ABSTRACT

Title of Document: ASSESSMENT OF PREHARVEST
                      MICROBIAL QUALITY OF CANTALOUPES
                      AND PUBLIC HEALTH RISKS ASSOCIATED
                      WITH CANTALOUPES CONTAMINATED
                      WITH *LISTERIA MONOCYTOGENES*

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Cantaloupe has been recognized as a common vehicle for foodborne infections among fresh produce commodities. A severe multistate outbreak of *Listeria monocytogenes* associated with the consumption of whole cantaloupe resulted in 33 deaths in 2011. Quantitative microbial risk assessment (QMRA) modeling in food safety risk analysis has been acknowledged as an efficient tool to estimate and provide knowledge needed to manage potential public health risks associated with foodborne pathogens. The objectives of this study were to (i) conduct a microbiological survey of pre-harvest cantaloupes from farms in mid-Atlantic region, and (ii) develop a "farm to table" QMRA model for *L. monocytogenes* in cantaloupe. The results of the regional microbiological survey indicated a 5.3% (2/38) prevalence

of generic *Escherichia coli* and negative for *L. monocytogenes* and *Salmonella* on cantaloupe during harvest season. A QMRA model was developed based on a thorough review of data from scientific publications and communications with fresh-cut processing industry. The model was simulated with Monte Carlo technique for 100,000 iterations in @Risk. The model estimated the public health risks associated with the consumption of both fresh-cut and whole cantaloupes in the U.S. The model demonstrated the risk associated with the consumption of a serving of fresh-cut cantaloupe is around 10 times higher than that for whole cantaloupe. Using the baseline model, the estimated median number of listeriosis cases per year associated with the consumption of fresh-cut cantaloupe among susceptible subpopulation and general healthy population are 0.0368 and 0.00134, respectively. Sensitivity analysis suggested temperature control during retail (correlation coefficient: 0.69) and home storage (correlation coefficient: 0.48) are two critical factors in mitigating the risk for fresh-cut cantaloupe consumption while home storage temperature (correlation coefficient: 0.79) after cutting is the most important factor for whole cantaloupe consumption. The QMRA model provided critical information for risk management and identified the critical data gaps including initial contamination and prevalence for future risk assessments of melon.

ASSESSMENT OF PREHARVEST MICROBIAL QUALITY OF CANTALOUPES
AND PUBLIC HEALTH RISKS ASSOCIATED WITH CANTALOUPES
CONTAMINATED WITH *LISTERIA MONOCYTOGENES*

By

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Chapter 1: Introduction

The risk of illness associated with the consumption of raw and minimally processed produce has drawn increased scrutiny in the past decade. It is estimated that produce commodities accounted for 46% of illnesses per year in the U.S. (Painter et al., 2013). As a species of the Family *Cucurbitaceae* with attractive natural flavor and abundant vitamin content, cantaloupe is also known as muskmelon and rock melon. It offers a perfect habitat for the human pathogens because of its low acidity (pH 5.2 to 6.7) and high water activity (0.97 to 0.99) (Golden et al., 1993). The 2011 *Listeria monocytogenes* outbreak associated with the consumption of whole cantaloupe grown on farm in Colorado resulted in 33 deaths and 145 hospitalizations amplified already substantial public health concerns about the microbiological safety of cantaloupes (Cosgrove et al., 2011). Hence, it is critical to know about the public health risk associated with the consumption of contaminated cantaloupe.

Quantitative microbial risk assessment (QMRA), a component of risk analysis process, is being increasingly used as a tool to estimate the risk of foodborne illnesses as well as to evaluate the different intervention strategies, aiming at protecting public health combined with risk management and risk communication.

The objectives of this study were to, (i) conduct a microbiological survey of pre-harvest cantaloupes and field environment of cantaloupe farms in mid-Atlantic region, and (ii) develop a QMRA model to estimate the risk of human listeriosis that could be acquired by the consumption of either fresh-cut or whole cantaloupe.

Chapter 2: Literature review

2.1 Public health burden of melon

In the United States, it is estimated that, among 37.2 million illnesses and 2,612 deaths each year caused by 31 pathogens, 9.4 million illnesses and 1,315 deaths were caused by the consumption of contaminated food (Scallan et al., 2011). Recent outbreaks and recalls associated with melons contaminated with *Salmonella* spp. and *Listeria monocytogenes* emphasized melons as emerging food vehicles for these two pathogens, highlighting these product-pathogen pairs as critical and emerging food safety issues. A total of 98 outbreaks linked to fresh fruits and vegetables occurred in the U.S. between 1996 and 2006, and melons - *Salmonella* ranks second most frequently implicated produce - pathogen pair, behind leafy greens - *Escherichia coli* O157:H7 (FAO/WHO, 2008).

Served as fresh-cut pieces or mixtures for salad, melon has become popular worldwide due to its attractive flavor and rich content in micronutrients. In 2011, the U.S. produced 1.9 billion pounds of cantaloupes, 322 million pounds of honeydew, and 3.9 billion pounds of watermelon. The production value of cantaloupe reached $350 million in 2011. In 2012, the estimated domestic consumption of melons reached 7.70 billion pounds which equated to an annual per capita consumption of 24.5 pounds (USDA, 2012). However, more than 35% of the consumers responded in a national survey that they do not wash their melons before consumption, and almost half of the respondents indicated handling fresh produce without washing hands (Li-Cohen et al., 2002). Since melon is almost always eaten raw or after minimal

processing, any level of contamination on the surface can present a potential risk to consumer.

The first report of an outbreak linked to melon involved sliced watermelon contaminated with *Salmonella* Miami in 1955 which resulted in 7 illnesses (Gayler et al., 1955). 34 outbreaks caused by melons were reported from 1973-2011, resulting in 3,601 illnesses and 45 deaths, among which 19 outbreaks caused by cantaloupe (Walsh et al., 2013) (Table 1). Although most of outbreaks are caused by *Salmonella* spp., a large-scale outbreak in 2011 caused by *Listeria monocytogenes* resulted in 33 deaths which has the highest number of deaths of any foodborne outbreak since 1998. This is also the first documented listeriosis outbreak associated with fresh whole melon in the United States (CDC, 2011; FDA, 2011). Most recently, an outbreak of 261 infections and 3 deaths caused by *Salmonella enterica* serotype Typhimurium was traced back to the cantaloupe from a farm in Indiana (CDC, 2012).

Progress toward the improving food safety practices for melons have been made by collaboration among industry, government agencies and academia. In 2005, the Produce Industry Food Safety Initiative published the document "Commodity Specific Food Safety Guidelines for the Melon Supply Chain", which provides voluntary guidelines from the melon industry on food safety practices that help minimize the microbiological hazards associated with fresh and fresh-cut melons. In 2008, a meeting report "Microbiological hazards in fresh fruits and vegetables" was crafted by the joint FAO/WHO to response the request for scientific advice from the 38th Codex Committee on Food Hygiene (CCFH). In June 2011, the report "Microbiological Hazards and Melons", which specifically addressed the microbial

safety of the melon supply chain, was also generated in response to the request of CCFH (FAO/WHO, 2011). In 2011, U.S. FDA issued a guidance for melon industry in response to the multistate listeriosis outbreak associated with cantaloupe from Colorado. In addition, National Commodity-Specific Food Safety Guidelines for Cantaloupes and Netted Melons was released in 2013. Developed by a broad national coalition of industry stakeholders, government and academia representatives, the guidance provides a comprehensive framework from farm to fork for ensuring the highest level of food safety in melon supply chain but no information involved in melon processing and retail consumption part. In February of 2013, U.S.FDA issued a letter to cantaloupe industry, strongly encouraging to follow the good agricultural practices, as well as informing the FDA's inspection toward packinghouses over the upcoming harvest season in this year.

Table 1. Recent foodborne outbreaks associated with the consumption of cantaloupe

Year	Microorganism	Deaths/Illnesses	Reference
2006	*Salmonella* Saintpaul	0/36	Munnoch et al., 2009
2007	*Salmonella* Litchfield	0/111	CDC, FOOD
2008	Norovirus	0/23	CDC, FOOD
2011	*Salmonella* Panama	0/20	CDC, FOOD
2011	*Listeria monocytogenes*	33/145	CDC, 2011
2012	*Salmonella* Typhimurium	3/261	CDC, 2012

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2.2 Melon and pathogens

Melon was identified as the second highest concern of microbiological hazards in fresh produce commodity due to its widespread production, vulnerability to contamination and associated outbreaks (FAO/WHO, 2008). Quite a few factors may explain its vulnerability to contamination. Firstly, melons grow directly on the ground, a large potential reservoir for human pathogens. Warm and humid environments that are beneficial for growing melon in summer are also favorable for the growth of pathogens (FAO/WHO, 2011). In addition, the neutral pH of melon flesh (6.1 to 7.1) with its high sugar content and water activity make it an ideal environment for pathogen growth (Golden et al., 1993). In addition, cantaloupe rinds have a topography of crevices and cracks can provide a "netty" surface where microorganisms can strongly attach and biofilm may be formed on the surface. This makes it difficult to be removed microorganisms by washing treatments (Gerchikov et al., 2008; Annous, 2004; Parnell et al., 2005). A study of the relationship between pathogen attachment on cantaloupe rind and both bacteria cell surface charge and hydrophobicity found that the rank of pathogen attachment to cantaloupe rind is *Salmonella*, *L. monocytogenes* and *E. coli*, (Ukuku and Fett, 2002c). The authors discussed this as an indication of the relatively inefficiency of washing treatments for the removal of pathogens from cantaloupe surface. Conversely, surfaces of honeydew and watermelon are relatively smooth, which result in fewer attached microorganisms than the cantaloupe (Ukuku and Fett, 2002b; Parnell et al., 2005). Apart from the netted surface, the stem scar, where melon is separated from the vine, can also be a potential area for contamination (Guidance, 2013).

Salmonella is the most common reported etiological agents for outbreaks associated with melons (Walsh et al., 2013), although some microbiological surveys showed a low prevalence of *Salmonella* spp. on cantaloupe (Table 2). *Listeria monocytogenes*, as one of the four most deadly foodborne pathogens, is estimated to be responsible for 19% fatality each year in the United States (Scallan et al., 2011). Ready-to-eat meat, dairy, and seafood products are generally considered as common vehicles and account for a relatively high percentage of foodborne listeriosis cases (Czuprynski et al., 2005). Although *L. monocytogenes* did not show a high prevalence in fresh produce (Abadias et al., 2008; Koseki et al., 2011) including cantaloupes (Table 2) and listeriosis is identified as low risk in fresh fruits (0.9 case per year) and vegetables (0.2 case per year) by U.S. FDA/FSIS (FDA, 2003), fresh produce including melon can also serve as a vehicle for listeriosis regarding to several outbreaks and recalls in recent years (Cosgrove et al., 2011; McCollum et al., 2013).

Table 2. Prevalence studies of human pathogens investigated for cantaloupes

Sample	Prevalence	Reference
40 Cantaloupes of two seasons	Negative for *Salmonella* and *Listeria*	Materon, 2003
1250 Cantaloupes; 185 water; 60 environmental samples	*Salmonella*: 1.8% (31/1735) in general; 0.56% (7/1250) in cantaloupes	Castillo et al., 2004
398 produce (90 cantaloupe)	*Salmonella*: 3.3% (3/90)	Johnston et al., 2005
1257 samples in total	*Salmonella*: 0/100 in field cantaloupes; 3/100 in shed cantaloupes; 16/170(9.4%) in irrigation; 6/280(2.1%) in equipment; 0/12 in soil; 0/165 in wash water	Duffy et al., 2005
466 produce (42 melons)	Negative for *E. coli* O157:H7 and *Salmonella*. 3/466 for *L. monocytogenes* (cabbage)	Johnston et al., 2006
499 whole cantaloupe	1/499 positive for Salmonella	CFIA, 2014
593 imported, 302 domestic whole cantaloupes and 312 imported fresh-cut cantaloupe	Negative for *Salmonella* spp. and *Shigella* spp.	CFIA, 2010

2.3 Risk factors in the supply chain of melon

Major identified risk factors contributing to foodborne illness outbreaks that have been attributed to cantaloupes include: water quality for both pre- and post-harvest, residual surface moisture, equipment and packing facility sanitation

(Guidance, 2013). Cantaloupes may become contaminated before harvest, during harvesting, packing and processing (Bowen et al., 2006).

2.3.1 Pre-harvest

Some systematic reviews shed a light on the contamination sources in pre-harvest stage including irrigation water, soil, animal or human feces, organic fertilizer, human activity (Park et al., 2012; Doyle et al., 2012). Soil is a natural reservoir for various human pathogens and appears to be the primary source of pathogens for fruits at the pre-harvest level (Beuchat, 1995; Tauxe et al., 1997), *L. monocytogenes* and *S. enterica* can survive in soil for up to 8 and 23 weeks, respectively (Bowen et al., 2006). Water is indispensable for crop growth and pathogens are at high levels in furrows that were flood irrigated, which make it possible for the pathogen internalization (Gagliardi et al., 2003). Water is also a vehicle for pesticide and liquid fertilizer applications (Ng et al., 2005). One study estimated that the average percent transfer of bacteria from water used to dilute pesticides sprayed onto the surfaces of cantaloupe was estimated to 2.1×10^{-4} % with a maximum value of 3.3×10^{-4} % (Stine et al., 2005).

Enteric pathogens could enter plant tissues through plant roots, flowers, stomata and damaged cracks with a short-term persistence (Erickson, 2012). Low transfer of pathogen cells from roots into cantaloupe and honeydew fruit was observed under greenhouse conditions (Suslow, 2010). Microorganisms survived longer on cantaloupe under low relatively humidity than lettuce and peppers in pre-harvest stage (Stine et al., 2005). Improper field work could also contribute to the melon surface contamination in pre-harvest stage (Bowen et al., 2006).

2.3.2 Post-harvest

Although the purpose of post-harvest stage is to eliminate the possible pathogens introduced from the field, cantaloupe can be contaminated with pathogens through washing and packing in post-harvest stage. Risk factors may contribute to the pathogen contamination and growing in unmonitored disinfectant level of water, packinghouse and equipment design, packing and holding practices (FDA, 2011).

In the shed-pack postharvest processing, cantaloupes were brought to packinghouse, washed, sorted, and hand packed into shipping cartons. Melons typically are cooled either by forced-air or chilled water drench before packing. Warm fruit without removal of field heat has an increased water activity that allows the formation of condensation which is favorable for pathogens survival and growth (FDA, 2009). Although melon cooling with chlorine water may reduce microbial loads on the melon surface by 2-3 log CFU (Annous et al., 2004), pathogen could be internalized from cooling water to the cantaloupe by prolonged soaking time which may serve as a source of contamination (FDA, 2009; Erickson, 2012). In addition, submersion of warm melons in cool dump tank water may facilitate the internalization of pathogens into the cantaloupe via their stem scars or netting surface by creating an infiltration driving force (FDA, 2009; Guidance, 2013). Investigation indicated that much of the melon contamination during postharvest processing could be traced to a primary wash tank or hydrocooler (Gagliardi et al., 2003).

Some of microbial investigations demonstrated a higher microbial contamination of cantaloupe at packing shed than that in the farm field (Johnston et al., 2005; Ailes et al., 2008). The increased water activity on the wet surface of melon after postharvest washing in packing shed may enable the survival and growth of

pathogens during subsequent storage and transport. In addition, unsanitary food contact surface where the biofilm formed during fresh cutting and packing could contaminate the melon and provide nutrients for pathogens to persist on the melon surface. Transmission of pathogens from hands to melons also plays a significant role in postharvest stage. Human pathogens may also be transmitted to the melon during washing and packing by direct contact with contaminated workers (Guidance, 2013).

Pathogens attached to the surface of the rind can be transferred to the interior edible flesh during postharvest cutting (Beuchat, 1996; Lin and Wei, 1997; Ukuku and Sapers, 2001; Ukuku et al., 2005), but no transfer was observed after washing with sanitizer (Ukuku et al., 2012; Rodgers et al., 2004). Vadlamudi et al. (2012) concluded that peeling the rind before cutting cantaloupe was more effective in reducing transfer of *Salmonella* to the internal flesh than cutting cantaloupes prior to rind removal.

2.4 Risk reduction strategies

Numerous studies have focused on the reduction of pathogen population on melon surface. Water is applied to remove soil, pesticide residues and other dirt from melon surface with minimum efficacy in removal of pathogens (Beuchat, 1996). Washing with water did not cause a significant reduction of either native microflora or human pathogens (Ukuku and Fett, 2002; Ukuku et al., 2005; Casillas et al., 2007). A difference of 0.9 log CFU/g of *L. monocytogenes* was observed between inoculated and water washed cantaloupe during storage at 4°C (Rodgers et al., 2004). Ukuku et al (2012) reported a 0.2 log CFU/cm^2 reduction of *L. monocytogenes* on surface of water washed cantaloupe.

Although the mild heat treatment, ozone, organic acid, irradiation and bacteriophage have been demonstrated to provide some degree of risk reduction (Palekar et al., 2004; Ukuku et al., 2005; Fan et al., 2006; Selma et al., 2008; Sharma et al., 2009; Mahmoud, 2012; Ukuku et al., 2012; Vadlamudi et al., 2012; Annous et al., 2013; Oliveira et al., 2014), chlorine washing is still the predominant practice in melon industry based upon its low cost, broad spectrum of antimicrobial activity and high efficacy. Combinations of different sanitizer concentrations and durations of washing have been evaluated for *Salmonella* and *L. monocytogenes* (Table 3).

However, limitations of chemical sanitizer on reduction of pathogens on surface of cantaloupe have been reported (Fan et al., 2009). Chlorine and hydrogen peroxide could corrode the tanks and steel equipment used in packing or processing operations, and human exposure of chlorine residue in fresh produce is reported to have the potential carcinogenicity. In addition, chlorine water can reduce but not

eliminate pathogens (Golden et al., 1993). Therefore, other disinfection alternatives of intervention strategies may be needed for further risk mitigation.

Table 3. Efficacy of different sanitizers in cantaloupe decontamination

Pathogen type	Sanitizer	Pathogen log reduction/cm²	Pathogen transfer from rind to flesh	Reference
Salmonella	1000 ppm chlorine for 5 min	3.4	Below detection	Ukuku et al., 2001
Salmonella	5% 70°C hydrogen peroxide; 97°C hot water	3.8; 3.4	No transfer	Ukuku et al., 2004
Listeria monocytogenes	2.5% hydrogen peroxide	2.8	Below detection	Ukuku et al., 2012
Listeria monocytogenes	1000 ppm chlorine and 5% hydrogen peroxide	2.0; 3.5	Occur if *L. monocytogenes* on the melon rind is 2 log/cm² or more.	Ukuku and Fett, 2002
Salmonella	60 s 200 ppm chlorine	1.8 per melon on rind	N/A	Parnell et al., 2005
Listeria monocytogenes	5 min/200 ppm chlorine	Around 6 log	N/A	Rodgers et al., 2004

Chapter 3

Microbiological survey of pre-harvest cantaloupe from farms in mid-Atlantic region

3.1 Introduction

Recent outbreaks of human salmonellosis and listeriosis associated with consumption of cantaloupe raised the awareness of public health risks associated with the cantaloupe. Studies investigated the risk factors associated with pre-harvest contamination of fresh produce and suggested that monitoring microbial contamination level of irrigation water and soil is the most effective strategies for the prevention and control of pre-harvest contamination (Olaimat and Holley, 2012; Park et al., 2012; Doyle and Erickson, 2012). Melons are grown on the ground with warm and humid environment where bacteria could survive and grow in the presence of abundant organic substrates and in protected conditions (e.g. shaded from UV radiation, neutral pH). If melons are grown directly on soil, soil particles and bacteria can attach to the fruit surface over the course of the growing season. Compared to the honeydew and watermelon with smooth rind, cantaloupes are particularly vulnerable to attachment of pathogens because of their rind topography of gullies and niches where microorganisms can attach and remain shielded (Ukuku et al., 2002). Once attached to the rind, microorganisms are very difficult or impossible to be removed or inactivate completely without compromising the fresh attribute of the fruit (Parnell et al., 2005). Plastic mulch is conventionally used to warm the soil, conserve water and control the weeds (Foord, 2009), but the impact of this pre-harvest operation on

prevention of contamination has not been fully assessed. The effects of pre-harvest operations on the microbiota level on the cantaloupe were not clarified yet. Pre-harvest contamination of pathogenic and non-pathogenic bacteria in cantaloupe field was not fully investigated in the mid-Atlantic region. To date, few studies have investigated the small-scale microbial ecology on the surface of cantaloupe from mid-Atlantic region, such as the difference in microbial community on stem scar, upper portion of the melon exposed to sunlight, and bottom portion of the melon in contact with soil or mulch.

3.2 Materials and methods

3.2.1 Sample collection

Five farms in the mid-Atlantic region of the United States (three in Maryland and two in Delaware) were visited from June to September 2013, for a total of seven sampling sessions. For each sampling session, an equal number (n=3-4) of melons growing in direct contact with soil and on plastic mulch were collected. Each melon was cut from the vine with sterile scissors. To avoid hand touch on the melon rind, a large plastic bag was used to coat the melon from top to bottom. The bag was then gently inverted, closed with a tie to prevent the melon from rolling inside the bag, and placed in a cooler. For melons in direct contact with the soil, soil samples of approximately 50 g were scooped using a sterile spatula. For melons in contact with plastic mulch, the portion of the mulch touching the fruit was swabbed by wiping a 10×10 cm^2 area of plastic mulch with a sterile swab previously dipped in Buffer Peptone Water (BPW), and then placed into centrifuge tubes filled with 10 mL of BPW. When feasible, one liter water sample was collected from the source of

irrigation water (groundwater well or pre-irrigation pond). All non-disposable tools used in the field were wiped or sprayed with 70% ethanol and air dried before each sampling. Samples were transported to the lab in cooler filled with ice and transferred to a 4 ºC freezing room. Water samples were processed within 24 hours, while other samples were processed within 48 hours from collection.

3.2.2 Sample preparation

Prior to microbiological testing, three 7-cm diameter discs (approximately 50 g) were cut out from following areas of each melon: around the stem, ground spot, and the upper surface. Each melon disc was placed in a separate stomacher bag, with 50 mL BPW. 10 gram soil was weighed and 90 mL BPW was added to each soil sample. Each melon disc sample was shaken with 250 rpm for 2 min to remove the attached bacteria from niches on the rind. Soil and swabs samples were vortexed for 1 min. All samples were incubated at room temperature for 1-1.5 hours prior to quantitative test.

3.2.3 Microbiological identification and quantification

After incubation at room temperature for 1-1.5 hours, serial dilutions were made for bacteria quantification. 100 μL of the appropriate dilution was plated with a sterile spreader onto TBX, mFC and OXA plates for the identification and quantification of *E. coli*, fecal coliform and *Listeria* spp., respectively. TBX and mFC plates were incubated at 44°C for 24 ± 2 hours, while OXA plates were incubated at 35-37°C for 24-48 ± 2 hours due to a probable longer recovery time of some environmental *Listeria* spp. BPW suspensions were incubated at 35-37°C for 24±2 hours for pathogen enrichment.

3.2.4 Pathogen testing

1 mL of 24h-enriched BPW suspension was transferred to 15 mL TT Hajna solution with 1.2 mL iodine. At the same time, 10 μL of enriched BPW suspension was streaked on a Chromagar Listeria plate. The TT solution was re-incubated for 24 hours, and then 10 μL of suspension was streaked on XLT4 agar for *Salmonella* isolation. Both Chromagar Listeria and XLT4 plates were incubated at 35-37°C for 24 ± 2 hours. Target colonies of blue with white halo on Chromagar Listeria plates and black colonies on XLT4 plates were picked and restreaked for confirmation, and streaked on TSA for further isolation and purification. Isolated colonies were archived in Brucella Broth with 15% glycerol at -80°C.

3.2.5 Statistical analysis

Microbiological plate count data from TBX, mFC and OXA plates were converted to log CFU per square centimeter for melon discs and mulch surface swab samples, and log CFU per gram for soil samples. The bacterial counts were statistically analyzed by analysis of variance (ANOVA) (two-way and one-way) for differences in response to melon surface type (ground spot (GS), upper surface (US), and stem (ST)) and growing type (soil and mulch). Student's t (Least Significant Difference; LSD) multiple comparison test was used to separate means at a significance level of $\alpha = 0.05$, when the overall F test indicated significant differences. Specifically, two-way ANOVA to evaluate the effects of melon surface type and growing type together, one-way ANOVA to evaluate the effects of melon surface type, and two sample t-test to evaluate the effects of growing type on differences in mean bacterial count were used in the analysis. All statistical analyses

were conducted using statistical software JMP Pro 10.0.2 (SAS Institute, Cary, NC, USA).
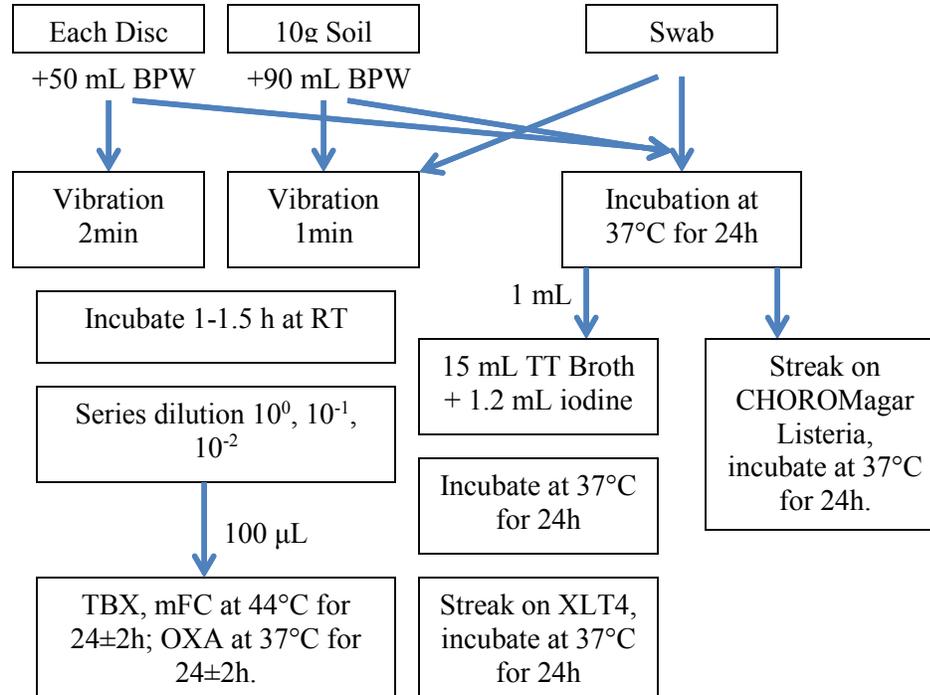
Figure 1. Experimental protocol of pre-harvest microbiological survey for cantaloupe in mid-Atlantic region.

3.3 Results

A total of 38 melons were collected during the harvest season in 2013, originated from five farms in Maryland and Delaware, among which 16 melons were growing on plastic mulch and 22 melons on ground soil. Thus, 48 and 66 melon discs of different melon surface areas were tested for plastic mulch and soil respectively. Besides, 41 environmental samples were obtained, including 22 soil samples, 16 swabs for swiping the mulch surface and 3 irrigation water (2 pond water and 1 ground water) samples.

3.3.1 Microbial quality of cantaloupe

Prevalence of generic *E. coli*, fecal coliforms, *Listeria* spp. and *Salmonella* are shown in Table 4. Generic *E. coli* was detected in two melon discs with 2.41 and 3.78 log CFU/cm^2. Fecal coliforms were detected on 27 of 114 melon discs ranging from 2.11 to 4.37 log CFU/cm^2, with 21/66 (31.8%) from melons growing on soil and 6/48 (12.5%) for melon growing on mulch. *Listeria* spp. was detected in 50 out of 114 melon discs, ranging from 2.11 to 4.21 log CFU/cm^2, among which 35/66 (53.0%) for soil-growing melon and 15/48 (31.2%) for mulch-growing melon. Both fecal coliform and *Listeria* spp. showed the highest prevalence on the ground spots of melon growing on soil while the lowest prevalence was seen on the upper surface of melon growing on plastic mulch.

All melon samples and environmental samples were analyzed for the presence of human pathogen *Salmonella* and *L. monocytogenes*. Apart from one positive of *Salmonella* in one soil sample resulting in a prevalence of 4.5%, all other samples are negative for these two pathogens.

Table 4. Prevalence of generic *E. coli*, fecal coliform, *Listeria* spp., *Salmonella* and *Listeria monocytogenes* in all cantaloupe and environmental samples

Microorganisms	Melon surface types						Environmental samples	
	Ground Spot (GS)		Upper Surface (US)		Stem (ST)		Soil	Swab
	Soil	Mulch	Soil	Mulch	Soil	Mulch		
Generic *E. coli*	4.5% (1/22)	0	0	0	0	6.3% (1/16)	4.5% (1/22)	6.3% (1/16)
Fecal coliform	54.5% (12/22)	18.8% (3/16)	22.7% (5/22)	6.3% (1/16)	18.2% (4/22)	12.5% (2/16)	45.4% (10/22)	37.5% (6/16)
Listeria spp.	72.7% (16/22)	56.25% (9/16)	45.4% (10/22)	18.8% (3/16)	40.9% (9/22)	18.8% (3/16)	100% (22/22)	62.5% (10/16)
Salmonella	0	0	0	0	0	0	4.5% (1/22)	0
Listeria monocytogenes	0	0	0	0	0	0	0	0

3.3.2 Effects of growing type and melon surface type on contamination levels of fecal coliform and Listeria *spp.*

Two-way ANOVA results indicated no significant interaction (P > 0.05) between two factors, growing type (soil and mulch) and surface type (GS, US, and ST), for both fecal coliform (Appendix A-1) and *Listeria* spp. (Appendix A-2). Hence, factors growing type and surface type can be treated and analyzed independently of one another. The main effect tests indicated significant differences (P < 0.05) in mean levels of bacteria, for fecal coliform (Appendix A-1) and *Listeria* spp. (Appendix A-2) for both factors, growing type and surface type. One-way ANOVA results indicated significant differences (P < 0.05) in mean bacterial levels for different surface types (GS, US and ST) for the whole dataset for growing type soil and mulch (fecal coliform: Appendix B-1, Figure 2; *Listeria* spp.: Appendix B-2, Figure 2). The levels of fecal coliform and *Listeria* spp. were significantly (P < 0.05) higher in GS compared to US and ST (Figure 2). Separate analysis for the melons growing either on soil (Figure 3) or mulch (Figure 4) indicated significant differences (P < 0.05) in mean bacterial levels for fecal coliform and *Listeria* spp. except for fecal coliforms in melons grown on mulch (Figure 4). Two sample t-test results showed significant differences (P < 0.05) in mean bacterial levels for melons growing on soil and mulch for both fecal coliform and *Listeria* spp. (Figure 5) for the whole data set for surface type (GS, US and ST). The levels of fecal coliform and *Listeria* spp. were significantly (P < 0.05) higher for melons growing on soil than melons growing on mulch (Figure 5). Separate analysis for the dataset for melon discs from different surface (GS, US, ST) indicated no significant differences (P > 0.05) in mean bacterial levels for fecal coliform and *Listeria* spp. between melons grown either on soil and

mulch (Figure 6-8), except for the ground spot melon discs indicating significant differences (P < 0.05) in the levels of fecal coliform (Figure 6).
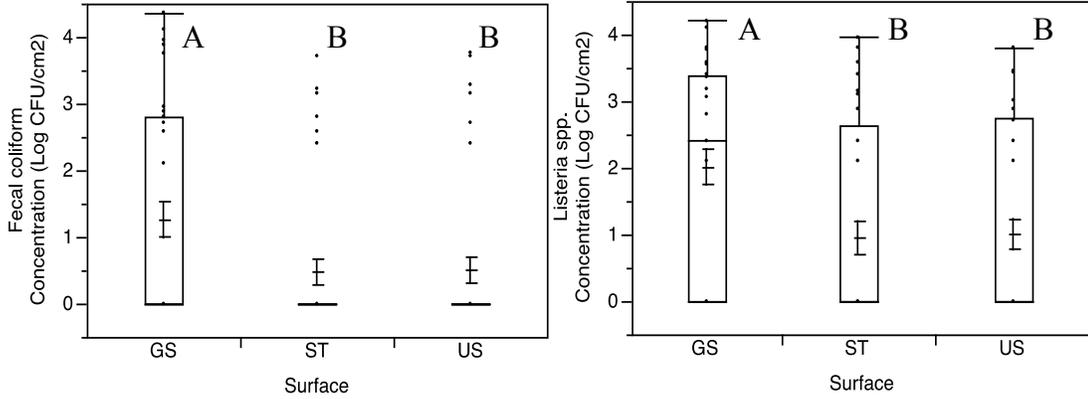
Figure 2. Contamination level of fecal coliform and *Listeria* spp. on three different melon surface types (GS, ST, US) from all melon samples. Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.
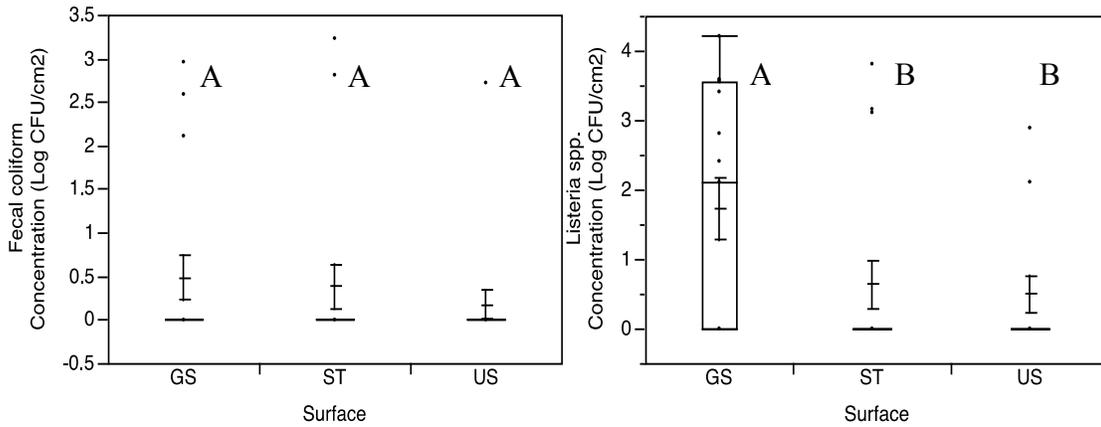
Figure 3. Contamination level of fecal coliform and *Listeria* spp. on three different melon surface types (GS, ST, US) from soil-growing melon. Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.

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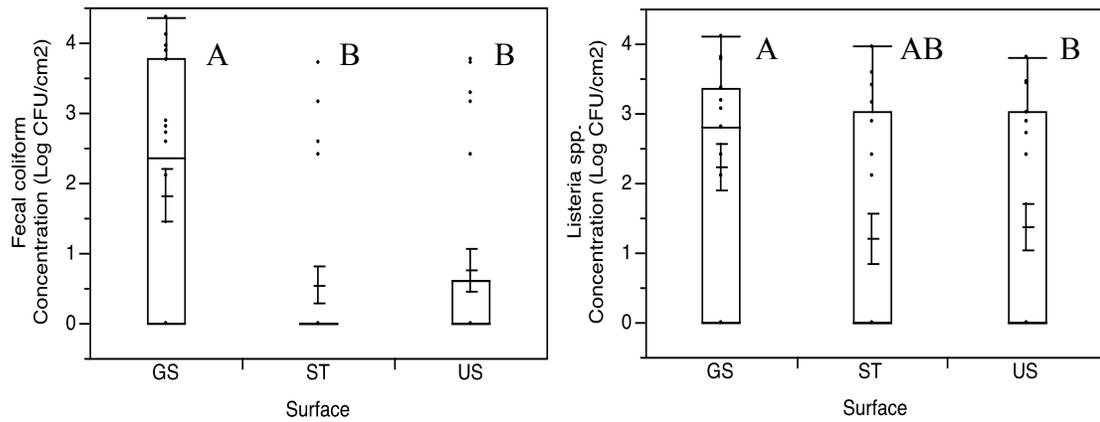
Figure 4. Contamination level of fecal coliform and *Listeria* spp. on three different melon surface types (GS, ST, US) from mulch-growing melon. Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.
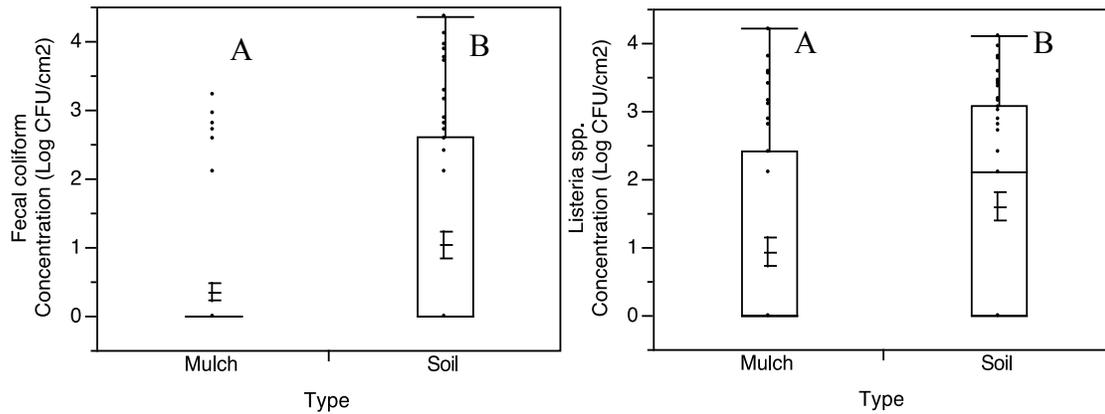
Figure 5. Contamination level of all surface type discs from melons growing on soil and mulch (left: fecal coliform; right: *Listeria*). Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.
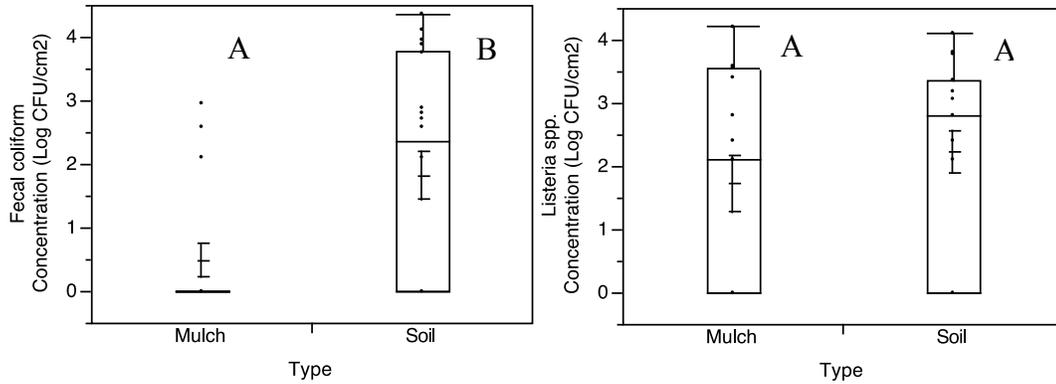
Figure 6. Contamination level of ground spot (GS) discs from melons growing on soil and mulch (left: fecal coliform; right: *Listeria*). Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.
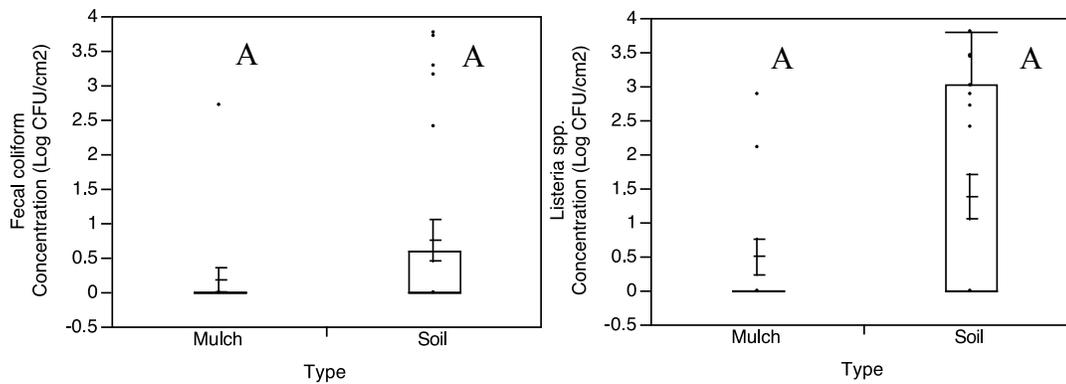
Figure 7. Contamination level of upper surface (US) discs from melons growing on soil and mulch (left: fecal coliform; right: *Listeria*). Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.
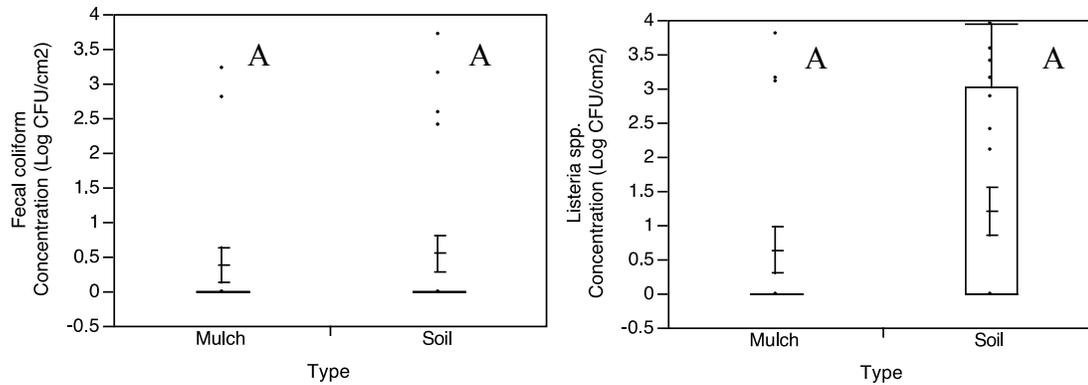
Figure 8. Contamination level of stem (ST) discs from melons growing on soil and mulch (left: fecal coliform; right: *Listeria*). Different upper-case letters indicate a statistically significant difference (P < 0.05) between groups.

3.3.3 Microbial ecology of field environment

Generic *E. coli* was detected in one soil (4.5%) and one mulch swab sample (6.3%), with concentration of 3.48 log CFU/g in soil and 1.70 log CFU/cm^2 on plastic mulch, respectively. Fecal coliform was positive in 10 out of 22 soil samples (45.4%) ranging from 3.30 to 5.38 log CFU/g, whereas in six out of 16 swab samples (37.5%) with the range of 1.00 to 3.15 log CFU/cm^2. *Listeria* spp. was detected in all soil samples with the concentration fluctuating between 2.30 to 5.34 log CFU/g, while positive in 10 out of 16 swab samples (62.5%) ranging from 1.00 to 3.46 log CFU/cm^2, which demonstrated that soil is the natural reservoir for *Listeria* spp. Irrigation water samples from two ponds and one ground water source were positive for generic *E. coli* with a concentration of 220, 310, and 30 CFU/L, respectively. Aerobic plate count (APC) was quantified as 5.13, 4.26, 5.17 log CFU/L, and with 5.16, 4.28, 5.21 log CFU/L total coliform.

3.4. Discussion

A pre-harvest microbiological evaluation was performed for cantaloupes and field environmental samples collected from five small-scale farms of mid-Atlantic region in the U.S. Numerous studies evaluate the pre-harvest microbial contamination of fresh produce commodities, but only a few contain cantaloupe as one of the target produce (Duffy et al., 2005; Materon, 2003). In general, our results demonstrated a low incidence of human pathogens present on cantaloupe farm which is consistent with some other studies. Johnston et al. (2005) reported a low incidence in a sample of 90 cantaloupes with 0, 0 and 3.3% for *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*, respectively, A bi-national study of Castillo et al. (2004) reported one (0.25%) and twelve (3.0%) out of 400 cantaloupes collected from the field in the U.S. to be positive for *Salmonella* and *E. coli*, respectively, and found 1.8% prevalence of *Salmonella* in a total of 1,735 samples including cantaloupes prior to and after washing, cantaloupes in cooler, river, water at field and out of irrigation pipe, reservoir tank, and surface of cooler wall. Strawn et al (2013) investigated the pre-harvest contamination associated with *Salmonella* and *L. monocytogenes* in produce fields and found a prevalence of 4.9% and 11% of *Salmonella* and *L. monocytogenes* in soil samples, which are higher than 1.9% and 8% in swab samples collected in this study. Although *Listeria* spp. is ubiquitous in the field environment, *L. monocytogenes* is rarely detected from fresh produce.

In addition, the distribution of microflora on the melon surface show that different areas of surface has different vulnerability for contamination although all rind areas has the "netted" surface with inherent roughness which is favorable for the

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attachment of microorganisms (Ukuku and Fett, 2002c) . Ground spot of melon rind is the most vulnerable for the contamination, while upper surface is the least and stem is intermediate. Similar results can be found in other studies as well. Ruengvisesh et al (2013) quantified the significant differences of native microbiota present on stem and rind of cantaloupe in association with different climate conditions in South Texas. Dobhal et al (2013) discovered the uneven distribution of microorganisms on the surface of field-grown cantaloupe from Central Oklahoma, with the ground spots areas being more contaminated than others. This is mainly because soil is the natural reservoir of various enteric bacteria and could be a primary source of pre-harvest contamination. However, Velasco et al. (2013) found the population behavior was not different between the upper rind and the yellow ground spot.

Post-harvest operation is critical in the prevention of contamination. It has been observed repeatedly that cantaloupes sampled at the packing shed (post-harvest stage) were more frequently contaminated than cantaloupes sampled at pre-harvest stage in the field (Gagliardi et al., 2003; Ailes et al., 2008; Johnston et al., 2005; Castillo et al., 2004). Cantaloupes sampled from the packing shed in Texas were positive for *Salmonella* at a prevalence of 3% (3/100), whereas all cantaloupes from field were all negative (Duffy et al., 2005). In addition, it also reported a prevalence of 24% (6/25) from swab samples of equipment in the packing shed in Texas. It was reported 2.2 and 0.2 log CFU/cm^2 of fecal coliforms in a packinghouse survey for cantaloupes before washing and after packed, respectively (Materon, 2003). As common in the mid-Atlantic region, all the farms included in our survey packed cantaloupes directly in the field without washing treatment although there is one farm

in Delaware using packing shed without postharvest washing, hence it was not possible to compare contamination levels in the field and in the packing shed.

Chapter 4

Quantitative microbial risk assessment for *Listeria monocytogenes* in cantaloupe

4.1 Introduction

Recent advances in quantitative microbial risk assessment (QMRA) provide efficient tools for modeling food supply chain in a systematic and objective way, which allows better identification of critical data gaps needed for both informed food safety decisions, and evaluation of the relative effectiveness of various risk-reduction strategies. Risk assessment is defined by Codex Alimentarius Commission as a science based approach consisting of four parts: hazard identification, dose-response assessment, exposure assessment, and risk characterization. QMRA can provide numeric expressions of risk and indication of the associated uncertainties.

Historically, human listeriosis was frequently associated with the consumption of contaminated RTE meat and dairy products (Czuprynski, 2005). With the increasing application of risk assessment in food safety area, a number of quantitative risk assessments have been developed to evaluate the risk of human listeriosis associated with the consumption of *L. monocytogenes* contaminated RTE food products such as leafy green vegetables (Ding et al., 2013), dairy products (Latorre et al., 2011), deli meats (Pradhan et al., 2010), pork sausage (Mürmann et al., 2011) and seafood (Pouillot et al., 2007). Most recently, an interagency risk assessment workshop for *L. monocytogenes* in retail delicatessens reported on a multi-agency collaboration by USDA, FSIS, FDA and CDC in the May of 2013.

Predicted risk for infection of human listeriosis associated with the consumption of fresh fruits is ranked as 15th among 23 ready-to-eat food categories, with 0.9 case per year in total, among which 0.2 case for intermediate-age, 0.6 case for elderly and 0.1 case for perinatal (FDA/FSIS, 2003). A draft qualitative risk assessment for on-farm contamination of fresh produce identified a high risk of illness for shed-packed cantaloupe while a low risk for cantaloupe packed in field (FDA, 2012). Another qualitative risk assessment of microbiological hazards in fresh fruits (including melon) and 14 significant bacteria (including *L. monocytogenes*) identified high human health risk associated with melons (Bassett and McClure, 2008). However, no quantitative risk assessment has been developed to estimate the risk associated with the consumption of melon.

Therefore, a QMRA model incorporating contamination transfer routes along cantaloupe supply is needed for *L. monocytogenes* in order to: (1) provide estimates of the expected current risks of human listeriosis per serving and per annum basis associated with the customer purchase of either fresh-cuts or whole cantaloupe in the U.S.; (2) identify the most important factors affecting the estimated risk which provide insights for risk management; and (3) evaluate the uncertainty of initial contamination and prevalence for their influence of risks associated with consumption of cantaloupe in the U.S.

4.2 Methods

4.2.1 Model overview

This QMRA model demonstrated the transfer route and transfer quantification of *L. monocytogenes* in fresh-cuts and whole cantaloupe supply within "farm to table" continuum. Final dose of *L. monocytogenes* was determined by the initial concentration on cantaloupe surface after harvested, postharvest farm storage, fresh cutting cross contamination, reduction by washing treatment, transfer of pathogen cell from inedible rind to flesh, and survival or potential growth of *L. monocytogenes* on the surface and fresh-cuts of cantaloupe during multiple transportation and storage stages. Pre-harvest contamination was generalized with an assumption of -1 log CFU/cm^2 upon harvest from field as baseline model due to the limited quantified information of *L. monocytogenes* on harvested cantaloupe. 1% prevalence was assumed for the baseline model considering the low prevalence on cantaloupe. Only postharvest farm operation without washing treatment was evaluated in this model. After postharvest farm storage, cantaloupe will be transported either to fresh-cut processing facility for fresh-cutting and packing, or to retailer for sale as whole melon. Either fresh-cut or whole cantaloupe can be sold to consumers after a certain time of retail storage. Having transported to individual households, whole cantaloupe would be cut before consumption while fresh-cut cantaloupe does not. Home storage of fresh-cut cantaloupe before consumption is likely to happen for the left-over of each serving. Two different models were developed for fresh-cut and whole cantaloupe supply, respectively. Figure 9 demonstrates the general framework of fresh-cut and whole cantaloupe consumption within "from farm to fork" continuum.
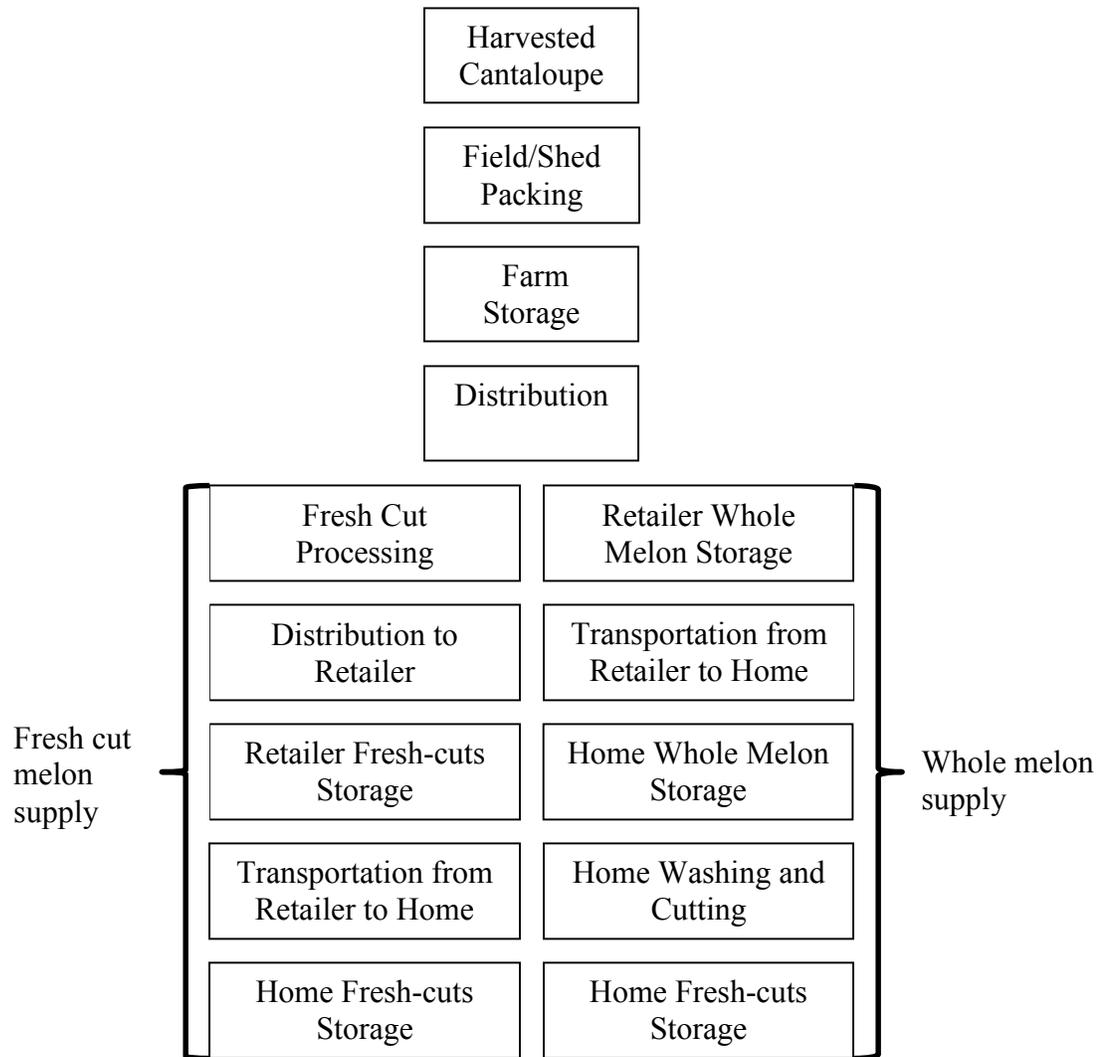
Figure 9. General framework of the QMRA model for *L. monocytogenes* in cantaloupe.

Table 5. Overview of variables, point-estimate values, statistical distributions, and formulas used in the QMRA model for fresh-cut cantaloupe consumption

Symbol	Variable	Distribution, Value or Formula	Unit	Source
C_0	Initial concentration	0.1	CFU/cm^2	Input
	Postharvest Farm Storage			
T_{post}	Temperature	4	°C	Assumed
t_{post}	Time	=RiskUniform(0,7)	day	Assumed
a	Survival percentage of LM[a] on melon surface	=0.9093exp(-0.156)×t_{post}	percent	See "Survival of LM on cantaloupe surface"
C_{post}	Number of LM after farm storage	=IF(t_{post}<1, C_0, C_0 × a)	CFU/cm^2	Calculated [b]
$Prev_0$	Initial Prevalence	1	%	Input
	Fresh-cut Processing			
W	Inactivation of chlorine washing	1	log	Input
C_w	CFU on rind after chlorine washing	=C_{post} − 10^(W)	CFU/cm^2	Calculated
$C_{surface}$	CFU on the food contact surface	=10^(RiskExtvalueMin(-4.7052, 0.36351))	CFU/cm^2	
b	Transfer percent from food contact surface to melon	=RiskTriang(80.298, 98.3, 98.3)	%	Jensen et al., 2013
C_{rind}	CFU on rind after cross contamination	=C_w + $C_{surface}$× b	CFU/cm^2	
c	Percentage of pathogen transfer from rind to fresh cuts	=RiskTriangle(2.89/6.81,0.5,4.31/4.81)	percent	Ukuku et al., 2012 Patil et al., 2013
C_{fre}	Number of LM after fresh cutting	=$log(C_{rind})$ × c	log CFU/g	Calculated
	Retail storage			
T_R	Retail Temperature	=RiskNormal(4.4441,2.9642,RiskTruncate(0,20.56))	°C	EcoSure, 2008
t_R	Retail Time	=RiskTriang(1,4,7)	day	Industry opinion
G_R	Growth of LM during retail storage	=((0.0186×(T_R + 0.5108))^2) × t_R × 24	log CFU/g	Danyluk et al., 2014
C_R	Log CFU of LM before selling	C_{fre} + G_R	log CFU/g	Calculated
	Transportation to household			
t_{Tran}	Transportation time, t_T	=RiskLognorm(1.421,0.46478,RiskTruncate(0.1833,3.8667), RiskShift(-0.24609))	hours	EcoSure, 2008
T_{bH}	Temperature before putting in home refrigerator	=RiskNormal(8.386,3.831,RiskTruncate	°C	EcoSure, 2008

		(0,20))		
T_{Tran}	Transportation temperature	$=1/2\times(T_R + T_{bH})$	°C	Calculated
G_{Tran}	Growth of LM during transportation	$((0.0186\times(T_{Tran} + 0.5108))^2) \times t_{Tran}$	log CFU/g	Danyluk 2014.
C_{Tran}	LogCFU of LM after transportation	$C_R + G_{Tran}$	log CFU/g	Calculated
	Household storage			
t_f	Time to first consumption (home storage)	$=RiskWeibull(0.905,2.32)\times24$	hours	Pouillot, 2010
t_l	Time to last consumption (home storage)	$=RiskWeibull(1.27,7.37)\times24$	hours	Pouillot, 2010
t_H	Time selected-home storage, t_H	$=1/2\times(t_f + t_l)$	hours	Calculated
T_H	Home storage temperature, T_H	$=RiskNormal(3.4517,2.4442,RiskTruncate(-5,17.22))$	°C	EcoSure, 2008
G_H	Growth of LM during household storage	$((0.0186\times(T_H + 0.5108))^2) \times t_H$	log CFU/g	Danyluk et al., 2014.
C_H	LogCFU of LM before consumption	$=IF(C_{Tran} + G_H<9, C_{Tran} + G_H, 9)$	log CFU/g	Calculated
	Serving			
Ser	Serving size	177	g	USDA, 2012
D	Dose per serving (CFU/serving)	$= C_H \times Ser$	CFU/serving	Calculated
	Dose response			
$R1$	r value for susceptible subpopulation	5.85×10^{-12}		FAO/WHO, 2004
$R2$	r value for general healthy population	5.34×10^{-14}		FAO/WHO, 2004
$P1$	Probability of illness per serving among susceptible population	$=(1-exp(-R1\times D)\times Prev$	probability	Calculated
$P2$	Probability of illness per serving among general healthy population	$=(1-exp(-R2\times D)\times Prev$	probability	Calculated
	Risk characterization			
N_{pop}	U.S. population	318,892,000		U.S.Census
A	Annual cantaloupe consumption	2386.7	million lbs	USDA-ERS, 2014
N_{serv}	No. of servings consumed per year in U.S. population	$= A/Ser$		Calculated
d	Percentage of susceptible among all population	0.2	percent	FAO/WHO, 2004
N_H	Number of cases per year among healthy	$= P2\times N_{serv}\times(1-d)$		Calculated

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Symbol	Variable	Distribution, Value or Formula	Unit	Source
	population			
Ns	Number of cases per year among susceptible population	$= P1 \times N_{serv} \times d$		Calculated
N_{cases}	Number of cases per year	$= N_H + N_S$		Calculated

[a] LM, *Listeria monocytogenes*.
[b] Calculated, values that are calculated in this QMRA model.

Table 6. Overview of variables, point-estimate values, statistical distributions, and formulas used in the QMRA model for whole cantaloupe consumption

Symbol	Variable	Distribution, Value or Formula	Unit	Source
C_0	Initial concentration	0.1	CFU/cm^2	Input
	Postharvest Farm Storage			
T_{post}	Temperature	4	°C	Assumed
t_{post}	Time	=RiskUniform(0,7)	day	Assumed
a	Survival percentage of LM[a] on melon surface	$=0.9093\exp(-0.156) \times t_{\text{post}}$	percent	See "Survival of LM on cantaloupe surface"
C_{post}	Number of LM after farm storage	$=IF(t_{post}<1, C_0, C_0 \times a)$	CFU/cm^2	Calculated [b]
$Prev_0$	Initial Prevalence	1	%	Input
	Retail Storage			
T_R	Temperature	20	°C	Assumed
t_R	Time	=RiskTriangle(1,4,7)	day	Assumed
b	Survival percentage of LM during retailer storage	$=1.0473\exp(-0.25 \times t_R)$		See "Survival of LM on cantaloupe surface"
C_R	Number of LM before selling	$=C_{post} \times b$	CFU/cm^2	Calculated
	Household processing			
W	Inactivation of plain water washing	=RiskTriangle(0,0.2,0.9)	log	

C_w	Number of LM after washing	$=log(C_R)-W$	CFU/cm^2 log CFU/cm^2	Calculated
c	Transfer from rind to flesh	=RiskTriangle(2.89/6.81,0.5,4.31/4.81)	percent	Ukuku et al., 2012. Patil et al., 2013.
C_{fres}	Number of LM in fresh-cuts after household cutting	$=C_w \times c$	log CFU/g	Calculated
	Household storage			
t_f	Time to first (home storage)	=RiskWeibull(0.905,2.32)×24	hours	Pouillot, 2010
t_l	Time to last (home storage)	=RiskWeibull(1.27,7.37)×24	hours	Pouillot, 2010
t_H	Time selected-home storage, t_H	$=1/2 \times (t_f + t_l)$	hours	Calculated
T_H	Home storage temperature, T_H	=RiskNormal(3.4517,2.4442,RiskTruncate (-5,17.22))	°C	EcoSure, 2008
G_H	Growth of LM during household storage	$((0.0186 \times (T_H + 0.5108))^2) \times t_H$	log CFU/g	Danyluk et al., 2014
C_H	Number of LM before consumption	$=IF(C_{fres} + G_H<9, C_{fres} + G_H, 9)$	log CFU/g	Calculated
	Serving			
Ser	Serving size	177	g	USDA
D	Dose per serving (CFU/serving)	$= C_H \times Ser$	CFU/serving	Calculated
	Dose response			
$R1$	r value for susceptible subpopulation	5.85×10^{-12}		FAO/WHO, 2004
$R2$	r value for general healthy population	5.34×10^{-14}		FAO/WHO, 2004
$P1$	Probability of illness per serving among susceptible population	=(1-exp(-$R1$×D)×$Prev$	probability	Calculated
$P2$	Probability of illness per serving among general healthy population	=(1-exp(-$R2$×D)×$Prev$	probability	Calculated
	Risk characterization			
N_{pop}	U.S. population	318,892,000		U.S.Census

A	Annual cantaloupe consumption	2386.7	million lbs	USDA-ERS, 2014
N_{serv}	No. of servings consumed per year in U.S. population	$= A/Ser$		Calculated
d	Percentage of susceptible among all population	0.2	percent	FAO/WHO, 2004.
N_H	Number of cases per year among healthy population	$= P2 \times N_{serv} \times (1-d)$		Calculated
Ns	Number of cases per year among susceptible population	$= P1 \times N_{serv} \times d$		Calculated
N_{cases}	Number of cases per year	$= N_H + N_S$		Calculated

[a] LM, *Listeria monocytogenes*.
[b] Calculated, values that are calculated in this QMRA model.

4.2.2 Survival of L. monocytogenes *on cantaloupe surface*

Without adequate nutrients and water activity, *L. monocytogenes* would not be expected to replicate on the melon surface. However, special topography of cantaloupe rind can provide niches for pathogen survival. Without the availability of any robust data and probability distributions regarding the distribution of *L. monocytogenes* on cantaloupe surface, it was assumed that *L. monocytogenes* is evenly distributed on the cantaloupe surface before cutting. Data extracted from Ukuku and Fett (2002) were used to fit the mathematic survival model of *L. monocytogenes* on the surface of cantaloupe by plot digitizer. Two exponential models provide a reasonable fit to the data under 4°C and 20°C up to 15 days storage, respectively.

$$4°C: \quad y = 0.9093e^{-0.156x} \quad R^2 = 0.9534 \tag{1}$$

$$20°C: \quad y = 1.0473e^{-0.25x} \quad R^2 = 0.9533 \tag{2}$$

Where x is the storage time (day), y is the survival percentage of CFU/cm^2 after storage which is defined as number of bacteria cells after storage divided by number of bacteria cells at the beginning of storage. No decline of pathogen cell number was observed within the first day storage, therefore x is supposed to be greater than 1. Since only two temperature conditions were considered in Ukuku and Fett (2002), 4°C and 20°C were applied to model refrigeration and room temperature storage, respectively.

4.2.3 Microbial growth kinetic of L. monocytogenes *in fresh-cut cantaloupe*

In terms of high water activity and nutrient content with neutral pH in fresh-cut cantaloupe, *L. monocytogenes* is capable to grow rapidly under temperature abuse

and tolerate the refrigeration temperature as well. In this QMRA model, a three phase linear model (Buchanan et al., 1997) and a secondary model (Ratkowsky et al., 1982) were used as primary and secondary growth kinetic model for *L. monocytogenes*, respectively.

The three-phase log-linear primary model consists of a lag phase, an exponential phase, and a stationary phase which was expressed as follows:

$$\log N_t = \begin{cases} \log N_0, \text{ (if } t < t_L) \\ \log N_0 + R \times (t - t_L), \text{ (if } t_L < t < t_m) \\ \log N_m, \text{ (if } t >= t_m) \end{cases} \tag{3}$$

$$R = (\log N_m - \log N_0) / (t_m - t_L) \tag{4}$$

Where N_t is the concentration at time t (CFU/g), R is the growth rate (log CFU g^{-1} h^{-1}), t_L is the lag time, N_0 is the concentration at 0 time (CFU/g), N_m is the Maximum Population Density (MPD; CFU/g), t_m is time at which the MPD is reached. Concentration of *L. monocytogenes* in cantaloupe prior to consumption was truncated at the MPD of 9 log CFU/g (Hong et al., 2014). Hong et al. (2014) reported the maximum growth of approximately 9 log CFU/g for both with or without cold-adapted *L. monocytogenes* in fresh-cut cantaloupes stored at temperatures less than 25°C (storage temperature in between 10°C and 25°C). At storage temperature of 30°C, the authors reported the maximum growth of approximately 11 log CFU/g for both with or without cold-adapted *L. monocytogenes* in fresh-cut cantaloupes. No lag phase was considered in this model as the worst case scenario.

The secondary model developed by Danyluk et al. (2014) was used to predict the maximum growth rate of *L. monocytogenes* in fresh-cut cantaloupe with the temperature fluctuation during distribution and storage.

$$\sqrt{\text{growth rate (log CFU/h)}} = b \times (T - T_0) \qquad (5)$$

($R^2 = 0.970$; SE=0.0256; D_f=16) where the parameter b is 0.0186, and T_0 = -0.5108°C which is the theoretical minimum growth temperature of *L. monocytogenes* in fresh-cut cantaloupe.

4.2.4 Exposure assessment: bacteria transfer from melon surface to flesh

Pathogen cells may be transferred from rind to interior flesh during the cutting process. Ukuku et al. (2012) investigated the *L. monocytogenes* transfer from cantaloupe rind surface to fresh cut melon pieces and found 2.2 log CFU/g *L. monocytogenes* in fresh-cut pieces which were transferred from 4.4 log CFU/cm^2 of inoculated cantaloupe surface. Patil et al. (2013) reported *L. monocytogenes* in fresh-cut cantaloupe ranging from 2.89 to 4.31 log CFU/g transferred from inoculated surface with 6.81 log CFU/g. A triangle (2.89/6.81, 2.2/4.4, 4.31/6.81) distribution was used to describe the percentage of bacteria transfer from melon rind (log CFU/cm^2) to fresh-cut pieces (log CFU/g).

4.2.5 Exposure assessment: cross-contamination

Cross contamination may occur in many stages during "from farm to fork" continuum. Only cross contamination between cantaloupe and food contact surface ("surface") when cutting in fresh-cut processing facility was considered for simplification. Model was developed based on the assumption that after chlorine washing, pathogens were firstly transferred from melon to zero-contaminated

"surface" and then transferred back from "surface" to fresh-cut melon during cutting and packing. It is also assumed that a proportion (20%) of cantaloupe surface touched with the "surface" when processing.

$$N_1 = N_0 \times c_1 \times 0.2 \qquad (6)$$

$$N = N_0 + N_1 \times c_2 \qquad (7)$$

Where N_0 and N represent the number of pathogen cells on the surface of cantaloupe before and after cross contamination, N_1 represents the number of pathogen cells on the "surface", c_1 is the bacteria transfer ratio from cantaloupe to food contact surface and c_2 is the transfer ratio from food contact surface to fresh-cut cantaloupe.

A separate model was developed to estimate the number of pathogen cells (N_1) on the "surface" due to cross contamination. The transfer from contaminated rind to surface was simulated with 100,000 Monte Carlo iterations of Eq.6. A Minimum Extreme Value distribution of pathogen cell number on "surface" (N_1) was fitted with the values obtained from the simulation. Subsequently, the distribution of N_1 was input to the base model to calculate the number of pathogen cells on cantaloupe after cross contamination (N) (Eq.7).

Data of bacteria transfer ratio between the "surface" and fresh-cut produce was extracted from a quantified cross contamination study Jensen at al. (2013). Transfer ratio from celery and carrot to the kitchen surfaces with drying time were fitted distributions to simulate the bacteria transfer from cantaloupe to the "surface". Data of transfer percent from kitchen surfaces to fresh-cut watermelon without drying time were extracted to fit distributions in @Risk, which is used to describe how *L.*

monocytogenes was transferred from the "surface" to fresh-cut cantaloupe when processing.

4.2.6 Exposure assessment: washing

4.2.6.1 Chlorine washing

Chlorine is widely used for cantaloupe washing in fresh cut processing facility. A six log reduction of *L. monocytogenes* on cantaloupe surface was observed with 200 ppm/5 min chlorine washing (Rodgers et al., 2004), while only 2.0- to 3.5-log reduction was found with 1000 ppm/2 min chlorine washing (Ukuku and Fett, 2002). Because of the different concentration and duration adopted in different processing line, variability and uncertainty existed in terms of the efficacy of chlorine washing. Information from a local fresh-cut processing facility indicated 4 ppm chlorine was used as washing sanitizer in order to minimize the chlorine residue on cantaloupe. Therefore, scenario analysis with input reduction of 1, 2, 3, 4, 5, 6 log CFU/cm^2 is applied for evaluating different efficacy of chlorine resulted from various concentration and duration of chlorine washing.

4.2.6.2 Plain water washing

Tap water is usually adopted in household washing of cantaloupe instead of chlorine. Washing with plain water did not demonstrate a significant effect in the reduction of *L. monocytogenes* on cantaloupe (Ukuku and Fett, 2002; Ukuku et al., 2012), whereas, 0.2 log CFU/cm^2 and 0.9 log CFU/cm^2 reduction of *L. monocytogenes* on cantaloupe surface were observed (Rodgers et al., 2004; Ukuku et al., 2012). Therefore, a triangle (0, 0.2, 0.9) distribution was used to estimate the

reduction of *L. monocytogenes* on cantaloupe surface (log CFU/cm^2) with plain water washing.

4.2.7 Exposure assessment: transportation and storage conditions

Data of all refrigerated food products were extracted from the EcoSure Cold Temperature Report (2007) and fitted to a normal distribution (μ=4.4441°C, σ=2.9642°C) which represented retail storage temperature for fresh-cut cantaloupe. This normal distribution was truncated within 0°C and 20.56°C, as refrigerator at retail refrigeration seldom falls below 0°C and 20.56°C is the maximum temperature reported in EcoSure report. Similarly, a normal distribution (μ=3.4517°C, σ=2.4442°C) truncated between -5°C and 17.22°C for minimum and maximum reported temperature was used to describe the temperature during household refrigeration storage based on the data from EcoSure Cold Temperature Report (EcoSure, 2008). Data from Pouillot et al. (2010) for fresh-cut fruits were used to define the household storage time of fresh-cut cantaloupe by averaging storage time before first and last consumption.

Temperature during transportation from retail to household was described by averaging retail storage temperature and temperature prior to household refrigeration (Latorre et al., 2011). Data of temperature for all refrigerated food products at the end of retail-home transport and just before household refrigeration were extracted from the EcoSure 2007, and fitted to a normal distribution (μ=8.3858°C, σ=3.8314°C) with truncation between 0°C and 20°C. In addition, transportation time (hours) for all refrigerated commodities were extracted from EcoSure report and fitted to a lognormal distribution (μ=1.421 h, σ=0.46478 h) with truncation at 0.1833 h and

3.8667 h, in order to model the transportation time of fresh-cut cantaloupe from retail to household.

Because of unavailability of any data regarding to the transportation conditions (time and temperature) during transportation of fresh-cut cantaloupe from processing facility to retailer, this segment was not included in the model. Similarly, for whole cantaloupe supply chain, time and temperature data are not available during transportation from farm to fresh-cut processor's storage facility or retailer and were not included. Since no decline of *L. monocytogenes* on cantaloupe surface was observed within one day based on the survival model, it can be assumed that the number of *L. monocytogenes* cells remain unchanged when the transportation time is one day or less.

4.2.8 Dose response relationship and risk characterization

Serving size in this model was set to be one cup (balls) which is 177 grams based on a USDA National Nutrient Database for Standard Reference. Dose (i.e., number of *L. monocytogenes* cells) ingested per serving was calculated by multiplying the concentration (CFU/g) prior to consumption with serving size. The exponential dose-response model was applied in this QMRA model to estimate the probability of illnesses due to the consumption of fresh-cut cantaloupe, with different r values for general healthy and susceptible subpopulation reported from "Risk assessment of *Listeria monocytogenes* in ready-to-eat foods (FAO/WHO, 2004)":

$$P = 1 - e^{(-r \times D)} \qquad (8)$$

Where P is the probability of illness, D is the number of microorganisms ingested per serving (i.e., dose), specific r values are derived for less susceptible (healthy) and

more susceptible population, which is 5.34×10^{-14} and 5.85×10^{-12}, respectively (FAO/WHO, 2004). Probability of illness per serving was calculated based on the contamination prevalence and the probability of illness from estimated ingested dose.

According to Food Availability (Per Capita) Data System (USDA, 2012), cantaloupe consumption reached 2386.7 million pounds in the year of 2012 with seven pounds (3.17 kg) per person in the U.S. Since there is no information about the ratio of fresh-cut and whole cantaloupe consumption, it is assumed that all cantaloupes were consumed either from fresh cut or whole melon supply.

Number of servings of cantaloupe per person per year in the U.S. was estimated to be 19 servings/person as calculated through annual consumption per person (3.17 kg) divided by serving size (177 g). Number of servings per year consumed in the U.S. is 6.12×10^9 servings/year based on the U.S. total population 314.268 million reported by U.S. Census Bureau. Percentage of listeriosis susceptible subpopulation was estimated as 20% among whole population (FAO/WHO, 2004). The estimated listeriosis cases per year for susceptible and general healthy population was calculated by the integration of dose-response model and the number of servings of cantaloupe in the U.S.

4.2.9 Scenario analysis

Designed to improve the decision-making process, scenario analysis is a process of evaluating possible future events by considering alternative possible outcomes. With the baseline model of 0.1 CFU/cm^2 initial contamination on cantaloupe surface, 1 log CFU/cm^2 reduction due to chlorine washing and 1% prevalence, different scenarios of initial contamination, efficacy of chlorine washing

and prevalence were run to analyze the possible final outcomes associated with different inputs.

4.2.9.1 Initial concentration

Because of unavailable information about the initial concentration of *L. monocytogenes* found on cantaloupe surface during pre-harvest stage, -3, -2, -1, 0 log CFU/cm^2 were input as initial contamination values on cantaloupe surface after harvest to apply scenario analysis.

4.2.9.2 Chlorine washing

Considering different industry practices in terms of the concentration and duration of chlorine washing, efficacies of chlorine washing with 1, 2, 3, 4, 5, 6 log CFU/cm^2 reduction of *L. monocytogenes* on cantaloupe surface were deemed as six scenarios for chlorine washing as six log reduction with 200 ppm chlorine washing for 5 minutes is the highest value reported from literature (Rodgers et al., 2004).

4.2.9.3 Prevalence

Since the prevalence of *L. monocytogenes* in cantaloupe before consumption is very low with no prevalence data available, the scenario analysis with 0.1%, 0.5%, 1%, 1.5% were run to predict the public health impacts of different prevalence scenarios.

4.2.10 Model simulations and analysis

The QMRA models were developed based on the incorporation of relevant data, information, probabilistic distributions, and mathematic equations as detailed in Table 5 and Table 6. The risk models for all scenarios were simulated with the one

dimensional Monte Carlo simulation technique by using risk modeling software @Risk 6.1 (Palisade Corp., Ithaca, NY). All models were simulated for 100,000 iterations (Danyluk and Schaffner, 2011; Latorre et al., 2011), and a Latin Hypercube sampling method was used to draw sample values for input parameters and variables. Sensitivity analyses were performed to identify important parameters affecting public health risk of listeriosis associated with cantaloupe consumption. Spearman's correlation coefficients were used for sensitivity analyses to determine the effect of input variables on the probability of illnesses per serving and the number of illnesses per year in the U.S. population.

4.3 Results

4.3.1 Dose of L. monocytogenes *per serving associated with consumption of fresh-cut and whole cantaloupe*

Distributions for number of *L. monocytogenes* cells (dose) per serving for fresh-cut and whole cantaloupes are shown in figures 10 and 11, respectively. Number of *L. monocytogenes* in cantaloupe associated with fresh-cut consumption ranged from 0.761 to 11.248 log CFU per serving with 5th percentile and 95th percentile of 1.344 and 6.215 log CFU per serving, respectively. Number of *L. monocytogenes* associated with consumption of whole cantaloupe ranged from 0.613 to 11.248 log CFU per serving with 5th percentile and 95th percentile of 1.126 and 4.219 log CFU per serving.
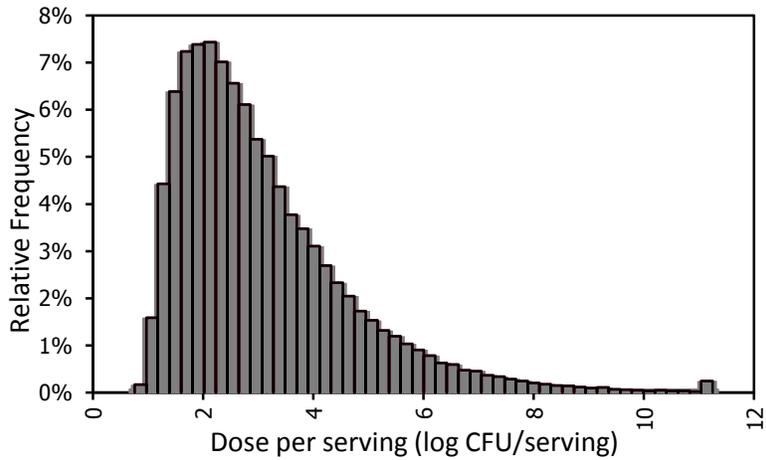
Figure 10. Distribution of relative frequency of *L. monocytogenes* cell number associated with per serving consumption of fresh-cut cantaloupe.
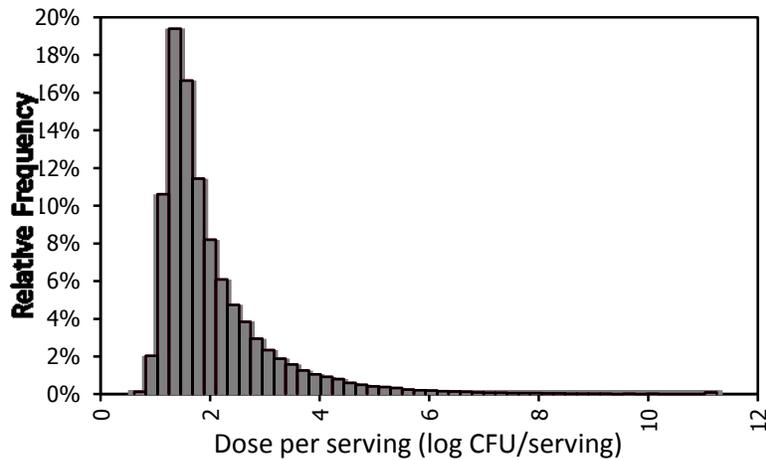
Figure 11. Distribution of relative frequency of *L. monocytogenes* cell number associated with per serving consumption of whole cantaloupe.

4.3.2 Probability of illnesses per serving and estimated number of listeriosis cases per year among different subpopulations in baseline model.

The ingested dose was input to the exponential dose-response model to calculate the probability of illness associated with consumption of a serving of cantaloupe from either fresh-cut or whole melon consumption in baseline model. By

using the r values for different subpopulations, the probability of illness per serving of cantaloupe from fresh-cut and whole cantaloupe consumption for two different populations was listed in Table 7 and Table 8. The cumulative density functions (CDF) of probability of illness per serving for the baseline model is provided in Figure 12. Probability of illness per serving are plotted on a logarithmic scale in CDF for the convenient comparison of fresh-cut and whole cantaloupe consumption among different subpopulations.

Table 7. Probability of illness per serving of fresh-cut cantaloupe consumption among different subpopulations

Population	Probability of illness per serving					
	Mean	1st percentile	5th percentile	Median (50th percentile)	95th percentile	99th percentile
General Healthy	2.68×10^{-7}	7.00×10^{-15}	1.18×10^{-14}	2.74×10^{-13}	8.76×10^{-10}	2.25×10^{-7}
Susceptible	1.98×10^{-5}	7.67×10^{-13}	1.29×10^{-12}	3.00×10^{-11}	9.60×10^{-8}	2.46×10^{-5}

Table 8. Probability of illness per serving of whole cantaloupe consumption among different subpopulations

Population	Probability of illness per serving					
	Mean	1st percentile	5th percentile	Median (50th percentile)	95th percentile	99th percentile
General Healthy	9.23×10^{-8}	4.97×10^{-15}	7.13×10^{-15}	2.65×10^{-14}	8.84×10^{-12}	1.69×10^{-9}
Susceptible	6.76×10^{-6}	5.44×10^{-13}	7.82×10^{-13}	2.91×10^{-12}	9.68×10^{-10}	1.85×10^{-7}
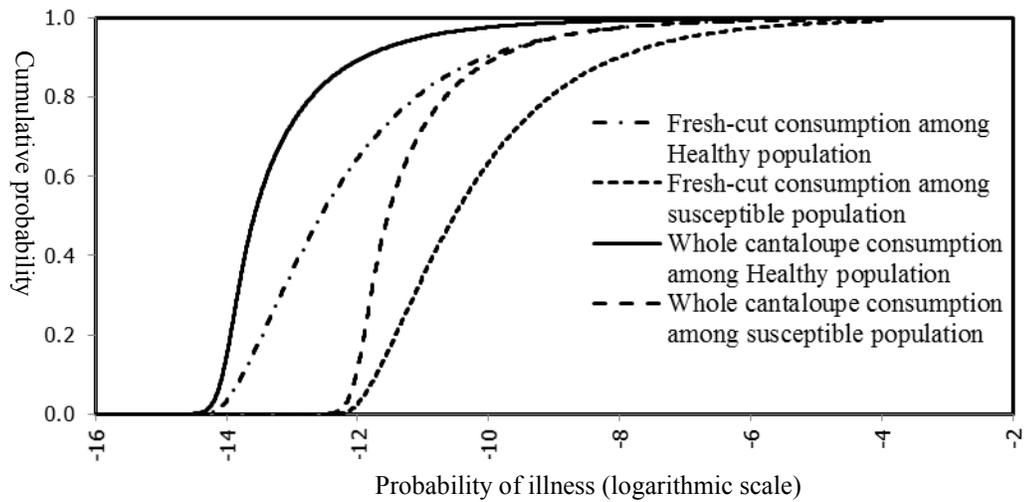
Figure 12. Cumulative density functions of probability of illness per serving associated with fresh-cut and whole cantaloupe consumption among healthy and susceptible subpopulation.

The number of cases per year was calculated by combining probability of illness per serving and cantaloupe consumption data from USDA Economic Research Sources. The calculations were based on the assumption that the highly susceptible population represents 20% of the whole population and the non-susceptible is 80%. The predicted number of cases was separately calculated for each subpopulation group. Total number of cases was the sum of both healthy and susceptible subpopulation. Median of total number of cases per year attributable to cantaloupes that is predicted by baseline model for associated with fresh-cut and whole cantaloupe consumption was 0.038 and 0.0037, respectively (Table 9 and 10).

Table 9. Number of listeriosis cases per year associated with consumption of fresh-cut cantaloupe among different subpopulations in the U.S.

Population	Number of cases per year					
	Mean	1st percentile	5th percentile	Median	95th percentile	99th percentile
General Healthy	1313.60	3.43×10^{-5}	5.77×10^{-5}	0.00134	4.29	1101.61
Susceptible	24186.27	0.000939	0.00158	0.0368	117.48	30135.21
Total	25499.86	0.000973	0.00164	0.0381	121.77	31236.88

Table 10. Number of listeriosis cases per year associated with consumption of whole cantaloupe among different subpopulations in the U.S.

Population	Number of cases per year					
	Mean	1st percentile	5th percentile	Median	95th percentile	99th percentile
General Healthy	451.82	2.43×10^{-5}	3.49×10^{-5}	0.00013	0.0432	8.29
Susceptible	8266.61	0.000666	0.000956	0.00355	1.18	226.91
Total	8718.43	0.000690	0.000991	0.00368	1.23	235.20

4.3.3 Scenario analysis for prevalence

Because of the low prevalence of *L. monocytogenes* found on cantaloupe, scenario analysis can be used to describe the public health impact caused by different possible prevalence. Apart from baseline model with 1% prevalence, 0.1%, 0.5% and 1.5% prevalence were run to compare with the baseline model. Average of total cases per year with 0.1% pre-harvest prevalence *L. monocytogenes* on cantaloupe is one tenth of baseline model (Table 11).

Table 11. Total number of cases per year due to the consumption of fresh-cut cantaloupe in the U.S. population for different scenarios of prevalence

Prevalence (%)		Number of Cases per Year					
	Mean	Fold change *	1st percentile	5th percentile	Median	95th percentile	99th percentile
0.1	2549.99	0.10	9.730×10^{-5}	0.000164	0.00381	12.18	3123.69
0.5	12749.93	0.49	0.000486	0.000819	0.0191	60.88	15618.44
1 (Baseline)	25499.86	-	0.000973	0.00164	0.0381	121.77	31236.88
1.5	38249.80	1.50	0.00146	0.00246	0.0572	182.65	46855.32

*Fold changes were calculated by comparing mean values of each scenario with the mean value of the baseline model.

4.3.4 Scenario analysis for initial concentration

Different scenarios of initial contamination with 0.001, 0.01, 1 CFU/cm^2 were evaluated their influence of relative risks on total number of cases compared with the baseline model which is assumed as 0.1 CFU/cm^2 initial contamination on cantaloupe. The predicted number of cases per year enlarged by 2.7 fold with the increase of initial concentration from 0.001 to 1 CFU/cm^2 (Table 12).

4.3.5 Scenario analysis for chlorine washing

Scenarios with different reduction of *L. monocytogenes* due to chlorine washing during fresh-cut processing were evaluated on their influence of relative risks compared to the baseline model. The predicted number of cases per year decreased by five folds with the reduction varying from 1 to 6 log CFU/cm^2 on cantaloupe (Table 13).

Table 12. Total number of cases per year due to the consumption of fresh-cut cantaloupe in the U.S. population for different scenarios of initial concentration

Initial Concentration (CFU/cm²)		Number of Cases per Year					
	Mean	Fold change *	1st percentile	5th percentile	Median	95th percentile	99th percentile
0.001	13720.43	0.54	7.332×10^{-5}	0.000141	0.00352	11.56	2933.29
0.01	18829.32	0.74	0.000270	0.000481	0.0115	37.15	9598.68
0.1 (Baseline)	25499.86	-	0.000973	0.00164	0.0381	121.77	31236.88
1	33997.85	1.33	0.00339	0.00549	0.125	401.41	101381.38

*Fold changes were calculated by comparing mean values of each scenario with the mean value of the baseline model.

Table 13. Total number of cases per year due to the consumption of fresh-cut cantaloupe in the U.S. population for different levels of *L. monocytogenes* reduction achieved by chlorine washing

Reduction due to chlorine washing (log CFU/cm²)		Number of cases per year					
	Mean	Fold change*	1st percentile	5th percentile	Median	95th percentile	99th percentile
1 (Baseline)	25499.86	-	0.000973	0.00164	0.0381	121.77	31236.88
2	18837.51	0.74	0.000271	0.000482	0.0116	37.20	9624.02
3	13720.43	0.54	7.332×10^{-5}	0.000141	0.00352	11.56	2933.29
4	9832.48	0.39	1.978×10^{-5}	4.057×10^{-5}	0.00107	3.53	908.92
5	7261.84	0.28	5.259×10^{-6}	1.155×10^{-5}	0.000327	1.09	277.36
6	5113.24	0.20	1.378×10^{-6}	3.250×10^{-6}	0.000100	0.34	85.97

*Fold changes were calculated by comparing mean values of each scenario with the mean value of the baseline model.

4.3.6 Sensitivity analysis

The sensitivity of output estimated listeriosis cases to input variables was determined using Spearman's rank order correlation. Number of cases per year associated with fresh-cut cantaloupe consumption was most sensitive to the following inputs (Figure 13): retail temperature (0.69), home storage temperature (0.48), time until last consumption at home (0.27), time of retail storage (0.22), time until first consumption at home (0.13). Similarly, number of cases per year associated with whole cantaloupe consumption was most sensitive to the following inputs (Figure 14): home storage temperature after cutting (0.79), time until last consumption at home (0.48), time until first consumption at home (0.19).

In this study, retail and home storage temperatures are the most important factors affecting the predicted number of cases per year associated with fresh-cut cantaloupe consumption while home storage temperature and time after cutting are the most sensitive for predicted number of cases associated with whole melon consumption. The results indicated that temperature control is critical during post processing storage, since *L. monocytogenes* is a psychrotrophic pathogen which cannot be completely inhibited at recommended storage temperature for fresh-cut melons of 0-5°C (FDA, 2009). This highlights the necessity for strict temperature control of fresh-cut cantaloupe during both retail and home storage.
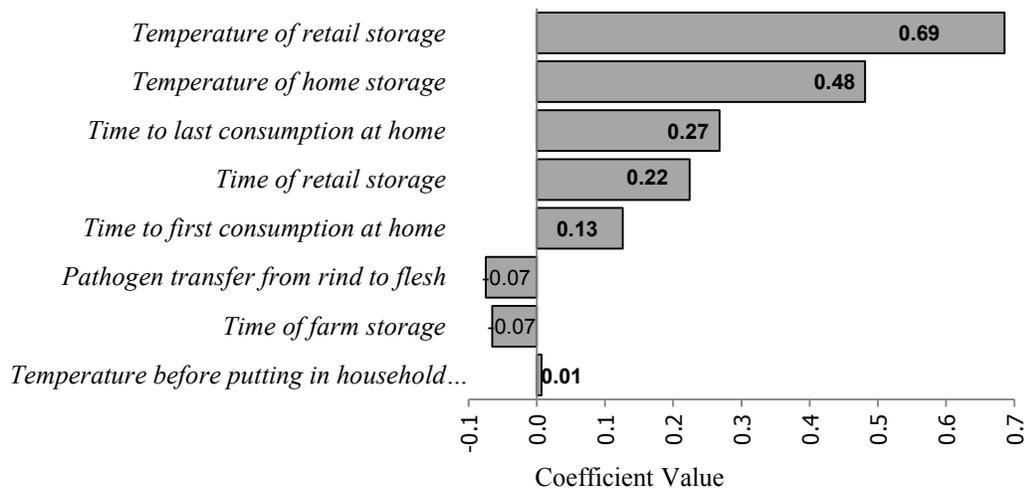
Figure 13. Tornado graph for correlation coefficients of input variables affecting the estimated number of cases per year associated with fresh-cut cantaloupe consumption. The Spearman correlation coefficients are shown next to each bar.
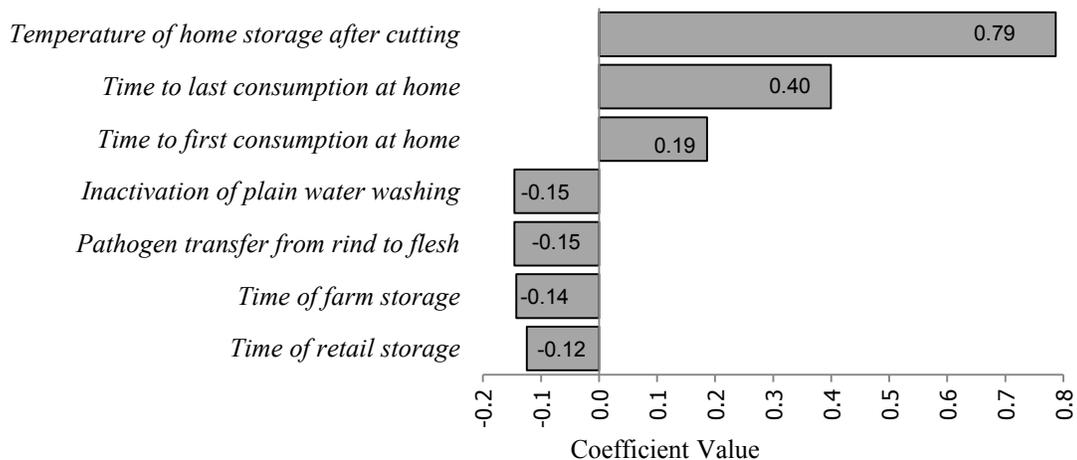
Figure 14. Tornado graph for correlation coefficients of input variables affecting the estimated number of cases per year associated with whole cantaloupe consumption. The Spearman correlation coefficients are shown next to each bar.

4.4 Discussion

This study developed a QMRA model for *L. monocytogenes* in cantaloupe consumed as either fresh-cut or whole cantaloupe. Although some QMRA have been

developed for fresh produce (Danyluk and Schaffner, 2011; Ding et al., 2013), this is the first QMRA model specifically developed for melon. Our results with 0.0381 median cases of listeriosis associated fresh-cut cantaloupe consumption, are in agreement with the predicted median cases of listeriosis for fresh fruits of 1.9×10^{-11} per serving basis and 0.9 per annum basis in risk assessment of listeriosis for 23 food categories carried out by FDA/FSIS (2003). There appears to be no other QMRA studies for listeriosis associated with fruits to which the current model can be compared.

Variability and uncertainty are two important factors in the development of QMRA models. Variability represents inherent heterogeneity of a population which is unavoidable, while uncertainty embodies the unknown of parameter values for variables. Separation of variability and uncertainty require large data availability and more complicated simulation techniques (Nauta, 2000). In this QMRA model, one dimensional modeling framework was applied with the combination of both variability and uncertainty which are described by probabilistic distributions.

Limitations and data gaps could be identified in this QMRA model. Since most of microbiological surveys for *L. monocytogenes* are qualitative test (presence/absence), concentration data is unavailable for initial contamination on cantaloupe after harvest. Little is known about the pre-harvest contamination of *L. monocytogenes* in irrigation water and soil for cantaloupe farming. Thus, an initial contamination value was inputted to generalize the pre-harvest contamination. Scenarios analysis was run to evaluate the impacts of different initial contamination after harvested and prevalence on the predicted number of listeriosis cases. Only post-

harvest operation without washing treatment was considered in this model because plenty of information was unknown for such operation on farm as all the farms we visited did not adopt postharvest washing. Nevertheless, it is reported that shed pack with postharvest washing demonstrated a higher risk than field pack (FDA, 2012).

Cantaloupe is chlorine washed and cut into chunks in fresh-cut processing facility. Reduction due to chlorine washing and cross contamination during fresh-cutting were included in this model. As data for efficacy of chlorine washing in the literature were achieved by a high concentration of 1000 ppm and 200 ppm chloride (Ukuku et al., 2002; Rodgers et al., 2004) which is impractical in melon industry and 4 ppm chlorine was implemented in a fresh-cut processing facility we visited, a scenario analysis with the reduction of *L. monocytogenes* from 1 to 6 log CFU/cm^2 was performed to evaluate different possible effectiveness of chlorine washing. Cross contamination is a potential risk factor during fresh-cut processing. For simplification, cross contamination was assumed as a result of transfer from contaminated melon rind to the food contact surface prior to cutting and subsequently, pathogen cells transferred from food contact surface back to the contaminated melon based on the assumption that pathogen cells are evenly distributed on cantaloupe rind as well as on food contact surface. A 20% proportion of melon rind was assumed to contact with the cutting board as such information is unknown. A distribution of pathogen cells on food contact surface was achieved from a separate cross contamination model to describe the contamination of food contact surface when contacted by the melon surface prior to cutting. Since no study has quantified the transfer ratio of *L. monocytogenes* between cantaloupe and food contact surface, data from Jensen et al.

(2013) on transfer ratio of *Salmonella* between watermelon, carrots, celery and kitchen surfaces were fitted with distribution to model cross contamination that could happen during fresh-cut processing.

Survival and growth of *L. monocytogenes* in fresh-cut and whole cantaloupe is critical for risk assessment. It is interesting that a 2-hour lag time was observed for the *L. monocytogenes* growing on the fresh-cut cantaloupes (Ukuku et al., 2012), whereas no lag phase was indicated for the growth of pathogens in fresh-cut melons in other studies (Fang et al., 2013; Li et al., 2013; Danyluk et al., 2014). To provide a worst-case scenario assumption, zero lag time was used to this QMRA model. As survival model in this QMRA is only based on data from 4°C and 20°C storage (Ukuku and Fett, 2002), a more accurate survival model is needed to describe how *L. monocytogenes* survives on cantaloupe surface during storage with temperature fluctuation. However, Behrsing et al. (2002) observed a significant growth of *Listeria innocua* on the inedible skins of cantaloupe during storage up to 7 days at 8°C. Martinez et al. (2013) noticed a significantly higher increase of *L. monocytogenes* on cantaloupe rind than on the flesh. In addition, water residue on the melon surface after postharvest washing could facilitate the growth of bacteria. Behavior of *L. monocytogenes* on cantaloupe with wet surface is worthy for more scrutiny.

Risk characterization was based on the preliminary assumption that the entire amount of cantaloupe consumed per year in the U.S. was either all consumed as fresh-cut, or as whole melon, since no more distinct consumption data for the two products is available. The ratio of fresh-cut and whole cantaloupe consumption could be applied in the future to achieve further estimation of public health impact

associated with cantaloupe consumption. In addition, this model does not consider the variability of consumption among individuals because of the unavailable information while different number of servings of fruits for three subpopulations were included in FDA/FSIS risk assessment (2003).

Quantitative risk assessment can provide a way to model the food system in a systematic way, which can provide risk managers a comprehensive picture of key factors that impact the contamination levels of a certain pathogen along the supply chain of a certain food product. The QMRA estimated the number of listeriosis cases per year associated with both fresh-cut and whole cantaloupe consumption among general healthy and susceptible population in the U.S. Median of risk per serving of fresh-cut cantaloupe consumption is around ten times higher than that of whole cantaloupe consumption which suggested fresh-cut consumption is riskier than whole cantaloupe. Median of risk per serving among susceptible population demonstrated 100 times higher than that among general healthy. In addition, this QMRA also compared the relative risks for different potential initial concentration, reduction of chlorine washing and prevalence based on the data gap as well as the uncertainty involved. Besides, sensitivity analysis provide a scientific basis for the necessity of strict temperature control along the melon processing and supply chain. Although limitations and assumptions lie within the model, the QMRA model provided a framework that is valuable to identify key factors and data gaps. The model is adaptable to provide better estimates as future research and available data could fill the gaps in the model.

Chapter 5: Conclusions and suggestions for future research

A microbiological survey of pre-harvest cantaloupe collected from farms in mid-Atlantic region demonstrated a good microbial quality of cantaloupe in this region. Good Agriculture Practice (GAP) is crucial in pre harvest operations to maintain a good quality of crop. The advantage of mulch in preventing contamination of pre-harvest operation was confirmed by this regional survey apart from the conventional application of mulch in weed control and moisture preservation. In the future study, enlargement of sample size could increase the robustness of our conclusion.

A QMRA model was developed in order to offer a systematic way for risk management and policy-making process to indicate the major risk factors along the farm-to-fork continuum of cantaloupe supply. Risk representing current supply chain for fresh-cut and whole cantaloupe was estimated based on a thorough review of available data in scientific publication as well as expert opinions from twice visits to a local fresh-cut processing facility. The predicted median of cases per year is reasonable and comparable to the risk assessment of FDA/FSIS (2003). Besides, the QMRA model developed in this study identify the risk factors along melon supply and compared the risk of fresh-cut and whole cantaloupe consumption which suggest a relatively safer way of whole cantaloupe consumption. The results reveal that retail and home storage temperature are the most important factors affecting the risk for cantaloupe consumption, suggesting risk management should take more efforts on temperature control at retail and home storage level for fresh-cut cantaloupe. Although limitations and data gaps exist, this QMRA model provided a first

framework of risk assessment for melon, described herein potential cross contamination during fresh-cut processing, and incorporated both survival and growth model that was able to quantify the decline of pathogen cells on cantaloupe rind as well as the multiplication of pathogen cells in flesh during transportation and storage.

Additional research for QMRA model development is needed on behavior of *L. monocytogenes* on cantaloupe surface with temperature and humidity fluctuation, efficacy of low concentrated chlorine washing, impacts of strain virulence and host susceptibility on dose response relationship. Critical data gaps were identified in this QMRA study including initial contamination and prevalence of *L. monocytogenes* on pre-harvest cantaloupe, ratio of fresh-cut and whole cantaloupe consumption, transfer ratio of *L. monocytogenes* between cantaloupe and food contact surface, farm and retail storage time, and time and temperature during transportation from farm to processing facility and from processing facility to retailer. Two dimensional Monte Carlo simulation to characterize both uncertainty and variability in the model need to be performed in future risk assessments.

Appendices

Appendix A

Appendix A-1: Two-Way ANOVA results of growing type and surface type factors affecting the level of fecal coliform on melon.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	5	34.65357	6.93071	4.2037	0.0016
Error	108	178.06208	1.64872		
C. Total	113	212.71565			

Effect tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Type	1	1	13.243645	8.0327	0.0055
Surface	2	2	11.71802	3.5537	0.032
Type*Surface	2	2	6.561718	1.9899	0.1417

Appendix A-2: Two-Way ANOVA results of growing type and surface type factors affecting the level of *Listeria* spp. on melon.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	5	38.51048	7.7021	3.4123	0.0068
Error	105	237.00484	2.25719		
C. Total	110	275.51532			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Type	1	1	11.556131	5.1197	0.0257
Surface	2	2	25.961974	5.751	0.0043
Type*Surface	2	2	0.712813	0.1579	0.8541

Appendix B

Appendix B-1: One-Way ANOVA results of three different surface types affecting the level of fecal coliform on melon.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	2	14.848	7.4241	4.1648	0.018
Error	111	197.87	1.7826		
C. Total	113	212.72			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Surface	2	2	14.848	4.1648	0.018

Appendix B-2: One-Way ANOVA results of three different surface types affecting the level of *Listeria* spp. on melon.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	2	26.121	13.06	5.6558	0.0046
Error	108	249.39	2.3092		
C. Total	110	275.52			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Surface	2	2	26.121	5.6558	0.0046

References

1. Abadias, M., Usall J., Anguera, M., Solsona, C. and Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology* 123: 121-129.

2. Ailes, E. C., Leon, J. S., Jaykus, L. A., Johnston, L. M., Clayton, H. A., Blanding, S., Kleinbaum, D. G., Backer, L. C. and Moe, C. L. 2008. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. *Journal of Food Protection* 71: 2389-2397.

3. Akins, E. D., Harrison, M. A. and Hurst, W. 2008. Washing practices on the microflora on Georgia-grown cantaloupes. *Journal of Food Protection* 71: 46–51.

4. Alvarado-Casillas, S., Ibarra-Sanchez, S., Rodriguez-Garcia, O., Martinez-Gonzales, N. and Castillo, A. 2007. Comparison of rinsing and sanitizing procedures for reducing bacterial pathogens on fresh cantaloupes and bell peppers. *Journal of Food Protection* 70: 655-660.

5. Annous, B. A., Burke, A., Sites, J. E. and Phillips J. G. 2013. Commercial thermal process for inactivating *Salmonella* Poona on surfaces of whole fresh cantaloupes. *Journal of Food Protection* 76: 420-428.

6. Annous, B. A., Burke, A. and Sites, J. E. 2004. Surface pasteurization of whole fresh cantaloupes inoculated with *Salmonella* Poona or *Escherichia coli*. *Journal of Food Protection* 67: 1876–1885.

7. Mahmoud, B. S. M. 2012. Effects of X-ray treatments on pathogenic bacteria, inherent microflora, color, and firmness on whole cantaloupe. *International Journal of Food Microbiology* 156: 296–300.

8. Bassett, J. and McClure, P. 2008. A risk assessment approach for fresh fruits. *Journal of Applied Microbiology* 104: 925–943.

9. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59: 204–216.

10. Bowen, A., Fry, A., Richards, G. and Beuchat, L. R. 2006. Infections associated with cantaloupe consumption: a public health concern. *Epidemiology and Infection* 134: 675-685.

11. Buchanan, R. L., Whiting, R. C. and Damert, W. C. 1997. When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology* 14: 313-326.

12. Canadian Food Inspection Agency (CFIA) Food Safety Action Plan Report. 2009-2010. Targeted surveys of bacterial pathogens in cantaloupes in the Canadian market.

    http://www.inspection.gc.ca/food/chemical-residues-microbiology/microbiology/cantaloupes/eng/1348592270745//1348592434455 accessed on 07/25/2014

13. Castillo, A. I., Mercado, L. M., Lucia, Y., Martinez-ruiz, J., Ponce de Leon, E. A. and Acuff, G. R. 2004. *Salmonella* contamination during production of cantaloupe: A binational study. *Journal of Food Protection* 67: 713-720.

14. Centers for Disease Control and Prevention. Foodborne Outbreak Online Database (FOOD). http://wwwn.cdc.gov/foodborneoutbreaks/ accessed on 07/24/2014.

15. Centers for Disease Control and Prevention. 2011. Multistate outbreak of *Salmonella* Panama infections linked to cantaloupe. http://www.cdc.gov/salmonella/panama0311/032911/ accessed on 06/11/2014.

16. Centers for Disease Control and Prevention. 2012. Multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe.   http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/ accessed on 06/11/2014.

17. Centers for Disease Control and Prevention. 2011. Multistate outbreak of Listeriosis linked to whole cantaloupes from Jensen Farms, Colorado. (Final Update)http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html accessed on 06/11/2014.

18. Czuprynski, C. J. 2005. *Listeria monocytogenes*: silage, sandwiches and science. *Animal Healthy Research Reviews* 6: 211-217.

19. Cosgrove, S. and Cronquist, A. 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe – United States, August – September 2011. *CDC Morbidity and Mortality Weekly Report* 60: 1-2.

20. Danyluk, M. D. and Schaffner, D. W. 2011. Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. *Journal of Food Protection* 74: 700-708.

21. Danyluk, M. D., Friedrich, L. M. and Schaffner, D. W. 2014. Modeling the growth of *Listeria monocytogenes* on cut cantaloupe, honeydew and watermelon. *Food Microbiology* 38: 52-55.

22. Ding, T., Iwahori, J., Kasuga, F., Wang, J., Forghani, F., Park, M. S. and Oh, D. H. 2013. Risk assessment for *Listeria monocytogenes* on lettuce from farm to table in Korea. *Food Control* 30: 190–199.

23. Doyle, M. P. and Erickson, M. C. 2012. Opportunities for mitigating pathogen contamination during on-farm food production. *International Journal of Food Microbiology* 152: 54-74.

24. Duffy, E. A., Lucia, L. M., Kells, J. M., Castillo, A., Pillai, S. D. and Acuff, G. R. 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. *Journal of Food Protection* 68: 70-79.

25. EcoSure. 2008. Cold temperature evaluation design and study summary. Available at: http://foodrisk.org/exclusives/ecosure, accessed on 06/13/2014.

26. Erickson, M. C. 2012. Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology* 3: 283-310.

27. Fan, X., Annous, B. A., Keskinen, L. A. and Mattheis, J. P. 2009. Use of chemical sanitizers to reduce microbial populations and maintain quality of whole and fresh-cut cantaloupe. *Journal of Food Protection* 12: 2453-2460.

28. Fan, X., Annous, B. A., Sokorai, K. J., Burke, A. and Mattheis, J. P. 2006. Combination of hot-water surface pasteurization of whole fruit and low-dose

gamma irradiation of fresh-cut cantaloupe. *Journal of Food Protection* 69: 912-919.

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

30. FAO/WHO. 2011. Microbiological hazards and melons. ftp://ftp.fao.org/ag/agn/.../jemra/Microbiological_hazards_and_Melons_Nov08.pdf accessed on 06/11/2014.

31. FAO/WHO. 2008. Microbiological hazards in fresh fruits and vegetables, meeting report. http://www.who.int/foodsafety/publications/micro/MRA_FruitVeges.pdf accessed on 06/11/2014.

32. FAO/WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical report. http://www.fao.org/docrep/010/y5394e/y5394e00.HTM accessed on 06/13/2014.

33. Foord, K., MacKenzie, J. 2009. Growing melons (Cantaloupe, Watermelon, Honeydew) in Minnesota Home Gardens. http://www.extension.umn.edu/distribution/horticulture/M1262.html accessed on 06/13/2014.

34. Gagliardi, J. V., Millner, P. D., Lester, G. and Ingram, D. 2003. On-farm and postharvest processing sources of bacterial contamination to melon rinds. *Journal of Food Protection* 66: 82–87.

35. Gayler, B. G. E., Maccready, R. A. and Reardon, J. P. 1955. An outbreak of Salmonellosis traced to watermelon. *Public Health Reports* 70: 311–313.

36. Gerchikov, N., Keren-Keiserman, A., Perl-Treves, R. and Ginzberg, I. 2008. Wounding of melon fruits as a model system to study rind netting. *Scientia Horticulture* 117: 115-122.

37. Golden, D. A., Rhodehamel, E. J. and Kautter, D. A. 1993. Growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons. *Journal of Food Protection* 56: 194-196.

38. Guidance. National commodity specific food safety guidelines for cantaloupes and netted melons. 2013. http://www.cantaloupe-guidance.org/docs/national-commodity-specific-food-safety-guidelines-cantaloupes-and-netted-melons accessed on 06/11/2014.

39. Hong, Y. K., Yoon, W. B., Huang, L. and Yuk, H. G. 2014. Predictive modeling for growth of non- and cold-adapted *Listeria monocytogenes* on fresh-cut cantaloupe at different storage temperatures. *Journal of Food Science* 79: 1168-1174.

40. Jensen, D. A., Friderich, L. M., Harris, L. J., Danyluk, M. D. and Schaffner, D. W. 2013. Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. *Journal of Food Protection* 76: 1530-1538.

41. Johnston, L. M., Jaykus, L. A., Moll, D., Martinez, M. C., Anciso, J., Mora, B. and Moe, C. L. 2005. A field study of the microbiological quality of fresh produce. *Journal of Food Protection* 68: 1840-1847.

42. Johnston, L. M., Jaykus, L. A., Moll, D., Martinez, M. C., Anciso, J., Mora, B. and Moe, C. L. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *International Journal of Food Microbiology* 112: 83-95.

43. Latorre, A. A., Pradhan, A. K., Van Kessel, J. A., Karns, J. S., Boor, K. J., Rice, D. H., Mangione, K. J., Grohn, Y. T. and Schukken, Y. H. 2011. Quantitative risk assessment of listeriosis due to consumption of raw milk. *Journal of Food Protection* 74: 1268-1281.

44. Li, D., Friedrich, L. M., Danyluk, M. D., Harris, L. J. and Schaffner, D. W. 2013. Development and validation of a mathematical model for growth of pathogens in cut melons. *Journal of Food Protection* 76: 953-958.

45. Li-Cohen, A. E. and Bruhn, C. M. 2002. Safety of consumer handling of fresh produce from the time of purchase to the plate: a comprehensive consumer survey. *Journal of Food Protection* 65: 1287-1296.

46. Lin, C. and Wei, C. 1997. Transfer of *Salmonella* Montevideo onto the interior surfaces of tomatoes by cutting. *Journal of Food Protection* 60: 858–862.

47. Lopez-Velasco, G., Pham, T., Wei, P., Tomas-Callejas, A., Sbodio, A. and Suslow, T. 2013. Abstract: Survival of *Listeria innocua*, *Listeria monocytogenes* and *Salmonella enterica* on watermelon surfaces during storage and postharvest washing. *Journal of Food Protection* 76 supplement*:* 46.

48. Materon, L. A. 2003. Survival of *Escherichia coli* O157:H7 applied to cantaloupes and the effectiveness of chlorinated water and lactic acid as disinfectants. *World Journal of Microbiology & Biotechnology* 19: 867-873.

49. McCollum, J. T., Cronquist, A. B., Silk, B. J., Jackson, K. A. et al. 2013. Multistate outbreak of listeriosis associated with cantaloupe. *New England Journal of Medicine* 369: 944-953.

50. Martinez, M. R., Siletzky, R. and Kathariou, S. 2013. Abstract: Survival and growth of outbreak strains of *Listeria monocytogenes* on cantaloupe. *Journal of Food Protection* 76 supplement: 135.

51. Munnoch, S. A., Ward, K., Sheridan, S., Fitzsimmons, G. J., Shadbolt, C. T., Piispanen, J. P., Wang, Q., Ward, T. J., Worgan, T. L. M., Oxenford, C., Musto, J. A., Mcanulty, J. and Durrheim, D. N. 2009. A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. *Epidemiology and Infection* 137: 367-374.

52. Mürmann, L. 2011. Quantitative risk assessment for human salmonellosis through the consumption of pork sausage in Porto Alegre, Brazil. *Journal of Food Protection* 74: 553-558.

53. Nauta, M. J. 2000. Separation of uncertainty and variability in quantitative microbial risk assessment models. *International Journal of Food Microbiology* 57: 9-18.

54. Ng, P. J., Fleet, G. H. and Heard, G. M. 2005. Pesticides as a source of microbial contamination of salad vegetables. *International Journal of Food Microbiology* 101: 237-250.

55. Olaimat, A. N. and Holley, R. A. 2012. Factors in influencing the microbial safety of fresh produce: A review. *Food Microbiology* 32: 1-19.

56. Oliveira, M., Vinas, I., Colas, P., Anguera, M., Usall, J. and Abadias, M. 2014. Effectiveness of a bacteriophage in reducing *Listeria monocytogenes* on fresh-cut fruits and fruit juices. *Food Microbiology* 38: 137-142.

57. Ottoson, J. R., Nyberg, K., Lindqvist, R. and Albihn, A. 2011. Quantitative microbial risk assessment for *Escherichia coli* O157 on lettuce, based on survival data from controlled studies in a climate chamber. *Journal of Food Protection* 74: 2000–2007.

58. Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J. and Griffin, P. M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases* 19: 407-415.

59. Palekar, M. P., Cabrera-Diaz, E., Kalbasi-Ashtari, A., Maxim, J. E., Miller, R. K., Cisneros-Aevallos, L. and Castillo, A. 2004. Effect of electro beam irradiation on the bacterial load and sensorial quality of sliced cantaloupe. *Journal of Food Science* 69: 267-273.

60. Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anciso, J. and Ivanek, R. 2012. Risk factors for microbial contamination in fruits and vegetables at the pre-harvest level: A systematic review. *Journal of Food Protection* 75: 2055-2081.

61. Parnell, T. L., Harris, L. J. and Suslow, T. V. 2005. Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *International Journal of Food Microbiology* 99: 59–70.

62. Pouillot, R., Lubran, M. B., Cates, S. C. and Dennis, S. 2010. Estimating parametric distributions of storage time and temperature of ready-to-eat foods for U.S. households. *Journal of Food Protection* 73: 312-321.

63. Pouillot, R., Miconnet, N., Afchain, A.-L., Delignette-Muller, M. L., Beaufort, A., Rosso, L., Denis, J.-B. 2007. Quantitative risk assessment of *Listeria monocytogenes* in French cold-smoked salmon: I. Quantitative exposure assessment. *Risk Analysis* 27: 683–700.

64. Pradhan, A. K., Ivanek, R., Grohn, Y. T., Bukowski, R., Geornaras, I., Sofos, J. N. and Wiedmann, M. 2010. Quantitative risk assessment of listeriosis-associated deaths due *to Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. *Journal of Food Protection* 73: 620–630.

65. Ratkowsky, D. A., Olley, J., McMeekin, T. A. and Ball, A. 1982. Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology* 149: 1-5.

66. Patil, R., Thorns, J. and Ryser, E. 2013. Abstract: Extent of *Listeria monocytogenes* transfer during cutting of cantaloupe and honeydew melon. *Journal of Food Protection* 76 supplement: 211.

67. Rodgers, S. L., Cash, J. N., Siddiq, M. and Ryser, E. T. 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. *Journal of Food Protection* 67: 721–731.

68. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V, Widdowson, M. A., Roy, S. L., Jones, J. L. and Griffin, P. M. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerging Infection Disease* 17: 7–15.

69. Selma, M. V. 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 Microbiology* 25: 162-168.

70. Sharma, M., Patel, J. R., Conway, W. S., Ferguson, S. and Sulakvelidze, A. 2009. Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettuce. *Journal of Food Protection* 72: 1481–1485.

71. Dobhal, S., Zhang, G., Gautam, D., Timmons, G. and Ma, Li. 2013. Abstract: Uneven distribution of microorganisms on the surface of field-grown cantaloupes. *Journal of Food Protection* 76 supplement: 225.

72. Koseki, S., Mizuno, Y., Kawasaki, S. and Yamamoto, K. 2011. A survey of iceberg lettuce for the presence of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* in Japan. *Journal of Food Protection* 74: 1543–1546.

73. Ruengvisesh, S., Villarreal, M., Anciso, J., Cisneros-Zevallos, L., Murano, E., Castillo, A. and Taylor, M. 2013. Abstract: Differential quantification of microorganisms on skin or rind and stem scar of tomatoes and cantaloupes harvested over two seasons in South Texas. *Journal of Food Protection* 76 supplement: 224.

74. Stine, S. W., Song, I., Choi, C. Y. and Gerba, C. P. 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection* 68: 913-918.

75. Strawn, L. K., Grohn, Y. T., Warchocki, S., Worobo, R. W., Bihn, E. A. and Wiedmann, M. 2013. Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Applied and Environmental Microbiology* 79: 7618-7627.

76. Suslow, T. 2010. California melon research board 2010 final report project title : Melon Food Safety. http://www.cmrb.org/documents/files/20110110085901.pdf accessed on 06/13/2014

77. Tauxe, R., Hedberg, H. K. C., Potter, M., Madden, J. and Wachsmuth, K. 1997. Microbial hazards and emerging issues associated with produce: A preliminary report to the National Advisory Committee on microbiologic criteria for foods. *Journal of Food Protection* 60: 1400–1408.

78. U.S. Food and Drug Administration (FDA). 2012. Draft Qualitative assessment of risk to public health from on-farm contamination of produce. http://www.nationalwatermelonassociation.com/pdfs/Qualitative_Assessment_of _Risk_to_Public_Health_From_On_Farm_Contamination_of_Produce[1].pdf accessed on 06/14/2014.

79. U.S. Food and Drug Administration (FDA). 2011. Environmental Assessment: Factors potentially contributing to the contamination of fresh whole cantaloupe Implicated in a multi-state outbreak of listeriosis. http://www.fda.gov/food/recallsoutbreaksemergencies/outbreaks/ucm276247.ht m accessed on 06/11/2014.

80. U.S. Food and Drug Administration (FDA). FDA/FSIS. 2003. Quantitative assessment of relative risk to public health from foodborne among selected

categories of ready-to-eat foods.

http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm183966.htm accessed on 06/13/2014

81. U.S. Food and Drug Administration (FDA). 2009. Guidance for Industry: Guide to minimize microbial food safety hazards of melons; Draft Guidance. http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ucm174171.htm accessed on 06/13/2014.

82. U.S. Food and Drug Administration (FDA). 2012. Information on the recalled Jensen Farms whole cantaloupe. http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm272372.htm accessed on 06/11/2014.

83. Ukuku, D. O. and Sapers, G. M. 2001. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *Journal of Food Protection* 64: 1286-1291.

84. Ukuku, D. O. and Fett, W. F. 2002a. Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection* 65: 924-930.

85. Ukuku, D. O. and Fett, W. F. 2002b. Effectiveness of chlorine and nisin-EDTA treatments of whole melons and fresh-cut pieces for reducing native microflora and extending shelf-life. *Journal of Food Safety* 22: 231-253.

86. Ukuku, D. O. and Fett, W. F. 2002c. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *Journal of Food Protection* 65: 1093-1099.

87. Ukuku, D. O., Bari, M. L., Kawamoto, S. and Isshiki, K. 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. *International Journal of Food Microbiology* 104: 225-233.

88. Ukuku, D. O., Olanya, M., Geveke, D. J. and Sommers, C. H. 2012. Effect of native microflora, waiting period, and storage temperature on *Listeria monocytogenes* serovars transferred from cantaloupe rind to fresh-cut pieces during preparation. *Journal of Food Protection* 75: 1912-1919.

89. USDA Economic Research Service. Food Availability (Per Capita) Data System http://www.ers.usda.gov/data-products/food-availability-(per-capita)-data-system.aspx#.U5kdel7PLuc accessed on 06/11/2014.

90. Vadlamudi, S., Taylor, T. Matthew, B. C. and Castillo, A. 2012. Effect of chemical sanitizers on *Salmonella enterica* Serovar Poona on the surface of cantaloupe and pathogen contamination of internal tissues as a function of cutting procedure. *Journal of Food Protection* 75: 1766-1773.

91. Walsh, K., Gould, L. H. and Bennett, S. 2013. Abstract: Melon-associated outbreaks of foodborne disease in the United States, 1973-2011. *Journal of Food Protection* 76 Supplement: 228.