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Prevalence of Indicator Organisms, Equipment Assessment of Risk, and Lexicon Development: An Analysis of the Tomato Packinghouse Environment

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To the Graduate Council:

I am submitting herewith a thesis written by Alexis Marie Hamilton entitled "Prevalence of Indicator Organisms, Equipment Assessment of Risk, and Lexicon Development: An Analysis of the Tomato Packinghouse Environment." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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Prevalence of Indicator Organisms, Equipment Assessment of Risk, and Lexicon Development: An Analysis of the Tomato Packinghouse Environment

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DEDICATION

I dedicate this thesis to my parents, who have sacrificed so much to enable me to pursue this work.

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ABSTRACT

As a result of previous outbreaks associated with packinghouse contamination and in conjunction with new regulatory requirements, environmental monitoring targeting *Listeria monocytogenes* has been recommended for packinghouses. However, there is an overall lack of knowledge regarding problem areas in the packinghouse. Absence of sufficient environmental monitoring programs have left growers and packinghouse operators ill-equipped to effectively monitor for *Listeria* species, a common indicator group for *L. monocytogenes*. A better understanding of *Listeria* spp. in the packing environment is required, in addition to an easily implemented method for conducting site-specific risk analysis to effectively target and eliminate foodborne pathogens during packing and between harvesting seasons. Three tomato packinghouses were sampled for presence of Gram-positive bacteria and *Listeria* spp. on zone 1 contact surfaces during the 2017 harvesting season. A designated surface area of 100 cm^2 [square centimeters] was sampled and stored in Dey Engley neutralizing buffer. Gram-positive bacteria were spiral-plated on Modified Oxford Medium (MOX) and incubated for 48 h [hours] at 35 °C [degrees Celsius]. A 1-ml [milliliter] sample was also enriched and streaked on MOX for basic detection of *Listeria* spp. Presumptive positive samples were confirmed with PCR. Additionally, common food-grade materials used in packinghouse environments were also collected and evaluated to describe

differences in attributes between materials that could affect microbial harborage or sanitation effectiveness. Materials were assigned numerical ratings for each value that were combined with microbial data to issue a resistance to clean score, which described cleanability of that material. While evidence of microbial harborage was not observed throughout sampling, several niche points were established as areas for potential attachment of *Listeria* spp. after sanitation. Additionally, a methodology was developed for growers and packinghouse operators to utilize to evaluate their equipment for areas that may be of greater risk to the integrity of their food safety system. This methodology can be implemented to enable the development of a more targeted approach to eliminating *Listeria* spp. in the packinghouse.

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Fruits and vegetables have been shown to be a vehicle for foodborne diseases. This is likely due to a combination of increased consumption of produce as part of a healthy diet, importation of products from various countries with varying levels of food safety rigor, and increased detection capabilities as part of the development of federal and local surveillance networks and reporting requirements. Additionally, the community of resident microorganisms associated with the plant structure, while commensal to the plant, have the potential to infect susceptible consumers if allowed to grow to infectious levels.

Produce-related Outbreaks

Of the 9.4 million annual foodborne illnesses in the United States, approximately 39% (3.6 million) were caused by bacteria *(80)*. Produce (fruits, nuts, and vegetables) accounted for almost 50% of foodborne illnesses in the United States over a ten-year period; of those, nearly 30% were due to bacterial causes *(74)*. Between 1998-2008, vegetables (fungi, leafy, root, sprout, and vine-stalk) accounted for three times more bacterial foodborne illnesses than fruits and nuts combined *(74)*. Vine-stalk vegetables (squash, tomatoes, etc.) were the implicated commodity in over half of these illnesses (57.8%) *(73, 74)*. Furthermore, of U.S. outbreaks with a known vehicle, the proportion due to produce increased by more than 5% over two decades *(62)*. The cause of this

increase is likely multifaceted, including increased consumption of fresh produce, elevated demand to consume produce outside of characteristic harvest seasons, different food safety and handling practices in foreign countries from which produce is sourced, and variations in transport conditions, among others. For example, between 1996-2014, imported produce was responsible for 33% of total outbreaks associated with imported foods, with Latin America and the Caribbean being the most common regions implicated *(41)*.

There are several challenges that the produce industry faces. Unlike many foods, produce is consumed without a heat treatment applied to reduce the microbial load *(18, 62)*. Additionally, washing postharvest results in minimal microbial reduction (90-99.9%) *(17, 92)*. Furthermore, due to the natural microbial ecology of the soil environment, fruits and vegetables grown in close contact with soil are at an increased risk for contamination. In addition to the rhizosphere microbial community, the use of manure, manure-based compost, and irrigation water have the potential to transmit unwanted microorganisms to the plant surface. Moreover, unsafe contact of this produce with wildlife *(57)*, livestock *(12)*, fly or bird populations *(4, 61, 90)*, and poor hygiene in human workers or operators *(11)* also provide risks for contamination.

Tomato associated Pathogens

Tomatoes (*Solanum lycopersicum*) have been implicated in 35 outbreaks in the United States from 1979-2011 *(89)*. In fact, in 2015, vine crops (*e.g.* tomatoes

and cucumbers) caused the most foodborne illnesses of all vegetable crops *(1)*. Viruses, parasites, and bacteria were all causative agents, with *Salmonella enterica* and *Listeria monocytogenes* as the only causative agents in domestically-grown tomatoes. The variation in serovars associated with outbreaks and tomato cultivars, in addition with current research findings, suggest that colonization of tomato surfaces by foodborne pathogens is cultivar-dependent *(8, 89)*. Additionally, some elements of the tomato anatomy are better colonized by bacteria. Barak, Kramer, and Hao have shown that pedicels and calyxes are more susceptible to *Salmonella* colonization via contaminated water *(8)*. These associations are just beginning to be uncovered as researchers have an expanding array of tools to evaluate the behavior of foodborne pathogens.

To the author's knowledge, the 1979 outbreak of *L. monocytogenes* is the only known instance of listeriosis in the U.S. due to contaminated tomatoes. Twenty cases of serotype 4b were confirmed from 8 hospitals in the Boston, Massachusetts area due to suspected tainted tomatoes *(47)*. This was one of the early instances of listerial contamination of produce, with cabbage being the first commodity implicated *(81)*. This point-source outbreak initiated the hypothesis that *L. monocytogenes* could be transmitted by the fecal-oral route. Subsequent studies about the organism and its relationship to tomato surfaces has shown the ability to grow on the tomato surface at near-room temperature conditions (21 °C), but not 10 °C, or slightly above refrigeration temperatures *(13)*. This research suggested that a major contributor to the growth of the pathogen was

temperature abuse during storage. If the organism is allowed to incubate at sufficient temperatures that encourage growth to levels threatening to public health, consumption of that food is much more likely to be followed by listerial infection.

Microbial Ecology of Produce

The microbiology of the plant environment is a diverse, complex, and interrelated community affected by plant and microbial activity. There are many factors that influence interactions between the plant surface and the microorganisms associated with it, including plant age and species, soil type, season, microbial colonization, root zone, and rhizodeposition *(32, 33, 43, 54, 100)*. Specifically, nutrients from sloughed plant cells, called rhizodeposits, affect the microbial community in the soil (rhizosphere) by providing sources of carbon, nitrogen, and gases that are differentially used by microorganisms *(27, 29, 30, 88)*. In fact, high populations of Gram-positive species have been detected and differentiated within a variety of plant soil environments *(37, 76)*. This contradicts existing research suggesting that Gram-negative organisms are better colonizers of the rhizosphere *(2, 52)*. Microbial communities associated with the soil and root environments vary based on and are perpetuated by local plant species *(86)*.

Listeria spp. were identified in both cultivated and uncultivated field settings *(32)*, indicating potential sources of contamination from the environment and cultivation practices. The use of soil amendments of animal origin, with most

focus on manure, is an established route of soil contamination *(3, 81, 91)*. Once present in the rhizosphere, *Listeria* spp. exhibit typical saprophytic bacteria behavior (recycling organic matter, colonizing root hair surfaces, etc.) *(21, 56)*.

II. *Listeria*-associated Outbreaks Linked to Fresh Produce

The prevalence of *Listeria monocytogenes* in the environment and in food products has led to increased awareness and monitoring of the organism across all levels of the food industry, particularly because of its high lethality rate among immunosuppressed populations. The organism is particularly suited for growth and survival across broad temperature ranges and food matrices, making it a significant issue in foods that are not heated prior to consumption. The zero-tolerance rule enacted by the Food and Drug Administration (FDA) emphasizes the industry's stance on eliminating the organism from the final product.

Properties of *Listeria* spp.

Listeriae are a group of Gram-positive, non-sporeforming, intracellular, rod-shaped bacteria. The abilities of *Listeria* spp. to survive extreme pH (4.2-9.6) *(20)*, high salt concentrations (10%) *(14)*, various antimicrobial agents (cinnamon oil, tetracycline, etc.) *(60)*, and grow across a wide temperature range (1-45 °C) *(20)*, coupled with its ubiquitous existence in the environment *(14)*, make this group extremely capable of persisting in the food handling environment. The

Listeria genus is composed of five major species: *L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri,* and *L. welshimeri*. These five are commonly characterized as the *sensu stricto* group; however, recent genetic evidence has surfaced to suggest that a new species, *L. marthii*, isolated from the Finger Lakes National Forest in 2010, should also be included within this classification *(42, 72)*. All other species are classified as *sensu lato*, including *L. grayi*, which was formally classified as *sensu stricto (72)*.

The *sensu stricto* group are motile via peritrichous flagella and exhibit tumbling motility. Flagellar function is temperature-dependent, expressed maximally from 4-30 °C and minimally at human body temperature (37 °C) *(75)*. These species are classified into serogroups based on expression of the A, B, C, D, or E flagellar (H) antigen. These six species are further classified into at least 17 serovars based on somatic (O) antigens, of which *L. monocytogenes* represents 13 *(50)*. The most common serotypes associated with listeriosis are 1/2a, 1/2b, and 4b, with 4b accounting for approximately 60-85% of all infections *(14, 66)*.

Listeria monocytogenes is the only known member of the *Listeria* genus to cause disease in humans, aside from a few isolated cases due to *L. ivanovii*, which typically causes illness in animals *(26, 44)*. *L. monocytogenes* is capable of infecting humans by crossing three barriers: intestinal lumen-blood (gastroenteritis), blood-brain (meningitis), and maternal-fetal (abortion/stillbirth). The gastrointestinal form of listerial infection occurs after ingestion of a sufficient

number of organisms. The infective dose for *L. monocytogenes* is currently unknown. Studies in normal adult mice identified a range of 50 to 10^{11} cells were necessary to achieve a 50% lethal dose (LD_{50}) *(14, 50, 78)*. Immune status likely plays a role in the infective dose. Children, the elderly, pregnant women, and immunosuppressed individuals are more susceptible to listerial infection than the normal, healthy population and so likely require fewer cells to become infected. However, disease manifestation is the same across the population, with fever, watery diarrhea, joint pain, and headache being the most common symptoms reported, in descending order *(70)*. The organism is responsible for 800 laboratory confirmed cases of listerial gastroenteritis each year, of which 94% are hospitalized and 15.9% succumbed to the illness *(80)*.

Once ingested, *L. monocytogenes* must survive several of the human body's defense mechanisms, including mucous membranes, stomach acid, pancreatic enzymes, bile, and intestinal secretions. *L. monocytogenes* can evade these host defenses through several adaptive mechanisms. The organism avoids trapping in the mucous membranes by persisting in foods in either sufficient numbers or encased within the food matrix *(97)*. Additionally, high fat, protein, and sugar foods can exhibit an insulator effect on microorganisms, protecting them from the effects of contact with stomach acid *(97)*. In addition to the increase of the pH of stomach acid due to neutralization by food particles, *L. monocytogenes* is able to protect itself against gastric acid through its glutamate decarboxylase (GAD) *(14, 24)* and acid tolerance response (ATR) systems *(68)*.

This adaptation to the gastrointestinal environment is modulated by the alternative sigma factor (Sigma B) regulon, which contains the genes that enable acid, bile, and salt adaptation *(20, 45, 69)*.

As an intracellular pathogen, the organism must translocate through the intestinal tract to survive and induce infection. Sigma B was discovered to be the chief regulator of virulence genes, including the internalin genetic spectrum (*inlA, inlB, inlC,* and *inlH*) *(87)*. Internalin, a surface protein, binds to an epithelial cell glycoprotein, E-cadherin, invading the mammalian cell *(65)*. Research shows that cells that do not produce internalin are not able to invade mammalian cells, and thus unable to induce infection *(58)*.

Once inside, *Listeria* cells lyse the host cell vacuole, reproduce in the cytosol, and polymerize an actin tail to move between host cells or into the bloodstream *(21, 39, 67)*. Another protein used during cell invasion is p60, encoded by the *iap* gene and present in all *Listeria* species. While the exact elucidation of the role of this invasion associated protein is unknown, preliminary studies indicate an integral role in cell division and the actin-mediated movement *(46, 77)*.

Upon entry into the bloodstream (septicemia), there are two major disease manifestations that could ensue. First, the organism could cross the blood-brain barrier, inducing bacterial meningitis, of which it is the third most common cause *(34)*. The second manifestation is entry into the umbilical cord and crossing the

maternal-fetal boundary, inducing spontaneous abortions and maternal complications or death.

Listeria and Produce

Ninety-nine percent (792 cases) of listeriosis infections in the United States were transmitted by food *(80)*. Several fruit and vegetable commodities have been implicated in outbreaks or recalls due to this microorganism, including apples *(5)*, cantaloupe *(19)*, celery *(40)*, and tomatoes *(47)*. Fruits and vegetables are likely to naturally encounter *Listeria* species in the environment during growing and harvesting, as the organism's ecological niche appears to be soil and decaying vegetation *(38, 51, 96)*; although, it has also been consistently found in avian intestinal tracts *(94)*. Isolation of these organisms increases with water availability *(95, 96)*, suggesting another potential route of dissemination.

Listeria and Ready-To-Eat Foods

Estimates of the cost of food safety measures in the United States related to *Listeria monocytogenes* mitigation range from 0.01 to 2.4 billion dollars annually, with the estimated benefits of those measures affording food companies 2.3 to 22 billion dollars in the same timeframe *(48)*. Batz et. al. (2014) found that the U.S. population suffers over 5,800 quality-adjusted life years (QALYs) for every 1,000 cases of *L. monocytogenes*, compared to 26 QALYs for *Escherichia coli* O157:H7, and 16 for *Salmonella* and *Campylobacter* spp. *(9)*. Due to the

extremely pathogenic nature of *Listeria monocytogenes*, the United States Food and Drug Administration (FDA) established a zero-tolerance policy for it in ready-to-eat (RTE) foods in 1985. The policy states that detection of the organism via an FDA-validated method in “any food that is normally eaten in its raw state or any other food, including a processed food, for which it is reasonably foreseeable that the food will be eaten without further processing that will significantly minimize biological hazards” is a violation of the Food, Drug, and Cosmetic (FD&C) Act, section 342(a)(1) and (4) *(6, 82)*. As a result, any produce contaminated with *L. monocytogenes* is characterized as adulterated and unable to be sold. Since this is an interpretation of the law, the USA vs Union Cheese Company case of 1995 is used as proof of an accurate explanation *(7)*. Under this law, recalls of RTE foods contaminated with *L. monocytogenes* are classified as a Class I recall by the FDA’s Health Hazard Evaluation Board. The passage of the Food Safety Modernization Act (FSMA) now gives the FDA the jurisdiction to initiate such a recall.

While the status of *Listeria monocytogenes* in ready-to-eat foods is solidly established in the United States, the global perspective is considerably more varied. For example, the International Commission on Microbiological Specification for Foods (ICMSF) states that a food is safe to consume by not-at-risk individuals if the organism is present in less than 100 CFU/g of food *(50)*.

Resiliency in the Food Processing and Packing Environment

Listeria monocytogenes has shown the ability to attach to a variety of surfaces found in the food processing environment, including stainless steel, glass, and plastics *(10, 15, 63)*. This ability to attach is one of the first steps of biofilm formation and a required step for transfer. Attachment is made possible through several routes, including flagella, surface adhesins, and the nature of liquid environments to bring organisms in contact with surfaces during processing. Initial attachment is followed by the development of microcolonies encased within an exopolysaccharide *(50)*.

Biofilms are complex communities of one or more microorganisms encased in an extracellular polymeric substance. Microorganisms naturally occur in biofilms, but these formations are problematic when they develop in the food industry due to their ability to protect bacterial cells from removal during cleaning and the lethal effects of sanitizing agents, allowing them to persist and potentially infect consumers *(35)*. Approximately 80% of hard-to-eliminate bacterial infections in the United States were associated with biofilm development *(49)*. Some studies show that biofilm formation varies by serotype, with 1/2a and 1/2b exhibiting strong biofilm forming abilities *(31)*, while others suggest these differences are the result of strain variations *(93)*. However, there has yet to be a clear correlation between either theory in describing biofilm forming abilities of *L. monocytogenes (28, 79)*. It is known that surface attachment and biofilm development are separate processes *(53)*.

Biofilm formation conveys an evolutionary advantage upon microorganisms that are capable of forming them; in fact, most organisms grow in single or multi-organism biofilm structures in the environment naturally *(23)*. In the food industry, biofilms have been shown to reduce cleaning efficiency *(84, 85)*, reduce heat transfer *(25, 55)*, and confer resistance to disinfectants and sanitizers *(16, 83)*. The surface topography of the food contact materials used in industry and at the farm have an effect on the ability of microorganisms to develop biofilms, and materials with hard-to-reach crevices or niche points tend to support biofilm formation *(98)*. Disruption of these surfaces during processing can result in sloughing off of part of the biofilm matrix, which can either be transplanted elsewhere along the processing line to grow (creating another niche or harborage point) or attach and contaminate to the food surface *(101)*. Due to the difficulty of removal of biofilms once they are attached to a food contact surface, it is preferable to prevent their development from the start.

III. Packinghouse Environment

Postharvest interventions implemented in the packinghouse are important for controlling resident *Listeria* spp. present on the surface of harvested vegetables. These practices are centered around controlling organisms on the vegetable surface and also controlling the spread of those organisms throughout or between lots. Poor construction of the packinghouse and processing line could

exacerbate the harborage of bacterial pathogens within or on equipment surfaces due to deterring processing or sanitation interventions. Therefore, control of the microorganism through processing interventions and hygienic design are of the utmost importance during packing.

Microbial Control During Packing

Intervention strategies for the control of foodborne pathogens in the packing environment have been explored as a means of mitigating risk during produce packing. For example, disinfectants have reduced bacterial load, but their success depends on the cultivar, surface characteristics, application method, and type of pathogen targeted *(71)*. For some commodities, the packinghouse is the last opportunity of pathogen reduction methods to be applied before consumption by the consumer. A minimum goal for packinghouse operations is to maintain the hygiene of the facility and equipment as to prevent these surfaces from contributing contamination to the produce being packed.

Packingline Structure and Hygienic Design

The purpose of food processing is to minimize the pathogenic and/or spoilage microorganisms on the food surface as the product moves along the processing line. There are specific areas along the processing line that are designed to significantly minimize or control for foodborne pathogens, called critical control points. But these food safety measures could be negated entirely by improper

management or design of the processing facility. The packinghouse itself can be as rudimentary as four posts and a roof or an intricate and highly automated, fully enclosed facility. Packinghouses are often open to the environment and include many different types of food contact materials. Additionally, the design decisions associated with the construction of packinghouses are largely up to the discretion of the owner of the packinghouse, and so can vary widely from packinghouse to packinghouse across commodities and regions.

Due to a lack of knowledge about hygienic design, insufficient funds to make necessary configuration adjustments, or the lack of available materials, the packing line could be constructed in such a way as to cause food safety problems during or after processing. For example, areas that cannot be easily cleaned could conceal microorganisms, protecting them from the lethal effects of sanitizers *(59)*. These areas that are more prone to collection of microorganisms, called niche points, could result in harborage of the microorganisms through survival and growth, as evidenced through repeated isolation when monitoring the food processing environment for the foodborne pathogen or associated indicator organisms. Harborage sites pose a significant risk to food safety as they present opportunities for foodborne pathogens to persist, multiply, and contaminate produce surfaces that encounter those sites.

Food safety can be enhanced by designing easy to clean materials and configurations, known commonly as hygienic design. Several engineering and material design groups have developed principles and associated metrics by

which equipment and food processing facilities can be evaluated, including the European Hygienic Engineering and Design Group (EHEDG), the National Sanitation Foundation (NSF), and 3-A Sanitary Standards, Inc (3-A). These organizations certify hygienically designed equipment and make recommendations for constructing easily cleaned facilities. A barrier to adopting hygienic design in packing facilities is the lack of certified equipment that could be used in the packinghouse. A piece of equipment can only be certified as hygienically designed if there is a standard already written for it (3-A), after a review of equipment design and clean-in-place (CIP) testing (EHEDG), or evaluated against developed standard and protocol requirements (NSF). Furthermore, any modifications to the material that occur after certification due to use, feasibility of cleaning, or wear render the certification null.

These difficulties to acquiring sanitary equipment and maintaining their certification have established a need for an alternative/more flexible method for ensuring produce safety through equipment design, establishing cleanability in-house. The process of establishing cleanability enables growers, packers, and manufacturers alike to construct and maintain a sanitary process by targeting hard-to-clean areas and adjusting their sanitation procedures accordingly. For example, a hard-to-clean surface like a joint (which often includes a three-dimensional surface and multiple material types) may require adjustments in sanitizer used (chemical components or concentration), contact time between the sanitizer and the surface, temperature at which the sanitizer is applied (either for

optimal sanitizer function or for loosening fatty or proteinaceous substances from materials), or adding a mechanical function to physically remove the debris (scrubbing, scraping, etc.) *(99)*. Additionally, some operators utilizing a four-step (rinse-alkaline detergent-rinse-acidic sanitizer) sanitation procedure could intensify their process to include a seven-step protocol (breakdown-sweep/flush-wash-rinse-sanitize-dry-validate), depending on the nature of their sanitation process *(36)*.

Outbreaks Linked to Packinghouses

While the relationship between *Listeria monocytogenes* and produce is important to delineate, this research required a targeted understanding of outbreaks of *L. monocytogenes* linked to packinghouse contamination and the factors that contributed to those outbreaks. In this section, two primary outbreaks are discussed: cantaloupe (2011) and apples (2014).

In the summer and fall of 2011, 147 cases of gastroenteritis-related illnesses were reported across the United States *(64)*. Through the use of *Listeria* Initiative surveillance data, culture-based, serotyping, and pulsed field gel electrophoresis (PGFE) testing of collected samples, *L. monocytogenes* was determined to be the causative agent of listeriosis linked to cantaloupe *(22, 64)*. The organism was traced back to a farm in Colorado that had recently installed new equipment and adjusted their cantaloupe processing methods. Equipment was installed that had been designed for the use of processing a separate raw

agricultural commodity. This equipment was neither adequately cleaned nor designed to be easily cleaned before the processing of cantaloupes *(64)*. In addition to eliminating a recirculating dump tank, municipal water without sufficient sanitizer was used to wash the cantaloupe surface *(64)*. The absence of current Good Manufacturing Practices and hygienic design failures prevented the safe processing of a ready-to-eat product intended for human consumption.

Another outbreak tied to packinghouse contamination occurred in the fall of 2014, resulting in 35 cases of listeriosis linked to consumption of caramel apples *(5, 22)*. Epidemiologists and laboratory personnel made use of structured interviews and whole-genome multilocus sequence typing (wgMLST) to implicate *Listeria monocytogenes* in the outbreak *(5)*. While outbreaks were associated with four major caramel apple manufacturers across many distributers, all manufacturers utilized whole apples obtained from the same packinghouse in California *(5)*. Upon further investigation of packinghouse conditions, FDA and California state officials discovered several isolates matching that of the outbreak strain from food contact and drain surfaces. Direct food contact belt surfaces were observed to have frayed edges, exposed absorbent padding, and damaged surfaces *(5)*.

Both packinghouses failed to follow basic current Good Manufacturing Practices and hygienic design principles. Through their management decisions, niche points in their processes developed into harborage of foodborne pathogens that were transmitted onto produce surfaces. These factors elevated the risk for

contaminating fresh produce and spreading that contamination across lots and commodities, depending on in-house sanitation practices in the packinghouses.

Rationale for Research

There is currently a lack of science-based information that can be utilized by produce growers and packers in the development of robust sanitation and environmental monitoring programs. Although studies have shown the relative abilities of food-grade materials to encourage biofilm formation, the industry needs studies designed to observe how these materials function with standard throughput during typical harvesting seasons. Additionally, while some materials may be better suited for the packinghouse environment, many operators will not be able to afford structural rearrangements to reflect these findings. Therefore, recommendations are needed to aid processors in the description of cleanability of current materials and also the design of adequate sanitation protocols that best suit their processes.

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CHAPTER II
FACTORS IMPACTING THE RECOVERY OF *LISTERIA* SPP. AND GRAM-POSITIVE BACTERIA FROM FOOD CONTACT SURFACES IN TOMATO PACKING OPERATIONS

I. Abstract

Given recent outbreaks and new federal regulatory requirements, a more targeted focus has been placed on identifying *Listeria monocytogenes* in packinghouse environments. However, there are still many gaps in knowledge with respect to the harborage and niche sites on packing equipment. *Listeria* spp. were used as an indicator for potential for *L. monocytogenes* on zone one surface samples (n=565) in three tomato packinghouses after sanitation practices were completed. Generic Gram-positive bacteria were enumerated from zone one food contact surfaces, and those samples were enriched to detect the presence of *Listeria* spp. Positive samples were PCR confirmed via presence of the *iap* gene. Sixty-two of 565 (10.97%) samples were confirmed as *Listeria* spp. Farm identity, sanitation personnel, and other sanitation practices were significantly associated with recovery of Gram-positive bacteria and *Listeria* spp. This research showed that site-specific sanitation characteristics were more likely indicators of bacterial presence than throughput. Further research should focus on designing tools to enable produce packers to develop sanitation protocols specific to their processes.

II. Introduction

Because produce contamination cannot be removed once it occurs and produce is consumed raw without a heat treatment applied to reduce microbial load on produce surfaces, it is important to reduce the risk of pathogen

contamination at all points of the continuum from the field through distribution *(6, 18)*. Due to the microbial ecology of the soil environment, fruits and vegetables grown in close contact with soil are at an increased risk for contamination. Fruits and vegetables are likely to naturally encounter *Listeria* species in the environment during growing and harvesting, as the organism's ecological niche appears to be soil and decaying vegetation *(11, 14, 28)*.

Of the 3.6 million annual foodborne diseases caused by bacteria, 1,591 (<1%) were caused by *Listeria monocytogenes (23)*. The zero-tolerance rule implemented by the Food and Drug Administration (FDA) in ready-to-eat (RTE) products requires RTE facilities (including packers of produce consumed raw) to control *L. monocytogenes* in the processing environment. Additionally, the Food Safety Modernization Act (FSMA) instituted several rules that aim to help strengthen the safety of the food industry, including Preventive Controls for Human Food, Produce Safety, Foreign Supplier Verification Program, and Accreditation of Third Party Auditors *(9, 10)*. These rules afford the Food and Drug Administration (FDA) the ability to more effectively manage food safety, including initiating recalls and requiring foreign suppliers to meet domestic standards. In particular, the Produce Safety Rule targets those growing, harvesting, packing, and holding produce by applying a series of pre- and postharvest minimum scientific standards to the management of agricultural water, soil fertilization, sprouts, domestic and wild animals, employees, and facilities (equipment, tools, and buildings) *(9)*. There is a lack of research

detailing how growers and packers can adhere to these rules and the areas in their processes that may pose a threat to the integrity of their food safety systems. The objectives of this study were to estimate the prevalence and persistence of *Listeria* spp. on packinghouse equipment surfaces and to characterize risks associated with design and construction of those surfaces.

III. Materials and Methods

Sample Collection

Packinghouse Design and Assignment of Zone 1 Contact Surfaces

Product flow was diagramed in three packinghouses in Tennessee prior to zone classification and sample identification (Figures 2.1-2.3). Food contact surfaces (zone 1) were selected based on likelihood to harbor microorganisms due to location, material, construction, and cleaning efficiency by packinghouse staff. Additional information was collected for samples by farm (100 from site one, 244 from site two, and 221 from site three) and material (20 from formica laminate, 12 from high density polyethylene, 128 from mixed materials, 166 from polyester nylon, 80 from polyethylene, 8 from polypropylene, 89 from polyvinylchloride, and 62 from stainless steel 304). Sample sites were labeled and photographed on the first sampling trip to ensure the same sites were visited on subsequent sampling events and parameters described in Table 2.1 were recorded for each.

Sample Collection

Samples were collected after sanitation (15-504 h) and against the flow of product through the facility. A 10 x 10 cm square (100cm^2) was sampled using a sponge-stick with 10 mL Dey/Engley Neutralizing Broth (3M, Saint Paul, MN) to neutralize any residual sanitizer. Samples were transported in an insulated bag in a refrigerated cooler.

Sample Processing

Direct Enumeration

Samples were eluted in 10 mL of Buffered Peptone Water (BPW; Becton Dickinson, Sparks, MD) and massaged by hand for 15 s. Samples were serially diluted in BPW and a 0.1 mL representative sample was spiral-plated (Eddy Jet 2 Spiral Plater, IUL Instruments, Barcelona, Spain) on Modified Oxford Medium (MOX; Becton Dickinson, Sparks, MD) and incubated at 35 °C for 48 h. Plates with characteristic *Listeria* spp. growth (gray-to-black colony with a black halo) were enumerated using a spiral plate counter to indicate the number of presumptive *Listeria* spp. present at the sample site.

Qualitative Detection

A 1-mL sample was removed from the eluted sample and enriched in Buffered Listeria Enrichment Broth (BLEB; Becton Dickinson, Sparks, MD) at 30 °C for 4 h. Three antibiotics were hydrated and filter-sterilized before being added to

select for *Listeria* spp. Acriflavine monohydrochloride (Acros Organics, Fair Lawn, NJ) was hydrated in sterile deionized water to a working concentration of 1mg/mL and aseptically added to achieve a final concentration of 10mg/L. Nalidixic acid (Alfa Aesar, Tewksbury, MA) was hydrated in sterile deionized water to a working concentration of 4mg/mL and aseptically added to achieve a final concentration of 40mg/L. Cycloheximide (Acros Organics, Fair Lawn, NJ) was hydrated in 190-proof (95%) ethanol (Acros Organics, Fair Lawn, NJ) to a working concentration of 5mg/mL and aseptically added to achieve a final concentration of 50mg/L. The sample was enriched for an additional 44 h at 30 °C. Each sample was streaked for isolation on MOX and incubated at 35 °C for 48 h. Plates showing characteristic *Listeria* spp. were recorded as either positive (+) or negative (-) and stored at 4 °C until isolation of presumptive *Listeria* spp. colonies.

Sample Confirmation

Isolation of Presumptive* Listeria *spp.

Colonies showing characteristic *Listeria* spp. morphology and reaction were removed from stored MOX plate surface using a 10 µL disposable inoculating loop/needle (Thermo Fisher Scientific, Foster City, CA) and streaked for isolation on MOX and incubated at 35 °C for 48 h. Colonies showing characteristic *Listeria* spp. growth after incubation were removed from the plate surface with a 10 µL disposable inoculating loop/needle and deposited in 10 mL of non-selective

Tryptic Soy Broth (Becton Dickinson, Sparks, MD) at 35 °C for 24 h to allow remaining potentially injured bacterial cells to recover to sufficient population numbers. A 1.5 mL sample of this overnight culture was saved in a 2 mL microcentrifuge tube and stored at -20 °C until DNA extraction.

DNA Extraction

Due to the stability of the Gram-positive cell membrane, DNA extraction was performed using an enzymatic kit, GenElute Bacterial Genomic DNA Kits (Sigma-Aldrich, St. Louis, MO). Prior to extraction, a 2.115 x 10^6 unit/mL lysozyme solution was prepared daily. A 46 mg sample of lysozyme from chicken egg white (Sigma-Aldrich, St. Louis, MO) was dissolved in 1 mL of Gram-Positive Lysis Buffer (Sigma-Aldrich, St. Louis, MO). Proteinase K (Sigma-Aldrich, St. Louis, MO) was hydrated with sterile molecular-grade water (Thermo Fisher Scientific, Foster City, CA). The Wash Solution Concentrate (Sigma-Aldrich, St. Louis, MO) was rehydrated with 200-proof (99.5+%) ethanol (Acros Organics, Fair Lawn, NJ). Binding columns were prepared by adding 500 µL Column Preparation Solution (Sigma-Aldrich, St. Louis, MO) to each column and centrifuging at 12,000 x *g* for 1 min. Flow-through was discarded.

Cells were harvested by centrifuging each 1.5 mL pure culture at 16,000 x *g* for 2 min. The supernatant was discarded, and the pellet left undisturbed. The pellet was resuspended in 200 µL lysozyme solution and incubated at 37 °C for 30 min in a noncirculating water bath to digest the cell wall. Cells were lysed by

adding 200 µL Proteinase K and 200 µL Lysis Solution C (Sigma-Aldrich, St. Louis, MO) to the cell suspension and vortexed to mix before being incubated at 55 °C for 10 min in a noncirculating water bath. Ethanol (200 µL) was added to the lysed cells, vortexed to mix, transferred to the binding column, and then centrifuged at 6,500 x *g* for 1 min to bind the DNA to the column.

The column was transferred to a new microcentrifuge tube and washed twice to remove remaining protein and salt residues. In the first wash, 500 µL Wash Solution 1 (Sigma-Aldrich, St. Louis, MO) was added to the column and centrifuged at 6,500 x *g* for 1 min. Flow-through was discarded before adding 500 µL Wash Solution Concentrate to the column and centrifuging at 12,000 x *g* for 4 min. Finally, DNA was eluted by transferring the column to a new collection tube, 200 µL Elution Solution (Sigma-Aldrich, St. Louis, MO) was added, and centrifuged at 6,500 x *g* for 1 min. Flow-through was retained and stored at -20 °C until PCR was performed.

PCR

DNA was amplified using traditional PCR. Due to the variance in opinions regarding members of the *sensu strictu* group of *Listeria* spp., two primers were used to isolate the most common *Listeria* species in the environment: *monocytogenes, ivanovii, innocua, seeligeri, welshimeri,* and *grayi*. The Food and Drug Administration (FDA) validated a PCR protocol for the isolation of *L. monocytogenes, L. ivanovii, L. innocua, L. seeligeri,* and *L. welshimeri* based on

a segment of the *iap* gene that encodes protein p60, an extracellular protein used for cell invasion *(17)*. However, since this method was not validated for the identification of *L. grayi*, a primer targeting a segment of 16S ribosomal DNA (rDNA) from that organism was also used*(24)*.

AmpliTaq Gold Fast PCR 2X Master Mix (Thermo Fisher Scientific, Foster City, CA) was used in a 20 µL reaction mixture. PCR reaction components are detailed in Table 2.2. Components were added to a sterile MicroAmp EnduraPlate Optical 96-Well Multicolor Reaction Plate (Thermo Fisher Scientific, Foster City, CA) and sealed using the associated sterile caps (Thermo Fisher Scientific, Foster City, CA). The PCR plate was inserted into the SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Foster City, CA) and a standard program (35 cycles, 20 µL reaction) was run at 96 °C for 3 s (annealing), 62 °C for 3 s (elongation), and 68 °C for 5 s (denaturation). The plate was kept at 4 °C until amplified products were analyzed.

Gel Electrophoresis

PCR products were examined for presence of *Listeria* spp. using an E-Gel EX 1% Agarose gel containing a proprietary stain as the fluorescing agent (Thermo Fisher Scientific, Foster City, CA) and read by the Invitrogen gel reader (Thermo Fisher Scientific, Foster City, CA) using a 1 Kb DNA ladder for reference (Thermo Fisher Scientific, Foster City, CA) and 10 µL 1 mL 1X E-Gel Sample Loading Buffer (Thermo Fisher Scientific, Foster City, CA). Electrophoresis was

conducted for 10 min at 48 V, 90 W and examined for characteristic bands at 108 bp (*iap* gene) or 400 bp (16S rDNA).

Enumeration of Gram-positive Bacteria versus Listeria *spp.*

Samples that exhibited characteristic reactions on MOX before enrichment but not confirmed as *Listeria* spp. after PCR were recorded as positive for generic Gram-positive bacteria and original enumeration data was maintained as such. Samples that exhibited characteristic reactions on MOX before enrichment and were confirmed as *Listeria* spp. after PCR were recorded as *Listeria* spp. and original enumeration data was maintained as such.

Presence of Listeria *spp.*

Samples that exhibited characteristic reactions on MOX after enrichment and were confirmed as *Listeria* spp. after PCR were recorded as positive for the presence of *Listeria* spp. qualitatively.

Statistical Analysis

A total of 565 samples were collected over four sample collection days from each farm: 100 from farm one, 244 from farm two, and 221 from farm three. Due to the nature of the data received, the following mixed methods analysis was performed. Significant differences between numbers of Gram-positive bacteria recovered from each site and farm were examined with analysis of variance and

a significance threshold of $p<0.05$. Additionally, a parametric assessment of the presence of *Listeria* spp. was also conducted.

IV. Results and Discussion

Quantification of Gram-positive bacteria

Three hundred thirty-seven of 565 (59.6%) samples had growth consistent with typical *Listeria* spp. characteristics on MOX agar. Due to the selective nature of MOX medium, these presumptive positive isolates were categorized as generic Gram-positive bacteria.

Effect of Farm

Farms one and three had lower mean populations than farm two (Table 2.3; $p\leq0.0001$). Farms two and three were similar in acreage at approximately 400-650 acres in tomato production while farm one had a much smaller production of approximately 20 acres in greenhouse and field-grown tomatoes. The sanitation programs utilized by the large-scale farms were essentially the same and differed greatly from farm one, while the sanitation program for farm one. While all farms utilized a detergent and sanitizing step during sanitation and visually monitored to determine the need for recleaning and sanitizing, farm one did not utilize water (in a wash or rinse capacity) during packing. Farms two and three had multiple sanitation crews that were exchanged out as needed, but farm two provided

education and training videos for all employees prior to beginning sanitation for the season. Farm one sanitized three times per season, while farms two and three self-reported sanitizing as harvest times allowed. The differences in Gram-positive bacterial recovery between farms suggest that count variations were more likely due to farm-specific characteristics discussed below.

Effect of Unit Operation

Amongst all farms, unit operation significantly affected differences in microbial counts ($p<0.0001$). Grading operations had the highest average bacterial counts (730.18 CFU/swab), followed by drying (625.33 CFU/swab), packing (559.73 CFU/swab), sorting/sizing (551.05 CFU/swab), conveying (493.25 CFU/swab), washing/rinsing (443.24 CFU/swab), and rolling (408.28 CFU/ml). Significant differences were observed between drying and rolling, drying and washing/rinsing, grading and rolling, grading and sorting/sizing, and grading and washing/rinsing ($p\leq0.05$; Table 2.4). These data suggest grading and drying operations provide a significant opportunity to control microbial load transfer from produce surfaces onto equipment and subsequent lots.

When viewed in context, these unit operations typically appear in the following order: washing/rinsing, drying, grading, rolling, sorting/sizing, conveying, and packing. If properly maintained, each unit along the packingline should have lower average bacterial counts than the previous unit operation. This pattern was not observed in this population of packinghouses, indicating

opportunities for improvement in sanitation practices. In interviews with packinghouse operators, they indicated that the grading equipment was one of the hardest pieces of equipment to clean and was not cleaned as frequently or as thoroughly as areas closer to the wet unit operations. We observed during sampling instances where plant matter and physical debris were lodged between belt and equipment surfaces or liquid residues were dried onto belt surfaces.

Effect of Material Type

Material type significantly affected recovery of Gram-positive bacterial counts ($p<0.0001$). Formica laminate (Table 2.5) showed the lowest average bacterial counts (57.28 CFU/ml), followed by polyvinylchloride (441.27 CFU/ml), high density polyethylene (HDPE; 461.29 CFU/ml), stainless steel 304 (482.70 CFU/ml), multiple material types joined together (mixed material; 554.58 CFU/ml), polyethylene (559.73 CFU/ml), polyester nylon (726.66 CFU/ml), and polypropylene (904.56 CFU/ml). Additionally, of the samples from which Gram-positive bacteria were isolated (Figure 2.4), 1% (6/903) were from formica laminate, followed by polypropylene (16/903; 2%) and HDPE (18/903; 2%), stainless steel 304 (89/903; 10%), PVC (116/903; 13%), polyethylene (131/903; 14%), mixed materials (206/903; 23%), and polyester nylon (321/903; 35%).

Formica laminate returned the lowest average and total Gram-positive bacterial counts; however, this material was utilized by farm one, which also did not use water during their sanitation procedure. While this study suggested that

formica laminate would function well in food processing environments, this material would not be compatible in operations that incorporate water consistently during processing and cleaning. Conversely, polypropylene and polyester nylon returned the highest and second highest average counts, respectively. Polypropylene was also used by only one farm in an interlocking conveyor belt to assist in transfer of tomatoes from the dump tank to a polyester nylon roller belt.

Mafu *et. al.* (1990) found that polypropylene surfaces supported the development of *L. monocytogenes* biofilm development better than rubber (ex. polyester nylon) or stainless steel surfaces *(20)*. While polyester nylon is a versatile material and used in a variety of conveyor belt and brush applications, these data suggest that this material has the potential to damage the integrity of the food safety system based on its potential to promote attachment of *Listeria* spp. This is supported by existing research about bacterial attachment. For instance, Allen (2003) showed *Salmonella* spp. were capable of surviving longer on conveyor belt surfaces than stainless steel 304 or PVC, with temperature and relative humidity playing a role in survival *(1)*. Additionally, this research showed *Listeria* spp. were able to bind indiscriminately to food contact surfaces, which is also supported by existing research studies *(3, 5, 20)*. A recent study showed that, although *L. monocytogenes* was able to bind to many types of materials, conveyor belt systems (PVC, polyurethane, and nitrile rubber) posed less of a threat than brushes (nylon and polyethylene) *(21)*. In our study, brushes

composed of polyester nylon were grouped with polyester nylon conveyor belt systems. While the conveyor belt systems used in the Nyarko *et. al.* study (2018) were composed of separate materials, these results do support our findings that polyvinylchloride supported lower microbial transfer than polyester nylon or polyethylene surfaces.

Effect of Surface Dimension

The surface dimension (Table 2.1) of the sample site had a significant effect on recovery of bacterial counts ($p<0.0001$). One- and two-dimensional surfaces were significantly less likely to return counts than three-dimensional surfaces. Three-dimensional surfaces, such as those shown in Figure 2.5, showed a greater likelihood to retain bacteria after sanitation than simpler surfaces. The distribution of one-, two-, and three-dimensional surfaces were: 196 (31.6%), 16 (2.6%), and 408 (65.8%), respectively. The small proportion of two-dimensional surfaces was due to the nature of design of these facilities, which lacked a ratio of surfaces distinguished as two-dimensional. These design features should be evaluated thoroughly to assess if improvements can be made, and sanitation programs should target these areas for monitoring.

To the author's knowledge, this is the first study to characterize microbial recovery explicitly by the dimension of the sample surface. However, several studies have alluded to the differences in microbial recovery based on roughness or surface topography *(7, 13, 26, 27)*. The consensus of these publications was

that rough surfaces or those with a high surface topography parameter retained more microorganisms than smoother surfaces, in agreement with this study.

Effect of Junction Type

Junction type (Table 2.1) did not have a significant effect on the model ($p=0.1295$). Essentially, while all surfaces returned bacterial counts, those counts did not differ based on the type or presence of a junction. Variations in junction type or presence of junctions do not pose a significant threat to the integrity of the food safety system.

Effect of Sanitizer Used

The sanitizer used in the operation did contribute significantly to recovery of Gram-positive bacteria; however, with the limited sample size this effect should be further evaluated to assure it is not driven by farm rather than sanitation performance. Farms that used peracetic acid had lower bacterial populations (465.8 CFU/swab) compared to those that used quaternary ammonium compounds (595.9 CFU/swab). Peracetic acid has a greater oxidizing capacity against bacterial species, which is associated with increased microbial lethality *(2, 16)*. However, this increased oxidative capacity also impacts the life of equipment, which should warrant consideration to find the right balance of inactivating microbial populations while not drastically impacting equipment performance.

Effect of Sanitizer Contact Time

The amount of time the sanitizer was in contact with the food contact surfaces significantly affected the recovery of Gram-positive bacteria after sanitation completion ($p \leq 0.0001$). Specifically, surfaces with a 0-hour contact time (sanitizer was sprayed onto surface and immediately removed with a cloth) had lower bacterial populations of bacteria than surfaces with a 2-hour sanitizer contact time that were allowed to dissipate on their own (Table 2.6). However, 0-hour and 2-hours both had higher Gram-positive bacterial populations than surfaces that had a continual application of a sanitizer (*e.g.* dump tank surfaces). The low and continual application of a sanitizer during production in the dump tank would continually sanitize this area, deterring microbial survival.

Effect of Hours Since Sanitation

Hours since sanitation (Table 2.1) significantly affected bacterial recovery ($p<0.0001$). As the number of hours after sanitation was completed increased, bacterial recovery also increased. This suggested that microbial recovery was associated with either recontamination of surfaces after sanitizing or microorganisms that did not succumb to the sanitizer were able to replicate once the sanitizer was exhausted or evaporated.

Effect of Sanitation Crew

The crew that performed the sanitation procedure had a significant effect on bacterial count ($p<0.0001$). Significant differences are shown in Table 2.7. The sanitation teams used at farm two showed a reduced ability to sanitize equipment to effectively reduce bacterial counts, which was also consistent with the distribution of bacterial counts observed across farms. This data supports the notion that farm-specific sanitation characteristics are an important factor in the efficacy of any sanitation event. A review of the results of effectiveness studies about food safety trainings offered worldwide found that safety trainings did have an impact on inspection and examination scores, specifically that staff members with training performed better overall than staff without *(8)*. Additionally, trainings provided on-site and in the workplace proved to be more effective than off-site education, which was likely due to difficulties in applying food safety theory in a workplace setting *(22, 25)*. Lastly, Jackson *et. al.* (1977) found that training of sanitation management personnel improved hygiene standards when it was supported by owners of the business and administered and followed up on regularly *(12)*.

Effect of Last Crop

The last crop packed before sanitation did not significantly affect Gram-positive bacterial recovery ($p=0.0595$). There were no significant differences between type of tomato packed (Roma versus round) and bacterial recovery. While this

information is based upon limited observations based upon what the cooperator farms were packing, there was not an observed difference in microbial populations based upon the type of tomato packed. This is consistent with other studies that did not find significant differences between microbial survival in Roma or round tomato surfaces *(4, 29)*.

Effect of Crop Cultivar

Tomato crop cultivar had a significant effect on bacterial recovery ($p \leq 0.0001$) as shown in Table 2.8. Of all tomato cultivars, Red Mountain returned the highest average Gram-positive bacterial counts (Table 2.8). Others have found differences in cultivars to support the survival of various pathogens *(15, 19)*. Further evaluation of the role of tomato cultivar may yield interesting insights to plant-microbe interactions and resulting microbial drift. To the author's knowledge, this is the only study to compare bacterial recovery from processing equipment by tomato plant variety.

Preliminary Conclusions

The effects of farm, unit operation, material type, surface dimension, sanitizer used, sanitizer contact time, hours since sanitation, sanitation crew, and crop variety were all shown to play a significant role in recovery of Gram-positive bacteria from zone 1 surfaces in tomato packing facilities. Factors such as farm, sanitizer used, sanitizer contact time, hours since sanitation, and sanitation crew

demonstrate that the general management practices associated with sanitation play a large role in the cleanliness of food contact surfaces. Additionally, design associated features such as unit operation, material type, surface dimension, and crop variety also played a role in retaining higher populations of Gram-positive bacteria. These factors should be targeted by equipment designers for potential improvement of hygienic design that could decrease opportunities for harborage across susceptible components.

Detection of Listeria species

Sixty-two of 565 (10.9%) samples were confirmed as general *Listeria* spp. after PCR confirmation. The results of these findings are shown by unit operation in Table 2.9. Further elucidation of differences in attributes that influenced *Listeria* species positives are discussed further below.

Effect of Farm

Farm had a significant effect on the likelihood of isolation of *Listeria* spp. ($p<0.0001$). Farm three was significantly more likely to contain *Listeria* spp. than farm two, but there were no other significant differences noted on frequency of *Listeria* spp. isolation. Generally speaking, there was a very low frequency of *Listeria* spp. isolated from farms one and two. Of the 62 positive samples, zero (0%) were recovered from farm one, one (1.6%) was recovered from farm two, and 61 (98.4%) were recovered from farm three. Each positive sample was

recovered from a different sample site within each farm and isolation was not repeated in subsequent evaluations.

While farm two returned the highest average Gram-positive bacterial count, farm three had the highest frequency of *Listeria* spp. samples. This finding is consistent with transient *Listeria* spp. contamination of zone one surfaces rather than harborage.

Effect of Sanitizer Contact Time

Contact time significantly affected the likelihood of recovery of *Listeria* spp. from food contact surfaces ($p=0.0005$; Table 2.10). The shortest contact time (when the sanitizer was wiped off immediately after spraying) was the only practice associated with *Listeria* spp. recovery. This suggested that the sanitizer may not have sufficient time to interact with the food contact surfaces prior to being wiped off. Additionally, the possibility of cross-contamination through wiping would be another potential source of contamination.

Effect of Hours Since Sanitation

The number of hours elapsed since sanitation occurred did significantly affect the recovery of *Listeria* spp. ($p \leq 0.0001$). Odds ratio estimates showed that the odds of a sample being confirmed as *Listeria* spp. were more likely after 72.6 hours. This suggested that packinghouse surfaces should be cleaned and sanitized at least once every 72 hours, regardless of structural or other sanitation factors.

This finding also aligns with the Gram-positive bacterial counts obtained on these surfaces.

Effect of Sanitation Crew

The crew that performed the cleaning and sanitation procedure did have a significant effect on the recovery of *Listeria* spp. ($p=0.0010$), with sanitation crews 2 and 3 contributing to this effect. These teams were from farms two and three (Table 2.7), which both returned confirmed *Listeria* spp. samples. Once more, this suggests that personnel can dramatically impact outcomes of sanitation. It would be important in future years that all packinghouse operations begin to invest in training for their employees and incorporate verification practices, such as direct observations during sanitation to assure personnel are implementing the sanitation program as intended. Additionally, future studies in this area should track sanitation crew as a variable that can impact outcomes.

Non-significant factors impacting the frequency of Listeria *spp. isolation*

Unit operation, wash step, material type, surface dimension, junction type, last crop or variety, and sanitizer type or concentration did not significantly alter the frequency of *Listeria* spp. isolation from food contact surfaces. Ultimately, the infrequent isolation of *Listeria* spp. from zone one may be contributing to the lack of statistical differences amongst those parameters. Through increased sampling

numbers in future studies, any limitations tied to statistical power may be overcome and further elucidate the role of these factors, if any.

Harborage

Harborage, the repeated isolation of microorganisms from designated sampling sites, of *Listeria* spp. on food contact equipment was not observed in any of the packinghouses evaluated over the course of a single packing season. Surfaces that supported the recovery of *Listeria* spp. after sanitation practices did not remain positive on subsequent sampling events. However, niche points identified for *Listeria* spp. show an ability to encourage growth of these organisms and thus should be routinely monitored.

Conclusions

In both Gram-positive and *Listeria* spp. data, the farm, sanitizer contact time, hours since sanitation, and sanitation crew all significantly impacted the likelihood of isolation of those organisms. This research showed that farm-specific sanitation characteristics and personnel play the most significant role in mitigating the risks associated with *Listeria monocytogenes* contamination in the packing environment. Additionally, these variables are factors that packinghouse operators have control over. Packinghouse operators should work closely with sanitation managers to design a sanitation protocol that best suits the facility, based on structural and processing needs. While studies have surfaced that

discuss hygienic design of processing equipment to enhance cleanability of packingline operations, there is a need to develop a protocol that growers and packers can use to evaluate existing processes for their potential to impede sanitation practices. Once implemented, operators can determine cleanability of the surfaces in their facilities and adjust their sanitation protocol to meet those needs. Lastly, the food safety culture within packinghouse facilities should shift to reflect a proactive attitude toward food safety that involves frequent educational trainings and is centered around prevention strategies. Frequent monitoring of hard-to-clean areas along the processing line should be implemented to ensure niche points do not become sources of frequent contamination (harborage).

Given the infrequent occurrence of *Listeria* spp. on zone one surfaces, a larger study evaluating a larger number of packinghouses over multiple growing seasons would be warranted. Additionally, sampling after startup would further assist with identification of potential harborage points that may remain negative until equipment has been operated for some time, allowing bacteria to work out of harborage points.

V. References

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and Roma tomato varieties. *In*, International Association for Food Protection, Lake Buena Vista, Florida.

VI. Appendix A

Table 2.1. Definitions of selected terms.

Term	Definition	Variables
Surface Dimension	The number of dimensions involved in the construction of a food contact surface.	1-dimensional 2-dimensional 3-dimensional
Junction Type	The number and type of materials involved in the construction of a food contact surface.	None 2 materials contacting Multimaterial surface Weld surface
Hours Since Sanitation	The number of hours lapsed since sanitation was completed.	*Continuous variable*
Surface Accessibility	The accessibility of a surface to complete drainage of food, cleaning, or sanitation materials.	Drainable Standing water

Table 2.2. PCR reaction mixture per reaction well with final concentration.

Component	Volume per Reaction (µl)	Final Concentration
Deionized water	5[a]	-
Primer Lisall-F (10 µM WS)	1	0.5 µM
Primer Lisall-R (10 µM WS)	1	0.5 µM
Primer LisGr-F (10 µM WS)	1	0.5 µM
Primer LisGr-R (10 µM WS)	1	0.5 µM
DNA template	1[b]	1 – 2 µg/reaction
AmpliTaq Gold Fast PCR	10	1X
Total volume	20	-

[a] Based on the assumption of adding 1 µl of DNA template per well.
[b] Based on the assumption of adding 100 – 200 ng gDNA per reaction well.

Table 2.3. Mean populations of Gram-positive bacteria obtained from all environmental swabs in three tomato packing facilities.

Farm	Population log CFU/swab[a]	Mean Separation
1	459.2±31.3[b]	A[c]
2	651.4±20.0	B
3	518.8±21.1	A

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.

[b] Least square mean population ± standard error

[c] Different letters denote significant differences ($p \leq 0.05$). Unit operations with the same letter are not statistically different from each other.

Table 2.4. Mean populations of Gram-positive bacteria obtained from all environmental swabs in three tomato packing facilities by unit operation.

Unit Operation	Population log CFU/swab[a]	Mean Separation
Washing/Rinsing	443.24±45.03[b]	A[c]
Drying	625.33±22.34	BC
Grading	730.18±47.04	C
Rolling	408.28±40.28	A
Sorting/Sizing	551.05±28.72	AB
Conveying	493.25±69.77	ABC
Packing	559.73±34.88	ABC

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.
[b] Least square mean population ± standard error
[c] Different letters denote significant differences ($p \leq 0.05$). Unit operations with the same letter are not statistically different from each other.

Table 2.5. Mean populations of Gram-positive bacteria obtained from all environmental swabs in three tomato packing facilities by surface material type.

Unit Operation	Population log CFU/swab[a]	Mean Separation
Formica laminate	57.28±64.40[b]	A[c]
HDPE	461.29±83.14	B
Mixed material	554.58±25.46	B
Polyester nylon	726.66±22.35	C
Polyethylene	559.73±32.20	B
Polypropylene	904.56±101.82	C
PVC	441.27±30.53	B
Stainless steel 304	482.70±36.58	B

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.
[b] Least square mean population ± standard error
[c] Different letters denote significant differences ($p \leq 0.05$). Material types with the same letter are not statistically different from each other.

Table 2.6. Mean populations of Gram-positive bacteria obtained from all environmental swabs in three tomato packing facilities by sanitizer contact time.

Sanitizer Contact Time (hours)	Population log CFU/swab[a]	Mean Separation
0	500.22±17.36[b]	A[c]
2	676.30±21.36	B
Continuous	486.27±54.98	A

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.
[b] Least square mean population ± standard error
[c] Different letters denote significant differences ($p \leq 0.05$). Sanitizer contact times with the same letter are not statistically different from each other.

Table 2.7. Sanitation team assignment by farm.

Sanitation Team	Farm
Team 1	1
Teams 3 and 4	2
Team 2	3

Table 2.8. Mean populations of Gram-positive bacteria obtained from all environmental swabs in three tomato packing facilities by crop variety.

Crop Variety	Population log CFU/swab[a]	Mean Separation
BHN-589	459.23±30.34[b]	AB[c]
FL-47	641.73±18.16	C
Red Mountain	679.29±38.22	C
Roma Express	506.07±39.17	B
Winter Haven	339.39±38.22	A

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.
[b] Least square mean population ± standard error
[c] Different letters denote significant differences ($p \leq 0.05$). Crop varieties with the same letter are not statistically different from each other.

Table 2.9. Number of *Listeria* spp. positive swabs obtained from all environmental swabs in three tomato packing facilities by unit operation.

Unit Operation	Frequency of *Listeria* spp. positive swabs[a]	Mean Separation
Washing/Rinsing	5	A[b]
Drying	34	A
Grading	0	A
Rolling	9	A
Sorting/Sizing	13	A
Conveying	0	A
Packing	0	A

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.

[b] Different letters denote significant differences ($p \leq 0.05$). Unit operations with the same letter are not statistically different from each other.

Table 2.10. Mean populations of confirmed *Listeria* spp. obtained from all environmental swabs in three tomato packing facilities by sanitizer contact time.

Sanitizer Contact Time (hours)	Frequency of *Listeria* spp. positive swabs[a]	Mean Separation
0	62/62	A[b]
2	0	B
Continuous	0	B

[a] 100cm^2 surface was targeted for all sites unless configuration would not permit.
[b] Different letters denote significant differences ($p \leq 0.05$). Sanitizer contact times with the same letter are not statistically different from each other.

VII. Appendix B

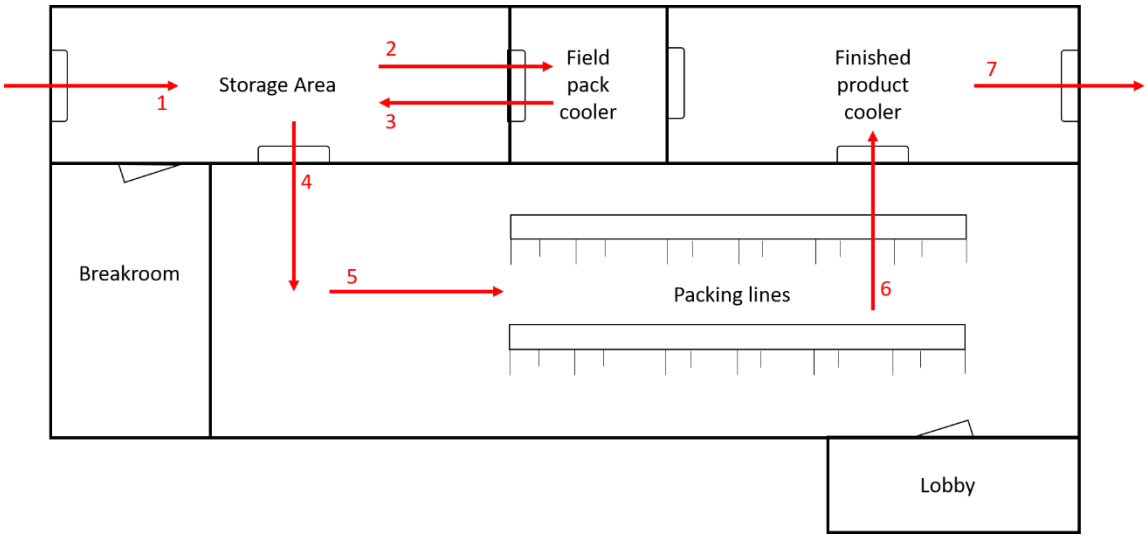

Figure 2.1. Packinghouse flow diagram for first site.

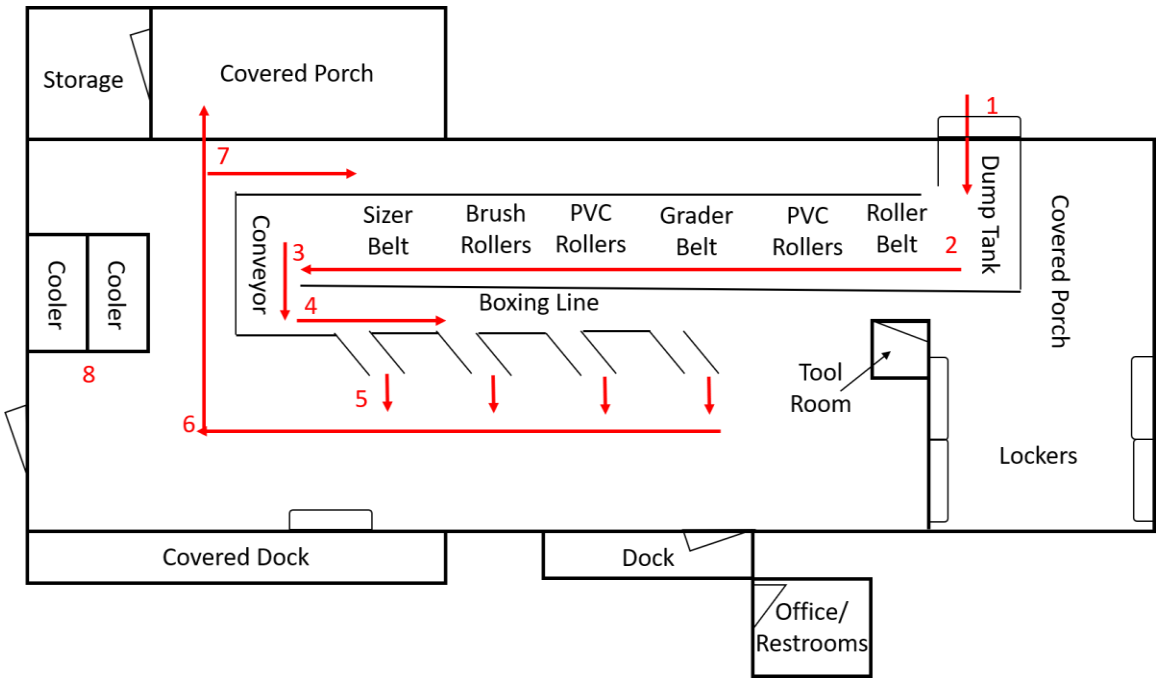

Figure 2.2. Packinghouse flow diagram for second site.

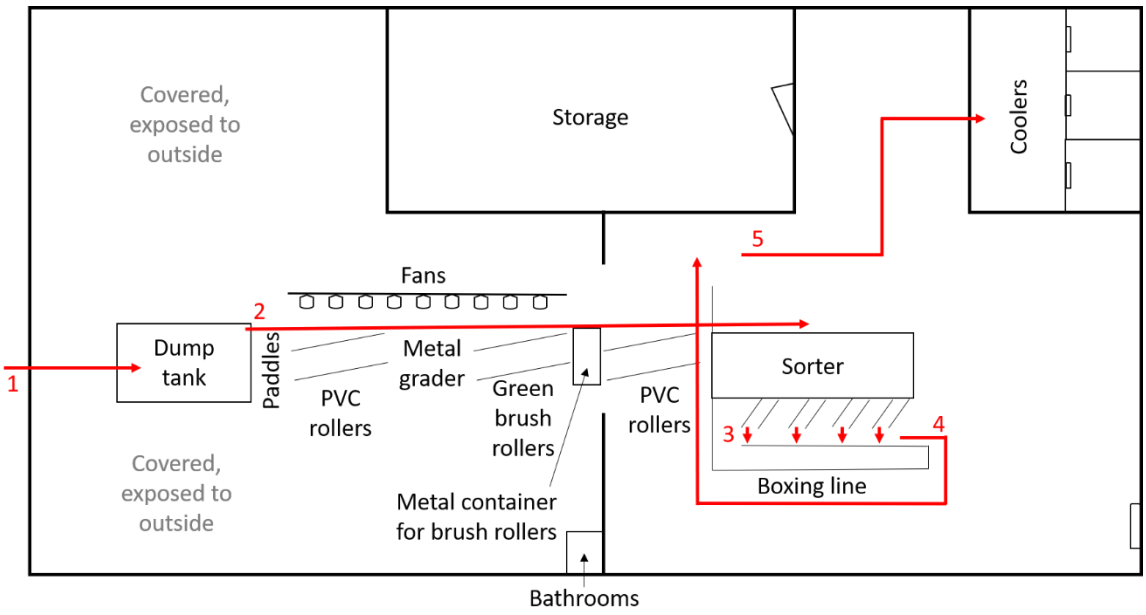

Figure 2.3. Packinghouse flow diagram for third site.

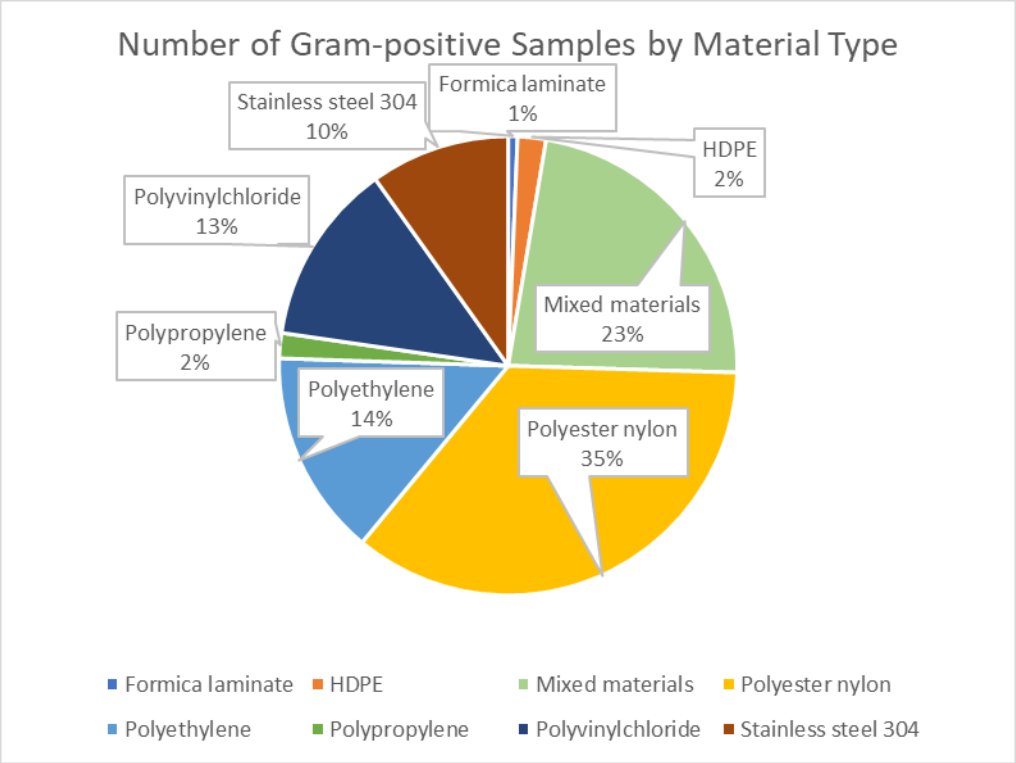

Figure 2.4. Frequency of Gram-positive isolation by material type.

Figure 2.5. Samples taken from each of three surface dimensions within a dump tank.

CHAPTER III
USING SENSORY SCIENCE DESCRIPTIVE ANALYSIS AS A NOVEL TECHNIQUE TO DESCRIBE CLEANABILITY OF FOOD-GRADE MATERIALS

I. Abstract

Due to regulations associated with the Food Safety Modernization Act (FSMA), greater emphasis is being placed on cleanliness of the packinghouse environment. Growers and packinghouse operators will be required to prove their adherence to the rule by meeting minimum scientific standards for the growing, harvesting, packing, and holding of produce. To prevent the contamination of this produce by equipment, tools, or lack of hygienic design in building construction, sanitation protocols and cleanability of materials should be verified. This research established a method for determining cleanability of food grade materials. Fifteen food grade material samples (12 solid surfaces and three weld surfaces) available from three tomato packinghouses were described via aesthetic and tactile observation to develop a method of calculating each material's resistance to clean score. Analysis of variance and partial least squares regression were used to evaluate the efficacy of the proposed methodology against Gram-positive bacteria recovered from those surfaces. High resistance to clean calculations were associated with higher bacterial populations. Surfaces with high resistance to clean scores had low cleanability and would require more targeted sanitation interventions to reduce the risk of microbial harborage. Future studies should include a wider array of food-grade materials and independent replication to determine utility of this approach.

II. Introduction

As Food Safety Modernization Act (FSMA) compliance dates go into effect, growers will be responsible for adhering to minimum standards for growing, harvesting, packing, and holding of produce intended for human consumption *(1)*. These requirements aim to prevent the contamination of the final product via equipment, tools, and buildings or improper sanitation. Before growers can prove their adherence to these standards, they will need to identify areas in their process that could pose contamination problems due to the presence of microorganisms. A poorly constructed process could create areas where microorganisms become trapped or protected from the lethal effects of sanitizers, resulting in repeated isolation of those organisms (referred to as microbial harborage). If foodborne pathogens can persist in these processes, the integrity of the food safety system would be at risk.

Many factors affect microbial harborage, including surface cleanability. Cleanability is a complex factor that describes how easy to clean a material surface is. The composition of the material, method of cleaning, and process construction, among other factors, affect the ease associated with cleaning and sanitizing a food contact surface. It is important to not only characterize each sample site by these factors, but also to define each factor individually to create a standardized method of site evaluation. Previous studies charged with elucidating differences in cleanability across materials have relied on laboratory

soil tests to determine the log reduction of bacteria possible on different material surfaces *(2, 7)*. However, these methodologies lack an interpretability at the farm level for growers to optimize their own processing systems. Proper adherence to FSMA guidelines will be incomplete without a basic methodology for identifying hard to clean areas on-site to enhance the efficacy of sanitation programs.

During the height of produce harvesting season, packinghouse operators can pack over 4,000 crates of produce per day or 200,000-300,000 crates of produce every year, depending on the size of the packinghouse *(3)*. This produce is then transported across the country and consumed fresh by healthy and immunocompromised individuals alike. Prevention of contamination can be accomplished through implementation of current Good Manufacturing Practices within the packinghouse accompanied with an adequate cleaning and sanitation protocol. Part of establishing an adequate cleaning and sanitation program requires understanding how cleanable the processing line is and which areas along that line pose a risk for microbial harborage. Additionally, wear from frequent use or improper sanitation practices (for example, inappropriate use of sanitizers with incompatible equipment) can change the finish or degrade the surfaces of previously hygienic equipment over time. Once identified through an established risk evaluation method, these problem areas can be overcome by redesigning the sanitation protocol to target these areas with advanced or more appropriate cleaning and sanitation measures.

This study used methods from sensory science to address this problem within produce packinghouses. A trained descriptive panel was used to describe the similarities and differences among a variety of products *(6)*. This type of sensory analysis typically employs a panel who work together to develop necessary lexicons or vocabularies for further evaluation of a series of products. The objective of the panel was to establish a lexicon, via aesthetic and tactile observation, for evaluating surface characteristics of various materials found in produce packinghouse environments. Important for ensuring validity and reproducibility of the larger experiment, this lexicon was also used to establish a resistance to clean rating for each sample site, which was compared to microbial recovery to assess the strategy's efficacy in evaluating microbial harborage risk.

III. Materials and Methods

Samples

A variety of food grade materials are used in vegetable packinghouses; therefore, the samples analyzed were based on common materials used in tomato packinghouses in Tennessee (Table 3.1). Samples were collected from Agri Machinery & Parts, Incorporated (Orlando, Florida) and Sparks Belting Company, Incorporated (Grand Rapids, Michigan). Upon receipt, samples were cut into easy to handle coupons (7.5 cm by 7.5 cm). The sensory panel leaders classified the samples into two groups: solid surfaces and weld surfaces. Materials that did not fall into these categories were eliminated from analysis.

Panel

A trained panel of 11 panelists from the Center for Sensory Science at the University of Tennessee (Knoxville, TN) was used for descriptive analysis. Each panelist underwent extensive training on food sensory evaluation techniques, and the panel had a combined 1,000 of hours experience in descriptive analysis. Aesthetic and tactile characteristics of material surface textures were evaluated. This experiment was conducted according to the Declaration of Helsinki for studies on human subjects and approved by the University of Tennessee IRB review for research involving human subjects (IRB 17-04044-XP).

Constructing Lexicon and References

Panelists were provided 15 commercially available food grade material samples (12 solid surfaces and three weld surfaces). They were initially asked to individually observe the visual and physical characteristics of each material and note words associated with those observations. Next, the panel openly discussed individual findings and, with assistance from the panel leader, constructed and reached consensus for a rudimentary collection of attributes. The panel leader synthesized the collection of words into a streamlined vocabulary by identifying themes and grouping similar descriptors together (Table 3.2).

The panelists established reference samples for each of the 12 identified attributes from a bank of possible materials commonly found in produce processing facilities. In most instances, references were established for high,

medium, and low expression of an attribute. In two cases for which it was important to distinguish between similar expression, references were also established for medium high and medium low attribute expression. This process was repeated for each attribute until a consensus was reached, with guidance from the panel leader. The reference samples for each attribute are listed in Table 3.2.

Evaluation Procedures

Panelists were instructed to observe the surface of material under white light using the naked eye and evaluate the physical structure by rubbing the food contact surface between their thumb and forefinger. Panelists were instructed to observe the visual and physical characteristics of all samples in regard to an attribute. Using a 150-point line scale, the panelists individually evaluated each sample by each attribute.

Microbial Sample Collection

Quantitative recovery of Gram-positive bacteria was collected from food contact surfaces at tomato packinghouses consisting of materials evaluated during lexicon development. Additionally, information detailing the specifics of the sampling site locations was also collected, including surface dimension, junction type, surface accessibility, hours since sanitation, and sanitizer concentration (Table 3.3). Surface dimension, junction type, and surface accessibility were

used to calculate a material's resistance to clean. Hours since sanitation and sanitizer concentration were used to understand discrepancies between differences in packinghouse sanitation programs and resulting variations in the interactions between microbial recovery and resistance to clean data.

Microbiological Analyses

Upon sample collection, a 1-ml sample was spiral-plated on Modified Oxford Medium (MOX) and incubated at 35 °C for 48 hours. Plates showing characteristic growth for organisms that were able to hydrolyze esculin via a black halo were counted using a spiral plate counter. These bacterial counts were log transformed and used to assess cleanability of each site.

Calculating Resistance to Clean and Cleanability

Resistance to clean, or the theoretical difficulty in adequately sanitizing a food contact surface, was calculated using the lexicon attributes, surface dimension, junction type, and surface accessibility data (Figure 3.1). Cleanability, or the ease of adequately sanitizing a food contact surface, is the opposite of resistance to clean. A highly cleanable surface has little to no microbial recovery.

Statistical Analysis

The statistical analysis program JMP Pro Version 13.2 (SAS Institute, Cary, NC) was used to analyze the data. Simple correlations and single linear regressions

were performed as exploratory methods. The relationship was considered significant when $p<0.05$. Due to significant correlations between predictors, a partial least squares regression (PLS-R) was used to determine the predictive value of hours since sanitation, sanitizer concentration, and resistance to clean on microbial count. Variables with variable importance factors (VIP) > 0.8 were considered influential.

IV. Results and Discussion

A total of 12 physical attributes were used to describe the cleanability of a variety of food grade materials used in vegetable processing (Table 3.2). To the authors' knowledge, no previous research has attempted to accomplish this task. While the lexicon was developed from a subset of materials used in tomato processing, the vocabulary and method of analysis is not specific to this industry and would likely have utility beyond this use to evaluate other types of food grade materials.

Surfaces with high microbial counts were correlated with higher resistance to clean calculations ANOVA ($r = 0.1645$; $p = 0.0056$), and therefore deemed to be more resistant to cleaning. While this correlation was statistically significant, other factors such as sanitation protocols at each site were not taken into account. To account for these factors, a partial least squares regression model was constructed to determine the effect of sanitation on the model (Figure 3.2). The model found two of the three predictors to be important in predicting

microbial count, resistance to clean, and time post-sanitation. Overall, the model was not able to substantially explain the variation in microbial count (R^2 = 0.0429). Resistance to clean was the primary predictor of microbial counts (β = 0.1677; VIP = 1.3416). More specifically, a more difficult to clean sample site was more likely to harbor potential pathogens.

Additionally, there was a weak negative association between hours since sanitation and microbial recovery. As time post-sanitation increased, microbial recovery also generally increased (β = -0.1419; VIP = 0.8777). It is thought that, with an increase in hours after sanitation, there was an increased likelihood that either contamination occurred to reintroduce microorganisms to the sample site or organisms that survived the sanitation process were able to grow.

Sanitizer concentration was not found to be important to understanding variation in microbial count (VIP = 0.6557). The data suggested that higher concentrations of sanitizer have a reduced lethal effect on microorganisms on food contact surfaces. This finding appears to contradict existing research on microbial death as a function of sanitizer concentration *(4, 5)*. In reality, there was a 70 parts per million (ppm)-increase in sanitizer concentration between the two packinghouses analyzed, indicating that the adjustment in active ingredient in sanitizer was so insufficient as to have a negligible effect on microbial death.

The inability of the three major variables used in this model (resistance to clean, sanitizer concentration, and hours since sanitation) highlight the importance of a wide variety in sanitation programs across packinghouses and

the methods and stringency with which they are implemented. Sanitation programs are one of the largest opportunities for mitigating risk in packinghouse settings, and yet seem to vary in scope through a multitude of factors. This data suggested that materials that have low cleanability and represent significant barriers to food safety could be improved by a more selective or strenuous sanitation program.

Conclusions

The lexicon and resistance to clean calculation could provide a reasonable framework for growers and packinghouse operators to implement in-house assessments of risk to establish cleanability of materials. Materials with low cleanability require a more targeted sanitation program than those with higher cleanability scores. Future studies should target a wider array of food processing materials and sanitation programs. Additionally, packinghouse operators should monitor sites with low cleanability to ensure established sanitation protocols are sufficient to reduce the risk of microbial harborage associated with more difficult to clean process points.

V. References

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2. Frank, J. F., and R. Chmielewski. 2001. Influence of surface finish on the cleanability of stainless steel. *Journal of Food Protection*. 64:1178-1182.
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4. Ruiz-Cruz, S., E. Acedo-Félix, M. Díaz-Cinco, M. A. Islas-Osuna, and G. A. González-Aguilar. 2007. Efficacy of sanitizers in reducing Escherichia coli O157: H7, Salmonella spp. and Listeria monocytogenes populations on fresh-cut carrots. *Food Control*. 18:1383-1390.
5. Scheusner, D., F. Busta, and M. Speck. 1971. Injury of bacteria by sanitizers. *Applied microbiology*. 21:41-45.
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7. Taylor, J., and J. Holah. 1996. A comparative evaluation with respect to the bacterial cleanability of a range of wall and floor surface materials used in the food industry. *Journal of Applied Microbiology*. 81:262-266.

VI. Appendix A

Table 3.1. Materials evaluated.

Material	Manufacturer
3 Ply Rib Cleat	Sparks Belting Company, Inc.
Automate I	Sparks Belting Company, Inc.
Econo Ruff Tan	Sparks Belting Company, Inc.
Endurothane 150 White	Sparks Belting Company, Inc.
Food King 1W	Sparks Belting Company, Inc.
Grip Tex	Sparks Belting Company, Inc.
Miscellaneous conveyor belt	Agri Machinery & Parts, Inc.
Painted mild steel	Agri Machinery & Parts, Inc.
Polyvinylchloride roller section	Agri Machinery & Parts, Inc.
Slip Top	Sparks Belting Company, Inc.
Stainless steel 304	Agri Machinery & Parts, Inc.
Thermoflex 2150 Black	Sparks Belting Company, Inc.

Table 3.2. Material lexicon as developed by the trained sensory panel via descriptive analysis.

Attribute	Definition	Reference	Attribute Score
Roughness (Jaggedness)	The quality or state of having an uneven or irregular surface. To measure, run your index finger over the surface of the sample. Example: natural limestone	Stainless steel 304	0
		Endurothane 150 White	8
		Grip Tex	15
Porous	The quality of having minute spaces or holes.	Stainless steel 304	0
		Endurothane 150 White	8
		Grip Tex	15
Depth of pores	*How deep the pores are recessed into the surface*	Stainless steel 304	0
		Slip Top	6
		Endurothane 150 White	9
		Grip Tex	15
Number of pores	*The quantity of pores observed*	Stainless steel 304	0
		Grip Tex	6
		Endurothane 150 White	10
		Slip Top	14
Diameter of pores	*The size of the pore opening*	Stainless steel 304	0
		Slip Top	6
		Endurothane 150 White	10
		Grip Tex	14
Hardness	The quality of being solid, firm, and resistant to pressure. To measure, place the sample between your thumb and forefinger, and squeeze.	Econo Ruff Tan	3
		Food King 1W	8
		Stainless steel 304	15
Slickness	The property of tending to slip from the hold or grasp or from position. To measure, place the sample on a hard surface and attempt to run your index finger over it.	Econo Ruff Tan	2
		Unlabeled conveyor belt	11
		Stainless steel 304	15
Ridges	The presence of the long and narrow upper edge, angle, or crest.	Stainless steel 304	0
		Automate I	4
		Unlabeled conveyor belt	8
		3 Ply Rib Cleat	15
Height of ridges	*The vertical amplitude of each ridge.*	Stainless steel 304	0
		Unlabeled conveyor belt	7
		3 Ply Rib Cleat	15

Table 3.2. Continued.

Attribute	Definition	Reference	Attribute Score
Number of ridges	*The quantity of ridges observed.*	Stainless steel 304	0
		3 Ply Rib Cleat	5
		Automate I	13
Coarseness	The quality of lacking in fineness or delicacy of texture, structure, etc. To measure, run your index finger over the surface of the sample. Example: sandpaper	Stainless steel 304	0
		Endurothane 150 White	10
		Slip Top	13
Weld Roughness		High quality weld sample	0
		Medium quality weld sample	7
		Low quality weld sample	15

Table 3.3. Definitions of additional surface attributes utilized in this study beyond the descriptive panel lexicon.

Attribute	Definition	Variables
Surface Dimension	The number of dimensions involved in the construction of a food contact surface.	1-dimensional 2-dimensional 3-dimensional
Junction Type	The number and type of materials involved in the construction of a food contact surface.	None 2 materials contacting Multimaterial surface Weld surface
Surface Accessibility	The accessibility of a surface to complete drainage of food, cleaning, or sanitation materials.	Drainable Standing water
Resistance to Clean	The theoretical difficulty in adequately sanitizing a food contact surface.	*Continuous variable*
Cleanability	The ease of adequately sanitizing a food contact surface.	*N/A*
Hours Since Sanitation	The number of hours lapsed since sanitation was completed.	*Continuous variable*
Sanitizer Concentration	The concentration of sanitizer used during sanitation.	*Continuous variable*

VII. Appendix B

$$(Jaggedness + Porous + Depth\ of\ Pores + Number\ of\ Pores +$$
$$Diameter\ of\ Pores + Ridges + Height\ of\ Ridges + Number\ of\ Ridges +$$
$$Coarseness + Surface\ Dimension + Junction\ Type + Surface\ Accessibility) -$$
$$(Hardness + Slickness) = Resistance\ to\ Clean$$

Figure 3.1. Resistance to Clean calculation.

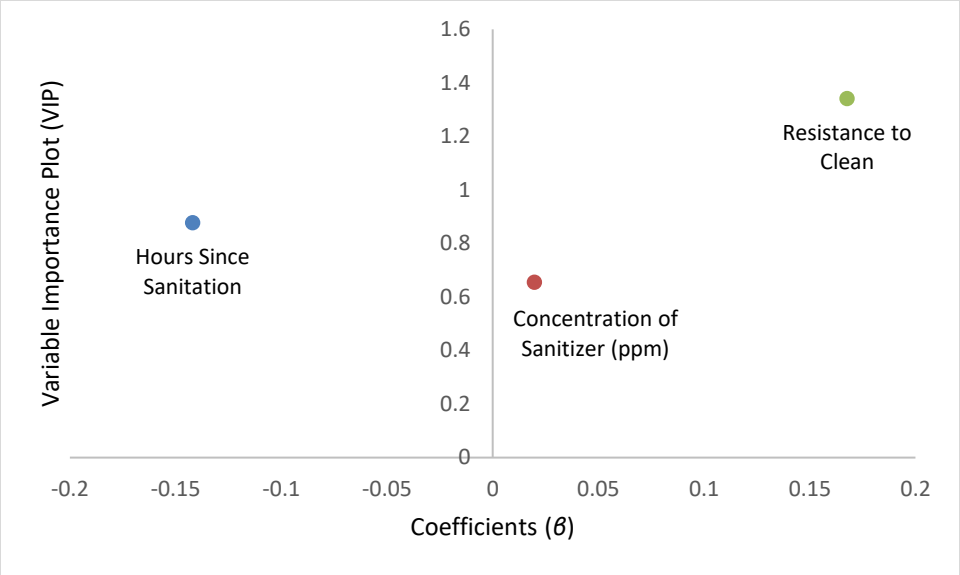

Figure 3.2. Partial least squares regression of resistance to clean, hours since sanitation, and sanitizer concentration on count.

CHAPTER IV
SUMMARY

Sanitation-related interventions significantly impacted the likelihood of detection of Gram-positive bacteria or *Listeria* spp. compared to structural components. Specifically, sanitizer contact time, hours since sanitation, and sanitation crew each provide farm-specific opportunities to reduce the risk of *Listeria monocytogenes* contamination. Since these are variables that packinghouse operators can control, efforts should be made to work with sanitation managers to construct a sanitation protocol and environment best suited to meet the needs of the individual packinghouse. Additionally, packinghouses should be cleaned and sanitized at least once every 72 hours during the packing season, regardless of throughput or structural design. Furthermore, packinghouse management personnel should work to establish and support a food safety culture within packinghouse facilities that proactively implements prevention strategies, including enhancing human hygiene during processing and regular monitoring hard-to-clean (low cleanability) areas on the packingline.

To help growers and packers design a better sanitation protocol that fits facility needs, this study also sought to construct a methodology for establishing cleanability of common materials used in packinghouses to reduce the risk of bacterial harborage due to structure-related design decisions. The lexicon and resistance to clean calculation showed the ability to aid packinghouse operators in assessing levels of risk within their facilities. Materials with high resistance to clean scores will require more strenuous cleaning and sanitation efforts to

overcome inherent structural or design barriers to adequate sanitation. Additionally, those sites or materials that are known to pose elevated risk for *Listeria monocytogenes* contamination should be frequently monitored to ensure the sanitation procedure decided upon and informed by the lexicon and resistance to clean calculation are sufficient to effectively clean the packingline equipment.

Due to the occasional isolation of *Listeria* spp. on zone one contact surfaces, a larger study equipped to evaluate a larger number of tomato packinghouses over several growing seasons is necessary to better understand the areas along the processing line and in packinghouses that pose the greatest risk for *Listeria monocytogenes* contamination and harborage. Sampling after sanitation may have prevented the detection of true harborage points by not allowing bacteria to work out of niche points. Additionally, many materials frequently used in packinghouse operations were not evaluated in the development of the lexicon and creation of the resistance to clean calculation, including formica laminate, polyethylene, and polypropylene. This exclusion of relevant materials may have affected interpretability of the lexicon and calculation.

The education and continued training of packinghouse personnel provides one of the most significant opportunities to reduce the risk of pathogen contamination in packinghouse facilities. These interventions should include considerations for developing sanitation protocols, human hygiene in the packing

environment, and the importance of cleaning and sanitizing properly. Sites that emerged as niche points for *Listeria* spp. or were constructed with materials that exhibited high resistance to clean scores should be routinely monitored for presence of foodborne pathogens. Future studies should assess more packinghouses across processing time points over several growing seasons, in addition to evaluating more food processing materials and sanitation programs commonly used in packinghouses to prevent the contamination of or harborage within processing equipment of *Listeria monocytogenes.*

VITA

Alexis Marie Hamilton was born in Lexington, Kentucky on March 25, 1994 to parents Steven and Renee Hamilton. Alexis grew up in Knoxville, Tennessee with her two younger siblings David and Jessica Hamilton. She graduated from Knoxville Catholic High School in 2012. She attended the University of Tennessee at Knoxville where she received her Bachelor of Science degree majoring in Food Science in December 2016. She began the Master of Science program at the University of Tennessee at Knoxville focusing in Food Microbiology and Food Safety and graduated in August 2018. Alexis has continued her education at Washington State University pursuing her doctorate in Food Microbiology.