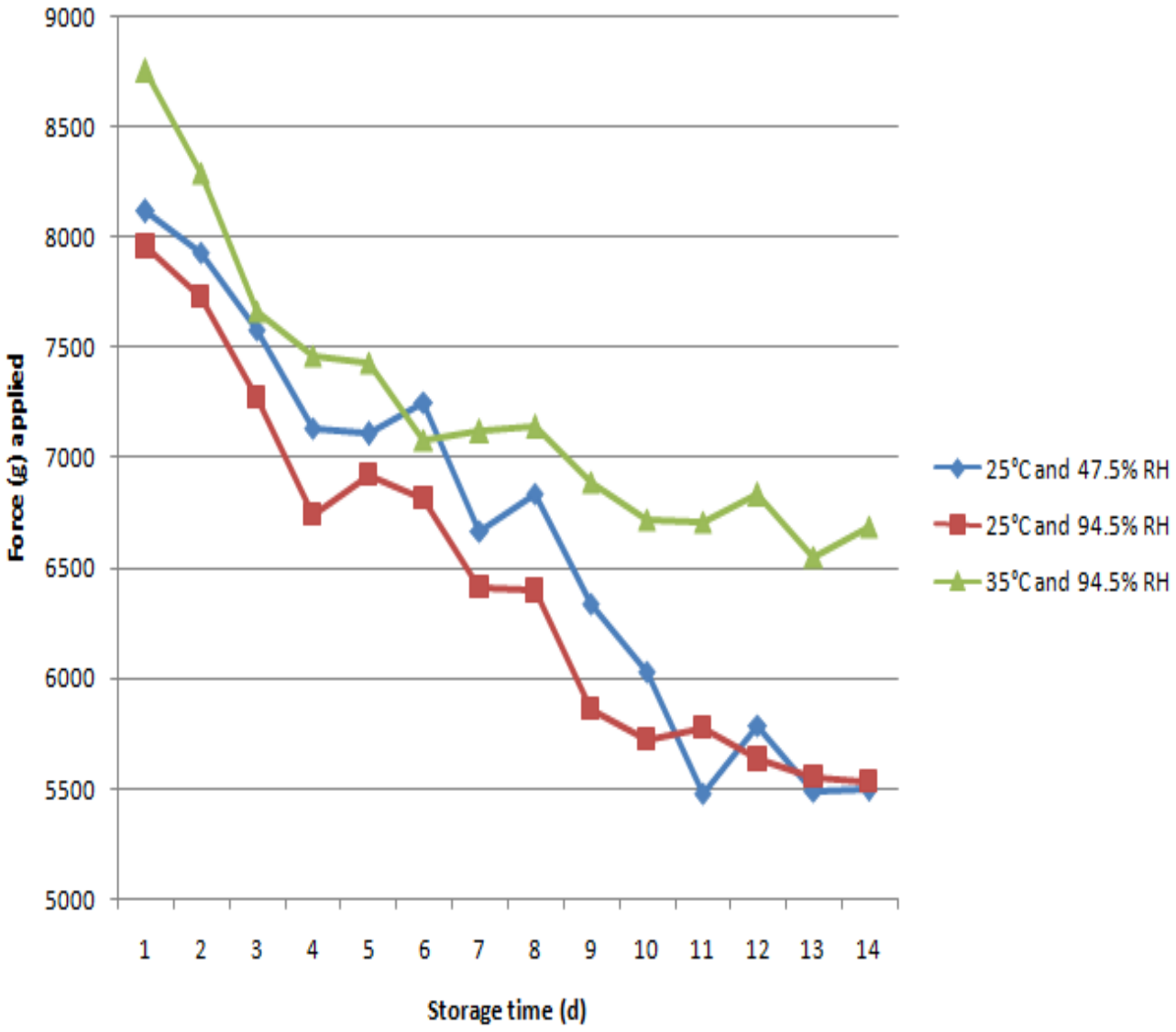

Figure C.1. Skin hardness test (force (g) applied) on whole 'Athena' cantaloupe fruit after 24 h storage at 25°C and 47.5% RH, 25°C and 94.5% RH, and 35°C and 94.5% RH followed by storage at 4°C for 2 weeks (n=9).