TRANSFER OF *LISTERIA MONOCYTOGENES* DURING CUTTING, SLICING, DICING, AND SUBSEQUENT STORAGE OF CANTALOUPE AND HONEYDEW MELONS

By

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ABSTRACT

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The 2011 multistate listeriosis outbreak associated with whole cantaloupe has heightened concerns regarding commercial handling and preparation practices for melons. In response, two separate studies were conducted to: (1) assess the transfer of *Listeria monocytogenes* during cutting, slicing, dicing in melons and (2) measure growth during subsequent storage of cantaloupes. Initially, cantaloupe and honeydew melon rinds containing *L. monocytogenes* populations (J22F, J29H, and M3) of 6.1 and 4.4 log CFU/cm^2, stored at 4 and 30^{o}C were cut with a sterile cork borer through the blossom scar, stem scar, and circumference regions of the rind to evaluate the *L. monocytogenes* transfer to the edible melon flesh. *L. monocytogenes* transfer ranged from 1.2 to 4.0 and 0.2 to 1.8 log CFU/cm^2 for cantaloupe and honeydew. Overall, no significant difference in *L. monocytogenes* transfer was seen at different depths or product temperatures ($P > 0.05$). *L. monocytogenes* transfer from one inoculated melon to subsequent uninoculated melons was evaluated after mechanical slicing (Vollrath Redco 401N) of the intact fruit and dicing (Nemco 55650 dicer) of the flesh. Diced cantaloupe samples were stored (4, 7, and 10^{o}C for seven days) and microbiologically analyzed for growth and survival of *L. monocytogenes*. During slicing, *L. monocytogenes* was transferred to uninoculated cantaloupe and honeydew melons were statistically similar ($P > 0.05$), ranging from 0.1 to 1.5 and 0.1 to 0.5 log CFU/g, respectively. During the storage of diced cantaloupes, *L. monocytogenes* generation time was 0.74 days for 10^{o}C, with minimal growth observed at 4 and 7^{o}C.

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Chapter 1: Introduction

Recently, people have been consuming more fresh produce in their diet due to its year round availability and health benefits (Pollack, 2001). This increased consumption of raw fruits and vegetables is not without risk. Due to common preharvest and postharvest production practices, fresh fruits and vegetables carry an inherent risk for pathogen contamination. Fresh produce contributed to approximately 46% of all foodborne outbreaks between 1998 to 2008 (Scallan et al., 2011). Melons, especially cantaloupes, have been a source of foodborne outbreaks due to their netted surfaces that have an increased risk of becoming contaminated from the environment and challenges in washing such surfaces during handling and processing.

Since 1994, there has been a drastic increase in the number of cantaloupe outbreaks associated with *Salmonella* contamination (Walsh et al., 2013). Cantaloupe surfaces are susceptible to microbial contamination due to their netted porous rind which allows for greater attachment of pathogens (Fan et al., 2006). Historically, *Listeria monocytogenes* has not been linked to cantaloupe outbreaks until 2011 when the "Rocky Ford" outbreak was documented in Colorado. This multistate outbreak caused 147 illnesses leading to 33 fatalities and 1 miscarriage (McCollum et al., 2013). The FDA concluded that contamination most likely occurred during the cooling step allowing for the growth and survival of *L. monocytogenes* on cantaloupes (FDA, 2012). The infectious dose of *L. monocytogenes* in healthy individuals has been estimated to be 10^7 CFU/g of food (Farber et al., 1996). In this outbreak, *L. monocytogenes* was most likely transferred from the rind to the edible flesh during postharvest cutting, slicing, dicing, or peeling.

These cantaloupe outbreaks have negatively affected the melon industry. Cantaloupe prices dropped 33.6% due to the adverse national media exposure in 2011 (Bottemiller, 2011). The outbreak from the "Rocky Ford" region of Colorado impacted the entire melon market even

though it only comprised 2% of the cantaloupe market. Overall, this outbreak heightened fears from consumers regarding the safety of fresh produce, questions were raised concerning how cantaloupes were processed and handled during preharvest conditions. These cantaloupe outbreaks have led to the development and implementation of melon processing guidelines to prevent microbial contamination of melon surfaces (FDA, 2005).

Cantaloupe and honeydew melons can be contaminated at any stage during preharvest and postharvest processing. During processing (e.g. cutting, slicing, and dicing), pathogens can be translocated from the rind inward to the edible flesh. In the food industry, there are no standardized practices for the cutting, slicing, and dicing of melons as companies use either manual or automated processes (Wang and Ryser, 2014). Problems can occur when contaminated produce is sliced or diced, transferring pathogens to subsequent slices or dices from the potentially contaminated blade or pusher of the equipment. Previous research has assessed the transfer of pathogens during slicing of delicatessen meats, tomatoes, and onions (Scollon et al., 2013; Vorst et al., 2006; Wang and Ryser, 2013). Additional studies have quantified the transfer of *L. monocytogenes* during mechanical dicing of celery and determined the effects of temperature on the growth of pathogens during storage (Kaminski et al., 2014). To my knowledge, no research has been conducted on the transfer of *L. monocytogenes* from contaminated to uncontaminated melons during mechanical slicing and dicing and subsequent growth in the melon flesh at normally encountered storage temperatures. Also, the bacterial transfer from the rind to the edible flesh on different rind regions and product temperatures was evaluated. Due to the recent multistate outbreak with *L. monocytogenes* in cantaloupe, the lack of research currently available on the transfer of *L. monocytogenes* in mechanical processing, handling and storage of cantaloupe, the impact of safety concerns to the produce industry and

public health agencies, and the increasing consumption of fresh produce in the public, there is a great need for this research.

Overall, knowledge gained from these studies will aid in the development and application of strategies to minimize the transfer of pathogens during the slicing, dicing, storage, and transportation of fresh-cut produce. Furthermore, the information gained from these experiments will help industry target interventions strategies that will aid in reducing pathogen cross-contamination during commercial processing, handling, and transportation of fresh-cut produce.

The objectives of this study were:

1. To assess the transfer of *L. monocytogenes* during cutting of cantaloupe and honeydew melon, impacted by three different regions of the rind (i.e., blossom scar, stem scar, and circumference of the rind) and product temperature.
2. To quantify *L. monocytogenes* transfer from one inoculated cantaloupe or honeydew melon to subsequent uninoculated melons during mechanical slicing and dicing.
3. To assess the growth of pathogens under commonly used storage conditions for diced cantaloupe.

Chapter 2: Literature Review

2.1 Foodborne Pathogens

Foodborne pathogens pose a hazard to the public as millions worldwide are affected by gastrointestinal diseases yearly resulting in a loss of work productivity and increased medical expenses. Also, foodborne pathogens are a greater risk to the youth, pregnant women, elderly, and immunocompromised individuals. Foodborne illnesses are still poorly understood and even its long term effects are relatively unknown even with its regularity in the population. The occurrence of foodborne illness annually in the U.S.A. is approximately 46 million incidents resulting in 250,000 hospitalizations and 3,000 deaths (Scallan et al., 2011). Recently, new foodborne pathogens have emerged from animal reservoirs that have unintentionally affected humans as the pathogen was identified as the source of the outbreak. Furthermore, genetic evolution in microorganisms along with changes in agricultural and manufacturing practices have led to the emergence of new foodborne pathogens. The emergence of new public health threats, complications in new global supply chain systems, and large multi-state foodborne outbreaks have led to the development of public health surveillance programs like FoodNet, PulseNet, National Antimicrobial Resistance Monitoring System (NARMS), and National Outbreak Reporting System (NORS) which track human infections caused by specific pathogens.

2.2 *Listeria monocytogenes*

Listeria monocytogenes is a rod shaped, gram positive facultative anaerobic bacterium which grows between -1.5 to 45^{o}C (Petran and Zottola, 1989). The typical size of *L. monocytogenes* is approximately 0.4 µm by 1 to 1.5 µm in length. *L. monocytogenes* is a unique pathogen which is widely found in nature due to its ability to survive at refrigeration temperatures and grow in high salt environments. Like many other foodborne pathogens, *L. monocytogenes* has a flagella which increases its infectivity during gastrointestinal cell invasion

in humans (Bigot et al., 2005). The regulation of the flagella is temperature-dependent as it is fully functional at temperatures between 20 to 25^{o}C, but at 37^{o}C, the motility genes are down regulated leading to decreased flagellin production inhibiting its movement (Peel et al., 1988). The genus *Listeria* consists of ten different species consisting of *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. fleischmannii*, *L. marthii*, *L. rocourtiae*, and *L. weihenstephanensis*. (den Bakker et al., 2013; Volokhov et al., 2002). The newly identified species of *Listeria* (*L. rocourtiae*, *L. marthii*, *L. weihenstephanensis*, and *L. fleischmannii*) are typically isolated from environmental samples at low occurrences. *L. monocytogenes* is virulent in humans and animals while *L. ivanovii* and *L. seeligeri* are only virulent in animals.

2.3 Foodborne Listeriosis

Foodborne illnesses have become a significant health concern leading to approximately 48 million illnesses and 3,000 deaths annually in the U.S.A. (CDC, 2011). Even if *L. monocytogenes* infection occurs in low frequency compared to other pathogens, it is widely found in food processing facilities and has the ability to survive in refrigerated temperatures making it difficult to control and making it one of the most hazardous concerns to our food supply. Annually in the United States, *L. monocytogenes* is responsible for approximately 2,500 listeriosis cases leading to a hospitalization rate of 91% and fatality rate of 20% (Mead et al., 1999). *L. monocytogenes* has been the causative agent in several foodborne outbreaks including the deadliest outbreak linked to cheese in 1985 (Linnan et al., 1988). Furthermore, several food products like poultry, meat, produce, and dairy products have been vehicles of transmission during *L. monocytogenes* outbreaks.

Human listeriosis is caused by the ingestion of foods containing *L. monocytogenes* with higher infection rates in immunocompromised individuals. Typically, human listeriosis is rare but if acquired, the fatality rate is approximately 20% (Low and Donachie, 1997). The infectious dose for *L. monocytogenes* varies widely depending on the immune status, the amount of contaminated food consumed and virulence of the particular *L. monocytogenes* strain (Vasquez-Boland et al., 2001). Contamination levels greater than 10^7 *L. monocytogenes* cells per gram of food are most often associated with listeriosis (Farber et al., 1996). These scientific findings prompted the European Food Safety Authority to establish a maximum threshold of 100 CFU/g for foods in which *L. monocytogenes* is unable to grow (EFSA, 2013). Overall, the safety limit established by this food regulatory agency holds food processors accountable to implement and establish good manufacturing practices (GMP) to prevent cross-contamination of *L. monocytogenes* during food preparation and handling.

Recently, consumption of produce has resulted in the most illnesses among food products with 25,222 cases and second highest number of outbreaks with (696 cases) from 2001 to 2010 (DeWaal and Glassman, 2013). Fresh fruits and vegetables can become contaminated at any stage during preharvest or postharvest. Furthermore, the increased availability of fruits and vegetables in the market and higher consumption rates of minimally processed produce in the population has led to increased occurrence of foodborne outbreaks (Buck et al., 2003). *Listeria spp.* has been detected and isolated from a wide range of products including bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushrooms, potatoes, radish, salad vegetables, and tomatoes (Buck et al., 2003). The increased number of outbreaks associated with fresh fruits and vegetables has heightened concerns about the safety of fresh produce resulting in the

development of food safety strategies to prevent contamination throughout the entire farm-to-fork continuum.

2.4 Human Listeriosis & Treatment

Listeria infections are primarily associated with immunocompromised and pregnant individuals who acquire it through the consumption of contaminated food. Immunocompromised and elderly individuals have a greater likelihood of acquiring *Listeria* infections as their immune systems have a larger proportion of helper T-cells that are unable to protect against foodborne pathogens (Swaminathan and Gerner-Smidt, 2007). Pregnant women who are infected with *Listeria* can transmit the disease to their fetus through the bloodstream causing fetal distress which could be fatal (Swaminathan and Gerner-Smidt, 2007). The FAO/WHO *Listeria* Risk Assessment Group that determined the elderly and fetuses were 2.6 and 14 times more likely to acquire invasive listeriosis compared to healthy individuals, respectively (Buchanan et al., 2004). Overall, invasive listeriosis has a greater likelihood of infecting individuals with predisposed risk factors.

The severity of foodborne illnesses primarily depends on the contamination level, the amount of food consumed and the health of the individual. Listeriosis can be a self-limiting diarrheal illness for which no antibiotic treatment is necessary, but individuals who contract invasive listeriosis are treated with ampicillin, penicillin, and amoxicillin (Temple and Nahata, 2000). Recently, *L. monocytogenes* has become resistant to antimicrobials like penicillin and tetracycline indicating that future resistance could occur to commonly administered treatments used for *Listeria* infections (Walsh et al., 2001). Prazak tested *Listeria* strains from water and environmental samples for antibiotic resistance and determined that approximately 95% of the

samples were resistant to two or more antibiotics (Prazak et al., 2002). These findings suggest that *Listeria* is acquiring antibiotic resistance reducing the effectiveness of current treatments.

2.5 Fresh-cut Produce Contamination Issues

Fresh produce is grown in non-sterile environments allowing pathogens to be present on the produce and is ultimately being identified as the source of foodborne outbreaks. *L. monocytogenes* has been recovered from minimally processed fruits and vegetables and survive and even grow under refrigeration conditions. The microbiological contamination of fresh-cut produce begins at food-processing facilities with increased efforts having been made to prevent *L. monocytogenes* contamination before it occurs due to the implementation of the Food Safety Modernization Act of 2010 (FDA, 2010).

Fresh-cut fruits and vegetables can be contaminated by pathogens during processing, packaging, or storage (Nguyen-the and Carlin, 1994). The growth of pathogens on fresh-cut produce depends on the level of microbial contamination during processing, the properties of the pathogen and the fresh produce. Fresh produce is susceptible to contamination during handling from unhygienic workers or improperly cleaned equipment. For example, contamination from handlers primarily depends on handling time and nature of the produce. Processors need to focus on personal hygiene of workers as excessive handling of produce can lead to potential cross-contamination and transmission of pathogens (Costa, 2015). In 1991, an outbreak caused by *Salmonella* Poona infection on precut cantaloupes resulted in 400 illnesses suggesting improved hygienic practices need to be adopted to prevent contamination at this level (CDC, 1991). Fresh-cut processors need to require workers in contact with produce to wear gloves and use hand dips before entering processing facilities. Overall, the implementation of GMPs and sanitation programs are important to curb poor worker hygiene.

During processing, equipment surfaces can be contaminated by inaccessible sites that promote bacterial growth and attachment on the surface of fresh-cut produce. The slicing and cutting of fresh produce results in the accumulation of liquid losses from the produce allowing for growth of microorganisms and potential cross-contamination of pathogens on the equipment surface. These slicing, dicing, and cutting processes can potentially lead to a seven-fold increase in the population of pathogens in fresh-cut produce (Brackett, 1996). These findings demonstrate that significant contamination occurs during minimal processing of fresh produce.

The application of sanitizer treatments and washing of fresh-cut produce is an important step to reduce the microbial load. Thorough washing of fresh produce is a necessary preventative measure to reduce pathogens as there are not any thermal treatments for raw foods. Water is used in fresh-cut processing during washing, cooling, and rinsing. Previous studies have documented that chlorinated wash water used in washing leafy greens has contained 10^3 bacteria/ml while the product contained 10^6 CFU/g (Nguyen-the and Prunier, 1989). This finding has shown that contamination of produce can occur during washing and potentially resulting in the transfer of pathogens during this process. If wash water is not properly sanitized, it can be the source of contamination to any produce during processing. Furthermore, increasing organic material in the wash water reduces the efficacy of the sanitizers in the wash water indicating the importance of changing wash water to prevent potential contamination (Davidson and Ryser, 2014).

Recently, the effects of packaging and storage temperatures have been evaluated on fresh-cut fruits and vegetables. Fresh-cut produce is held at refrigerated temperatures to prevent the growth of pathogens during storage. Temperature is known to alter the respiration rate of produce in packages, changing the gaseous composition of the environment, and affecting the

growth rate of pathogens (Nguyen-the and Carlin, 1994). Storage of fresh-cut produce under refrigerated temperatures inhibits the growth of pathogens especially psychotropic organisms. Temperature abuse of fresh-cut produce leads to the proliferation of microorganisms as there is significant decrease in the lag and generation times (Gil and Selma, 2006).

2.6 Outbreaks Associated with Melons

Fresh-cut melons have been increasingly implicated in foodborne outbreaks. Cantaloupes and honeydew melons can be microbiologically contaminated during the preharvest, processing, distribution, and preparation stages. Since 1994, there has been a significant increase in foodborne outbreaks associated with cantaloupes primarily due to increased consumption of raw produce in diets and its year round availability (Bowen et al., 2006). Melon outbreaks have been predominantly linked to microbiological contamination from *Salmonella* species accounting for 56% of the outbreaks (Walsh et al., 2013).

Recently, studies have been conducted to evaluate *Salmonella* and *Listeria* contamination in cantaloupe production facilities in Mexico and United States. A survey of cantaloupes from eight cantaloupe farms and packaging sheds determined that 1.8% of the samples were positive for *Salmonella* (Castillo et al., 2004). In another survey for cantaloupe contamination in California, there were no melons contaminated with *Salmonella* in the fields from 1999 to 2001 (Suslow, 2004). During 2013, environmental samples were collected from production field sites in Arizona to detect for the presence of *L. monocytogenes* from air, soil, cantaloupe, water, and rhizosphere biomass (Kumar et al., 2015). An evaluation determined *S. enterica* and *L. monocytogenes* was primarily absent on cantaloupes and honeydew melons after pre-shipment from production sites in Arizona and California from 2011 to 2014 (Suslow et al., 2015). The FDA has imposed strict regulations on imported melons to the United States as these melons

have been linked to several foodborne outbreaks. The CDC determined that three consecutive multistate outbreaks linked to imported cantaloupes from Mexican farms resulted in 155 cases and two fatalities from 2000 to 2002 (CDC, 2002). The FDA concluded the source of those outbreaks was primarily due to unsanitary conditions in the packaging facilities in Mexico. The FDA conducted a survey comparing domestic and imported melons to assess the levels of surface contamination on the melons (FDA, 1999). Overall, higher levels of contamination were seen for imported as compared to domestic melons as pathogens were detected on 5.3% to 2.4%, respectively. These findings led to the implementation of the "Melon Safety Plan" which called for the use of chlorinated water to wash melons and any surfaces contacting melons during transportation.

Melons especially cantaloupes have been the source of foodborne outbreaks associated with *Salmonella* infections. Cantaloupes are inclined to surface contamination, because they grow on the ground allowing pathogens to attach to the rind potentially leading to the internalization and formation of biofilms (Annous et al., 2005; Fan et al., 2006). A study determining the prevalence of *Salmonella* in produce in South Texas found that only cantaloupes tested positive for *Salmonella* indicating its rind was more susceptible to surface contamination compared to other fruits and vegetables (Duffy et al., 2005). The first melon outbreak associated with *Salmonella* was recorded in 1955 when 17 cases were linked to the consumption of sliced watermelon from a local retailer (Gaylor et al., 1955). In 1990, a multistate outbreak associated with *Salmonella* Chester led to 245 cases including two deaths in 30 states (Ries et al., 1990). This outbreak was determined to be caused by the transfer of pathogens from the rind to the edible flesh during cutting as the melons were not washed prior to consumption. In 1991, another multistate outbreak caused by *Salmonella* Poona was linked to contaminated cantaloupes

from Texas. This outbreak led to 400 cases in 23 states and two provinces in Canada (CDC, 1991). These foodborne outbreaks associated with melons have been predominately caused by unsanitary washing of whole melons and temperature abuse to cut melons leading to the proliferation of pathogens in the edible flesh.

L. monocytogenes was not implicated in any melon-associated cases of illness until the 2011 "Rocky Ford" cantaloupe outbreak in Colorado. This outbreak resulted in 147 illnesses leading to 33 deaths and 1 miscarriage affecting consumers in 28 states (McCollum et al., 2013). The genomic characterization of this *L. monocytogenes* strain consisted of 1/2a and 1/2b serotype which shown unique traits associated with the 1/2a (Laksanalamai et al., 2012). The majority of listeriosis cases were observed in immunocompromised individuals above the age of 60 (CDC, 2011). Based on FDA investigations, possible factors contributing to this outbreak included improper facility design and packing facility equipment, and postharvest processing during the cooling step causing the proliferation and survival of *L. monocytogenes* in cantaloupes (FDA, 2012).

Identification of potential sources of contamination during melon processing can be helpful in future risk assessments to prevent the growth and survival of *L. monocytogenes*. Adherence to strict food safety regulations and learning from past outbreaks can help the food industry to prevent future outbreaks. Melon outbreaks occur more frequently during the summer months while imported melons are the source of melon contamination during December to April (Walsh et al., 2013). Multistate foodborne outbreaks have been more frequently linked with cantaloupes compared to honeydew melons as shown in Table 2.1 and 2.2 (Danyluk et al., 2014). Melon outbreaks have increased in recent years in both domestic and imported melons

suggesting increased food safety programs and implementation of strategies to prevent contamination during the entire farm-to-fork continuum.

Year	Location	Pathogen	Location of Consumption	Cases (Deaths)	Food Vehicle
1998	US (IA)	Norovirus	Restaurant	41(0)	Honeydew, strawberries*
2001	US (CO)	Norovirus	Restaurant	100(1)	Honeydew, pineapple*
2002	US (DC)	*Staphylococcus aureus*	Not reported	8(0)	Honeydew, cheese (pasteurized), potato (fried)*
2003	US (multistate)	*Salmonella* Newport	Grocery store, hospital, nursing home, restaurant	68(2)	Honeydew
2003	US (CO)	*Shigella sonnei*	Hotel restaurant	39(0)	Honeydew*
2007	US	*Salmonella* Litchfield	Private home, restaurant	11(0)	Honeydew*

*Denotes a suspected food vehicle which was epidemiologically linked

Table 2.1: Foodborne illness outbreaks associated with honeydew melons adapted from Danyluk et al. (2014).

Year	Location	Pathogen	Location of Consumption	Cases (Deaths)	Food Vehicle
1990	US (multistate)	*Salmonella* Chester	Restaurant salad bars	245(2)	Cantaloupe*
1991	US (IL, MI), and Canada	*Salmonella* Poona	Grocery stores, restaurants	400(0)	Cantaloupe*
1997	US (CA)	*Salmonella* Saphra	Private home, grocery store, restaurant	24(0)	Cantaloupe
1997	US (OR)	*E. coli* O157:H7	Restaurant	9(0)	Cantaloupe*
1998	Canada (ON)	*Salmonella* Oranienburg	Supermarket	22(0)	Cantaloupe*
2000	US (multistate)	*Salmonella* Poona	Nursing home, home care, private home, restaurant, school	47(0)	Cantaloupe*
2000	US (MN)	Norovirus	Workplace	33(0)	Cantaloupe, sandwich (turkey)*
2001	US (OR)	*Salmonella* spp.	Nursing home, home care, restaurant	2(0)	Cantaloupe*
2001	US (multistate)	*Salmonella* Poona	Private home	50(2)	Cantaloupe*
2001	US (MN)	Norovirus	Workplace	42(0)	Cantaloupe, pineapple*
2001	US (WA)	Not reported	Restaurant	4(0)	Cantaloupe, pineapple*
2002	US (multistate), Canada	*Salmonella* Poona	Nursing home, home care, private home	58(0)	Cantaloupe*
2004	US (Not reported)	*E. coli* O157:H7	Not reported	6(0)	Cantaloupe*
2005	US (UT)	*Salmonella* spp.	Private home	126(0)	Cantaloupe, chicken, corned beef*
2007	US (CA)	*Salmonella* Litchfield	Private home	11(0)	Cantaloupe*
2008	US (CO)	*Salmonella* Newport	Private home	5(0)	Cantaloupe, hamburger meat*
2008	US (multistate)	*Salmonella* Javiana	Not reported	10(0)	Cantaloupe
2008	US (multistate), Canada	*Salmonella* Litchfield	Hospital, private home	51(0)	Cantaloupe*
2008	US (CA)	Norovirus	Restaurant	23(0)	Cantaloupe*
2011	US (multistate)	*Salmonella* Panama	Private home	20(0)	Cantaloupe*
2011	US (multistate)	*L. monocytogenes*	Grocery retailer	147(33)	Cantaloupe
2012	US (multistate)	*Salmonella Typhimurium* and *Salmonella* Newport	Not reported: all source from same farm	261(3)	Cantaloupe

*Denotes a suspected food vehicle which was epidemiologically linked

Table 2.2: Foodborne illness outbreaks associated with cantaloupes adapted from Danyluk et al. (2014).

2.7 *Listeria* spp. in Produce

Listeriosis outbreaks are typically associated with dairy and meat products, but recently a few were triggered by the consumption of fresh produce like cantaloupe and celery (CDC, 2011; Gaul et al., 2013). *L. monocytogenes* has been detected and isolated in agricultural fields, soil, water, fruits and vegetables, suggesting that contamination can occur during preharvest. After 2008, all six *listeriosis* outbreaks have been associated with produce grown in the United States suggesting the possible source of contamination was the result of structural defects or inadequate cleaning and handling of produce (Jackson et al., 2015). The majority of the outbreaks associated with cantaloupes have been linked to *Salmonella* rather than *Listeria*. A few studies have investigated the properties of *Listeria* to determine why it is a less frequent cause of foodborne outbreaks. Ukuku demonstrated that the attachment ability of *Listeria* to the surface of whole cantaloupes was less compared to *Escherichia* and *Salmonella* (Ukuku and Fett, 2002). Furthermore, transfer studies from the rind to the edible melon flesh of cantaloupe indicated that lower populations of *Listeria* were transferred compared with *Salmonella* (Ukuku et al., 2012). *Listeria* outbreaks involving produce occur relatively sporadically, but minimal processing of produce increases the products susceptibility to potential surface contamination.

Recently, outbreaks associated with fresh-cut produce have been steadily increasing with increased availability of produce and consumption by consumers. Microbial contamination of produce can occur at any stage during the farm-to-fork continuum, and it is difficult to completely remove *Listeria* once contamination has occurred. *L. monocytogenes* is commonly associated with fruits and vegetables due to its contact with the soil which could potentially harbor any pathogenic microorganisms. Chemical disinfectants are only marginally effective in reducing microbial contamination on the surface of fresh produce (Rees et al., 2013). Some

pathogenic organisms can penetrate and internalize in the plant tissue of produce which can protect them from the application of sanitizers. *L. monocytogenes* can enter plant tissue by though natural opening like the stomata or be taken up by the roots through water movement. If *Listeria* enters the internal tissue, it would most likely occur during postharvest.

There are several risk factors associated with potential *Listeria* contamination in produce fields emphasizing the importance of developing and implementing GAPs to prevent surface contamination of minimally processed fruits and vegetables. The potential risk factors associated with produce fields are difficult to pinpoint as there are many environmental factors like climate, land interaction, and topographical variations (Strawn et al., 2013). A greater understanding of the possible environmental risk factors can aid in changing field practices to reduce contamination risks. One research group identified potential field practices that can increase the likelihood of *Listeria* contamination which included manure application, presence of wildlife, worker activity, quality of irrigation water, soil, and buffer zone presence (Strawn et al., 2013). Furthermore, the timing of these environmental factors significantly impacted the presence of *Listeria* contamination in produce fields. These findings can help farmers to evaluate their current farming practices and implement preventative measures to minimize contamination associated with harvesting.

2.8 Cantaloupe Rind Structure

The cantaloupe rind protects the interior flesh from contamination. However, unlike the smooth surface of honeydew melon, the netted surface of cantaloupe allows pathogens to attach to the rind, form biofilms on its surface and internalize into the edible flesh (Annous et al., 2005). For example, the cantaloupe surface is netted allowing for more bacterial attachment sites compared with the smooth surface of honeydew melons. The cantaloupe rind prevents the

effective removal of pathogens due to its netted surface (Annous et al., 2005). Furthermore, the ability of pathogens to attach to the netted rind leads to higher transfer of pathogens by cutting into the edible flesh, suggesting the importance of effective removal of pathogens from the cantaloupe surface (Ukuku and Sapers, 2001). The contamination levels on honeydew melons are lower compared with cantaloupe leading to reduced microbial transfer into the edible flesh during cutting (Ukuku and Sapers, 2005). The netted rind of the cantaloupe indicates the disruption of the cuticle leads to higher levels of pathogen internalization into the edible flesh in the presence of contaminated water during cantaloupe processing. Overall, cantaloupe's netted rind allows for higher levels of bacterial attachment compared with the non-netted structure of honeydew melon leading to different pathogen transfer rates to the edible flesh during processing.

2.9 Sources of Contamination during Cutting

Cantaloupes are susceptible to higher levels of bacterial contamination due to their netted surface. Contamination can readily occur during cutting when pathogens are transferred from the rind by the knife to the edible melon flesh (Beuchat, 1996). Even after the application of sanitizers to the melon rind, bacterial transfer can still occur by cutting but at lower levels compared without prior sanitization. A study conducted by Selma demonstrated that fresh-cut cantaloupe pieces were still positive for *E. coli* when the rind contained 4.3 to 8.3 log CFU/g before cutting. However, *E. coli* was not detected in the fresh-cut cantaloupe when the rind contained 3.3 log CFU/g (Selma et al., 2008). The potential transfer of pathogens to the edible flesh is the result of contamination that has occurred during production as there are no further microbial treatments to reduce bacterial transfer introduced by cutting. Previous bacterial transfer studies involving cantaloupe and honeydew melons indicated that approximately 3.3 and

2.0 log/CFU/g of aerobic mesophilic bacteria were transferred to fresh-cut pieces during cutting (Ukuku and Fett, 2004). Recently, a quantitative microbial risk assessment (QMRA) was created for *L. monocytogenes* which concluded that the risk of consuming fresh-cut cantaloupes was ten-fold higher compared to whole cantaloupes (Wang et al., 2015). Furthermore, the manner of cantaloupe processing can affect the transfer of pathogens as it was determined that peeling the rind before cutting resulted in less contamination instead of first cutting then peeling (Castillo, 2009). Presently, there is limited data on the transfer of pathogens during peeling, slicing, and dicing of melons during processing.

2.10 Commercial Slicing and Dicing of Fresh-Cut Produce

Foodborne outbreaks have been attributed to the commercial processing of fresh-cut produce as bacterial transfer can occur during processing. Slicing and dicing of fruits and vegetables can lead to the transfer of *L. monocytogenes* from the surface of contaminated produce to subsequent uncontaminated produce during processing. Commercial slicing and dicing can lead to potential cross-contamination of pathogens during processing due to contamination of blades and pushers of the equipment. Currently in the food industry, there are no standardized practices for slicing or dicing fruits and vegetables as companies employ manual cutting with knives or semi-manual practices with hand-operated slicers to make fresh-cut produce (Wang and Ryser, 2014).

Previous studies have indicated that inoculated fresh produce can contaminate subsequent sliced/diced produce due to contamination of the slicer's blades and pusher resulting in extended bacterial transfer during processing. One study comparing the transfer of *Salmonella* during manual slicing and electric slicing of tomatoes showed that *Salmonella* transfer was greater with the manual slicer as it decreased 2.7 and 4.4 log CFU/tomato, respectively (Wang and Ryser,

2013). Another study assessing transfer of *L. monocytogenes* during mechanical slicing of onions indicated that bacterial transfer was quantifiable up to the 20th onion with 0.3 log CFU/g when one onion was initially inoculated to contain 6.1 log CFU/g (Scollon et al., 2013). A study on the dicing of celery showed that *L. monocytogenes* easily spread from inoculated to previously uninoculated celery during processing (Kaminski et al., 2014). These studies clearly show that bacterial transfer can occur from contaminated fresh-cut produce to subsequent uncontaminated fresh-cut produce during slicing and dicing and reinforce the importance of applying sanitizers to slicers and dicers during processing to minimize the transfer of pathogens to fresh-cut produce.

2.11 Contamination of Melons

2.11.1 Preharvest Contamination of Melons

The factors responsible for contamination of melons are due to indirect and direct contact with fecal material in the preharvest environment. Potential indirect routes of fecal contamination result from irrigation water, airborne particles from animal production, and field processing (Ukuku et al., 2005). Irrigation water has a high risk of potentially contaminating fruits and vegetables. The CDC indicated that contamination of cantaloupe rinds occurred from contact with contaminated water during the production and handling (CDC, 2002). The survival of *L. monocytogenes* in soil depends on several factors including soil type, presence of decaying vegetation, and climate conditions (Sauders and Wiedmann, 2007). Furthermore, direct fecal contamination can occur by wild animals like reptiles, birds, and rodents defecating in the fields and on the produce, respectively (Geldreich and Bordner, 1971). The external rind of cantaloupe has a porous, netlike structure allowing for the attachment of microorganisms making it difficult to remove during the cleaning process with sanitizers or antimicrobial agents (Park and Beuchat,

1999). Consequently, watermelon and honeydew melon are less likely to microbial attachment due to their smoother surfaces compared to cantaloupe. Also, poor hygiene of field workers during harvesting can lead to the contamination of melons. The outbreaks associated with melons during the preharvest stage have led to the formation and implementation of Good Agricultural Practices (GAP) which reduces the risk of contamination.

2.11.2 Postharvest Contamination of Melons

Melons can be contaminated during several stages of postharvest processing like handling, packaging, transportation, and distribution of the product as depicted in Figure 2.1 (FDA, 2009). Typically, cantaloupes and other melon varieties are field packaged and shipped directly to the market without prior washing. Field packaging of cantaloupes reduces the risk of contamination by limiting the transfer of pathogens to other cartons by not introducing water. It is critical that field packaging equipment and facility packinghouses are sanitary to prevent the microbial load from increasing during processing (Castillo et al., 2004). Furthermore, FDA strongly recommends that packers clean, sanitize, and remove pests from packing equipment before operations begin.

If melons are shed-packaged, the melon dump operations and cooling methods can be potential sources of contamination. In packinghouse operations, melons are transferred from bins either by dry or water dump operations possibly leading to cross-contamination between melons or from the equipment surface. The purpose of washing the melons during the rinsing process is to remove soil or any organic matter instead of removing pathogens from the cantaloupe surface. Some facilities have water systems that recycle water which increase the risk of potential contamination of produce. After the melons are removed from the dump operations, the melons are cooled by either forced-air cooling or chilled water drench which

could result in approximately a two to three-fold reduction of pathogens on the surface of the melon (Annous et al., 2004). Furthermore, melons should be cooled promptly after harvesting to minimize any pathogen growth on the surface of the melons. During the distribution and transportation of melons, processers need to follow Good Manufacturing Practices (GMPs) to minimize the cross-contamination and growth of pathogens. Fresh-cut melons should be stored at refrigeration temperatures between 0 to 5^{o}C to prevent the growth of pathogenic microorganisms (FDA, 2009).

Several outbreaks associated with melons have occurred at food service establishments due to contamination during cutting and peeling. Previous studies have determined that microbial contamination from the rind can be transferred by a knife while cutting into the edible portion of the melon posing a hazard to consumers (Ukuku and Fett, 2002). The FDA suggests that melons with external damage or decay be disposed as there is an increased risk of contamination from foodborne pathogens in the edible flesh (FDA, 2009). Furthermore, retail employees should wash their hands and not directly touch cut melons to prevent cross-contamination. All equipment that comes in contact with the melon should be thoroughly washed and sanitized prior to use. Overall, these guidelines are helpful in assessing risks regarding transfer of pathogens while cutting.

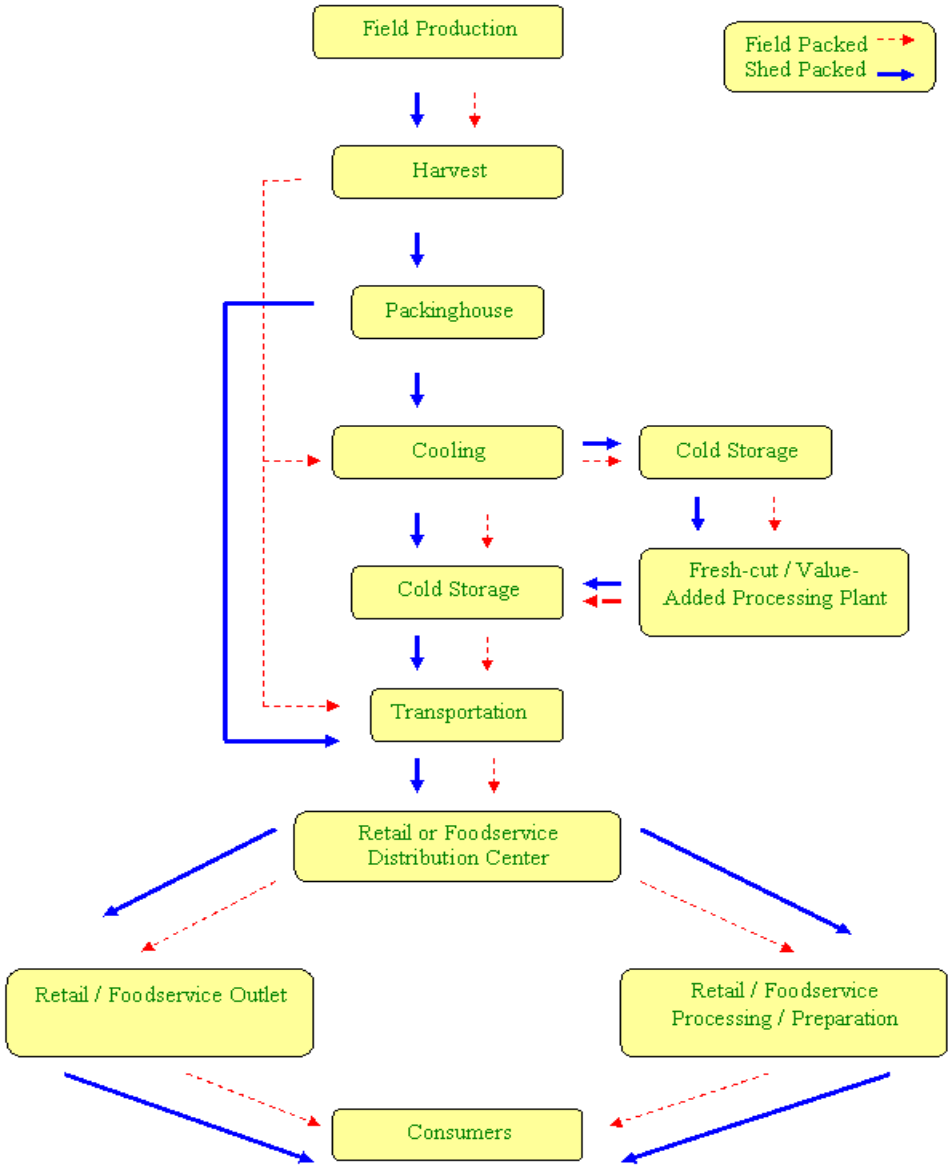

Figure 2.1: Flow diagram of melon processing from field to consumer for retail and foodservice establishment using field pack and shed pack methods adapted directly from the Food and Drug Administration (FDA, 2009).

2.12 Industry Practices to Minimize Contamination during Melon Processing

Governmental agencies and food industries understand the importance of maintaining the safety of melon processing during each stage in the farm-to-fork continuum. It is essential that Good Manufacturing Practices (GMPs), Standard Operating Procedures (SOPs), and Sanitation Standard Operating Procedures (SSOPs) be strictly followed during melon production,

processing, packaging, distribution and retail display because each stage can introduce microbial contamination. Some major sources of microbial contamination occur primarily due to temperature abuse during storage and infected food processors. Preventative measures should be implemented to prevent contamination of melons; because once contaminated during production pathogens may be difficult to remove at each successive step.

Melons are primarily grown in non-sterile environments that can allow pathogens to attach to the rind. Cantaloupes and honeydew melons are in contact with soil, irrigation water, and animal feces. The FDA recommends melon producers conduct environmental assessments and evaluate field practices involved with melon production to determine any possible sources of contamination. The agency suggests producers determine the presence of wildlife, flooding possibilities, and land history as these are environmental factors that could introduce pathogens during initial production (FDA, 2009). For example, the geographical features of the melon production site can introduce potential contamination due to water runoff from other fields, waste hazards, or production facilities. Climate conditions of excess rainfall or prevailing winds could transport pathogens from the soil to the cantaloupe rind. Also, humidity can increase the activity of animals around the production sites and this climate favors the growth of pathogens. Furthermore, melon production sites should be properly maintained to minimize these risk factors.

The establishment of proper hygienic policies for workers to follow during melon production and processing is essential to minimize the transfer of pathogens. Companies need to have GMPs and SOPs for worker health and hygienic standards. It is vital that workers who have direct contact with cantaloupes either in the production and processing stage maintain a high level of personal hygiene. These workers should refrain from actions that could

contaminate cantaloupes like sneezing or spitting in the production or handling sites. Any worker showing symptoms of foodborne illness should be relieved of their duties. Also, sanitary facilities should be located away from the production sites and readily available to workers to prevent any human contamination in the fields.

The characteristics of the surface of the melon rind have played a significant role in pathogens adhering and growing on the rind. Cantaloupes have been the source of the most foodborne outbreaks in melons due to their netted rinds (Harris et al., 2003). Melons with netted surfaces are more susceptible to microbial contamination as it is difficult to remove pathogens from the surfaces compared with smoother surfaces (Ukuku and Fett, 2002). The FDA strongly suggests to minimize risk factors associated with melon production and processing, because once surface contamination occurs it is difficult to remove pathogens on melons with netted rinds. Also, the stem scar can be a source of contamination as it may allow for the infiltration of pathogens into the edible melon flesh (Richards and Beuchat, 2004). Recently, a study by Macarisin et al. (2015) used dye to mimic *L. monocytogenes* during hydro-cooling showed pathogen infiltration occurred at the stem scar resulting in the contamination of the hypodermal mesocarp and calix of the cantaloupe. Melon processors should focus on implementing and adhering to postharvest practices that minimize contamination especially on the stem scar. In postharvest operations, sanitation programs have to be properly enforced during field packing and in the packaging facilities to minimize surface contamination of melons. FDA recommends melon processors to determine if the cleaning processes of removing pathogens from the melons are potentially a source of contamination (FDA, 2009). It is essential that all field packaging equipment be properly cleaned especially any surfaces in contact with the melons to prevent any

cross-contamination. Also, field packing equipment needs to be protected from any potential pest infestations to minimize pathogen exposure.

A possible source of cross-contamination during melon processing occurs during the melon dump operation when melons are transferred from field containers to dry or water dump operations. In this operation, contamination can occur between produce, water, and equipment surfaces (Akins et al., 2005). FDA recommends that packinghouses find alternative methods for transporting melons from the containers to dump units to reduce the transfer of pathogens. Furthermore, processing equipment that is in contact with melons should be regularly cleaned and sanitized to reduce contact contamination. If melons are transferred to wet dump units, it is critical that the water is monitored for disinfectant levels to ensure that the water is not contaminated from pathogens from the surface of the melon rind as it could recontaminate incoming melons. Also, the time that melons are in the wet dump units should be limited to prevent any cross-contamination. If the melons are hot from field heat when dumped into cold wet dump tanks, the large temperature differential created can lead to infiltration of pathogens. Therefore, pre-cooling of the cantaloupes or warming of the water is necessary before dump tank washing.

After melon dump operations, melons have to be cooled to minimize microbial growth on the rind. Melons are typically cooled by flume immersion, a chilled water drench, or forced-air cooling (Park and Beuchat, 1999). For forced-air cooling, the FDA strongly recommends that the equipment be regularly sanitized to prevent cross-contamination. If the melons are cooled by submersion in cold water, processors need to decrease the contact time to reduce potential infiltration by pathogens into the edible flesh due to the increased temperature gradient. Any delays in cooling could allow for the multiplication of pathogens on the surface of the rind

(Behrsing et al., 2003). To prevent this issue, operators need to enforce regulations that ensure proper cooling to reduce moisture as this could lead to the proliferation of pathogen growth on melons.

During fresh-cut operations, the edible melon flesh can be contaminated during cutting, slicing, dicing, or peeling from surface microbial contamination. Whole melons with any visible damage to the rind should not be used for fresh-cut produce as they have a higher risk of transmitting pathogens to the edible melon flesh. Also, thorough washing of melons prior to cutting or peeling operations can reduce microbial surface contamination by 2 to 3 logs (Park and Beuchat, 1999). Other methods to remove potential pathogens from the surface include scrubbing the crevices with sanitizer or applying hot water or steam to reduce pathogens on the rind. The removal of pathogens from the surface primarily depends on the sanitizers and contact time with the melons, but excessive contact time can lead to the infiltration of pathogens through the stem scar or rind into the edible melon flesh. During fresh-cut processing, knives should be regularly sanitized to reduce the likelihood of pathogens being transferred to the melon flesh. Refrigerated storage of fresh-cut and whole melons is necessary to maintain quality and prevent the growth of pathogens during distribution. FDA recommends that fresh-cut melons be held at 0 to 5^{o}C to prevent growth of pathogens (Del Rosario and Beuchat, 1995). Other whole melon varieties are stored at different temperatures to maintain optimal quality as cantaloupes and honeydew melons are stored from 2.2 to 5^{o}C and 7 to 10^{o}C, respectively.

Recently, foodborne outbreaks have been caused by user handling in retail and foodservice operations by accidental contamination to edible melon flesh during cutting or rind removal. Furthermore, a survey indicated that approximately 6% of the population does not wash their produce and 35% do not wash melons before consumption (Li-Cohen and Bruhn,

2002). These findings suggest that more educational programs need to emphasize safe handling and preparation of fresh produce before consumption to prevent possible foodborne outbreaks. The FDA recommends the following guidelines during the preparation of fresh-cut melons to minimize microbial contamination. It is recommended that before cutting melons that individuals wash their hands thoroughly to prevent transfer of human pathogens. Melons that have any visible damage or decay to the rind should not be used to prepare fresh-cut melons. The FDA Food Code recommends that the outer surface of melons be washed under cool water before being cut or added to other ingredients in retail establishments (FDA, 2009). Fresh-cut melons should be immediately refrigerated between 0 to 5^{o}C and should be consumed within seven days.

2.13 Temperature

Temperature abuse during storage of fresh-cut melons can increase microbial growth and amplify the risk to consumers. The FDA recommends that fresh-cut melons be stored between 0 to 5^{o}C to prevent the proliferation of foodborne pathogens (FDA, 2009). Previous studies have indicated that higher levels of contamination on the rind can result in increased pathogen transfer allowing melon flesh to be contaminated and grow during refrigeration if temperature abuse occurs during storage (Golden et al., 1993). In a study conducted by Ukuku and Fett (2002) assessing *L. monocytogenes* growth after the direct application of sanitizers like chlorine or hydrogen peroxide, *L. monocytogenes* growth was inhibited at 4^{o}C until 15 days of storage while populations reached up to 4.9 logs at 8 and 20^{o}C after 15 days, respectively. In another experiment, the growth of pathogens in fresh-cut melons increased significantly between 4 to 6 h of incubation at 25^{o}C reaching 6.8 logs, but no significant change was observed on fresh-cut cantaloupes at 5^{o}C (Delrosario and Beuchat, 1995). In a study assessing the effects of storage

temperature of fresh-cut melons inoculated with *Salmonella* populations remained unchanged and decreased 1 log at 5°C during 12-day storage for cantaloupe and honeydew melon, respectively (Ukuku and Sapers, 2007). Also, statistically greater growth of *Salmonella* was seen at 10 and 22°C compared to 5°C during 12 days of storage. During storage of fresh-cut cantaloupe and honeydew melon, *Salmonella* populations increased from 2.0 to 3.6 log and 1.9 to 3.0 log during storage at 10°C, respectively (Ukuku and Sapers, 2007). Overall, it is critical to store fresh-cut melons at temperatures between 0 to 5°C to minimize pathogen and reduce the risk of infection to consumers.

Delayed storage time can also lead to increased microbial growth. In one study, *Salmonella* populations increased 0.3 log and 1.3 log when the fresh-cut melons were held at 22°C for 3 and 5 hours before storage at 5°C, respectively (Ukuku and Sapers, 2007). The study indicated that holding fresh-cut melons at room temperature for an extended period of time leads to proliferation of pathogens, and suggests that fresh-cut melons should be stored immediately at 5°C to prevent further microbial contamination. Recently, a QMRA conducted on *L. monocytogenes* on fresh-cut cantaloupes and determined temperature at retail and household storage after cutting was the most significant factor in the risk assessment (Wang et al., 2015). Overall, these scientific studies have led to guidelines being adopted in the U.S.A. to minimize proliferation of foodborne pathogens by storing fresh-cut produce below 5°C. (FDA, 2009).

2.14 Application of Sanitizers to Reduce Contamination

2.14.1 Application of Sanitizers on Surface of Melons

In the food industry, melons are initially washed with water before sanitizers are applied to the surface of the melons. The cantaloupe netting poses some difficulties in the removal of pathogens from the rind even after the application of chemical sanitizers. Sanitizer efficacy

varies in the removal of pathogens from the rind of cantaloupes. Recently, a study determined spraying octenidine hydrochloride on cantaloupes at preharvest resulted in a 2 log CFU/cm^2 reduction of *Listeria* (Keelara et al., 2015). A study by Craigshead et al. (2015) assessed the efficacy of *Bacillus subtilis* UD 1022 on cantaloupe rinds as there was a reduction of 2 to 3 log at 22^{o}C which are possibly encountered in temperature abuse conditions during storage and transport. For example, a study conducted by Rodgers indicated that pathogen reduction on cantaloupe rind was approximately 6 log CFU/g when dipped in a solution consisting of chlorine dioxide, ozone, chlorinated trisodium phosphate, and peroxyacetic acid (Rodgers et al., 2004). There have been conflicting results from other studies suggesting that bacteria removal from cantaloupe rind is relatively difficult. For example, Ukuku and Fett (2004) observed a reduction of 2.5 log when cantaloupes were washed in 2.5% or 5.0% hydrogen peroxide. Another study by Alvarado-Casillas determined that there was a reduction of up to 2.9 log when cantaloupes were dipped in hypochlorite at a concentration of 1000 mg/L (Alvarado-Casillas et al., 2007). Researchers have determined that immersing cantaloupes in hot water at 90^{o}C for 60 seconds can effectively reduce pathogens up to 4 logs while maintaining the integrity of the rind (Ukuku and Fett, 2004). A study conducted by Ukuku et al. (2004) determined the presence of natural microflora on the cantaloupe rind inhibits the growth and survival of *L. monocytogenes* suggesting that possible recontamination can occur if natural microflora is removed by the application of sanitizers. Recently, a nisin-based sanitizer was effective in the reduction of *L. monocytogenes* transfer from the cantaloupe rind positive only by enrichment (Ukuku et al., 2015). Sanitizers reduce the potential contamination on the surface of melons, reducing the potential pathogens transferred from the rind to the edible melon flesh ultimately reducing a consumer's risk for illness.

2.14.2 Inactivation Treatments for Surface of Cantaloupes

Recently, new inactivation treatments were evaluated to determine if there was significant reduction of *L. monocytogenes* on the cantaloupe surface while still maintaining the quality of the melon. A study performed by Mahmoud evaluated the effects of X-ray treatments on *L. monocytogenes* and quality of the whole cantaloupe. It was determined 5 log reduction of *L. monocytogenes* occurred when 2.0 kGY X-ray was exposed on the cantaloupe rind while color and firmness of the melons were maintained during storage at 22^{o}C for 20 days (Mahmoud, 2012). The use of alginate coatings with 2.0 % cinnamon bark oil and 0.5% soybean oil on the surface of cantaloupes resulted in the reduction of *L. monocytogenes* to 1.3 log CFU/cm^2 while also preventing any color change or softening of the melon during 15-day storage (Zhang et al., 2015). Kaminski performed surface pasteurization of cantaloupes by using a heated wash at 65^{o}C for 45 seconds or heated wash followed by an application of peroxyacetic acid spray that resulted in a reduction in *L. innocua* of 2.3 to 4.3 log CFU/cm^2 (Kaminski et al., 2015). Mukhopadhyay et al. (2015) applied high pressure processing of 500 MPa at 8^{o}C to achieve a 6 log reduction of *L. monocytogenes* in cantaloupe puree which had minimal impact to the quality. Overall, these inactivation treatments have demonstrated significant reduction of pathogens on the rind and edible flesh while maintaining the quality of the cantaloupe.

2.15 Food Safety Regulation to Minimize Cantaloupe Contamination

Regulatory agencies like the Food and Drug Administration (FDA) impose strict legislation on the importation and distribution of cantaloupes to the ensure safety of imported products. These agencies realize that food safety programs are necessary to implement preventative strategies to minimize the risks associated with melon processing. Furthermore, regulatory agencies understand that strict enforcement of GMPs is essential to eliminate potential

microbial contamination on melons; because once contaminated removal of these pathogens with sanitizers can no longer be assured. The FDA considers fresh-cut cantaloupe to be a potentially hazardous food due to its ability to support bacterial growth because of its high water activity and mild acidity (Bhagwat, 2006). This finding suggests that the contamination of cantaloupes can occur at any stage of postharvest melon processing.

The *Salmonella* outbreaks traced to cantaloupe that included two deaths in California (Green et al., 2005) led the FDA to ban the importation of cantaloupes from Mexico during 2000 to 2002. These contamination events have led to economic repercussions against Mexican cantaloupe exporters as the production of Mexican cantaloupes declined by 92% and only made up 3% of all U.S. cantaloupe imports (SAIP-SAGARPA, 2002). The cantaloupe import ban from Mexico has improved food safety standards, but not all growers are restricted by the legislation as growers are judged by their past safety performance (Anonymous, 2005). Overall, the majority of outbreaks associated with cantaloupes are due to cross-contamination and direct contact of pathogens. The documentation and implementation of regulatory actions has instilled the practice of GMPs in melon processing to prevent microbial contamination and future outbreaks.

CHAPTER 3: Extent of *Listeria monocytogenes* Transfer during Cutting of Cantaloupe and Honeydew Melon

3.1 Materials and Methods

3.1.1 Experimental Design

One cantaloupe or honeydew melon was inoculated with a 3-strain avirulent cocktail of *L. monocytogenes* (J22F, J29H, and M3), stored at 4 or 30°C for 24 h mimicking temperatures encountered in processing facilities or field heat, respectively. After inoculation, melons were cut through the stem scar, blossom scar, and rind midpoint (i.e., between the stem and blossom scar) with a sterile, stainless steel cork borer to obtain five 25 mm length and 18 mm diameter core samples in each region. All core samples were aseptically cut into five 5 mm-long pieces, yielding five separate depth sections (0-5 mm, 6-10 mm, 11-15 mm, 16-20 mm, and 21-25 mm). Each depth section was sampled using composite sampling from the 5 core samples taken from each separate rind location. All experiments were performed in three replicates.

3.1.2 Produce

Cantaloupes (5- inch diameter) (*Cucumis melo* var. cantalupensis) and honeydew melons (6 – 7 inch diameter) (*Cucumis melo* var. inodorus) free of any visible bruising or defects were purchased from a local retailer, stored in 4°C refrigerator and used within 2 days of purchase. Melons were washed under running tap water to remove any debris before any experimentation proceeded.

3.1.3 Bacterial Strains & Inoculation

Three avirulent strains of *Listeria monocytogenes* (J22F, J29H, and M3) were obtained from the laboratory of Dr. Sophia Katharian (North Carolina State University, Raleigh, NC). J22F is a transposon mutant of H7550 and purine biosynthesis (*purB*) that are attenuated for systemic infection. J29H is a non-hemolytic, transposon mutant of H7550 and *hly* gene. M3 is a non-hemolytic, transposon mutant consisting of TN916 transposon in the *hly* gene inhibiting

Listeriolysin O (LLO) secretion. Stock cultures were maintained at -80°C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE; Becton and Dickinson, Sparks, MD) and 10% (v/v) glycerol (Sigma Chemical Co., St. Louis, MO). The stock cultures of each strain were streaked onto trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE; Becton and Dickinson) and incubated at 37°C for 24 h to prepare working cultures. Single colonies from the TSAYE plates were subjected to two successively transfers in 9 ml of TSBYE at 37°C for 24 h. The cultures were combined in equal volumes to obtain a 3-strain avirulent *L. monocytogenes* cocktail containing 10^7 and 10^9 CFU/ml for cantaloupe inoculation and 10^9 CFU/ml for honeydew melon inoculation, respectively. Cantaloupe and honeydew melons were dip-inoculated in 4.5 L *L. monocytogenes* cocktail. Cantaloupe inoculation levels reached approximately ~6 log CFU/cm^2 and ~4 log CFU/cm^2. Honeydew melon inoculation levels reached near ~5 log CFU/cm^2. Intact inoculated melons were air-dried for 1 h in a biological safety cabinet and stored at 4 and 30°C for 24 h to simulate processing plant and field temperatures. Prior to experimentation, rind concentrations of *L. monocytogenes* were confirmed by direct plating.

3.1.4 Cantaloupe and Honeydew Melon Coring

An 18-mm diameter sterile, stainless steel cork borer was used to obtain five 25 mm length core samples from each of the blossom scar, stem scar, and circumference regions of the rind depicted in Figure 3.1. After aseptically cutting each core sample into five 5 mm-long pieces (0-5 mm, 6-10 mm, 11-15 mm, 16-20 mm, and 21-25 mm), each of the five pieces from the same depth and regions were composited in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) and assessed for numbers of *L. monocytogenes* within 2 h.

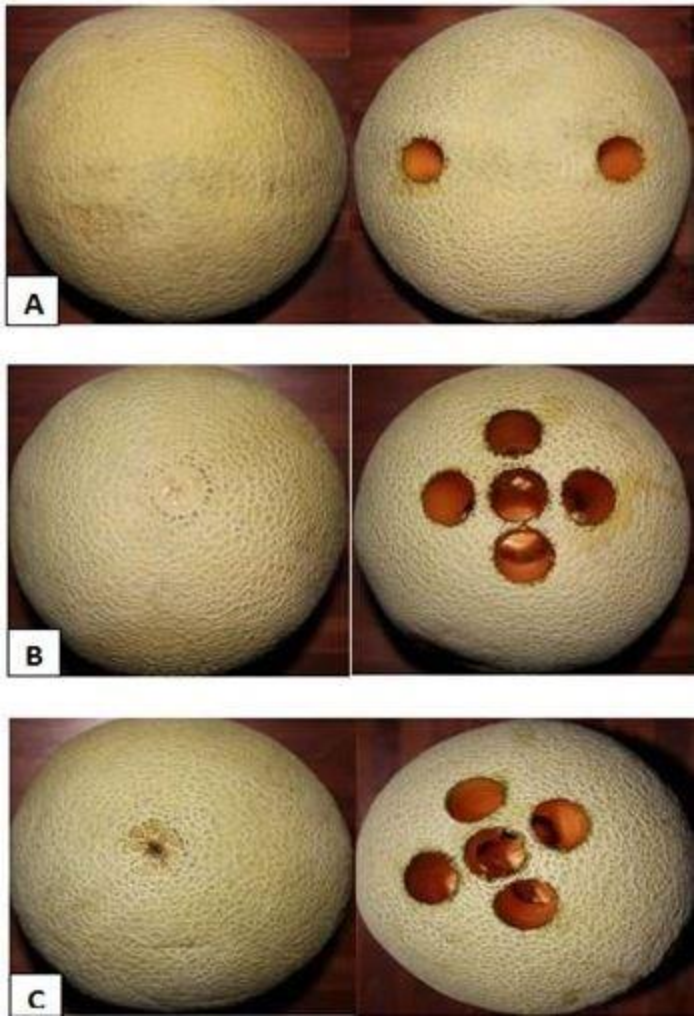

Figure 3.1: The coring locations at the A) circumference of the rind B) blossom scar and C) stem scar region.

3.1.5 Comparison of Coring Tools

Experiments were performed on cantaloupe alone using the 18-mm diameter sterile stainless steel cork borer and 18-mm diameter sterile stainless steel apple borer. The apple borer had a serrated blade section, corer head which was 30-mm in length and 18-mm in diameter and is pictured in Figure 3.2. The cork borer and apple corer were used to obtain five 30 mm-long core samples from the circumference of the rind to determine any potential differences in transfer between the two coring tools at 4^{o}C.

Figure 3.2: "Farberware Classic Apple Corer." Amazon. n.p., n.d. Web. 24 Oct. 2016.

3.1.6 Microbiological Analysis

The five cantaloupe or honeydew melon pieces weighing ~ 25 g were diluted 1:2 in sterile phosphate-buffered solution (PBS), homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 1 min, serially diluted in PBS and then plated with or without membrane filtration using 0.45-µm-pore-size membrane filters (Millipore Corp., Billerica, MA) on *Listeria* differential agar base, trypticase soy agar containing 0.6% yeast extract, 0.1% esculin, and 0.05% ferric ammonium citrate (FAC), or *Listeria* differential/selective agar base, Modified Oxford Agar (MOX, Neogen Corporation, Lansing, MI) consisting of moxalactam and colistin sulfate as selective agents to obtain black *Listeria* colonies from esculin hydrolysis which were counted after 24 to 48 h of incubation at 37^{o} C. Any samples negative for *Listeria* by direct plating were subsequently enriched for 48 h and then streaked on Modified Oxford Agar (MOX).

3.1.7 Surface Population Calculations

The surface population of *L. monocytogenes* on cantaloupe and honeydew rinds were determined by Eq. (1):

$$N = \frac{C \times D}{n \times A}$$

where N is the Colony Forming Units per square centimeter (CFU/cm^2), C is the number of colonies plated, D is the dilution factor, n is the number of surface rind samples (n = 5), and A is the rind surface area sampled ($A = \pi r^2$) in square centimeters.

The population of *L. monocytogenes* on the side surface area of the cantaloupe and honeydew melon flesh cores were determined by Eq. (2):

$$N = \frac{C \times D}{n \times S}$$

where N is the Colony Forming Units per square centimeter (CFU/cm^2), C is the number of colonies plated, D is the dilution factor, n is the number of core melon flesh samples (n = 5), and S is the surface area of each corer section, side surface area, ($SA = 2\pi rh$) of melon flesh in square centimeters.

3.1.8 Statistical Analysis

All experiments were performed in triplicate and reported as log CFU/cm^2. Results were analyzed using a two-way mixed design analysis of variance (ANOVA) in SAS 9.4 (SAS Institute Inc., Cary NC) were the temperature was designated as between factor and depth as within factor. The Tukey's test was used to determine significant differences in *L. monocytogenes* transfer between the five core depths, three rind regions, and two temperatures during simulated cutting with a cork borer. The Tukey's test was used to determine if significant differences in coring methodologies between the cork borer and apple corer were present. Different letters above the bars from Tukey's test comparisons indicate statistical significance ($P < 0.05$) between surface population means.

3.2 Results

3.2.1 *L. monocytogenes* populations on rind surfaces of cantaloupe and honeydew melon

Higher populations of *L. monocytogenes* were attached to the surface of dip-inoculated cantaloupe compared to honeydew melon ($P < 0.05$) (Figure 3.3). *L. monocytogenes* populations on the cantaloupe rind were not significantly different at any localized regions of the stem scar, blossom scar, or circumference of rind ($P > 0.05$). For honeydew melon, significantly lower *L. monocytogenes* were seen on the rind circumference compared to the blossom and stem scar regions ($P < 0.05$). A two-way ANOVA examined the effect of melon type and localized regions of the rind, blossom scar, and stem scar on the attachment of *L. monocytogenes* on the rind surface in Figure 3.3. There was a significant interaction between the melon type and localized region on surface populations of *L. monocytogenes* on the rind surface ($P < 0.05$). The analysis of the fixed effects indicated there were significantly greater attachment of *L. monocytogenes* on cantaloupe compared to honeydew melon ($P < 0.05$). There were significant differences in the attachment of *L. monocytogenes* in the localized regions of the rind surface ($P < 0.05$).

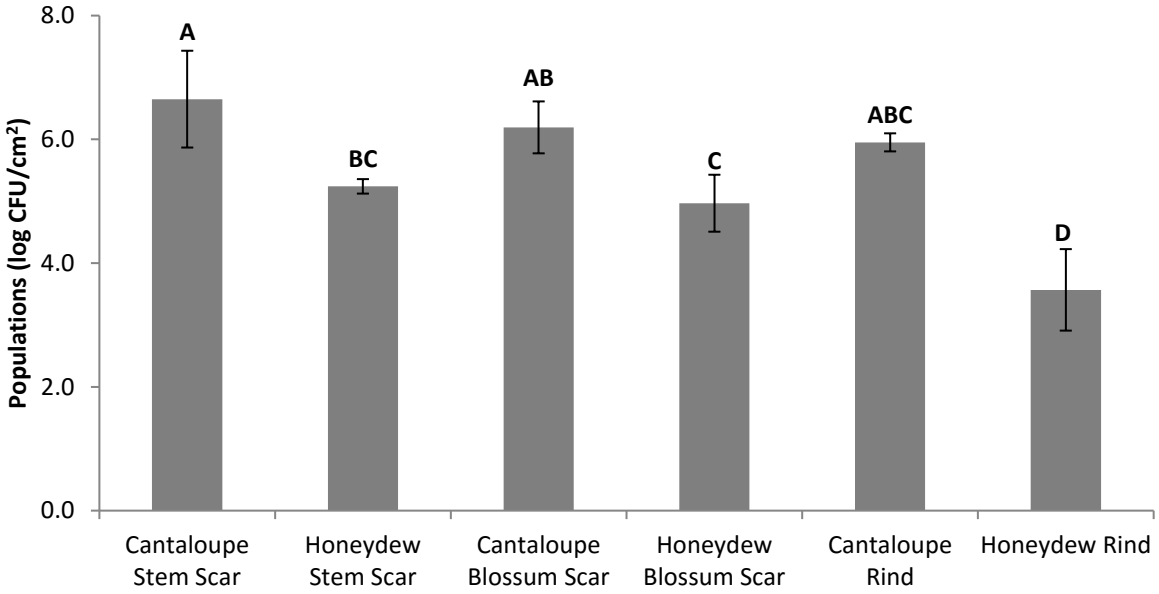

Figure 3.3: Mean (±SE) *L. monocytogenes* populations on surface of cantaloupe and honeydew melon at the stem scar, blossom scar, and rind dip-inoculated and stored for 24 h at 4°C. Different letters above the bars from Tukey-Kramer HSD comparisons indicate statistical significance ($P < 0.05$) between surface population means.

3.2.2 *L. monocytogenes* transfer from rind to cantaloupe and honeydew melon flesh

After surface inoculation, the difference in *L. monocytogenes* populations on the rind affected the numbers of *L. monocytogenes* to the edible flesh during cutting. There was ~3 log/cm^2 reduction in *L. monocytogenes* populations transferred from the rind to the 5 mm melon flesh depth ($P < 0.05$) (Figure 3.4 – 3.12). By assessing the factor of depth, these results indicate there is a significant difference of populations of *L. monocytogenes* transferred due to cutting depth after the depth zero, rind, populations ($P < 0.05$). However, core samples taken from the same region, similar *L. monocytogenes* populations were seen at the five different depth populations after the rind to 5 mm population decreases were observed ($P > 0.05$).

3.2.3 Impact of melon region and temperature on *L. monocytogenes* transfer

For honeydew melon, greater *L. monocytogenes* transfer occurred for core samples taken from the stem and blossom scar regions compared to the circumference of rind ($P < 0.05$) (Figure 3.4 – 3.6). A two-way ANOVA was performed to determine the effect of temperature

and depth on the transfer of *L. monocytogenes* by cutting. There was no statistically significant interaction between temperature and depth for cutting in both cantaloupe and honeydew melons within the depths of the rind, blossom scar, and stem scar ($P > 0.05$). An analysis of the fixed effects at the stem scar of the cantaloupe showed there was a significant difference of the bacterial transfer due to temperature and depth ($P < 0.05$). However, the effects at the rind circumference and blossom scar indicated that there was a statistically significant difference in transfer of *L. monocytogenes* due to depth ($P < 0.05$). There was no significant difference due to storage temperature at either 4 or 30°C ($P > 0.05$).

However, these same differences were not seen for cantaloupe (Figure 3.7 - 3.9). All core samples yielded statistically similar *Listeria* populations for cantaloupe cut at 4 or 30°C ($P > 0.05$). By comparing the cutting of cantaloupe at 4 and 30°C by two-way analysis, there was no significant difference in *L. monocytogenes* populations transferred by temperature ($P > 0.05$). There was statistical difference in the transfer of *L. monocytogenes* due to depth in the stem scar region ($P < 0.05$); however greater *L. monocytogenes* transferred by cutting in the blossom scar and circumference of the rind at 4°C compared with 30°C ($P < 0.05$). There was also a significant difference in the transfer of *L. monocytogenes* due to depth ($P < 0.05$).

There was a reduction in enumerated cells from the surface inoculated *L. monocytogenes* populations to the edible melon flesh in all the transfer studies (Figure 3.4 – 3.12) performed at 4 and 30°C. The initial surface contamination ranged from 5.2 to 7.0 log CFU/cm^2 in cantaloupe ($P > 0.05$) and 3.4 to 5.2 log CFU/cm^2 in honeydew melon ($P < 0.05$). The level of *L. monocytogenes* transfer from rind surface to 5 mm depth varied from 2.8 to 3.6 log CFU/cm^2 in cantaloupe ($P < 0.05$) and 1.1 to 1.8 log CFU/cm^2 in honeydew melon ($P < 0.05$) at 4°C. The level of *L. monocytogenes* transfer from the rind surface to 5 mm depth varied from 1.9 to 4 log

CFU/cm^2 in cantaloupe ($P < 0.05$) and 0.7 to 1.8 log CFU/cm^2 in honeydew melon ($P < 0.05$) stored at 30^{o}C. There was no statistical significant difference observed in the transfer populations compared with the storage temperature conditions of either the cantaloupe or honeydew melons ($P > 0.05$).

Transfer of *L. monocytogenes* between the various depths varied without any statistical significance observed between the depths ($P > 0.05$). *L. monocytogenes* was detected at the depths of 5 mm to 25 mm from 1.2 to 4.0 log CFU/cm^2 in cantaloupe and 0.2 to 2.1 log CFU/cm^2 in honeydew melon at 30^{o}C ($P > 0.05$). *L. monocytogenes* was detected at the depths of 5 mm to 20 mm from 2.2 to 3.6 log CFU/cm^2 in cantaloupe and 0.3 to 1.8 log CFU/cm^2 in honeydew melon stored at 4^{o}C ($P > 0.05$).

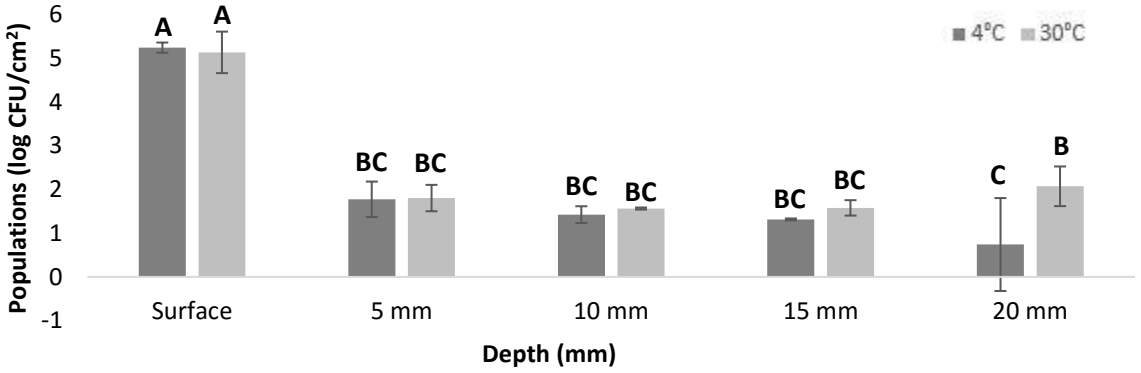

Figure 3.4: Mean (±SE) *L. monocytogenes* populations transferred from the honeydew rind dip-inoculated and stored at 4 and 30^{o}C for 24 h to the honeydew flesh from the stem scar.

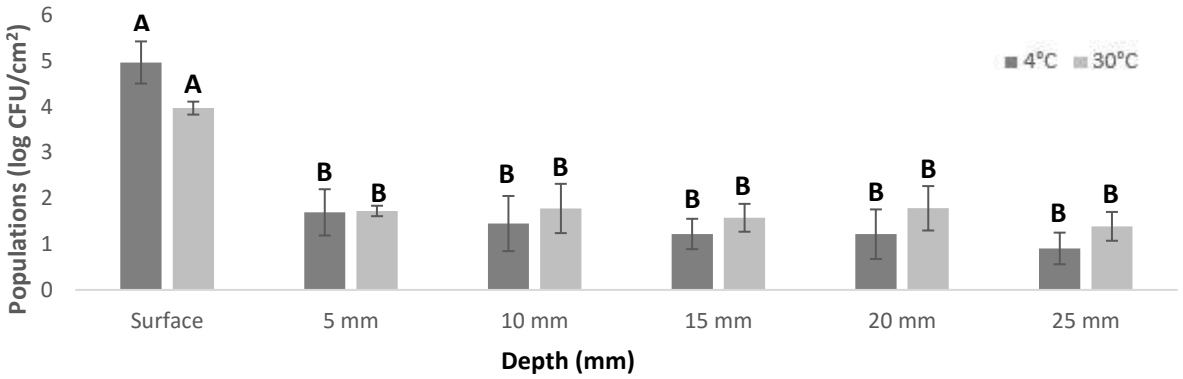

Figure 3.5: Mean (±SE) *L. monocytogenes* populations transferred from the honeydew rind dip-inoculated and stored at 4 and 30°C for 24 h to the honeydew flesh from the blossom scar.

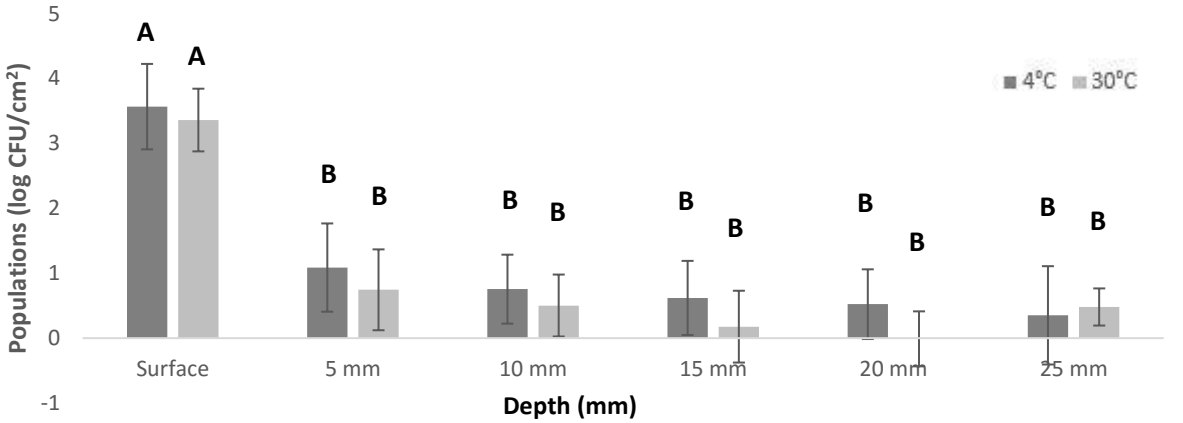

Figure 3.6: Mean (±SE) *L. monocytogenes* populations transferred from the honeydew rind dip-inoculated and stored at 4 and 30°C for 24 h to the honeydew flesh from the rind circumference area.

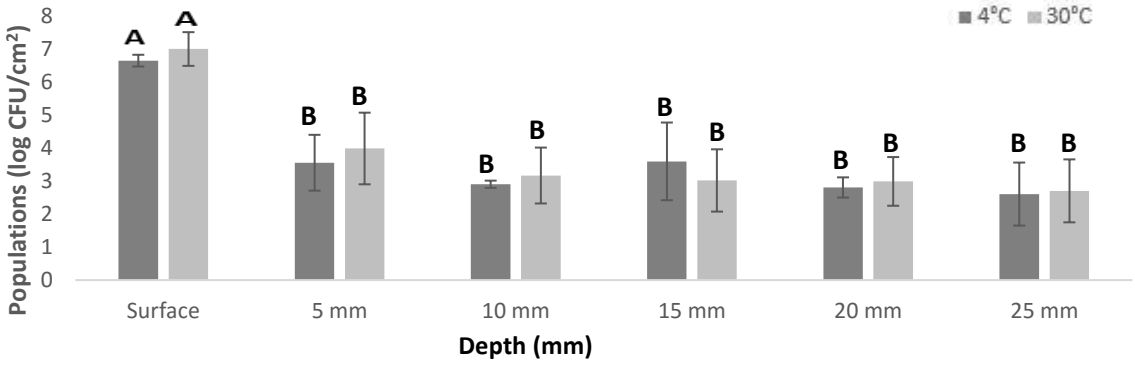

Figure 3.7: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from stem scar.

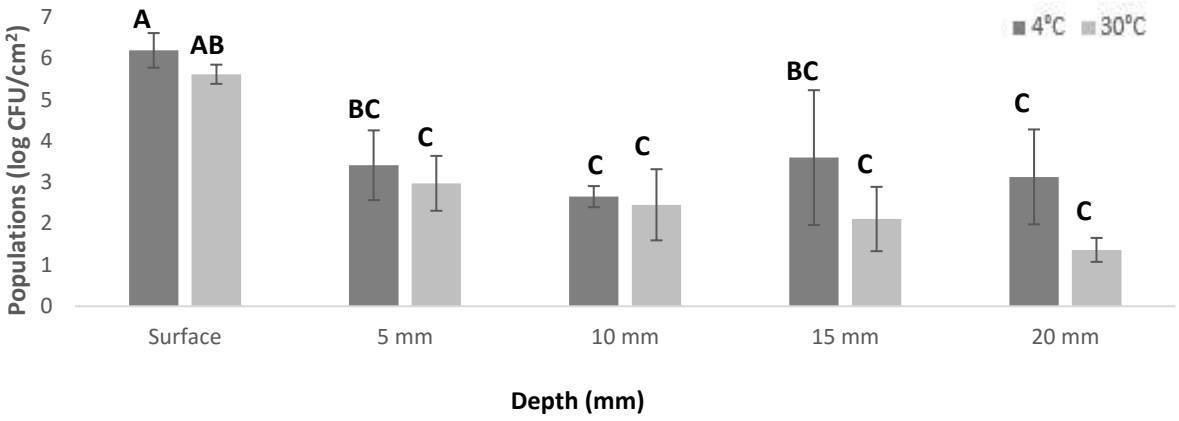

Figure 3.8: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from blossom scar.

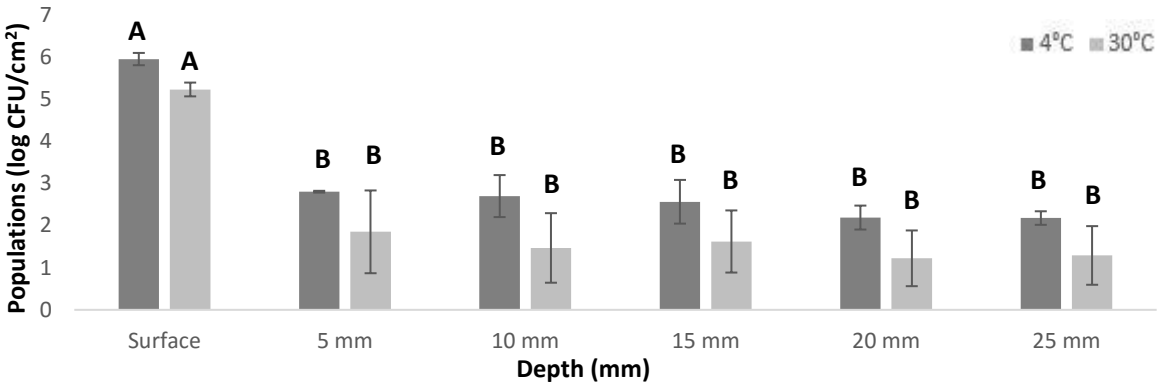

Figure 3.9: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from rind circumference area.

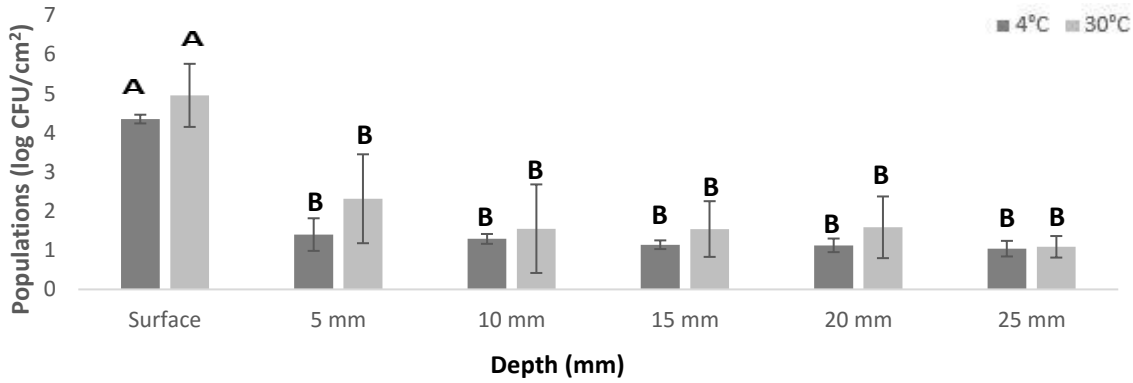

Figure 3.10: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30ºC for 24 h to the cantaloupe flesh from the stem scar.

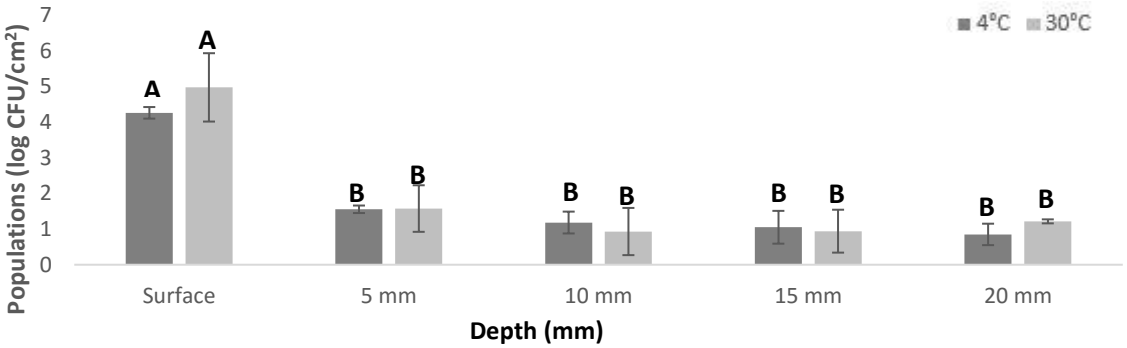

Figure 3.11: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30ºC for 24 h to the cantaloupe flesh from the blossom scar.

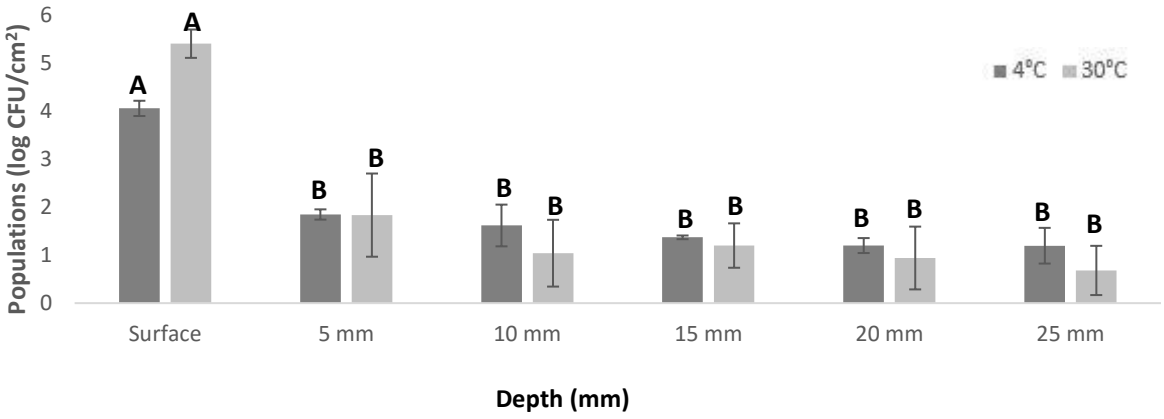

Figure 3.12: Mean (±SE) *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30ºC for 24 h to the cantaloupe flesh from the rind circumference area.

3.2.4 Comparison of coring tools

Overall, statistically similar *L. monocytogenes* populations were seen at the six different core depth samples for the two coring implements ($P > 0.05$) (Figure 3.13). Subsequent transfer of *L. monocytogenes* varied from 3.0 to 3.9 log CFU/cm^2 using the cork borer and 3.1 to 4.1 log CFU/cm^2 using the apple corer in the cantaloupe ($P > 0.05$). A two-way ANOVA was performed to determine if there was any effect of the different coring tools and transfer of *L. monocytogenes* populations by depth. There was no significant interaction between the effect of the coring method and depth ($P > 0.05$). The analysis of the fixed effects showed that there was no difference in transfer of *L. monocytogenes* due to the use of different coring tools ($P > 0.05$).

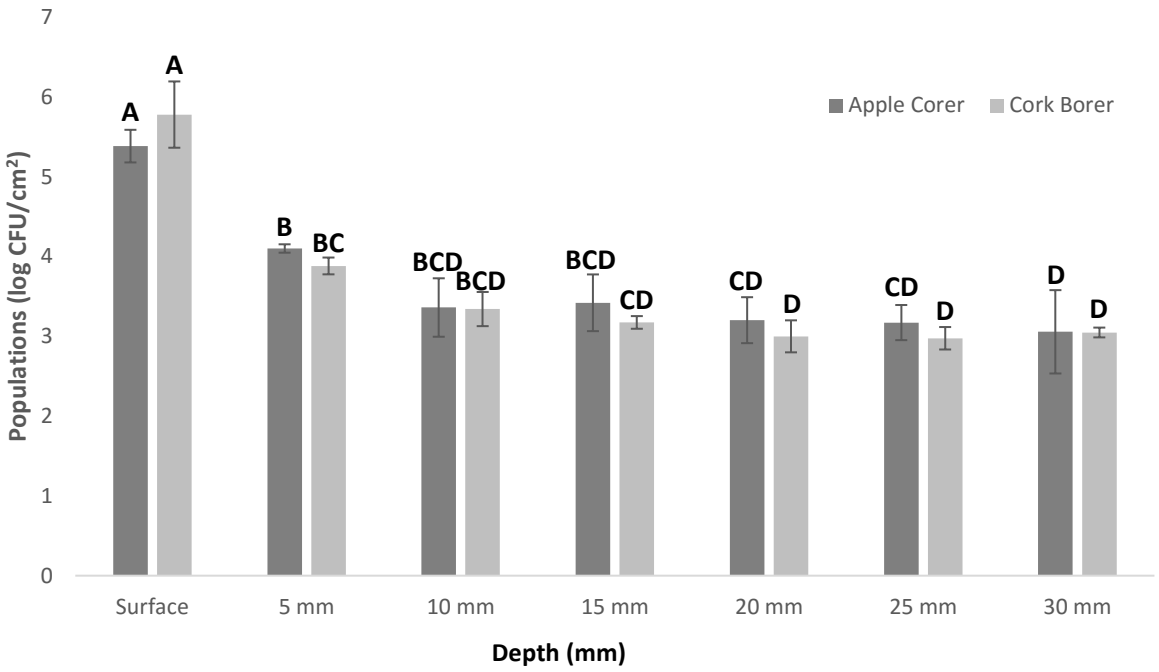

Figure 3.13: Mean (±SE) transfer of *L. monocytogenes* populations from the cantaloupe rind to the interior melon flesh by using an apple corer and cork borer to compare bacterial transfer.

3.3 Discussion

Previous studies mimicking procedures used in commercial and home preparation of fresh-cut melons have shown that bacteria can readily transfer from the rind to the edible melon flesh during cutting, with the extent of contamination on the rind determining the numbers of organisms transferred (Beuchat, 1996; Ukuku and Fett, 2004; Selma et al., 2008). Other studies have evaluated the ability of chemical sanitizer to reduce microbial contaminants on the rind, thereby reducing transfer during cutting (Ukuku and Fett, 2002; Ukuku et al. 2005, 2015).

One of the objectives of this study was to evaluate transfer of *L. monocytogenes* from inoculated cantaloupe and honeydew melon into the edible flesh at 5 mm increments using a cork borer. Cantaloupe and honeydew melons were dip-inoculated with *L. monocytogenes* to simulate surface contamination possibly occurring during dump tank washing, cooling, and subsequent storage (Parnell et al., 2005). These potential sources of melon rind contamination

emphasize the need to follow recently revised GAPs to minimize both preharvest and postharvest contamination (FDA, 2011). Furthermore, insanitary kitchen operations can lead to potential cross-contamination as pathogens can be transferred from the rind to edible flesh by improper cleaning and sanitizing of melons before preparation. Cantaloupe rind is more susceptible to surface contamination due to its porous and netted nature compared with the honeydew melon's smoother surface (Annous et al., 2005). Previous studies have demonstrated that cantaloupe rind can support increased pathogen growth compared to honeydew melon with total coliform counts being 1.4 log higher on cantaloupe (Cabrera 2003, unpublished data). The extent of *L. monocytogenes* can be transferred observed in this study was based on initial populations on the rind at the time of cutting with less transfer seen at the lower inoculation level.

Structural differences between the rind of cantaloupe and honeydew melon largely dictate the extent of surface contamination. Previous bacterial attachment studies have shown less bacteria attachment to the rind of honeydew melon compared to cantaloupe (Ukuku et al., 2005). According to Barak et al. (2003), netted cantaloupe rind provided greater opportunities for *S.* Poona attachment. Furthermore, Suslow (2004) has shown that infiltration of pathogens can occur through the stem scar and ground spot area as pathogens were present 5 mm below the melon rind. In our study, *L. monocytogenes* attachment to the cantaloupe rind was not statistically different at either the stem car, blossom scar, or circumference of the rind ($P > 0.05$). Statistically similar transfer of *L. monocytogenes* during coring was observed. For honeydew melon, initial *L. monocytogenes* populations were 1 log CFU/cm^2 higher on the stem and blossom scar regions compared with the smoother portions of the rind which led to higher numbers of *L. monocytogenes* in melon flesh from the stem and blossom scar regions. This

research supports that the inherent differences in melon structure can truly impact the microbial safety and risks present with different melon types.

As a result of worker mishandling and poor hygienic practices, cantaloupe and honeydew melons can be readily contaminated during the postharvest stage of production. Previous studies have suggested as few as 150 bacteria per cm^2 are needed on the cantaloupe rind for contamination to occur while cutting (Suslow and Cantwell, 2001). During cutting of cantaloupe and honeydew melon, the initial level of surface contamination directly impacted the number of *L. monocytogenes* transferred to the edible flesh during coring. When contaminated produce is cut with a knife, bacteria are first transferred from the produce surface to the knife and then to the cut flesh with the number of bacteria transferred decreasing as cutting continues (Zilelidou et al., 2015). A previous study performed by Vorst et al. (2006) indicated that knives inoculated with *L. monocytogenes* at 5 and 3 log CFU per blade were able to contaminate 20 and 5 slices of deli meat during slicing, respectively. Despite obvious surface differences between cantaloupe and honeydew melon, an initial 3 log CFU/cm^2 decline in *L. monocytogenes* populations was seen from the rind to the first 5 mm section of melon flesh with no significant difference seen thereafter. This suggests that all edible flesh is susceptible to contamination from the rind surface during cutting. Hence, *L. monocytogenes* can be readily transferred from the rind to the interior edible flesh of cantaloupe and honeydew melon during cutting.

As part of our study, the effect of cantaloupes and honeydew melon holding temperature prior to preparation on transfer of *L. monocytogenes* during coring was also evaluated. In this study, the melons were dip-inoculated, held at 4 or 30^{o}C for 24 h and then enumeration prior to mechanical preparation. Recently, a study performed by Nyarko et al. (2016) shows no difference in *L. monocytogenes* populations based on cantaloupe cultivars over 15-day storage

period at different temperatures. In this same study, *L. monocytogenes* populations decreased on the rind sections and increased on the stem scar region during 15 days of storage while there was no difference in *L. monocytogenes* populations after 1 day of storage. This study contributes to the field of food safety and supports enumeration of surface *L. monocytogenes* populations would be by impacted significantly by 1 day of storage at various temperatures. Nyarko et al. (2016) indicated that a difference in *L. monocytogenes* surface populations between the two cantaloupe cultivars tested was not statistically different. Our study examines two different melon types (cantaloupe and honeydew) and observed a statistically significant difference in the *L. monocytogenes* populations enumerated from the surfaces of the stem scar, blossom scar, and rind regions ($P < 0.05$). Findings from this study demonstrate that no statistical difference in bacterial transfer was seen with temperature prior to coring at 4 or 30^{o}C in the honeydew melon rind, blossom scar, and stem scar ($P > 0.05$). However, this study shows there was greater bacterial transfer observed in 4^{o}C compared with 30^{o}C in the cantaloupe rind and blossom scar ($P < 0.05$). These findings and those by Nyarko et al. (2016) suggest that further studies are needed to investigate differences in survival of *L. monocytogenes* on the surfaces of other commonly consumed melon types.

Biofilm formation and bacterial attachment of *L. monocytogenes* is impact by knife blade materials present in a production facility. This can ultimately lead to an impact on transfer rate of *L. monocytogenes* during cutting and processing of fresh produce (Rodriguez et al., 2007). The force and angle of the knife while cutting could also impact the number of bacteria transferred as previously demonstrated during slicing of delicatessen meats (Vorst et al., 2006). In this study, a stainless steel cork borer and apple corer were used to simulate cutting since both yielded core samples for *L. monocytogenes* quantification at 5 mm increments. Using the cork

borer, core samples were retained inside the cork borer which were removed by pushing core samples with sterile forceps. In contrast, cores obtained using the apple corer could be easily removed without the risk of further bacterial transfer. The two-way analysis indicate comparison of the cork and apple borers does not differ in the *L. monocytogenes* transfer in the depth within the cantaloupe flesh while cutting. Overall, no significant difference in *L. monocytogenes* was seen between the cork and apple borers ($P > 0.05$). This suggests that use of either the cork or apple borer methodologies can be used to illicit statistically similar transfer results in subsequent experiments.

In conclusion, this study demonstrates that *L. monocytogenes* can readily transfer from the melon surface into the interior melon flesh during cutting in commercial, restaurant and home settings. This study uses a cork borer to simulate cutting processes that allow for the quantification of *L. monocytogenes* from the rind to specific depths in the edible melon flesh. Appropriate chemical sanitizers and washing methods must be used to minimize surface contamination, which in turn may reduce the likelihood of bacteria being transferred to the edible flesh during cutting. The quantitative findings from this research should be helpful in current risk assessments and help determine best practices for fresh-cut preparation cantaloupe and melons.

CHAPTER 4: Transfer of *Listeria monocytogenes* during Mechanical Slicing and Dicing and Growth during Subsequent Storage of Cantaloupe and Honeydew Melon

4.1 Materials and Methods

4.1.1 Experimental Design

Two cantaloupe or honeydew melon halves inoculated with a 3-strain avirulent cocktail of *Listeria monocytogenes* were sliced followed by subsequent slicing of eight uninoculated melon halves of the same type to assess bacterial transfer during preparation. Two cantaloupe halves without its rind were inoculated with a 3-strain avirulent cocktail of *L. monocytogenes* followed by subsequent dicing of eight uninoculated cantaloupe halves were stored for 7 days at 4, 7, or 10^{o}C to evaluate growth of *L. monocytogenes*.

4.1.2 Produce

Cantaloupes (5-inch diameter) (*Cucumis melo* var. cantalupensis) and honeydew melons (6 – 7-inch diameter) (*Cucumis melo* var. inodorus) were selected free of any visible bruising or rind defects and purchased from a local retailer and stored for no longer than 2 days at 4^{o}C prior to experimentation. Before surface inoculation, all melons were washed under cool running tap water to remove any debris.

4.1.3 Bacterial Strains

Three avirulent strains of *L. monocytogenes* (J22F, J29H, and M3) were obtained from the laboratory of Dr. Sophia Katharian (North Carolina State University, Raleigh, NC). J22F is a transposon mutant of H7550 and purine biosynthesis (*purB*) that are attenuated for systemic infection. J29H is a non-hemolytic, transposon mutant of H7550 and *hly* gene. M3 is a non-hemolytic, transposon mutant consisting of TN916 transposon in the *hly* gene inhibiting Listeriolysin O (LLO) secretion. Stock cultures were maintained at -80^{o}C in Trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE; Becton and Dickinson, Sparks, MD) and 10% (v/v) glycerol (Sigma Chemical Co., St. Louis, MO). The stock cultures of each strain were

streaked onto Trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE; Becton and Dickinson) and incubated at 37ºC for 24 h to obtain working cultures. Singles colonies from the TSAYE plates were subjected to two successive transfers in 9 ml of TSBYE at 37ºC for 24 h. Thereafter, the cultures were combined in equal volumes to obtain one 3-strain avirulent *L. monocytogenes* cocktail.

4.1.4 Cantaloupe and Honeydew Melon Inoculation for Slicing

Retail cantaloupe and honeydew melons were washed then dip-inoculated in a 3-strain avirulent cocktail of *L. monocytogenes* containing ~10^9 CFU/ml for 10 min to contain ~5 log CFU/cm^2 and ~4 log CFU/cm^2 for slicing, air-dried for 1 h and then stored at 4ºC for 24 h. Prior to slicing, the whole melons were cut in half starting at the stem end after which the seeds were removed aseptically with a knife from the cavity of melons. The initial *L. monocytogenes* populations on the melon were confirmed by direct plating as detailed below.

4.1.5 Cantaloupe Inoculation for Dicing

Retail cantaloupe was washed then followed by removal of the cantaloupe rind with a sterile knife. The whole rind-free melon was dip-inoculated in a 3-strain avirulent cocktail for 30 s to contain ~3 log CFU/cm^2 and immediately diced. Prior to dicing, the whole melons were cut in half starting at the stem end after which the seeds were removed from the cavity of melons without rind. The initial *L. monocytogenes* populations on the melon were confirmed by direct plating as detailed below.

4.1.6 Cantaloupe and Honeydew Melon Slicing

Two cavity-cleaned inoculated melon halves were manually sliced with the rind on using an aluminum manual slicer with bladed contact area of 233 cm^2 (Vollrath Redco 401N, Vollrath Company, Sheboygan, WI) to obtain nine 0.75 inch slices followed by eight cavity-cleaned

uninoculated melons halves (Figure 4.1). After slicing, alternate melon slices (~150 to 250 g/slice) had its rind aseptically removed by a sterile knife before being transferred into a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI) for the enumeration of *L. monocytogenes* within 2 hours.

Figure 4.1: "Vollrath 401N Redco Lettuce King I ¾" Cut Vegetable Shredder and Slicer – 10 Blades." Webstaurant Store. n.p., n.d. Web. 10 Aug. 2016.

4.1.7 Cantaloupe Dicing and Storage

Two cavity-cleaned, inoculated rind-free melon halves were manually diced using an aluminum manual dicer with bladed contact area of 498 cm^2 (Nemco 55650 dicer, Nemco Inc., Hicksville, OH) to obtain 1 inch cubes followed by eight cavity-cleaned uninoculated rind-free melon halves (Figure 4.2). After dicing, 100 g of diced cantaloupe from each melon half were weighed into sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) to quantify *L. monocytogenes*. Additionally, 100 g of diced cantaloupe from each half was equally divided into three sterile

PLA trays (Nature Works, Minnetonka, MN) and stored at 4, 7, or 10°C for 3 and 7 days to evaluate *L. monocytogenes* growth during storage.

Figure 4.2: "Nemco 55650 Easy Lettuce Kutter (Cutter)" Webstaurant Store. n.p., n.d. Web. 10 Aug. 2016.

4.1.8 *L. monocytogenes* Growth on Diced Cantaloupe

One whole cantaloupe had its rind aseptically removed by cutting with a knife, after which the melon flesh was inoculated and mechanically diced using the Nemco dicer. Inoculated cantaloupe pieces (100 g) were placed in sterile PLA, trays which were then stored at 4, 7, or 10°C for 7 days and were tested daily for number of *L. monocytogenes*. Since little if any growth was seen at 4 or 7°C, generation times were only calculated for cantaloupe samples stored at 10°C using the following Eq(1):

$$G = \frac{t}{3.3 \log b/B}$$

where t is time in days, *b* is the *L. monocytogenes* population at time 1, and B is the *L. monocytogenes* population at time 2.

4.1.9 Microbiological Analysis

One hundred-gram samples of cantaloupe or honeydew melon pieces were added to sterile Whirl-Pak filter bags, diluted 1:1 in sterile UVM, and homogenized in a stomacher (Stomacher 400 Circulator, Seward, Worthington, UK) at 260 rpm for 2 minutes. The sample homogenates were serially diluted in phosphate-buffered solution (PBS) and surface-plated on *Listeria* differential agar base, Trypticase soy agar containing 0.6% yeast extract, 0.1% esculin, and 0.05% ferric ammonium citrate or *Listeria* differential/selective agar base, Modified Oxford Agar (Neogen Corporation, Lansing, MI) consisting of moxalactam and colistin sulfate as selective agents or processed with membrane filtration using 0.45-µm-pore-size membrane filters (Millipore Corp., Billerica, MA) and plated Modified Oxford agar to obtain black *L. monocytogenes* colonies from esculin hydrolysis which were counted after 24 to 48 h of incubation at 37^{o} C. Any samples negative for *L. monocytogenes* by direct plating were subsequently enriched for 48 h and then streaked on Modified Oxford Agar to determine presence or absence of *L. monocytogenes*.

4.1.10 Surface Population Calculations

The surface population of *L. monocytogenes* for honeydew melon with rind and cantaloupe with or without rind were determined by Eq. (2):

$$N = \frac{C \times D}{n \times A}$$

where N is the Colony Forming Units per square centimeter (CFU/cm^2), C is the number of colonies plated, D is the dilution factor, n is the number of surface samples with or without rind

(n = 4), and A is the surface area (A = length x width, 5 x 5 cm) of the rind or melon flesh in square centimeters.

4.1.11 Statistical Analysis

The direct plate counts for *L. monocytogenes* from triplicate experiments were converted into log CFU/g for each. Sliced or diced melon half was subjected to one-way analysis of variance by using SAS 9.4 (SAS Institute Inc., Cary NC). The Tukey's test was used to determine significant differences in *L. monocytogenes* transfer between sliced cantaloupe and honeydew melon halves subsequent uninoculated melon halves were sliced. The same comparison test was used to determine if significant differences also existed in *L. monocytogenes* transfer during dicing and growth on diced cantaloupes during storage. The letters within the bar graphs indicate statistical significance from the Tukey's test comparison ($P < 0.05$) between the sliced or diced halve population means.

4.2 Results

4.2.1 *L. monocytogenes* transfer during melon slicing

Cantaloupes and honeydew melons inoculated with a 3-strain avirulent *L. monocytogenes* contained 5.2 ± 0.4 and 4.2 ± 0.2 log CFU/g on the rind, respectively, after 1 h of air-drying followed by 24 h storage at 4^{o}C. Thereafter, manually slicing two cavity-cleaned inoculated melon halves resulted in 2.2 ± 0.4 log and 1.7 ± 0.6 log CFU/g in the edible flesh of cantaloupes and honeydew melons after slicing (Figure 4.3 & 4.4). When two inoculated cantaloupe halves were sliced followed by 8 uninoculated halves, *L. monocytogenes* transfer was not significantly different ($P > 0.05$) for halves 3 through 9 (0.2 - 1.5 log CFU/g) while only halve 10 was significantly different compared to halve 3 ($P < 0.05$). When honeydew melon was sliced, *L. monocytogenes* transfer was not statistically different for halves 3 through 10 (0.1 – 0.5 log

CFU/g). All uninoculated cantaloupe and honeydew melon halves yielded *L. monocytogenes* by either direct plating or enrichment.

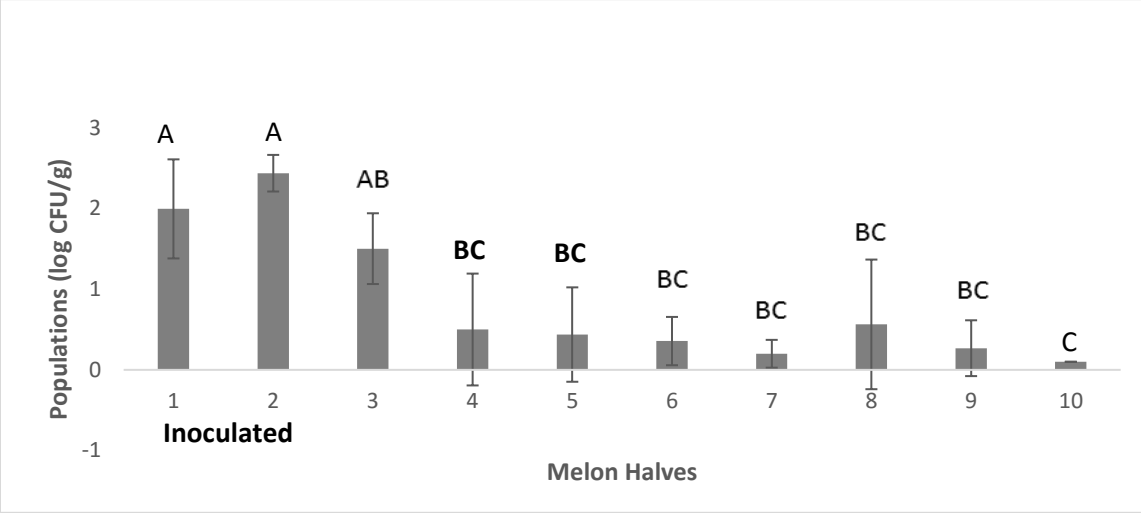

Figure 4.3: Mean (±) SE *L. monocytogenes* populations on cantaloupe halves that were sliced. Inoculation level at (5.2 ± 0.4 log CFU/cm^2).

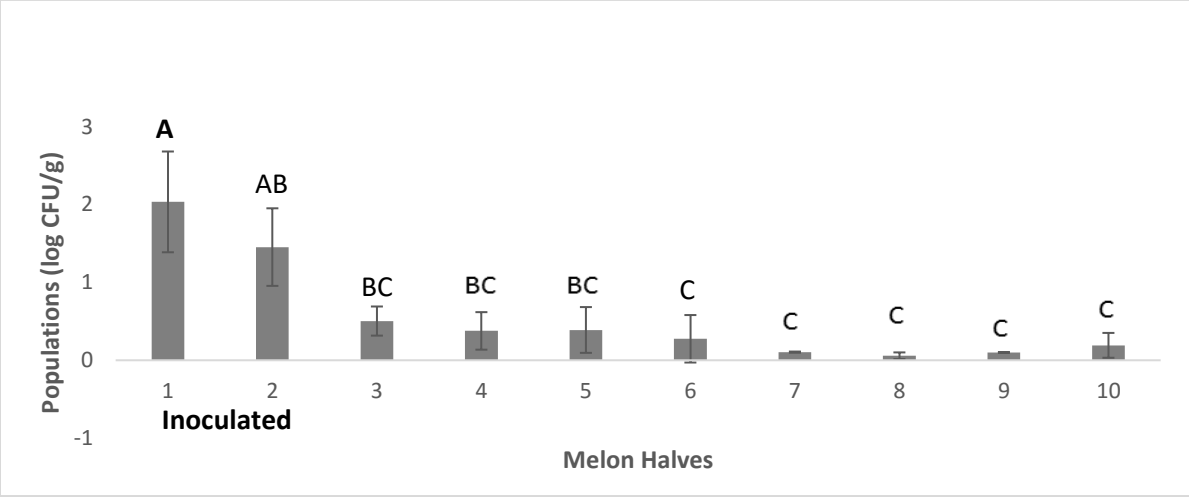

Figure 4.4: Mean (±) SE *L. monocytogenes* populations on honeydew melon halves that were sliced. Inoculation level at (4.2 ± 0.2 log CFU/cm^2).

4.2.2 *L. monocytogenes* transfer from inoculated melons to subsequent uninoculated melons during dicing

Dip-inoculation of rind-free cantaloupes with 3-strain avirulent *L. monocytogenes* cocktail containing of *L. monocytogenes* yielded populations of 5.5 ± 0.1 log and 3.3 ± 0.2 log

CFU/cm^2 on the surface. Manual dicing of the two cavity-cleaned inoculated cantaloupe halves led to *L. monocytogenes* populations of 4.8 ± 0.3 log CFU/g and 3.3 ± 0.4 log CFU/g in the edible melon flesh (Figure 4.5 & 4.6). Significantly greater *L. monocytogenes* transfer ($P < 0.05$) from the inoculated to uninoculated melon halves was seen for 3rd and 4th halves (2.6 and 3.7 log CFU/g) compared to halves 5 through 10 (0.7 – 1.6 log CFU/g) with *L. monocytogenes* again found in all samples by either direct plating or enrichment (Figure 4.5).

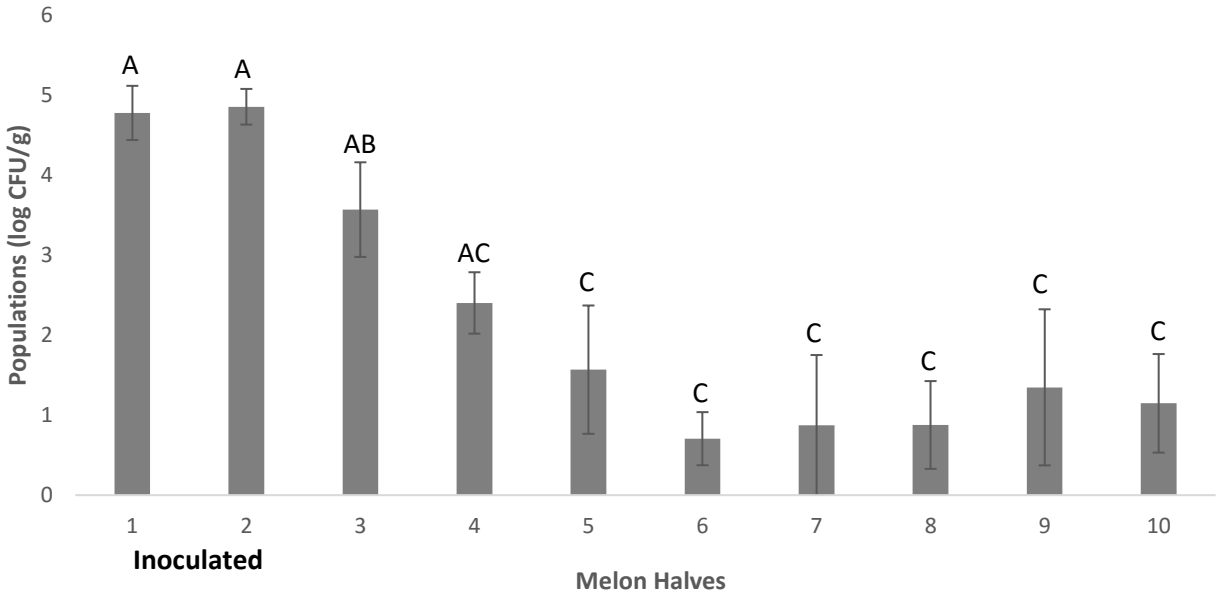

Figure 4.5: Mean (±SE) *L. monocytogenes* populations on cantaloupe halves that were diced. Inoculation level at (5.5 ± 0.1 log CFU/cm^2).

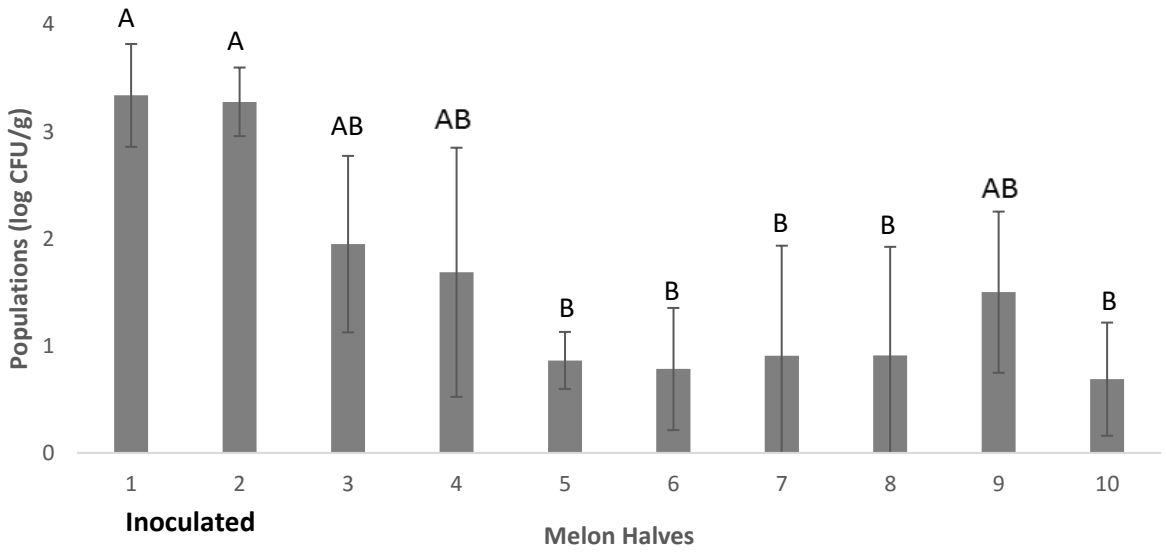

Figure 4.6: Mean (±) SE *L. monocytogenes* populations on cantaloupe halves that were diced. Inoculation level at (3.3 ± 0.2 log CFU/cm^2).

4.2.3 *L. monocytogenes* growth in ten diced cantaloupe halves during storage

Diced cantaloupe pieces stored at 4 and 7^{o}C for 7 days resulted in the average increased growth of *L. monocytogenes* population by 0.5 and 3.3 log CFU/g, respectively (Figure 4.7 & 4.8). During storage of diced cantaloupe at 4^{o}C, there was no significant difference in the growth of *L. monocytogenes* during the 7-day storage ($P > 0.05$) except for halve 6 which had more growth at 7 days compared with 3 days ($P < 0.05$) (Figure 4.7). When compared to diced cantaloupe stored at 7 and 10^{o}C, there were no diced cantaloupe halves with more growth during the storage at 4^{o}C, respectively (Figure 4.7 – 4.9). There was significant growth observed with *L. monocytogenes* growth at 7 and 10^{o}C ($P < 0.05$) with increases of 3.3 and 5.4 log CFU/g on the uninoculated cantaloupe dices compared at 4^{o}C. *L. monocytogenes* population after growth in uninoculated diced cantaloupe pieces ranged from 4.8 to 5.9 log CFU/g in melon halves from 3 to 10 at 10^{o}C (Figure 4.9). At 7^{o}C storage, there was significant difference in the growth of *L. monocytogenes* populations transferred to the uninoculated cantaloupe halves during dicing ($P < 0.05$). For example, all the cantaloupe dices from halves 3 to 10 had significant growth at 7 days

compared with 0 day ($P < 0.05$). In cantaloupe dices (halves 3, 4, 8-10), growth at 7 days was significantly greater than at 0 and 3 days ($P < 0.05$) (Figure 4.8). For halves 5 and 6, there was significant difference in growth between 0, 3, and 7 days ($P < 0.05$). At 10°C storage, there was a significant difference in growth of *L. monocytogenes* populations transferred to uninoculated cantaloupe halves 3 and 4 because there was more significantly more growth at 3 and 7 days compared to 0 day ($P < 0.05$) (Figure 4.9). For cantaloupe halves 5 to 10, there was a significant difference in growth between all the storage day durations ($P < 0.05$).

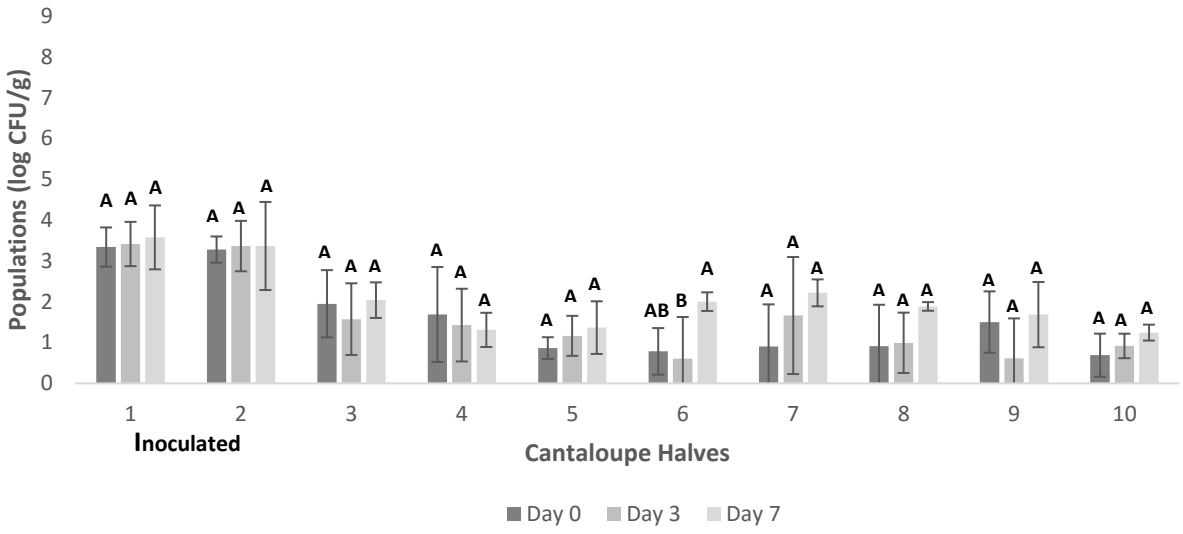

Figure 4.7: Mean (± SE) *L. monocytogenes* populations on cantaloupe halves that were diced and stored at 4°C.

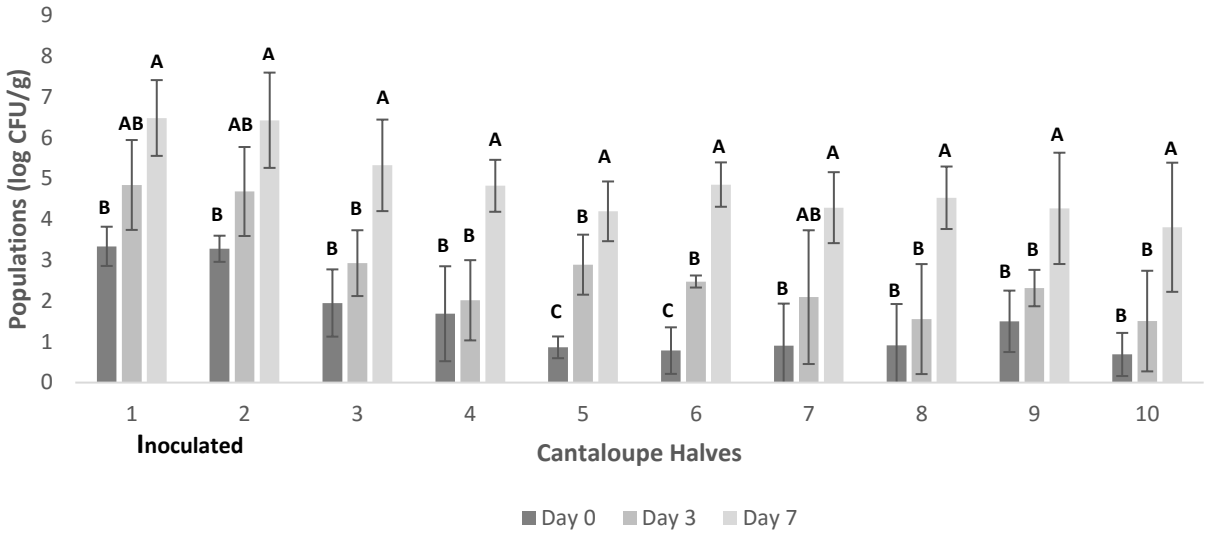

Figure 4.8: Mean (± SE) *L. monocytogenes* populations on cantaloupe halves that were diced and stored at 7°C.

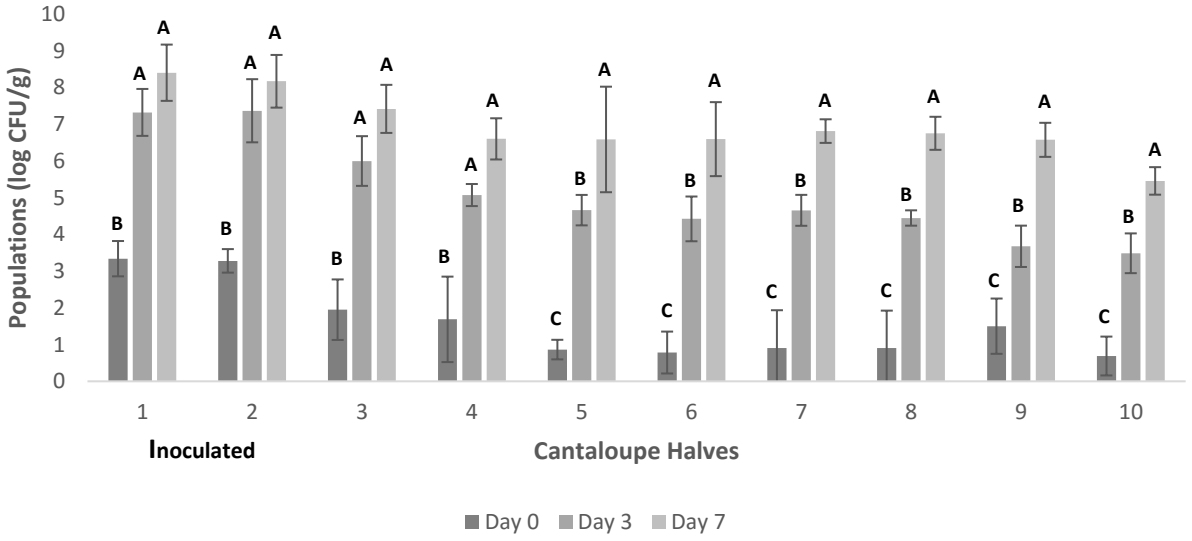

Figure 4.9: Mean (± SE) *L. monocytogenes* populations on cantaloupe halves that were diced and stored at 10°C.

4.2.4 *L. monocytogenes* growth on inoculated diced cantaloupe pieces

A whole cantaloupe without the rind was dip-inoculated in a 3-strain avirulent cocktail of *L. monocytogenes* of ~5 log CFU/ml to contain 3.8 ± 0.1 log CFU/cm^2 on the melon surface which was immediately followed by mechanical dicing. During the seven day storage at 4, 7,

and 10°C, *L. monocytogenes* counts reached maximum levels of 4.7 ± 0.1, 6.2 ± 0.1, and 9.2 ± 0.1 log CFU/g, respectively (Figure 4.10). *L. monocytogenes* populations had minimal growth during the seven day storage at 4°C as there was an increase of ~1 log CFU/g. At 7 and 10°C storage, there was an increase of 2.4 and 5.4 log CFU/g during the seven day storage. During the storage of diced cantaloupes, the *L. monocytogenes* generation time was calculated to be 0.74 days at 10°C as it exhibited significant growth.

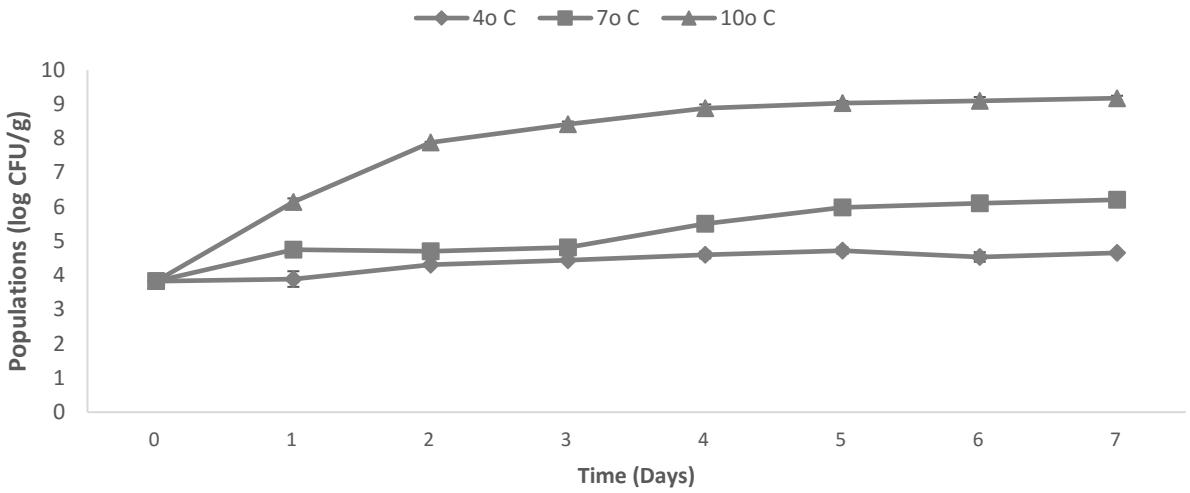

Figure 4.10: Mean (± SE) *L. monocytogenes* population growth on cantaloupe dices during subsequent storage of seven days and stored at 4°C, 7°C, and 10°C.

4.3 Discussion

This study assessed the transfer of *L. monocytogenes* from inoculated to uninoculated melon halves during mechanical slicing and dicing. The results from these studies demonstrate that bacterial transfer readily occurs during mechanical slicing and dicing similar to cross-contamination observed in previous experiments with onions (Scollon et al., 2013; Scollon and Ryser, 2014), tomatoes (Wang and Ryser, 2013), and celery (Kaminski et al., 2014). Cantaloupe and honeydew melons were dip-inoculated in the slicing experiment to simulate surface contamination from soil or irrigation water, whereas in the dicing study, rind-free cantaloupe

halves were dip-inoculated to simulate contamination during rind removal (Chimbombi et al., 2013). Chimbombi et al. (2013) demonstrated in cantaloupe flesh that *Salmonella* growth occurred between 10 to 30 h at 23ºC and no growth was observed up to 5 h after inoculation. This study supports the use of dip inoculation procedures for cantaloupe used in the present study with sampling procedures immediately, within 10 minutes, following inoculation. However, unusually high inoculation levels were needed to obtain quantitative data that could be modeled for future risk assessments.

Surveys have shown *Salmonella* to be present on field-grown cantaloupe in Mexico (Bhagwat, 2006) indicating that proper implementation of Good Agricultural Practices (GAP) is necessary to minimize contamination of melons during processing. Cantaloupes and honeydew melon rinds have opposing textural compositions with cantaloupe rind far more inclined to pathogen contamination compared with honeydew melon due to its netted surface (Barak et al., 2003).

Previous studies have demonstrated the importance of applying sanitizer treatments to reduce surface contaminants on fresh produce since any remaining pathogens can be transferred to previously uncontaminated product during subsequent processing. In one study evaluating *Escherichia coli* O157:H7 transfer during pilot-scale production of fresh-cut lettuce (Buchholz et al., 2012) showed that pathogen spread to previously uncontaminated product was relatively random especially when the initial contamination level was below 3 log CFU/cm^2. In slicing experiment involving onions, Scollon et al. (2013) determined that transfer of *L. monocytogenes* in onions throughout subsequent slicing regardless of the initial inoculation level. In our study, the starting population of *L. monocytogenes* determined the extent of subsequent transfer to uninoculated melon halves.

In the dicing experiments, the initial inoculation level also impacted the extent of *L. monocytogenes* transfer to subsequently diced product. These findings are similar to those seen for celery with a 2 log decrease in *L. monocytogenes* observed after dicing 8 batches of previously uncontaminated product (Kaminski et al., 2014). In this study, *L. monocytogenes* growth was also assessed in the diced cantaloupe halves during subsequent storage at 4, 7, and 10°C. After seven days of storage, *L. monocytogenes* populations were significantly greater in diced cantaloupe stored at 10°C compared to 4 and 7°C ($P < 0.05$) as these findings were similar to the growth of *L. monocytogenes* on fresh-cut cantaloupe at temperature abuse conditions performed by Huang et al. (2015) and data could be used to model growth kinetics of *L. monocytogenes* transfer at different storage temperatures (Fang et al., 2013; Yoon et al., 2014). Nyarko et al. (2016) presented similar finding at 4°C with an artificially created cantaloupe rind juice suggesting *L. monocytogenes* was able to grow ~3 log CFU/ml, after 7 days of storage when adequate nutrients and moisture were available at lower temperatures. Our study noticed modest growth after 4°C. The difference could have been caused by Nyarko et al. (2016) having prepared artificially macerated rind juice, which would have damaged cellular tissues of cantaloupe making nutrients readily available for microbial growth, liquid growth medium. In our study microbial growth was determined over 7 days on diced intact pieces of cantaloupe in which nutrients may not have been as readily available, station growth medium. These findings indicate the proper temperature storage of fresh-cut cantaloupe is necessary to prevent growth of pathogens during storage.

The results obtained during dicing and subsequent storage of cantaloupe are similar to those observed in several previous studies indicating that temperature is the primary factor in the growth of *L. monocytogenes*. During 12 days of storage, Ukuku (2007) reported no significant

growth of *Salmonella* on cubes of cantaloupe at 5°C while an increase of 3.6 logs was observed at 10°C. The growth of diced cantaloupes at 4°C in our study was similar to the growth model predicted by Danyluk et al. (2014), which noted 1 log growth over 6 days of storage. Another fresh-cut cantaloupe study by Ukuku and Fett (2002) showed that even after exposing cantaloupes inoculated with *Salmonella* at 1000 ppm chlorine-based sanitizer or 5% H_2O_2 for 2 min, the pathogen was not eliminated during cantaloupe storage at 4 or 8°C. Furthermore, Lamikanra et al. (2000) compared the biochemical changes of cantaloupe during storage at 4 and 20°C, respectively. The study indicated that prolonged storage at 20°C resulted in increased acidity and lactic acid production and loss of 17% of soluble solids leading to increased microbial growth. These findings emphasize that storage of fresh-cut cantaloupe pieces at temperatures less than 5°C can inhibit the growth of most pathogens, while higher temperatures that may be encountered during retail display can lead to amplification of microorganisms overtime.

In conclusion, this is the first study to show that *L. monocytogenes* can be transferred from an inoculated melon halve with or without its rind to uninoculated melon halves during subsequent mechanical slicing and dicing. Furthermore, when stored under temperature abuse conditions, *L. monocytogenes* can reach potentially hazardous levels in diced cantaloupe. These results reinforce proper washing and use of chemical sanitizers or other treatments such as hot water to decrease microbial populations on the rind before slicing or dicing with thorough washing of honeydew melons also recommended. Sliced and diced melon remains a perishable product that needs to be properly refrigerated and consumed in a timely manner to minimize the risk of foodborne illness.

Chapter 5: Conclusions and Future Recommendations

The increased availability and health benefits offered by the consumption of fresh fruits and vegetables are not without its risks as half of all foodborne outbreaks are associated with fresh produce. Melons, especially cantaloupes, have been a major source of outbreaks due to their netted surface that increases the risk of contamination during preharvest and challenges with cleaning during postharvest processing. The application of sanitizers to the rind surface is necessary to reduce the transfer of *L. monocytogenes* into the edible melon flesh during commercial processing.

The first objective of this study was to quantify the transfer of *L. monocytogenes* from the rind into the edible melon flesh by cutting the rind in three different areas and storing the melon at different product temperatures. The results demonstrated *L. monocytogenes* can be readily transferred from the rind to interior flesh during preparation. *L. monocytogenes* transfer ranged from 1.2 to 4.0 and 0.2 to 1.8 log CFU/cm^2 for cantaloupe and melon rind when rinds were inoculated with *L. monocytogenes* populations of 6.1 and 4.4 log CFU/cm^2, respectively. Overall, no significant difference in *L. monocytogenes* transfer was seen at different depths, or product temperatures ($P > 0.05$). Therefore, these findings emphasize thorough washing of the rind with a sanitizer is important to minimize translocation of *L. monocytogenes* to the interior melon flesh.

The second study quantified the transfer of *L. monocytogenes* from one inoculated cantaloupe or honeydew melon to an uninoculated melon during mechanical slicing and dicing followed by storage at 4, 7, 10^{o}C for seven days. *L. monocytogenes* populations in subsequently uninoculated cantaloupe and honeydew melons were similar ($P > 0.05$). The transfer of *L. monocytogenes* reached a maximum of 1.5 and 0.5 log CFU/g in cantaloupe and honeydew melons, respectively. During the storage of diced cantaloupes, the *L. monocytogenes* generation

time was calculated to be 0.74 days at 10^{o}C. This study demonstrates that *L. monocytogenes* transfer was similar between cantaloupe and honeydew melons while slicing. These findings reinforce the importance of refrigeration of diced cantaloupes in an effort to prevent the growth of *L. monocytogenes* during storage and transport. Overall, these findings will be helpful in current risk assessments associated with commercial processing, storage and transportation of melons.

Future work includes conducting experiments to gain a better understanding of *L. monocytogenes* transfer during industry specific commercial melon processing. In the cutting experiments, *L. monocytogenes* transfer due to the maturity and ripeness of the melons or the application force of the core borer should be assessed. *L. monocytogenes* transfer comparing mechanical and non-mechanical peeling from inoculated melons to subsequent uninoculated melons would add value to the field of food microbiology. In the dicing experiment, the melon flesh was directly inoculated to obtain consistent *L. monocytogenes* populations in an effort to determine the transfer to uninoculated melons during dicing. Furthermore, the growth of *L. monocytogenes* during extended storage at different temperatures was assessed. The addition of humidity and modified atmosphere parameters could also add value and should be investigated in future studies. These experiments demonstrate that *L. monocytogenes* transfer can occur during processing and that sanitizer application to the melon rind and equipment is necessary to mitigate contamination, recontamination and cross-contamination during commercial melon processing.

APPENDICES

Appendix A

Coring of Cantaloupe and Honeydew Melon

	Replicate 1	Replicate 2	Replicate 3
Cantaloupe Stem Scar	6.6	6.5	6.8
Honeydew Stem Scar	5.1	5.3	5.3
Cantaloupe Blossom Scar	6.6	6.2	5.8
Honeydew Blossom Scar	4.5	5.0	5.4
Cantaloupe Rind	6.1	5.9	5.8
Honeydew Rind	2.8	4.1	3.8

Table 3: Raw data for *L. monocytogenes* populations on surface of cantaloupe and honeydew melon at the stem scar, blossom scar, and rind dip-inoculated (log CFU/cm^2) and stored for 24 h at 4^{o}C.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	5.1	5.3	5.3
4	5 mm	1.4	1.7	2.2
4	10 mm	1.6	1.2	1.4
4	15 mm	1.3	1.3	1.3
4	20 mm	< 1.0 CFU/g*	1.6	1.1
30	Surface	5.6	5.1	4.7
30	5 mm	2.2	1.6	1.6
30	10 mm	1.5	1.5	1.6
30	15 mm	1.8	1.5	1.5
30	20 mm	1.9	1.8	2.6

*LOD/2 = < 1.0 CFU/g

Table 4: Raw data for *L. monocytogenes* populations transferred from the honeydew rind dip-inoculated and stored at 4 and 30^{o}C for 24 h to the honeydew flesh from the stem scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	4.5	5.0	5.4
4	5 mm	1.2	1.8	2.2
4	10 mm	0.8	2.0	1.5
4	15 mm	0.9	1.6	1.1
4	20 mm	0.6	1.7	1.3
4	25 mm	0.5	1.2	0.9
30	Surface	4.1	3.8	4.0
30	5 mm	1.6	1.7	1.9
30	10 mm	1.4	1.5	2.4
30	15 mm	1.2	1.6	1.9
30	20 mm	1.6	1.4	2.3
30	25 mm	1.6	1.5	1.0

Table 5: Raw data for *L. monocytogenes* population transferred from the honeydew rind dip-inoculated and stored at 4 and 30°C for 24 h to the honeydew flesh from the blossom scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	2.8	4.1	3.8
4	5 mm	0.4	1.8	1.1
4	10 mm	0.2	1.2	1.0
4	15 mm	0.2	1.3	0.5
4	20 mm	< 1.0 CFU/g*	1.1	0.5
4	25 mm	< 1.0 CFU/g*	1.1	0.5
30	Surface	3.9	3.0	3.2
30	5 mm	1.1	< 1.0 CFU/g*	1.1
30	10 mm	1.0	< 1.0 CFU/g*	0.5
30	15 mm	0.4	< 1.0 CFU/g*	0.6
30	20 mm	0.4	< 1.0 CFU/g*	< 1.0 CFU/g*
30	25 mm	0.7	0.2	0.6

*LOD/2 = < 1.0 CFU/g

Table 6: Raw data for *L. monocytogenes* population transferred from the honeydew rind dip-inoculated and stored at 4 and 30°C for 24 h to the honeydew flesh from the rind circumference area.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	6.6	6.5	6.8
4	5 mm	3.2	3.0	4.5
4	10 mm	2.8	3.0	3.0
4	15 mm	3.1	2.8	5.0
4	20 mm	3.0	2.5	2.9
4	25 mm	1.7	3.6	2.5
30	Surface	6.6	7.6	6.9
30	5 mm	4.5	2.7	4.7
30	10 mm	3.6	2.2	3.7
30	15 mm	3.2	2.0	3.9
30	20 mm	3.2	2.2	3.6
30	25 mm	2.8	1.7	3.6

Table 7: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from stem scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	6.6	6.2	5.8
4	5 mm	4.4	3.1	2.8
4	10 mm	2.9	2.4	2.6
4	15 mm	3.1	5.4	2.3
4	20 mm	2.9	4.4	2.1
30	Surface	5.5	5.5	5.9
30	5 mm	2.5	2.7	3.7
30	10 mm	1.7	2.2	3.4
30	15 mm	1.7	1.7	3.0
30	20 mm	1.2	1.2	1.7

Table 8: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from blossom scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	6.1	5.9	5.8
4	5 mm	2.8	2.8	2.8
4	10 mm	2.4	3.3	2.5
4	15 mm	2.2	3.2	2.3
4	20 mm	2.5	1.9	2.2
4	25 mm	2.4	2.0	2.1
30	Surface	5.0	5.3	5.3
30	5 mm	1.0	1.6	2.9
30	10 mm	0.9	1.2	2.4
30	15 mm	1.0	1.4	2.4
30	20 mm	0.5	1.2	1.9
30	25 mm	0.5	1.4	1.9

Table 9: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30^{o}C for 24 h to the cantaloupe flesh from rind circumference area.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	4.4	4.2	4.4
4	5 mm	1.3	1.0	1.9
4	10 mm	1.4	1.2	1.3
4	15 mm	1.0	1.2	1.2
4	20 mm	1.0	1.0	1.3
4	25 mm	1.0	1.2	0.9
30	Surface	5.6	4.0	5.2
30	5 mm	3.5	1.2	2.2
30	10 mm	2.8	0.5	1.3
30	15 mm	2.4	1.2	1.0
30	20 mm	2.5	1.2	1.0
30	25 mm	0.8	1.2	1.2

Table 10: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30^{o}C for 24 h to the cantaloupe flesh from stem scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	4.3	4.1	4.4
4	5 mm	1.7	1.5	1.5
4	10 mm	1.2	0.9	1.5
4	15 mm	1.5	0.5	1.2
4	20 mm	1.2	0.9	0.5
30	Surface	5.1	4.0	5.9
30	5 mm	2.3	1.2	1.2
30	10 mm	1.7	0.5	0.5
30	15 mm	1.3	1.2	0.2
30	20 mm	1.2	1.2	1.2

Table 11: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from blossom scar.

Temperature	Depth	Replicate 1	Replicate 2	Replicate 3
4	Surface	4.1	3.9	4.2
4	5 mm	1.8	2.0	1.8
4	10 mm	1.7	2.0	1.2
4	15 mm	1.4	1.3	1.4
4	20 mm	1.0	1.3	1.2
4	25 mm	1.2	1.6	0.9
30	Surface	5.2	5.7	5.3
30	5 mm	2.5	2.2	0.9
30	10 mm	1.5	1.3	0.2
30	15 mm	1.7	1.0	0.9
30	20 mm	1.5	1.0	0.2
30	25 mm	1.2	0.5	0.2

Table 12: Raw data for *L. monocytogenes* populations transferred from the cantaloupe rind dip-inoculated and stored at 4 and 30°C for 24 h to the cantaloupe flesh from rind circumference area.

Method	Depth	Replicate 1	Replicate 2	Replicate 3
Apple Corker	Surface	5.2	5.5	5.5
Apple Corker	5 mm	4.1	4.2	4.1
Apple Corker	10 mm	3.8	3.1	3.2
Apple Corker	15 mm	3.8	3.4	3.1
Apple Corker	20 mm	3.5	3.2	2.9
Apple Corker	25 mm	3.4	3.2	2.9
Apple Corker	30 mm	3.2	3.5	2.5
Cork Borer	Surface	5.5	6.3	5.5
Cork Borer	5 mm	3.8	3.8	4.0
Cork Borer	10 mm	3.6	3.1	3.3
Cork Borer	15 mm	3.3	3.1	3.2
Cork Borer	20 mm	3.1	2.8	3.1
Cork Borer	25 mm	3.1	2.9	2.9
Cork Borer	30 mm	3.0	3.0	3.1

Table 13: Raw data for transfer of *L. monocytogenes* populations from the cantaloupe rind dip-inoculated and stored at 4ºC to the interior melon flesh by using an apple corer and cork borer to compare bacterial transfer.

Appendix B

Slicing and Dicing of Cantaloupe and Honeydew Melon

	Replicate 1	Replicate 2	Replicate 3
Inoculated Cantaloupe Halve 1a	1.4	2.7	2.2
Inoculated Cantaloupe Halve 1b	1.4	2.6	1.6
Inoculated Cantaloupe Halve 2a	2.6	2.7	2.4
Inoculated Cantaloupe Halve 2b	1.8	2.6	2.5
Uninoculated Cantaloupe Halve 3a	1.3	2.1	1.3
Uninoculated Cantaloupe Halve 3b	1.2	1.9	1.2
Uninoculated Cantaloupe Halve 4a	< 0.25 CFU/g	1.3	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 4b	< 0.25 CFU/g	1.3	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 5a	< 0.25 CFU/g	1.1	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 5b	< 0.25 CFU/g	1.2	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 6a	< 0.25 CFU/g	0.7	0.9
Uninoculated Cantaloupe Halve 6b	< 0.25 CFU/g	0.6	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 7a	< 0.25 CFU/g	0.4	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 7b	< 0.25 CFU/g	0.4	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 8a	< 0.25 CFU/g	1.7	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 8b	< 0.25 CFU/g	1.3	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 9a	< 0.25 CFU/g	0.6	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 9b	< 0.25 CFU/g	0.8	0.2
Uninoculated Cantaloupe Halve 10a	< 0.25 CFU/g	< 0.25 CFU/g	< 0.25 CFU/g
Uninoculated Cantaloupe Halve 10b	< 0.25 CFU/g	< 0.25 CFU/g	< 0.25 CFU/g

*LOD/2 = < 0.25 CFU/g

Table 14: Raw data for *L. monocytogenes* populations transferred from inoculated cantaloupe to uninoculated melon halves during mechanical slicing. Inoculation level at (5.2 ± 0.4 log CFU/cm^2).

	Replicate 1	Replicate 2	Replicate 3
Inoculated Honeydew Halve 1a	2.7	1.8	1.5
Inoculated Honeydew Halve 1b	2.5	2.5	1.2
Inoculated Honeydew Halve 2a	1.5	0.8	2.3
Inoculated Honeydew Halve 2b	1.5	1.1	1.6
Uninoculated Honeydew Halve 3a	0.6	0.5	0.9
Uninoculated Honeydew Halve 3b	0.8	0.2	< 0.25 CFU/g
Uninoculated Honeydew Halve 4a	0.4	0.3	1.5
Uninoculated Honeydew Halve 4b	0.3	< 0.25 CFU/g	< 0.25 CFU/g
Uninoculated Honeydew Halve 5a	0.6	0.8	1.0
Uninoculated Honeydew Halve 5b	0.3	< 0.25 CFU/g	0.4
Uninoculated Honeydew Halve 6a	< 0.25 CFU/g	< 0.25 CFU/g	1.1
Uninoculated Honeydew Halve 6b	< 0.25 CFU/g	< 0.25 CFU/g	0.2
Uninoculated Honeydew Halve 7a	< 0.25 CFU/g	< 0.25 CFU/g	0.6
Uninoculated Honeydew Halve 7b	< 0.25 CFU/g	0.2	< 0.25 CFU/g
Uninoculated Honeydew Halve 8a	< 0.25 CFU/g	< 0.25 CFU/g	0.4
Uninoculated Honeydew Halve 8b	0.2	< 0.25 CFU/g	< 0.25 CFU/g
Uninoculated Honeydew Halve 9a	< 0.25 CFU/g	< 0.25 CFU/g	< 0.25 CFU/g
Uninoculated Honeydew Halve 9b	< 0.25 CFU/g	< 0.25 CFU/g	< 0.25 CFU/g
Uninoculated Honeydew Halve 10a	< 0.25 CFU/g	< 0.25 CFU/g	0.4
Uninoculated Honeydew Halve 10b	< 0.25 CFU/g	< 0.25 CFU/g	0.3

*LOD/2 = < 0.25 CFU/g

Table 15: Raw data for *L. monocytogenes* populations transferred from inoculated honeydew melon to uninoculated melon halves during mechanical slicing. Inoculation level at (4.2 ± 0.2 log CFU/cm^2).

	Replicate 1	Replicate 2	Replicate 3
Inoculated Cantaloupe Halve 1	4.9	4.4	5.0
Inoculated Cantaloupe Halve 2	5.0	4.6	5.0
Uninoculated Cantaloupe Halve 3	4.1	2.9	3.7
Uninoculated Cantaloupe Halve 4	2.7	2.0	2.6
Uninoculated Cantaloupe Halve 5	1.7	0.7	2.3
Uninoculated Cantaloupe Halve 6	1.1	0.5	0.6
Uninoculated Cantaloupe Halve 7	1.9	0.3	0.5
Uninoculated Cantaloupe Halve 8	1.5	0.5	0.7
Uninoculated Cantaloupe Halve 9	1.6	0.3	2.2
Uninoculated Cantaloupe Halve 10	1.8	1.1	0.6

Table 16: Raw data for *L. monocytogenes* populations transferred from cantaloupe halves during mechanical dicing. Inoculation level at (5.5 ± 0.1 log CFU/cm^2).

	Replicate 1	Replicate 2	Replicate 3
Inoculated Cantaloupe Halve 1	3.4	2.8	3.8
Inoculated Cantaloupe Halve 2	3.0	3.6	3.2
Uninoculated Cantaloupe Halve 3	1.3	1.7	2.9
Uninoculated Cantaloupe Halve 4	1.0	1.0	3.0
Uninoculated Cantaloupe Halve 5	1.2	0.7	0.7
Uninoculated Cantaloupe Halve 6	1.2	1.0	0.1
Uninoculated Cantaloupe Halve 7	0.7	2.0	< 0.5 CFU/g
Uninoculated Cantaloupe Halve 8	0.7	2.0	< 0.5 CFU/g
Uninoculated Cantaloupe Halve 9	1.1	2.4	1.0
Uninoculated Cantaloupe Halve 10	0.7	1.2	0.1

*LOD/2 = < 0.5 CFU/g

Table 17: Raw data for *L. monocytogenes* populations transferred from inoculated cantaloupe halves to uninoculated cantaloupe halves during mechanical dicing. Inoculation level at (3.3 ± 0.2 log CFU/cm^2).

Day	Cantaloupe Halves	Replicate 1	Replicate 2	Replicate 3
0	Inoculated Cantaloupe Halve 1	3.4	2.8	3.8
3	Inoculated Cantaloupe Halve 1	3.8	2.8	3.7
7	Inoculated Cantaloupe Halve 1	4.1	2.7	4.0
0	Inoculated Cantaloupe Halve 2	3.0	3.6	3.2
3	Inoculated Cantaloupe Halve 2	3.9	2.7	3.4
7	Inoculated Cantaloupe Halve 2	3.8	2.1	4.2
0	Uninoculated Cantaloupe Halve 3	1.3	1.7	2.9
3	Uninoculated Cantaloupe Halve 3	1.4	0.8	2.5
7	Uninoculated Cantaloupe Halve 3	2.1	1.6	2.4
0	Uninoculated Cantaloupe Halve 4	1.0	1.0	3.0
3	Uninoculated Cantaloupe Halve 4	1.0	2.4	0.8
7	Uninoculated Cantaloupe Halve 4	1.0	1.8	1.1
0	Uninoculated Cantaloupe Halve 5	1.2	0.7	0.7
3	Uninoculated Cantaloupe Halve 5	1.0	1.7	0.7
7	Uninoculated Cantaloupe Halve 5	0.6	1.7	1.7
0	Uninoculated Cantaloupe Halve 6	1.2	1.0	0.1
3	Uninoculated Cantaloupe Halve 6	1.8	< 0.5 CFU/g	< 0.5 CFU/g
7	Uninoculated Cantaloupe Halve 6	1.7	2.1	2.2
0	Uninoculated Cantaloupe Halve 7	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 7	2.3	2.6	< 0.5 CFU/g
7	Uninoculated Cantaloupe Halve 7	2.6	2.2	1.9
0	Uninoculated Cantaloupe Halve 8	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 8	1.8	0.9	0.3
7	Uninoculated Cantaloupe Halve 8	2.0	1.8	1.8
0	Uninoculated Cantaloupe Halve 9	1.1	2.4	1.0
3	Uninoculated Cantaloupe Halve 9	1.7	0.1	< 0.5 CFU/g
7	Uninoculated Cantaloupe Halve 9	2.6	1.0	1.5
0	Uninoculated Cantaloupe Halve 10	0.7	1.2	0.1
3	Uninoculated Cantaloupe Halve 10	1.2	1.0	0.6
7	Uninoculated Cantaloupe Halve 10	1.1	1.5	1.2

*LOD/2 = < 0.5 CFU/g

Table 18: Raw data for *L. monocytogenes* populations transferred from inoculated cantaloupe halves to uninoculated halves during mechanical dicing and stored at 4ºC.

Day	Cantaloupe Halves	Replicate 1	Replicate 2	Replicate 3
0	Inoculated Cantaloupe Halve 1	3.4	2.8	3.8
3	Inoculated Cantaloupe Halve 1	5.1	3.6	5.8
7	Inoculated Cantaloupe Halve 1	6.3	5.6	7.5
0	Inoculated Cantaloupe Halve 2	3.0	3.6	3.2
3	Inoculated Cantaloupe Halve 2	4.7	3.6	5.8
7	Inoculated Cantaloupe Halve 2	5.5	6.1	7.7
0	Uninoculated Cantaloupe Halve 3	1.3	1.7	2.9
3	Uninoculated Cantaloupe Halve 3	2.5	2.4	3.9
7	Uninoculated Cantaloupe Halve 3	4.2	6.5	5.3
0	Uninoculated Cantaloupe Halve 4	1.0	1.0	3.0
3	Uninoculated Cantaloupe Halve 4	2.2	0.9	2.9
7	Uninoculated Cantaloupe Halve 4	4.4	5.5	4.6
0	Uninoculated Cantaloupe Halve 5	1.2	0.7	0.7
3	Uninoculated Cantaloupe Halve 5	3.6	2.1	3.0
7	Uninoculated Cantaloupe Halve 5	3.6	5.0	4.0
0	Uninoculated Cantaloupe Halve 6	1.2	1.0	0.1
3	Uninoculated Cantaloupe Halve 6	2.6	2.3	2.5
7	Uninoculated Cantaloupe Halve 6	5.1	5.2	4.2
0	Uninoculated Cantaloupe Halve 7	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 7	4.0	1.2	1.1
7	Uninoculated Cantaloupe Halve 7	4.5	5.0	3.3
0	Uninoculated Cantaloupe Halve 8	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 8	2.8	0.1	1.8
7	Uninoculated Cantaloupe Halve 8	4.7	5.2	3.7
0	Uninoculated Cantaloupe Halve 9	1.1	2.4	1.0
3	Uninoculated Cantaloupe Halve 9	2.8	1.9	2.2
7	Uninoculated Cantaloupe Halve 9	4.2	5.7	3.0
0	Uninoculated Cantaloupe Halve 10	0.7	1.2	0.1
3	Uninoculated Cantaloupe Halve 10	2.7	0.3	1.5
7	Uninoculated Cantaloupe Halve 10	2.9	5.6	2.9

*LOD/2 = < 0.5 CFU/g

Table 19: Raw data for *L. monocytogenes* populations transferred from inoculated cantaloupe halves to uninoculated halves during mechanical dicing and stored at 7^{o}C.

Day	Cantaloupe Halves	Replicate 1	Replicate 2	Replicate 3
0	Inoculated Cantaloupe Halve 1	3.4	2.8	3.8
3	Inoculated Cantaloupe Halve 1	7.7	6.6	7.7
7	Inoculated Cantaloupe Halve 1	8.0	7.9	9.3
0	Inoculated Cantaloupe Halve 2	3.0	3.6	3.2
3	Inoculated Cantaloupe Halve 2	7.8	6.4	7.9
7	Inoculated Cantaloupe Halve 2	7.8	7.7	9.0
0	Uninoculated Cantaloupe Halve 3	1.3	1.7	2.9
3	Uninoculated Cantaloupe Halve 3	6.1	5.3	6.6
7	Uninoculated Cantaloupe Halve 3	7.2	6.9	8.1
0	Uninoculated Cantaloupe Halve 4	1.0	1.0	3.0
3	Uninoculated Cantaloupe Halve 4	5.3	4.8	5.1
7	Uninoculated Cantaloupe Halve 4	7.2	6.1	6.6
0	Uninoculated Cantaloupe Halve 5	1.2	0.7	0.7
3	Uninoculated Cantaloupe Halve 5	4.2	4.8	5.0
7	Uninoculated Cantaloupe Halve 5	4.9	7.5	7.4
0	Uninoculated Cantaloupe Halve 6	1.2	1.0	0.1
3	Uninoculated Cantaloupe Halve 6	5.1	3.9	4.3
7	Uninoculated Cantaloupe Halve 6	5.5	6.9	7.4
0	Uninoculated Cantaloupe Halve 7	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 7	5.1	4.4	4.4
7	Uninoculated Cantaloupe Halve 7	6.7	7.2	6.6
0	Uninoculated Cantaloupe Halve 8	0.7	2.0	< 0.5 CFU/g
3	Uninoculated Cantaloupe Halve 8	4.6	4.5	4.2
7	Uninoculated Cantaloupe Halve 8	6.4	7.3	6.6
0	Uninoculated Cantaloupe Halve 9	1.1	2.4	1.0
3	Uninoculated Cantaloupe Halve 9	3.9	4.1	3.0
7	Uninoculated Cantaloupe Halve 9	6.2	7.1	6.5
0	Uninoculated Cantaloupe Halve 10	0.7	1.2	0.1
3	Uninoculated Cantaloupe Halve 10	3.9	3.7	2.9
7	Uninoculated Cantaloupe Halve 10	5.1	5.8	5.4

*LOD/2 = < 0.5 CFU/g

Table 20: Raw data for *L. monocytogenes* populations transferred from inoculated cantaloupe halves to uninoculated halves during mechanical dicing and stored at 10ºC.

Temperature	Days	Replicate 1	Replicate 2	Replicate 3
4	0	3.9	3.9	3.8
4	1	3.9	4.1	3.6
4	2	4.3	4.2	4.3
4	3	4.5	4.4	4.5
4	4	4.7	4.6	4.5
4	5	4.7	4.8	4.7
4	6	4.4	4.5	4.7
4	7	4.7	4.7	4.6
7	0	3.9	3.9	3.8
7	1	4.8	4.7	4.7
7	2	4.6	4.8	4.6
7	3	5.0	4.7	4.7
7	4	5.6	5.5	5.4
7	5	6.0	6.0	5.9
7	6	5.9	6.2	6.2
7	7	6.3	6.1	6.2
10	0	3.9	3.9	3.8
10	1	6.1	6.1	6.3
10	2	7.9	7.9	7.9
10	3	8.4	8.5	8.4
10	4	9.0	8.9	8.8
10	5	9.0	9.0	9.1
10	6	9.0	9.2	9.1
10	7	9.3	9.1	9.1

Table 21: Raw data for *L. monocytogenes* population growth on cantaloupe dices during subsequent storage of seven days at 4^{o}C, 7^{o}C, and 10^{o}C.

REFERENCES

REFERENCES

1. Akins, E. D., M. A. Harrison and W. C. Hurst. 2005. Microflora on Georgia-grown cantaloupes related to packaging and handling practices. IAFP 92nd Annual Meeting, International Association for Food Protection, Des Moines, IA.

2. Alvarado-Casillas, S., S. Ibarra-Sánchez, O. Rodríguez-García, N. Martínez-Gonzáles, and A. Castillo. 2007. Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *J. Food Prot.* 70(3):655-60.

3. Annous, B. A., A. Burke, and J. E. Sites. 2004. Surface pasteurization of whole fresh cantaloupes inoculated with *Salmonella* Poona or *Escherichia coli*. *J. Food Prot.* 67(9):1876-1885.

4. Annous, B. A., E. B. Solomon, P. H. Cooke, and A. Burke. 2005. Biofilm formation by *Salmonella* spp. on cantaloupe melons. *J. Food Saf.* 25(4):276-287.

5. Anonymous. 2005. Memorandum of understanding between the Food and Drug Administration Department of Health and Human Services of the United States of America and Servico Nacional de Sanidad, Inocuidad y CaIidad Agroalimentaria of the United Mexican States concerning entry of Mexican cantaloupes into the United States of America.

6. Barak, J. D., B. Chue, and D. C. Mills. 2003. Recovery of surface bacteria from and surface sanitization of cantaloupes. *J. Food Prot.* 66(10):1805-1810.

7. Behrsing, J., J. Jaeger, F. Horlock, N. Kita, P. Franz, and R. Premier. 2003. Survival of *Listeria innocua, Salmonella salford* and *Escherichia coli* on the surface of fruit with inedible skins. *Postharvest Biol. Technol.* 29(3):249-256.

8. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 2:204-216.

9. Bhagwat, A. 2006. Microbiological safety of fresh-cut produce: Where are we now?, p. 121-165 *In* A. Press., ed., *Microbiology of fresh produce*. Washington D.C.

10. Bigot, A., H. Pagniez, E. Botton, C. Fréhel, I. Dubail, C. Jacquet, A. Charbit, and C. Raynaud. 2005. Role of FliF and FliI of *Listeria monocytogenes* in flagellar assembly and pathogenicity. *Infect Immun.* 73:5530-5539.

11. Bottemiller, H. 2011. Rocky road ahead for cantaloupe after *Listeria* outbreak. Available at http://www.foodsafetynews.com/2011/11/rocky-road-ahead-for-cantaloupe-after-listeria-outbreak/#.Vbgk0unJDAU. Accessed 26 July 2015.

12. Bowen, A., A. Fry, G. Richards, and L. R. Beuchat. 2006. Infections associated with cantaloupe consumption: a public health concern. *Epidermiol. Infect.* 134(4):675-685.

13. Brackett, R. E. 1996. Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables, 269-312. In R. C. Wiley (ed.), Minimally Processed Refrigerated Fruits and Vegetables. Springer, U.S.A.

14. Buchanan, R., R. Lindqvist, T. Ross, M. Smith, E. Todd, and R. Whiting. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods interpretative summary. *Microbiological Risk Assessment Series 4 ©WHO / FAO*.

15. Buchholz, A. L., G. R. Davidson, B. P. Marks, E. C. Todd, and E. T. Ryser. 2012. Quantitative transfer of Escherichia coli O157:H7 to equipment during small-scale production of fresh-cut leafy greens. *J. Food Prot.* 75(7):1184-97.

16. Buck, J. W., R. R. Walcott, and L. R. Beuchat. 2003. Recent trends in microbiological safety of fruits and vegetables. Online. *Plant Health Progress*.

17. Castillo, A., I. Mercado, L. M. Lucia, Y. Martinez-Ruiz, J. Ponce de Leon, E. A. Murano, and G. R. Acuff. 2004. *Salmonella* contamination during production of cantaloupe: A bi-national study. *J. Food Prot.* 67(4):713-720.

18. Castillo, A. 2009. Melons, p. 189-221. *In.* G. Sapers, E. Soloman, and K. Matthews (ed.), The produce contamination problem: causes and solutions, 1st Ed. Academic Press, U.S.A.

19. Costa, R. 2015. Current issues in produce safety: The packinghouse. Available at http://www.foodsafetynews.com/2015/02/current-issues-in-produce-safety-the-packinghouse/#.VbgTAunJDAU. Accessed 26 July 2015.

20. CDC. 1991. Multistage outbreak of *Salmonella* Poona infections – U.S. and Canada. *MMWR* 40(32):549-552.

21. CDC. 2002. U.S. multistate outbreaks of *Salmonella* Poona infections associated with eating cantaloupe from Mexico, United States, and Canada, period 2000-2002. *MMWR* 51(46):1044-1047.

22. CDC. 2011. Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado. Available at http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/. Accessed 26 July 2015.

23. CDC. 2011. Estimates of foodborne illness in the United States. Available at http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed 26 July 2015.

24. Chimbombi, E., R. G. Moreira, E. M. Castell-Perez, and A. F. Puerta-Gomez. 2013. Assessing accumulation (growth and internal mobility) of *Salmonella Typhimurium* LT2 in fresh-cut cantaloupe (*Cucumis melo* L.) for optimization of decomination strategies. *J. Food Control.* 32(2):574-581.

25. Craigshead, S., S. Markland, K. Kniel, and H. Bais. 2015. *Bacillus subtilis* increases crop yield and reduces contamination by *Listeria monocytogenes* on cantaloupes. P3-163: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

26. Danyluk, M. D., R. McEgan, A. N. Turner, and K. R. Schneider. 2014. Outbreaks of foodborne illness associated with melons. Available at http://edis.ifas.ufl.edu/fs258. Accessed 26 July 2015.

27. Davidson, G. R. and E. T. Ryser. 2014. Impact of organic load on *Escherichia coli O157:H7* survival during pilot-scale processing of iceberg lettuce with acidified sodium hypochlorite. *J. Food Prot.* 77(10):1669-1681.

28. Del Rosario, B. A. and L. R. Beuchat. 1995. Survival and growth of enterohemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. *J. Food Prot.* 58(1):105-107.

29. den Bakker, H. C., C. A. Desjardins, A. D. Griggs, J. E. Peters, Q. Zeng, S. K. Young, C. D. Kodira, C. Yandava, T. A. Hepburn, B. J. Haas, B. W. Birren, and M. Wiedmann. 2013. Evolutionary dynamics of the accessory genome of *Listeria monocytogenes*: PLoS One 8(6): e67511.

30. Dev Kumar, G., J. Pinzon-Avila, T. Suslow, and S. Ravishankar. 2015. Baseline assessment of the prevalence of *Salmonella* and Listeria on cantaloupe and in select melon production environments in Arizona. T7-06: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

31. DeWaal, C. S. and M. Glassman. 2013. Outbreak alert! 2001-2010, Center for Science in the Public Interest. Available at http://cspinet.org/new/pdf/outbreak_alert_2013_final.pdf. Accessed 26 July 2015.

32. Duffy, E. A., L. M. Lucia, J. M. Kells, A. Castillo, S. D. Pillai, and G. R. Acuff. 2005. Concentrations of *Escherichia coli* and genetic diversity and antibiotic resistance profiling of *Salmonella* isolated from irrigation water, packing shed equipment, and fresh produce in Texas. *J. Food Prot.* 68(1):70-79.

33. European Food Safety Authority. 2013. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates. EFSA Journal 11(6):3241.

34. Fan, X., B. A. Annous, K. J. Sokorai, A. Burke, and J. P. Mattheis. 2006. Combination of hot water surface pasteurization of whole fruit and low-dose gamma irradiation of fresh-cut cantaloupe. *J. Food Prot.* 69(4):912-919.

35. Fang, T., Y. Liu, and L. Huang. 2013. Growth kinetics of *Listeria monocytogenes* and spoilage microorganisms in fresh-cut cantaloupe. *Food Microbiology.* 34(1):174-181.

36. Farber, J. M., W. H. Ross, and J. Harwig. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. *Int. J. Food Microbiol.* 30(1-2):145-156.

37. FDA. 1999. Survey of imported fresh produce. Available at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm. Accessed 26 July 2015.

38. FDA. 2005. FDA Industry Commodity Specific Food Safety Guidelines for the Melon Supply Chain. Available at http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM168625.pdf. Accessed 14 August 2016.

39. FDA. 2009. Food Code 2009. Available at http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm2019396.htm. Accessed 26 July 2015.

40. FDA. 2009. Guidance for industry: Guide to minimize microbial food safety hazards of melons; draft guidance. Available at http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm174171.htm. Accessed 26 July 2015.

41. FDA. 2011. Full text of the Food Safety Modernization Act (FSMA). Available at http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm247548.htm. Accessed 28 December 2015.

42. FDA. 2012. Information on the recalled Jensen Farms whole cantaloupe. Available at http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm272372.htm. Accessed 26 July 2015.

43. Gaylor, G. E., R. A. MacCready, J. P. Reardon, and B. F. McKernan. 1955. An outbreak of salmonellosis traced to watermelon. *Pub. Health Rep.* 70(3):311-313.

44. Gaul, L. K., N. H. Farag, T. Shim, M. A. Kingsley, B. J. Silk, and E. Hyytia-Trees. 2013. Hospital-acquired listeriosis outbreak caused by contaminated diced celery--Texas, 2010. *Clin Infect Dis.* 56(1):20-26.

45. Geldreich, E. E. and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market. *J. Milk Food Technol.* 94(4):184-195.

46. Gil, M. I. and M. V. Selma. 2006. Overview of hazards in fresh-cut produce production: control and management of food safety hazards, p. 156-198. *In* J. James (ed.), Microbial hazard identification in fresh fruit and vegetables. John Wiley and Sons, Inc., Hoboken, NJ.

47. Golden, D. A., E. J. Rhodehamel, and D. A. Kautter. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. *J. Food Prot.* 56(3):194-1296.

48. Green, T., L. Hanson, and L. Lee. 2005. North American approaches to regulatory coordination, p. 9-47. In K. Huff, K. D. Meilke, R. D. Nuston, R. F. Ochoa, J. Rude (ed.), *Agrifood regulatory and policy integration under stress*, Proc. 2nd Workshop of the North American Agri-food Market Integration Consortium, San Antonio, Texas.

49. Harris, L. J., J. N. Farber, L. R. Beuchat, M. E. Parish, T. V. Suslow, E. H. Garrett, and F. F. Busta. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Rev. Food Sci. Food Safety* 2(1):78-141.

50. Huang, J., Y. Luo, and X. Nou. 2015. Growth of *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut cantaloupe under different temperature abuse scenarios. *J Food Prot.* 78(6):1125-31.

51. Jackson, K., A. Conrad, L. H. Gould, and B. Jackson. 2015. Emergence of listeriosis outbreaks associated with produce in the United States, 1983-2014. P3-233: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

52. Kaminski, C. N., G. R. Davidson, and E. T. Ryser. 2014. *Listeria monocytogenes* transfer during mechanical dicing of celery and growth during subsequent storage. *J. Food Prot.* 77(5):765-71.

53. Keelara, S., J. Patel, D. Macarisin, and K. Venkitanarayanan. 2015. P1-165: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

54. Laksanalamai, P., L. A. Joseph, B. J. Silk, L. S. Burall, L. Tarr, P. Gerner-Smidt, and A. R. Datta. 2012. Genomic characterization of *Listeria monocytogenes* strains involved in a multistate listeriosis outbreak associated with cantaloupe in US. *PLoS One* 7(7):e42448.

55. Lamikanra, O., J. C. Chen, D. Banks, and P. A. Hunter. 2000. Biochemical and Microbial Changes during the Storage of Minimally Processed Cantaloupe. *J. Agric. Food Chem.* 48:5955-5961.

56. Li-Cohen, A. E. and C. M. Bruhn. 2002. Safety of consumer handling of fresh produce from the time of purchase to the plate: a comprehensive consumer survey. *J. Food Prot*. 65(8):1287-1296.

57. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, and R. Weaver. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319(13):823-828.

58. Low, J. C. and W. Donachie. 1997. A review of *Listeria monocytogenes* and listeriosis. *Vet. J.* 153:9-29.

59. Nguyen-the, C. and J. P. Prunier. 1989. Involvement of *Pseudomonas* in the deterioration of "ready-to-use" salads. *Int. J. Food Sci. Technol.* 24(1):47-58.

60. Nguyen-the, C. and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* 34(4):371-401.

61. Macarisin, D., Y. Chen, A. J. de Jesus, I. Tchagou, and P. Evans. 2015. Effect of postharvest practices on cantaloupe colonization by *Listeria monocytogenes*. T7-05: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

62. Mahmoud, B. S. 2012. Effects of X-ray treatments on pathogenic bacteria, inherent microflora, color, and firmness on whole cantaloupe. *Int. J. Food Microbiol.* 156(3):296-300.

63. McCollum, J. T., A. B. Cronquist, B. J. Silk, K. A. Jackson, K. A. O'Connor, S. Cosgrove, J. P. Gossack, S. S. Parachini, N. S. Jain, P. Ettestad, M. Ibraheem, V. Cantu, M. Joshi, T. DuVernoy, N. W. Fogg, J. R. Gorny, K. M. Mogen, C. Spires, P. Teitell, L. A. Joseph, C. L. Tarr, M. Imanishi, K. P. Neil, R. V. Tauxe, and B. E. Mahon. 2013. Multistate outbreak of listeriosis associated with cantaloupe. *N. Engl. J. Med.* 369:944-953.

64. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.*, 5(5):607-625.

65. Mukhopadhyay, S., D. Ukuku, and V. Juneja. 2015. Inactivation of *Salmonella enterica* and *Listeria monocytogenes* in cantaloupe puree by high pressure processing. P3-176: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

66. Nyarko, E., K. E. Kniel, P. D. Millner, Y. Luo, E. T. Handy, R. Reynnells, C. East, and M. Sharma. 2016. Survival and growth of *Listeria monocytogenes* on whole cantaloupes is dependent on site contamination and storage temperature. *Int. J. Food Microbiol.* 234:65-70.

67. Pollack, S. 2001. Consumer demand for fruit and vegetables: the U.S. example. In: Regmi A, ed. Changing Structure of Global Food Consumption and Trade. Washington,

DC: Economic Research Service/United States Department of Agriculture. WRS01-1:49–54.

68. Park, C. M. and L. R. Beuchat. 1999. Evaluation of sanitizers for killing *Escherichia coli* O157:H7, *Salmonella*, and naturally occurring microorganisms on cantaloupes, honeydew melons, and asparagus. *Dairy, Food Environ. Sanitation* 19:842-847.

69. Parnell, T., L. J. Harris, and T. Suslow. 2005. Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *Int. J. Food Microbiol.* 99:59-70.

70. Peel, M., W. Donachie, and A. Shaw. 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. *J. Gen. Microbiol.* 134:2171-2178.

71. Petran, R. and E. Zottola. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes*. *J. of Food Sci.* 54:458-460.

72. Prazak, M. A., E. A. Murano, I. Mercado, and G. R. Acuff. 2002. Antimicrobial resistance of *Listeria monocytogenes* isolated from various cabbage farms and packing sheds in Texas. *J. Food Prot.,* 65(11):1796-1799.

73. Rees, C., C. Dodd, and O. Nwaiwu. 2013. Risk of *Listeria*: fresh produce. Available at *http://www.nottingham.ac.uk/mic/documents/risk-of-listeria-fresh-produce.pdf*. Accessed 26 July 2015.

74. Richards, G. M. and L. R. Beuchat. 2004. Attachment of *Salmonella* Poona to cantaloupe rind and stem scar tissues as affected by temperature of fruit and inoculum. *J. Food Prot.* 67(7):1359-1364.

75. Ries, A. A., S. Zaza, C. Langkop, R. V. Tauxe, and P. A. Balke. 1990. A multistate of *Salmonella* Chester linked to improper cantaloupe. Abstr. p. 238. Prog. Abst. 30th Intersci. Conf. Antimicrob. Agents Chemother., Am. Soc. Microbiol., Washington D.C.

76. Rodgers, S. L., J. N. Cash, M. Siddiq, and E. T. Ryser. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J. Food Prot.* 67(4):721-31.

77. Rodriguez, A., W. R. Autio, and L. A. McLandsborough. 2007. Effect of biofilm dryness on the transfer of *Listeria monocytogenes* biofilms grown on stainless steel to Bologna and hard salami. *J. Food Prot.* 70(11):2480–2484.

78. Sauders, B. D. and Wiedmann M. 2007. Ecology of *Listeria* species and *L. monocytogenes* in the natural environment, p 21–53. *In* E. T. Ryser and E. H. Marth (ed.), *Listeria*, listeriosis, and food safety, 3rd E. CRC Press, Boca Raton, FL.

79. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerg. Infect. Dis.* 17(1):7-15.

80. Scollon, A., H. Wang, and E. T. Ryser. 2013. Transfer of *Listeria monocytogenes* during mechanical slicing of onion. P3-98: Abstr.102nd Annu. Meet. IAFP 2013. International Association for Food Protection, Des Moines, IA.

81. Scollon, A. and E. T. Ryser. 2014. Transfer of *Listeria monocytogenes* during pilot-scale dicing of onions, T4-01. Abstr.103rd Annu. Meet. IAFP 2014. International Association for Food Protection, Des Moines, IA.

82. Selma, M. V., A. M. Ibáñez, A. Allende, M. Cantwell, and T. Suslow. 2008. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli O157:H7* during cutting. *Food Microbiol.* 25(1):162-168.

83. SIAP-SAGARPA, 2002, SIACON.

84. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67(10):2342-53.

85. Strawn, L. K., Y. T. Grohn, S. Warchochki, R. W. Worobo, E. A. Bihn, and M. Wiedmann. 2013. Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Appl. Environ. Microbiol.* 79(24):7618-7627.

86. Suslow, T. V. and M. Cantwell. 2001. Recent findings on fresh-cut cantaloupe and honeydew melon. *Fresh Cut* 18-20.

87. Suslow, T. V. 2004. Minimizing the risk of foodborne illness associated with cantaloupe production and handling in California. Available at http://ucfoodsafety.ucdavis.edu/files/26308.pdf. Accessed 26 July 2015.

88. Suslow, T. V., A. Sbodio, J. Pinzon, G. Lopez-Velasco, R. Leong, P. Wei, L. Derby, G. Kumar, S. Ravishankar, and L. A. Richmond. 2015. On-farm Evaluation of the Prevalence of Human Enteric Bacterial Pathogens during the Production of Melons in California and Arizona. T7-04: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

89. Swaminathan, B. and P. Gerner-Smidt. 2007. The epidemiology of human listeriosis. *Microbes Infect*. 9(10):1236-1243.

90. Temple, M. E. and M. C. Nahata. 2000. Treatment of listeriosis. *Ann. Pharmacother.* 34(5): 656-61.

91. Ukuku, D. O. and G. M. Sapers. 2001. Effect of sanitizer treatments on *Salmonella stanley* attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *J. Food Prot.* 64(9):1286-91.

92. Ukuku, D. O. and W. F. Fett. 2002. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *J. Food Prot.* 65(6):924-930.

93. Ukuku, D. O. and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J. Food Prot*. 65(7):1093-1099.

94. Ukuku, D. O. and W. F. Fett. 2004. Method of applying sanitizers and sample preparation affects recovery of native microflora and *Salmonella* on whole cantaloupe surfaces. *J. Food Prot.* 67(5):999-1004.

95. Ukuku, D. O., W. F. Fett, and G. M. Sapers. 2004. Inhibition of *Listeria monocytogenes* by native microflora of whole cantaloupe. *J. Food Safety.* 24(2):129-146.

96. Ukuku, D. O., M. L. Bari, S. Kawamoto, and K. Isshiki. 2005. Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces. *Int. J. Food Microbiol.* 104(2):225-33.

97. Ukuku, D. O. and G. M. Sapers. 2005. Microbiological safety issues of fresh melons, p. 231-251. *In* G. M. Sapers, J. R. Gorny, and A. E. Yousef (ed.), Microbiology of fruits and vegetables. CRC Press, Boca Raton, Florida.

98. Ukuku, D. O. and G. M. Sapers. 2007. Effect of time before storage and storage temperature on survival of *Salmonella* inoculated on fresh-cut melons. *Food Microbiol.* 24(3):288-95.

99. Ukuku, D. O., M. Olanya, D. J. Geveke, and C. H. Sommers. 2012. Effect of native microflora, waiting period, and storage temperature on *Listeria monocytogenes* serovars transferred from cantaloupe rind to fresh-cut tissues during cutting practices. *J. Food Prot.* 75(11):1912-1919.

100. Ukuku, D. O., L. Huang, and C. Sommers. 2015. Efficacy of sanitizer treatments on survival and growth parameters of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on fresh-cut pieces of cantaloupe during storage. 2015. *J. Food Prot.* 78(7):1288-1295.

101. [USDA] U.S. Dept. of Agriculture, Economic Research Service. 1999 Oct. Fruit and Tree Nut Tree Nut Situation and Outlook Report. FTS-287.

102. Vázquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev.* 14(3):584-640.

103. Volokhov, D., A. Rasooly, K. Chumakov, and V. Chizhikov. 2002. Identification of *Listeria* species by microarray-based assay. *J. Clin. Microbiol.* 40(8):4720-4728.

104. Vorst, K. T., E. C. Todd, and E. T. Ryser. 2006. Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. *J. Food Prot.* 69(3):619-26.

105. Walsh, D., G. Duffy, J. J. Sheridan, I. S. Blair, and D. A. McDowell. 2001. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J. Appl. Microbiol.,* 90(4):517-522.

106. Walsh, K., L. H. Gould, and S. Bennett. 2013. Melon-associated outbreaks of foodborne disease in the United States, 1973-2011. P3-150: Abstr. 102nd Annu. Meet. IAFP 2013. International Association for Food Protection, Des Moines, IA.

107. Wang, H. and E. T. Ryser. 2013. Quantitative transfer of *Salmonella* during commercial slicing of tomatoes as impacted by multiple processing variables. T8-04: Abstr. 102nd Annu. Meet. IAFP 2013. International Association for Food Protection, Des Moines, IA.

108. Wang, H. and E. T. Ryser. 2014. Microbiological safety of fresh-cut produce from the processor to your plate. *Food Safety Magazine*. Available at http://www.foodsafetymagazine.com/magazine-archive1/augustseptember-2014/microbiological-safety-of-fresh-cut-produce-from-the-processor-to-your-plate/. Accessed 26 July 2015.

109. Wang, M., E. Lambertini, S. Micallef, and A. Pradhan. 2015. Quantitative risk assessment for *Listeria monocytogenes* in Cantaloupe. P2-231: Abstr. 104th Annu. Meet. IAFP 2015. International Association for Food Protection, Des Moines, IA.

110. Yoon-Ki, H. W. B. Yoon, and L. Huang. 2014. Predictive modeling for growth of non- and cold-adapted *Listeria monocytogenes* on fresh-cut cantaloupe at different storage temperatures. *J. Food Sci.* 79(6):M1168-M1174.

111. Zhang, Y., M. Qiumin, F. Critzer, P. M. Davidson, and Q. Zhong. 2015. Effect of alginate coatings with cinnamon bark oil and soybean oil on quality and microbiological safety of cantaloupe. *Int. J. Food Microbiol.* 215(23):25-30.

112. Zilelidou, E. A., V. Tsourou, S. Poimenidou, A. Loukou, and P. N. Skandamis. 2015. Modeling transfer of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during preparation of fresh-cut salads: impact of cutting and shredding practices. *Food Microbiol.* 45(Pt B):254-65.