EFFECTIVE STRATEGIES TO SANITIZE HARVEST BINS AND PICKING BAGS USED IN THE TREE FRUIT INDUSTRY

by

HEMA SAI SAMHITHA CHALAMALASETTI

(Under the Direction of Faith Critzer)

ABSTRACT

Tree fruits have been linked with the outbreaks associated with foodborne illnesses, with recent associations with *Listeria monocytogenes* and *Salmonella*. These organisms spread through environmental, animal, or human sources, which can occur at any point of the farm-to-fork chain. This study evaluated commercially available sanitizers in reducing *Listeria monocytogenes, Salmonella,* and *Escherichia coli* in nylon harvest bags as well as wood and plastic bins commonly used in the apple industry. Bacterial inoculated surfaces of nylon, plastic, and wood were treated with peracetic acid (500 ppm), chlorine (500 ppm), silver dihydrogen citrate, for contact times of 1 and 2 min, steam (80-90 psi) for 0.5 and 1 min and chlorine dioxide (70 ppm maximum concentration) for 24 h. Log reduction was compared to the control using analysis of variance with post hoc analysis for significant factors using Tukey's HSD ($p \leq 0.05$). Peracetic acid outperformed all sanitizers for *L. monocytogenes*, while chlorine dioxide showed maximum efficacy for *Salmonella* and STEC.

INDEX WORDS: Sanitizers, *Listeria monocytogenes*, Tree fruits, Foodborne illnesses

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CHAPTER 1

INTRODUCTION

Produce is typically cultivated outside in soil under the influence of weather and other environmental conditions, and there are no interventions that can be applied postharvest to get rid of pathogens that can make people ill, referred to as microorganisms of public health significance [11]. Biofilms form when bacteria are left on packaging containers or bags. Although non-pathogenic bacteria make up the majority of biofilms, they serve as habitats where any pathogenic bacterial cell passing by can attach and reproduce. It is crucial to prevent the formation of biofilms on harvest, wash, and packing equipment and other food contact surfaces by regularly cleaning and sanitizing, thus removing plant debris, soil, and associated microorganisms. This is because once biofilms are developed, they increase the possibility that harmful bacteria will remain on a surface and multiply [129].

A key component of sustainable food production is maintaining collected product's quality, safety, and nutritional value [124]. Fresh produce, such as tree fruits, has been traditionally regarded as non-supportive to the growth of human pathogens [36]. Foodborne pathogens are not frequently associated with the epiphytic populations of fresh produce [57]. Improper sanitation and hygiene of food contact surfaces are risk factors that contribute to outbreaks [32]. Contamination of fresh produce is a major concern because it is to be consumed raw or with minimal processing or preparation without heating. So, prevention is the key to eliminating microbes [120].

Consumption of fresh fruits and vegetables has been associated with an increase in foodborne microbial illness outbreaks over time [44]. The safety of these food products for consumers is essential because they are recognized as critical elements of a healthy diet [62]. In relation to fresh produce, notable foodborne diseases include bacteria like *Escherichia coli, Listeria monocytogenes,* and *Salmonella* sp. [22]. These organisms make their way through environmental, animal, or human causes and can happen at any stage of the farm-to-fork chain and result in illness and outbreaks [178].

Previous research has emphasized postharvest hygienic needs for *Listeria* management. But still, there is a need to develop on-farm and packinghouse cleaning and sanitary measures for harvest bins and picking bags to prevent cross-contamination. This study aims to provide science-based information regarding the efficiency of sanitizers in reducing microorganisms of public health importance concerning food contact materials of nylon, plastic, and wood commonly encountered in picking bags and harvest bins. The industry can use the results to support science-based best practices for managing risk from surfaces commonly faced during harvest.

CHAPTER 2

LITERATURE REVIEW

2.1 The prevalence of foodborne illness in the USA

Fresh produce is an excellent source of nutrients like fiber, carbs, proteins, vitamins, and minerals [132]. Consequently, These are essential aspects of a balanced diet [29]. Additionally, they offer a variety of health advantages, from a lower risk of developing chronic conditions like cancer and cardiovascular disease to an improvement in the general well-being of the consumer [117].

Every year in the USA, millions fall ill, and hundreds of foodborne outbreaks occur due to consuming contaminated food [151]. Fresh produce has been increasingly implicated as a source of foodborne outbreaks in many world regions [120]. Produce is more frequently related to multistate foodborne outbreaks compared with other food categories [27]. Infections linked to fresh produce include bacteria *Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella* sp., and *Staphylococcus aureus*, protozoan parasites *Cryptosporidium* spp. and *Cyclospora* spp., and viruses Hepatitis A and Norovirus [22].

Various bacterial foodborne pathogens can thrive if given proper conditions for growth [67]. Before eating, raw produce is not put through any processing stages to ensure the effective inactivation of pathogenic microorganisms [122]. Given that outbreaks linked to fresh produce are a burden to both individual's health and the economy in general, mitigation strategies that reduce the likelihood of illness can have significant impacts [10].

2.2 *Listeria monocytogenes*

2.2.1 *Listeria* Habitat and Pathogenicity

The genus *Listeria* presently contains 17 species *Listeria monocytogenes, Listeria seeligeri, Listeria ivanovii, Listeria welshimeri, Listeria marthii, Listeria innocua, Listeria grayi, Listeria fleischmannii, Listeria floridensis, Listeria aquatica, Listeria newyorkensis, Listeria cornellensis, Listeria rocourtiae, Listeria weihenstephanensis, Listeria grandensis, Listeria riparia, and Listeria booriae* [139]. Out of these *L. monocytogenes* and *L. ivanovii* are considered the two pathogenic species. *L. monocytogenes* contributes to the foodborne pathogenicity that may be fatal to humans and animals and thus the virulence mechanism has been extensively studied since the mid-1980s *[169]. L. ivanovii* is economically significant due to its pathogenicity in livestock and is seldom linked to human listeriosis [88].

Listeria monocytogenes is inherently found in agricultural habitats such as soil, manure, and water [99]. It is a gram-positive, non-spore-forming Firmicutes bacterium that adheres to surfaces and grows at temperatures between 0 and 45 °C[56]. The bacterium causes listeriosis in both humans and animals which is defined as the isolation of the organism from blood, cerebrospinal fluid, or another ordinarily sterile source [147], and food is a primary source of contamination [42].

L. monocytogenes is a genetically diverse species with 13 serotypes. Serotypes 1/2a (lineage II), 1/2b (lineage I), and 4b cause over 95% of human illnesses (mostly lineage I and some lineage III or IV) [46]. This bacterium has an intriguing ability for life cycle adaptation. When it enters human or animal cells, its role changes from that of a saprophyte in the soil to an intracellular pathogen [80]. Pathogen contamination between agriculture and wildlife occurs

primarily in public waterways such as rivers, lakes, and ponds [119]. Environmental strains can cause produce to become contaminated before it is harvested. These strains can then enter packing facilities and processing plants.

Wildlife nesting in produce fields is a potential source of preharvest contamination, so growers in some areas are encouraged to use fencing or clear riparian areas near produce fields to discourage wildlife nesting [159]. *L. monocytogenes* is a particular problem as a postharvest contaminant in food processing facilities and packing houses due to its ability to grow at refrigeration temperatures, ability to resist acid and osmotic stress, and join biofilms [75].

Each year, an estimated 1,600 people contract listeriosis, with approximately 260 dying [83]. Pregnant women and their newborns, adults 65 and older, and people with weakened immune systems are the most vulnerable to the infection [175]. When a pathogen enters a mammalian cell via phagocytosis, it is released from the membrane-bound vacuole and begins to multiply. The pathogen infects a wide range of host tissues using actin polymerization for intracellular movement and cell-to-cell spread, with the liver being the primary site of infection [65]. For these reasons, *L. monocytogenes* infections have a mortality rate of 20-30%, despite the fact that the number of cases appeared to be small in comparison to the estimated illnesses associated with salmonellosis and campylobacteriosis and are considered a pathogen that must be mitigated through effective food safety programs [167].

Two different disease syndromes can be introduced by *L. monocytogenes.* Listeriosis is caused by invasive *L. monocytogenes*. The organism commonly infects sterile organs such as the liver [176], spleen [15], cerebral spinal fluid [50], and blood [33]. The primary symptoms in health individuals are diarrhea and fever [149], in pregnant women fever, diarrhea, abortion, or

stillbirth [142], and in newborns sepsis, pneumonia, and meningitis [37, 41, 76, 110, 138]. *L. monocytogenes* can also produce non-invasive disease, most commonly febrile gastroenteritis or non-invasive gastroenteritis, and it has been linked to outbreaks caused by contaminated deli meat [82, 116], chocolate milk [145], cheese [179], smoked fish [148, 166], and corn [19].

2.2.2 *Listeria* Foodborne Outbreaks

The first case of foodborne listeriosis was recognized in 1981 with the consumption of contaminated coleslaw following an outbreak in Canada. The Maritime Provinces of Canada experienced an outbreak between March and September 1981 that involved 41 cases (34 neonatal and 7 adults). The mortality rate was 28.6% for adults and 27% for infants [156]. A assessment of the farmer's agronomic procedures showed that fields where cabbage was grown had received application of both composted and raw sheep manure [31].

2.2.3 *Listeria* linked to produce

Freshly harvested produce is highly susceptible to *Listeria* spp. contamination because these saprophytes are frequently found in the soil of different agricultural landscapes, this is especially true for crops that are close to the topsoil [45]. The crops carry *Listeria* cells as they are harvested and carried into the packing or processing facility, if the cells establish themselves at the facility, they could contaminate handled or processed food frequently [100].

In 2010, a listeriosis outbreak connected to chopped celery was reported by the Texas Department of State Health Services (DSHS), five of the hospital's 10 affected patients, who ranged in age from 56 to 93, passed away within three months [179]. The *L. monocytogenes* contamination at the produce facility may have started as an environmental issue, such as tainted water in a floor crack and believed that after being introduced to the facility, the bacterium

propagated throughout the processing facility, lingered for months, colonized hard-to-clean machinery (such as a dicer), and contaminated celery as a result of processing [77].

In 2011, a listeriosis multistate outbreak impacting 28 states was caused by eating cantaloupe grown by Jensen Farms in Colorado. The cantaloupe-borne outbreak was the deadliest foodborne illness outbreak to hit the United States in the previous 25 years, resulting in 147 illnesses and 33 fatalities [52]. Unhygienic activities and cross-contamination from surfaces of cantaloupe packaging machinery, such as brush and felt rollers that were previously used for potato processing, were shown to be the causes of contamination [130].

In 2014–2015, was the first to link outbreaks of foodborne illnesses in the United States and Canada to whole apples in commercially produced caramel apples, leading to 35 hospitalizations and 7 fatalities [121]. Inserting a stick into an apple causes juice to seep into the area between the skin and the caramel, creating an ideal environment for *L. monocytogenes* growth, especially at room temperature [43]. During the FDA and California state inspection of a grower's apple packing factory, 110 environmental samples were collected; seven of those samples produced *L. monocytogenes*, of which six isolates came from food contact surfaces (such as the polishing brush, drying brush, conveyor, and inside a wooden bin) and one from the main packing line floor drain [13]. In the 2017 listeriosis outbreak, was again linked to eating caramel apples, three people fell ill [174]. The epidemiological data suggested that the presumed source of this incident was caramel apples [127].

In January 2016, nine states in the USA were impacted by a multistate outbreak of *L. monocytogenes* in packaged salad in which one person from Michigan died from listeriosis, and 19 of the sick people were hospitalized [179]. Laboratory and epidemiological data demonstrated

that packed salads made in Ohio were to cause for the outbreak where processing and manufacturing environment were likely the cause of contamination [153].

In 2020, the CDC investigated the first known *Listeria* outbreak in the United States linked to Enoki mushrooms which resulted in three hospitalizations. Epidemiologic, traceback, and laboratory data identified that Enoki mushrooms provided by Green Co. LTD, a company based in the Republic of Korea, as the likely cause of this outbreak.[1].

2.3 *Salmonella*

2.3.1 Introduction

Salmonella has been identified as the primary contributor to foodborne illnesses and a significant global public health issue, with increasing concern related to the development and spread of antimicrobial-resistant forms [66]. It accounts for 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year [51]. Nontyphoidal *Salmonella* are the most prominent zoonotic bacterial foodborne diseases of humans in nature [108]. *Salmonella* has been identified as a prevalent source of infection in fresh produce due to the bacteria's tendency to attach and internalize in vegetables. [89].

2.3.2 Serovars and Pathogenicity

There are more than 2,500 known serotypes of the bacteria *Salmonella,* [135] which are classified into two species: *Salmonella enterica* (*S. enterica*) and *Salmonella bongori* (*S. bongori*) [87]. *S. enterica* is divided into six subspecies, and more than 150 serotypes are linked to foodborne salmonellosis [63], out of which *S.* Typhimurium and *S.* Enteritidis are most common [164] . *Salmonella* is a gram-negative, rod-shaped, motile (with the exception of *S.* Gallinarum and *S.* Pullorum), mesophilic, facultative anaerobe of the *Enterobacteriaceae* family

[40]. *Enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* IIIb, *houtenae* (IV), and *indica* are the six subspecies of *S. enterica*. (IV). Previously known as subspecies V, *S. bongori* is now regarded as a species [59]. The majority of human pathogenic serotypes are found in *S. enterica* subspecies *enterica* (I) [71].The majority of *Salmonella* infections, commonly known as salmonellosis, are brought on by eating food contaminated with the *Salmonella* group [68]. Foodborne *S. enterica* infections account for 94% of cases in the United States [92]. The majority of the symptoms include gastrointestinal, such as nausea, vomiting, pains in the stomach, and bloody or greenish-watery diarrhea that is mucous-filled [9] typically starts 12–72 hours after consuming food and last for 4–7 days. [58]. *Salmonella* infection with microfold cells in Peyer's patches and localized damage without septicemia are the characteristics of enteric infection, which is made possible by fimbriae adhesions then the target cell membrane is disturbed, causing the bacteria to get internalized in membrane-bound vacuoles where the ruffles aid in bacterial absorption in membrane-bound vacuoles or vesicles, which frequently congregate [9].

One of the most significant epidemiological factors is the prevalence of common carrier status among both domestic and wild animals [26] [91]. Pathogenic *Salmonella* species have been found to grow at temperatures ranging from 8 to 45 °C, pH levels ranging from 4.0 to 9.5, and water activity levels as low as 0.94, raising concerns in the food industry [47]. Although the pathogen has been detected in many other foods, poultry [21], pigs, and cattle [16], as well as their byproducts such meat, eggs [91] [150], and milk [162], are most frequently recognized as food sources responsible for outbreaks of human salmonellosis[91]. *Salmonella* outbreaks associated with contaminated produce are frequently widely distributed, which implies that

contamination is happening immediately after the production process, whether in the field or a processing factory [156]. In humans it has been linked to consumption of raw or unsafe food, cross-contamination, inadequate cooling and reheating of food items, poor personal hygiene practices, and a protracted gap between food preparation and consumption [66]

2.3.3 *Salmonella* associated with fresh produce

Fresh produce outbreaks of *Salmonella* are most commonly associated with alfalfa sprouts, cantaloupes, mangoes, cucumbers, bean sprouts, and papayas [51]. Fresh produce is more likely to become contaminated with bacterial infections before it reaches consumer as the distance from farm to fork has increased over time [44]. Pathogens such as *Salmonella* may live in protected areas on the surface of fresh vegetables and persist for extended periods of time beyond the intended shelf-life [53]. *Salmonella* contamination in fresh food prior to eating is difficult to eliminate or diminish after contamination during processing [35]. Moreover, pathogens can cling to surfaces by interacting with epiphytic microflora, and they may become even more protected by internalizing, which depends on numerous factors and results in phyllo sphere features [72]. By controlling their internal pH, *Salmonella* may survive for long periods of time in an acidic environment like apple and orange juice [12]. Hence, the early stages of production should be used to control *Salmonella* contamination in fresh products [72]. The stomata of plant leaves [97], hydathodes [146], and roots [57] can all be penetrated by members of the *Enterobacteriaceae* family. Pre- or post-harvest infected plants don't show evidence of deterioration [25], while pre- or post-harvest contaminated product does [3].
Salmonella produces periplasmic enzymes with the ability to break plant surface barriers.

However, the penetration of these enzymes into plant systems is dependent on pectin and polygalacturonate processing (level of ripening) and physiological wounds [7].

2.3.4 *Salmonella* linked to outbreaks

Serotypes Newport [38], Enteritidis [35], and Javiana [28] were responsible for the majority of *S. enterica* infection outbreaks with a single etiology. Between 1989 and 1991, two successive melon epidemics sickened over 400 people due to contaminated cantaloupe imported from Mexico and Texas, and a review of farms and processing plants conclude that the majority of *Salmonella* contamination was caused by the rind's immersion in tainted wash water at post-harvest facilities [74]. A cantaloupe outbreak in 2012 of *Salmonella* Typhimurium and *S.* Newport caused 261 infections in 24 states, a 36.0% hospitalization rate, and three fatalities [53]. At the farm, poor agriculture and sanitation practices contributed to the outbreak [155].

Alfalfa sprouts were the cause of two significant epidemics in 1995 and 1996 that sickened over 700 people and resulted in one fatality in USA, Canada and Finland [30]. Alfalfa sprouts grown from contaminated seed are found to be responsible for the surge of both infections of *S.* Stanley in 1995 and *S.* Newport [123]. The first known salmonellosis outbreak of raw mung bean sprouts caused by *S.* Enteritidis in the United States in 2000, involving 45 illnesses [134]. Contact with birds or rats during storage or shipping could be a possible cause of contamination for seeds [123] . The greatest *Salmonella* outbreak linked to alfalfa sprouts was in 2010, resulting in 140 infections across 26 states [54]. Identification of unhygienic circumstances, and also an presence of outbreak strain in one environmental sample (water runoff) is concluded for the contamination of seeds [104] .

A foodborne illness outbreak at first linked to tomatoes in 2008 resulted in 1442 confirmed cases of salmonellosis in 29 of the 50 states of the United States with 21% were hospitalized, and 2 died where the serrano peppers and jalapeno peppers were both significant sources of contamination and tomatoes may have also been a contributor, especially early on in the pandemic [2]. Irrigation water used at a farm in Mexico to hydrate crops of jalapeno and Serrano pepper was also considered the cause of contamination [89].

The 2015 multistate outbreak of *S.* Poona was at least the fifth multistate outbreak resulting from contaminated cucumbers since 2010 causing 907 illnesses and 6 deaths reported from 40 states and the outbreak was caused by cucumbers grown in Baja California Sur, Mexico, which were distributed by a Andrew & Williamson Fresh Produce but the source of contamination by distributor has not been identified . [115]

In 2017, the salmonellosis outbreak by *Salmonella* Thompson was connected with imported papaya and covered 23 states after the prior papaya outbreak in 2011, resulting in 220 illnesses, 68 documented hospitalizations, and one fatality [55]. The infected food came from a single farm in Mexico [90].

The largest foodborne *Salmonella* outbreak in more than a decade occurred in 2020 and was caused by red onions contaminated with *S.* Newport causing 1127 cases in 48 states [49]. Red onions from Thomson International Inc. were the most likely source of this outbreak, according on epidemiologic and trace back data [131] . Because the onions were produced and harvested at the same time, other onion varieties (such white, yellow, or sweet yellow) were also likely to be affected [2]. One primary hypothesis is that contaminated irrigation water used in a

growing area near Holtville, California may have contributed to the contamination of the onions [6].

2.4 Shiga-toxigenic *Escherichia coli*

Escherichia coli is an innocuous member of the gut microbiota of warm-blooded animals and people, but pathogenic strains can cause infections of the intestines and other organs and tissues [79]. The presence of STEC is utilized as a sign of fecal contamination in food safety and hygiene [165].

2.4.1 Habitat and pathogenicity

E. coli is a coliform bacterium and one of the six kinds of *Escherichia* species (*E. adecaroxylate, E. blattae, E. fergusonii, E. hermannii,* and *E. vulneris*), one of the 30 members of the bacterial family Enterobacteriaceae [165]. It is a rod-shaped, gram-negative, facultative, anaerobic, mesophilic bacteria that thrives in temperatures between 7 and 45 °C [96]. *Escherichia, Citrobacter, Enterobacter*, and *Klebsiella* comprise the coliform bacteria family [86]. Along with coliform bacteria also known as fecal bacteria, there are also bacteria with plant origins like *Enterobacter aerogenes, Citrobacter freundii*, and *Klebsiella pneumonia* [114]. Vegetable produce is frequently connected with certain coliforms (Klebsiella), which multiplies in a favorable environment [101].

E. coli is common in feces and the environment and is the dominant bacteria in the facultative anaerobic microbiota of the intestines [160]. *E. coli* is the most common gram-negative bacilli bacterium that causes meningitis, particularly in neonates [168]. Some of its pathogenic strains both produce toxins that make people intoxicated and induce infection-type food poisoning through gastroenteritis, pathologic kidney, and brain damage through cellular

expansion [58]. There are seven different pathotypes of *E. coli*, which comprises entero-hemorrhagic *E. coli* (EHEC), entero-aggregative *E. coli* (EAEC), entero-pathogenic *E. coli* (EPEC), entero-toxicogenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC), entero-invasive *E. coli* (EIEC), and adherent-invasive *E. coli* (AIEC) [84]. Shiga toxin-producing *E. coli* (STEC) and verotoxin-producing STEC (VTEC) are other names for EHEC [144].

In Canada, the United States, and Europe, STEC is one of the main causes of bacterial gastrointestinal disease [137]. The hemolytic uremic syndrome (HUS), which causes low blood cell and platelet counts as well as acute kidney failure, can develop from the serious and potentially fatal EHEC toxic infection [163]. *E. coli* O157:H7 is regarded as a serious contributor to foodborne illnesses and is the most commonly isolated in North America of multiple serotypes of STEC [70]. Among the additional STEC serogroups involved in foodborne illnesses are O26, O45, O103, O111, O121, and four more serogroups O145 ,O91, O113, and O128 have the capacity to develop biofilms on abiotic surfaces [61]. Shiga-like toxins produced by STEC O157:H7 are cytotoxic to the human colon and duodenum. This toxin causes ulcers in the colon and fluid accumulation in the intestines by damaging the crypt epithelia. [165]. Intimin facilitates intestinal canal adhesion [68]. STEC strains can live on fresh leafy green vegetables and ground beef [105].

Many plants that are consumed by humans, including spinach, lettuce, alfalfa, cress, beans, arugula, tomatoes, and radish, have had some STEC strains isolated from them [118]. Physical barriers present on these plants include wax, cuticle, cell wall, trichomes, and stomata (respiratory opening) [173]. EHEC has the capacity to diffusely cling to the epidermis, aggregate around the stomata, and penetrate cut lettuce leaves to a depth of 20 to 100 m into the junction

zones (spongy mesophyll) and stomata [140]. Additionally, it has been demonstrated that *E. coli* O157: H7 can enter a plant through the root system and make its way to the plants like lettuce, spinach, and tomato [177]. Microorganism internalization is influenced by variables, including the specificity of bacterial strains or serotypes, plant age, the composition of root exudates, soil type, and environmental conditions [143]. Numerous human pathogenic bacteria including STEC can survive on and enter the apoplast of plants, where they can continue to exist with little metabolic activity and withstand extreme fluctuations in temperature, pH, osmolality, and nutrition availability [157].

2.4.2 STEC linked to outbreaks

The outbreak of *E. coli* O157:H7 infection linked to unpasteurized fruit juice is thought to be the first one to have been recorded in 1980 in Canada [109]. Bacteria in the apple's subsurface structures and core may be protected from removal during washing but are released into the juice during crushing and pressing, according to a high level of cross contamination from the hammer mill and/or press cloth used to process apples into cider [14].

E. coli O157:H7 was isolated in 40 individuals (13 hospitalized) in the United States in 1995, and 70% of these patients reported eating leaf lettuce [8]. In 1996, *E. coli* O157:H7 poisoning was most likely caused by white radish sprouts, and 7,996 people became ill, 398 were hospitalized, and three died, cattle feces in contact with irrigation water was a potential source of the outbreak [8, 133]. In the Western United States, 66 people were ill and one person passed away in October 1996 as a result of an outbreak of *E. coli* O157:H7 infections linked to unpasteurized commercial apple juice [4]. The contamination of the juice may have come from

three lots of apples: two lots were from an orchard where deer visited and were later found to carry *E. coli* O157:H7, and one lot included waxed, decaying apples [48].

In 2006, a deadly *E. coli* O157:H7 outbreak in bagged spinach was attributed to California's Central Coast region, which produced more than 70% of the salad vegetables marketed in the United States [98]. Although no cause of the outbreak was identified, wildlife has been considered as a disease vector and feral pig feces was considered a source [106]. In 2018 and 2019, two multistate outbreaks of California region were linked to Romaine lettuce were triggered by a recurring strain of *E. coli* O157, resulting in 234 cases in 33 states. Cattle grazing on nearby land in 2019 and a contaminated agricultural water reservoir in 2018 were thought to be the contributors of these outbreak [172].

Late in the spring of 2011, there was a large *E. coli* outbreak, this time linked to serotype O104:H4 in central Europe that affected approximately 4,000 people, mostly in Germany with more than 900 cases of HUS, and 54 fatalities [39]. Evidence suggests the outbreaks are related to a 15,000 kg seed shipment from Egypt that landed in Germany in December 2009, and with the source of the illnesses epidemiologically linked to tainted fenugreek sprouts [85].

The first known instance of the outbreak of *E. coli* O157:H7 linked to strawberries was identified from an Oregon farm that caused at least 15 illnesses and one fatality [94]. The analysis confirmed deer feces as the source of contamination and identified fresh strawberries as a novel carrier for *E. coli* O157:H7 [34].

2.5 Sanitizers

To lessen the chance of microbial contamination, sanitizers can be used to clean surfaces that come into touch with food and eliminate food residues [69]. The produce safety guideline

mandates that surfaces that come into touch with food be cleaned frequently to avoid cross-contamination [102] . Prior to reuse, wet-cleaned surfaces need to be fully dried and sanitized, to remove dirt and loosen the soil containers with food-contact surfaces should be cleaned or immersed in fresh water [60]. When selecting a sanitizer for food contact surfaces, it's important to take into account factors such a product's ability to reduce microbial contamination under particular circumstances, affordability, ease of use, the need for rinsing, worker protection, and compatibility with readily available water in the area [78].

Sanitizers are known to be used in the food industry for disinfecting surfaces or equipment. Some of the industrially used sanitizers include chlorine (Cl), chlorine dioxide (ClO_2), peracetic acid (PAA), silver dihydrogen citrate (SDC), and steam. Chlorine is the most common, inexpensive, and strong oxidizing agent which is effective against broad spectrum germicides [93, 113]. PAA is a stronger oxidizing agent than chlorine which is effective against bacteria, yeast, and virus [141]. It decomposes the byproducts into harmless and it has application over a wide range of temperatures 0-40˚C and pH 3-7.5 [64]. SDC is a highly soluble form of silver stabilized in citric acid, the antimicrobial property of silver had its application throughout the industry for centuries [128]. SDC is a ready-to-use colorless, non-flammable liquid that covers a wide range of spectrum of materials, furthermore the most concentric form is not classified as hazardous [5]. SDC has been demonstrated to have antimicrobial effect against bacteria like *Listeria monocytogenes* and is a combination of electrolytically produced silver ions (0.003%) in citric acid (4.846%) [125]. Chlorine dioxide has been identified to be a necessary and adaptable disinfectant due to its efficiency against a variety of microbes in both liquid and gaseous forms, throughout a wide pH range, and at extremely low concentrations [103]. Gas

sanitizers are generally advantageous over liquid ones because of their ability to cover a wider area [81]. It is also effective for biofilms and can be considered as a more powerful disinfectant than bleach [17]. Steam is known as a natural disinfectant and degreaser has the properties of easy cleaning, quick in action, has broad kill range, reduces the potential of cross-contamination, is non-toxic, and friendly about reaction onto surfaces. The temperature and pressure relation is essential for effective application [112].

2.6 Food Contact Surfaces

Tree fruits like pears or apples are storage crops and may require handling and storage for several months prior to distribution [161]. Physical injuries and inappropriate handling during storage and distribution are to blame for almost half of the losses [171]. Wood, plastic, and nylon [73] are the three primary materials used to make produce containers [20] . Wood bins have been traditionally used in the industry due to their superior strength and low cost [107]. Wood is a hydro stable cellulosic material that is strong, durable, and retains its strength when damp. Plastic bins have been mentioned as being more weather resistant and have surfaces that are easier to clean and sanitize [111]. Nylon is a synthetic polymer composed of repeating units joined by amide bonds, In addition to having strong resistance to abrasion, rot, fungal, mold, mildew, and many chemicals, nylon also possesses great abrasion resistance [170].Nylon is used as harvesting bags mainly for tree fruit picking [126].

A study stated that bins can be considered as food contact surfaces which are a potential source of contamination with the microbial load. The most common methods of cleaning and sanitation of bins include immersion in a chlorinated dump tank system, hydro cooler, and pressure washing which can be automated [111]. Wood bins are primarily used for storage of

apples and pears in bulk prior to packing and distribution. Plastic storage containers are frequently used for cherries, and soft fruits like apricots, peaches, and plums as well as organic apples and pear. While fruit does not spend the same quantity of time in contact with harvest bags made of cordura nylon fabric the opportunity for cross-contamination still exists. Given their role as a common food contact surface for tree fruit, there is a need to determine effective strategies to sanitize harvesting bins and picking bags.

CHAPTER 3

MATERIAL AND METHODS

3.1. Food Contact Surface Selection

The food contact surfaces were selected based on the commercial application in the tree fruit industry. These materials are selected based on their usage in the industry which has the ability to serve as surfaces for microorganism growth and attachment. The wood-type basswood (Hobby Lobby, Oklahoma, OK), plastic-type HDPE (BioSurface Technologies Corporation, Bozeman, MT), and nylon (donated by partnering packaging houses) are known to be used for the picking and harvesting operations of produce. The coupons were cut in the dimensions of 1.4 cm by 5 cm. The nylon bags were washed with Tergazyme (Alconox Inc., White Plains, NY) in water before use and air dried. All the coupons were sterilized using UV light in a biosafety cabinet for 10 minutes to eliminate background microflora.

3.2. Bacteria growth Conditions and Culture Enrichment

L.monocytogenes strains were grown using Tryptic Soy Broth with Yeast Extract (0.6% w/v; TSBYE; Difco, Becton Dickinson Co, Sparks, MD) at 35 ◦C for 24 h with three consecutive transfers at 24 h intervals. At the third serial transfer, 250 μL was plated onto Tryptic Soy Agar with Yeast Extract (TSAYE; Difco, Becton Dickinson Co, Sparks, MD) and incubated at 35 °C for 24 h for the creation of a bacterial lawn in duplicates. *Salmonella* and STEC strains were similarly grown using Tryptic Soy Broth (0.6% w/v; TSB; Difco, Becton Dickinson Co, Sparks, MD) at 37 °C for 24 h with three successive transfers at 24 h intervals and at the third serial transfer, 250 μL was plated onto Tryptic Soy Agar with Yeast Extract (TSAYE; Difco, Becton

Dickinson Co, Sparks, MD) and incubated at 37 °C for 24 h for the harvesting of a bacterial lawn in duplicates. On the fifth day, for all bacterial strains 10ml of Buffered Peptone water (BPW; Difco, Becton Dickinson Co, Sparks, MD) was added and bacteria was collected by scraping the surface of the inoculated Tryptic Soy Agar plates with hockey stick. The cocktail for *L. monocytogenes* includes an equal volume of the strains 390-6 (serotype 1/2a),390-2 (serotype 1/2b), 573-035 (serotype 4b), for *Salmonella* Enteritidis ATCC BAA-1045, Agona LJH 517, Newport ATCC 6962 and for STEC (STEC) O157:H7, O154:H4, and O45:H2.

Table 1. Strains used in this study with information pertaining to the outbreak where originally isolated.

Bacteria	Strain
L. monocytogenes	390-2 Environmental isolate 2011 cantaloupe outbreak
L. monocytogenes	390-6 Environmental isolate 2011 cantaloupe outbreak
L. monocytogenes	573-035 Clinical isolate 2014 caramel apple outbreak
Salmonella enterica Enteritidis	ATCC BAA-1045 Raw almond outbreak
Salmonella enterica Agona	LJH 517 Alfalfa sprouts outbreak
Salmonella enterica Newport	ATCC 6962 clinical isolate
STEC	O157:H7 KSU 31 Apple juice outbreak
STEC	O45:H2 96-3285 Clinical isolate

STEC	O104:H4 Clinical isolate from the 2011 German sprouts outbreak

3.3. Inoculation and Treatments

The surfaces were inoculated with 100 µL of cocktail (8.5 log CFU/mL) and each coupon was inoculated with 10 µL size droplets with a total of ten droplets. The surfaces were dried in a biosafety cabinet at room temperature (22˚C) for a period of 1.5 h.

Total number of treatments: 11

The inoculated coupons of treatment T_1-T_6,T_{10} and T_{11} were treated by immersing the inoculated coupons in 30 mL of the sanitizer solution for the determined contact times.

Treatment 1 (T_1: Peracetic acid [PAA] contact time 1 min): PAA at a concentration of 500 ppm was prepared by diluting 3 mL of Shield Brite® PAA 15% solution (Pace International, Wapato, WA) in 1 L deionized water. The concentration was monitored using a PAA titration test kit (AquaPhoenix Scientific, Hanover, PA).

Treatment 2 (T_2: Peracetic acid [PAA] contact time 2 min): Treatment application was followed as described in T_1, altering the contact time by 2 min.

Treatment 3 (T_3: Chlorine [Cl] contact time 1 min): Chlorine at a concentration of 500 ppm was prepared by diluting 7.063 mL of Pac-Chlor 12.5% (Pace International, Wapato, WA) and Hypochlorite (Diversey, Inc, Charlotte, NC) in 1 L deionized water. The concentration was tested using a FAS-DPD chlorine/bromine test kit (LaMotte, Chestertown, MD).

Treatment 4 (T_4: Chlorine [Cl]) contact time 2 min): Treatment application was followed as described in T_3, altering the contact time by 2 min.

Treatment 5 (T_5: Silver Dihydrogen Citrate [SDC] contact time 1 min): A ready-to-use SDC solution (PURE Bioscience, EI Cajon, CA) solution at 0.003% of silver ions stabilized with citric acid at 4.846% was used for the treatment.

Treatment 6 (T_6: Silver Dihydrogen Citrate [SDC] contact time 2 min): Treatment application was followed as described in T_5, altering the contact time by 2 min.

Treatment 7 (T_7: Chlorine dioxide [ClO_2] contact time): The generated StayFresh™, mini tub set chlorine dioxide gas (ICA Trinova, Newnan, GA) at a concentration of 70 ppm was monitored using a D-16 PortaSens III gas detector of 0-1000 ppm (GasSensing, Inwood, IA**).** The relative humidity was also assessed using a datalogging traceable™ hygrometer (Fisherbrand™ Excursion-Trac™; Fisher Scientific, Waltham, MA). The treatment was applied by exposing the inoculated surfaces for 24 h in an enclosed area.

Treatment 8 (T_8: Steam contact time 0.5 minutes): Hill injection™ Steam cleaner (dupray, Newark, USA) of 80-90 psi was used and the temperature was evaluated using a Traceable 4-Input Data Logging Thermocouple Probe Thermometer, Type K/J (Cole-Parmer, Vernon Hills, IL) introduced to the food contact surface containing the inoculated bacteria for a contact time of 0.5 minutes enclosed in a chamber.

Treatment 9 (T_9: Steam contact time 1 min): Treatment application was followed as described in T_8, altering the contact time by 1 min.

Treatment 10 (T_{10}: Water): 30 mL of water was added to the inoculated coupons for a contact time of 2 min.

Treatment 11 (T_{11}: Control): For the control treatment, coupons were inoculated with the respective bacteria cocktail only.

3.4. Enumeration of Bacteria

After treatment, 30 mL of Dey/Engley (D/E) neutralizing broth containing 1% tween 80 (NEOGEN, Lansing, MI) was added to inactivate the effect of the sanitizers peracetic acid, chlorine, Silver Dihydrogen Citrate, steam, and Difco™ Neutralizing Buffer (Becton Dickinson Co, Sparks, MD) was used for chlorine dioxide. Coupons and neutralizers were vortexed for 30 s. A 100 µL aliquot was plated on microbiological media using a spiral plater in duplicate. Modified Oxford Medium (MOX; Difco, Becton Dickinson Co, Sparks, MD) was used for *L. monocytogenes,* and the colonies were enumerated based on characteristic esculin hydrolysis having black halo formation. Mac Conkey (MAC; Difco, Becton Dickinson Co, Sparks, MD) was used for STEC, the colonies were pink due to lactose fermentation. Xylose Lysine Deoxycholate (XLD; Difco, Becton Dickinson Co, Sparks, MD) was used for *Salmonella,* the colonies being black centered because of thiosulphate metabolizing to hydrogen sulfite. Plates were incubated at 35-37 ˚C for 24-48 h and the results were recorded as log CFU/surface. In case the values are below the limit of detection bacteria counts are reported as 2.43 log CFU/surface. This was calculated based on the growth of 1 colony-forming unit (CFU) in the lowest dilution on MOX, XLD, and MAC, and 30ml was considered for the dilution factor of the neutralizer (Dey/Engley (D/E) neutralizing broth containing 1% tween 80 for peracetic acid, chlorine, Silver Dihydrogen Citrate, steam, and Neutralizing Buffer for chlorine dioxide).

3.5. Statistical Analysis

A complete randomized design was used with treatments across coupons in each replicate and all the experiments were performed in triplicates. All the data were presented graphically as the mean of triplicate replications, and the standard deviation of the mean was represented by

error bars. Tukey test was conducted for making multiple comparisons of log reduction of bacteria (Log CFU/surface) among treatments by type of surface. JMP® pro 17 was used for the analysis and significance at $p \leq 0.05$.

CHAPTER 4

RESULTS

The initial population of untreated control of *L.monocytogenes, Salmonella,* and STEC were determined to be 7-8 log CFU/coupon for the nylon, plastic, and wood contact surfaces (Fig. 1, 2, 3). The limit of detection was set as 2.43 log CFU/coupon. When referencing log reduction, the reference is the untreated controls. Significant differences in population recovered were observed across chemical or physical sanitizers (SDC, chlorine, PAA, steam, chlorine dioxide), material type (nylon, plastic, wood) and contact time demonstrated a significant difference for *Salmonella* throughout PAA treatments for wood and STEC for chlorine and steam for nylon surface. Fig. 1, 2, and 3 shows where significant differences existed within a microorganism. Treatments not followed by the same letter were found to be significantly different ($p \leq 0.0001$). Control showed limited efficacy and had resulted in values close to no treatment ($p > 0.0001$).

4.1. *L.monocytogenes*

Among all the treatments PAA and SDC had the highest reduction of *Listeria* for all three materials with a nearly 3 log reduction per coupon. Plastic surfaces of *L.monocytogenes* showed a maximum log reduction of 5 log CFU/coupon when treated with SDC, PAA, chlorine, and chlorine dioxide. PAA 1 minute of wood showed variation to nylon and plastic at different contact times equal to SDC, and steam. Overall analysis of treatments provided maximum reduction in nylon of 4.7 log CFU/coupon while wood obtained 3.7 log CFU/coupon.

Chlorine dioxide and PAA application of wood at 2 minutes displayed mean values of 3.6$\pm$1 log CFU/coupon similar to nylon surfaces of SDC and PAA 1 minute of 4$\pm$ 0.3 log CFU/coupon. Steam demonstrated minimal effect of 2 log CFU/coupon with no significant difference ($p>0.05$; Fig. 1) on contact time on all the three materials. Chlorine dioxide at 24 hours with nylon had the least reduction <1 log CFU/coupon which was equivalent to control and no treatments. Chlorine nylon showed a similar effect of inactivation to steam at a lower contact of 0.5 min.

Steam at 0.5 minutes of plastic and Chlorine of nylon at 2 minutes exhibited a limited efficacy of 1.8 log CFU/coupon comparison to steam on wood of 1.3$\pm$ 0.2 log CFU/coupon. Chlorine dioxide at 24 hours and chlorine 2 minutes application caused significant difference across three surfaces of nylon, wood, and plastic ($p\leq0.0001$; Fig. 1). Food contact surface had no statistical difference when treated with PAA at 2 minutes. Contact time showed no remarkable impact on the sanitizer application and materials tested for *L.monocytogenes*.

4.2. *Salmonella*

Among all the sanitizers chlorine dioxide was the most effective against *Salmonella* with approximately 5.5 log CFU/coupon reduction with no significant difference on all food contact surfaces ($p>0.05$; Fig. 2). Similarly, plastic coupons of chlorine, SDC and PAA showed maximum efficacy. Chlorine exhibited least efficiency treatment across all the sanitizers at two contact times with the exception for plastic. Chlorine dioxide of nylon at 24 h exhibited reduction analogous to steam at a less contact of 0.5-1 minute likewise wood of chlorine dioxide at 24 hours displayed inactivation similar to 2 minutes of PAA.

The effect of PAA enhanced on wood and nylon when exposure increased from 1 minute to 2 minutes and showed significant difference ($p \leq 0.0001$; Fig. 2). Steam showed the greater performance of 5 log CFU/coupon reduction equivalent to chlorine dioxide at 24 hours on nylon. SDC, PAA 1 minute, and steam application on wood showed similar outcome and minimal activation of nearly 2 log CFU/coupon inactivation among all sanitizers.

Steam at 0.5 minutes on wood and plastic displayed limited results of 2.7 log CFU/coupon equal to nylon of chlorine 1 minute of 1.9 log CFU/coupon ($p \leq 0.0001$; Fig. 2). Chlorine 2 minutes of nylon material demonstrated least impact analogous to control of plastic and wood materials. Majority of sanitizers were effective on the nylon, wood, and plastic surfaces and showed a mean of 3 log CFU/coupon reduction at both the contact times.

4.3. STEC

Chlorine dioxide at 24 hours was the most effective against STEC with 6 log CFU/coupon reduction on nylon and plastic like SDC 2 minutes of plastic ($p<0.0001$; Fig. 3). Chlorine exhibited efficacy similar to SDC and showed no significant difference towards plastic and wood across contact times of 1 and 2 minutes. PAA of wood and plastic showed higher efficacy of greater than 5 log CFU/coupon similar to plastic surface of chlorine and SDC, and steam of nylon surface all at 1 minute.

PAA 2 minutes of nylon exhibited average reduction similar to that of steam 1 minute treatment of plastic coupons of 3.2 ± 0.1 log CFU/coupon. PAA 1 minute for nylon displayed 1.7 log CFU/coupon inactivation analogous to steam of 1.5 ± 0.4 log CFU/coupon on wood and steam 0.5 minutes on nylon coupons with no significant difference. Steam of plastic coupons,

and wood of chlorine and SDC treatments showed limited outcome equivalent to nylon of PAA, SDC, and chlorine at 2 minutes.

Chlorine at 2 minutes for nylon showed same effect of reduction when treated with steam at lower contact time of 0.5 minutes. SDC was the least effective sanitizer among all the sanitizer applications except on plastic surfaces. Control and no treatment showed similar inactivation on all the three surfaces and showed no significant difference ($p>0.0001$; Fig. 3).

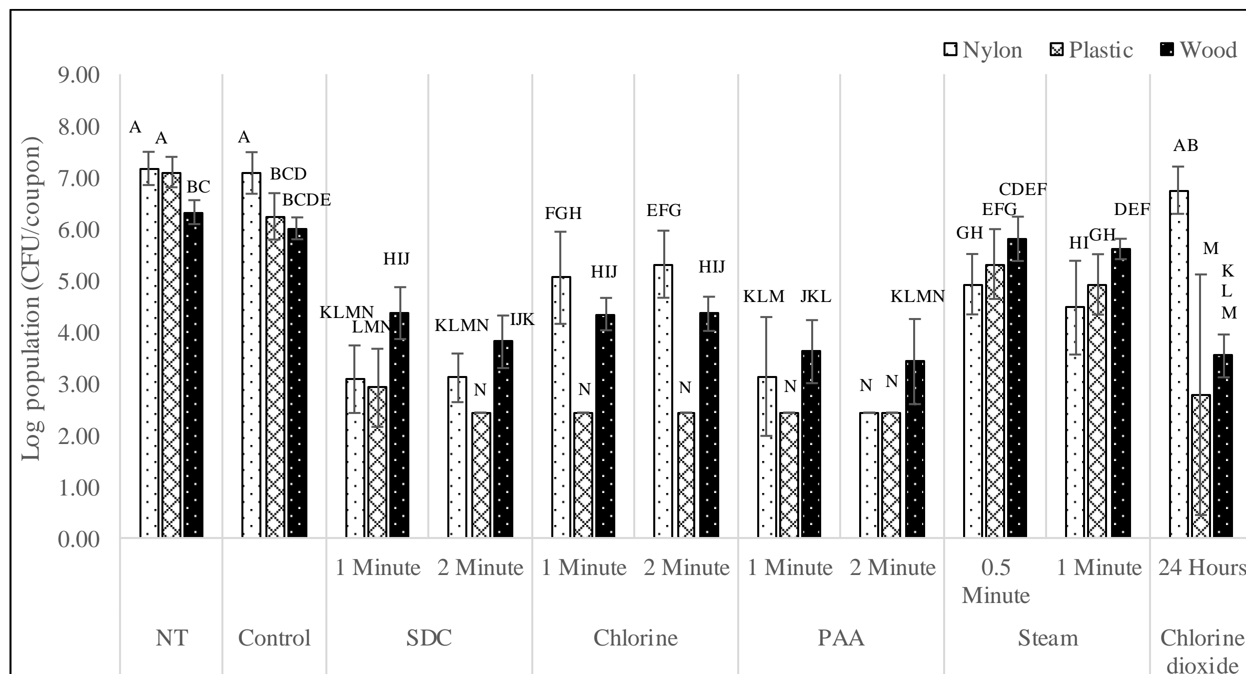

Figure 1. *Listeria monocytogenes* Log population recovered from common food contact surfaces when treated with sanitizers at two different contact times (NT=no treatment control, control=water only, PAA=peracetic acid, SDC=silver dihydrogen citrate). Mean populations recovered with different letters indicate a significant difference $p \leq 0.05$.

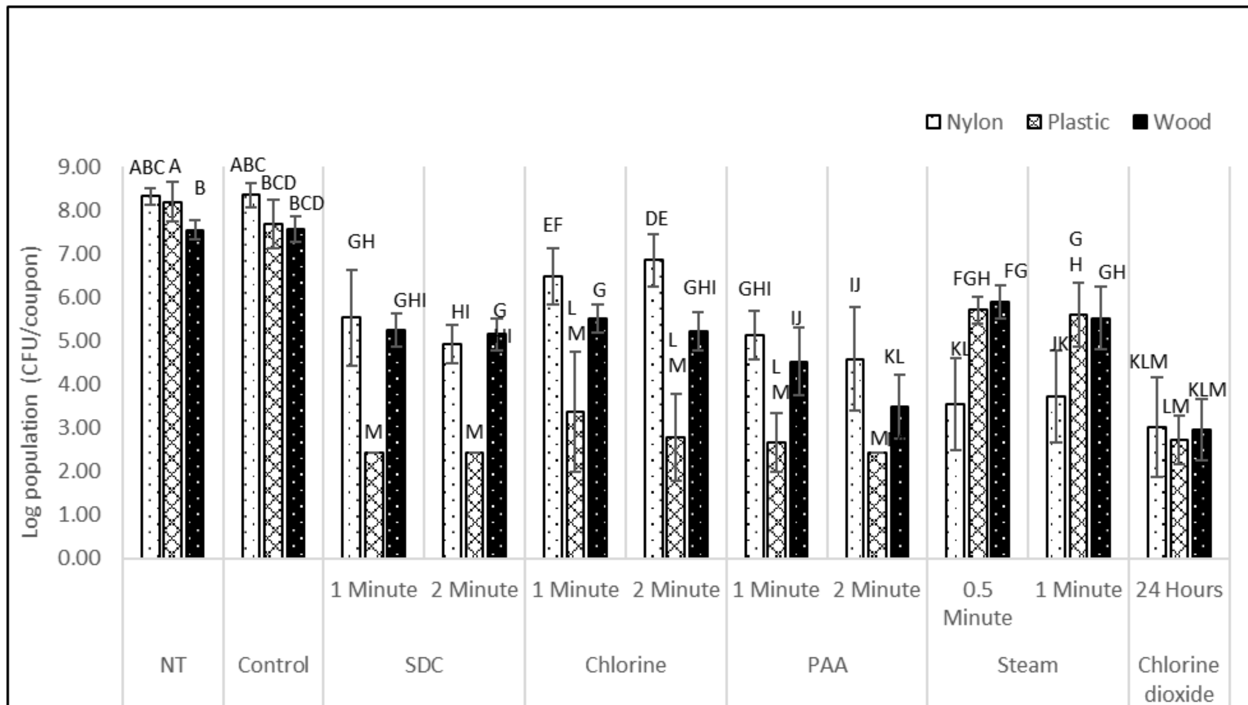

Figure 2. *Salmonella* Log population recovered from common food contact surfaces when treated with sanitizers at two different contact times (NT=no treatment control, control=water only, PAA=peracetic acid, SDC=silver dihydrogen citrate). Mean populations recovered with different letters indicate a significant difference p<u><</u>0.05.

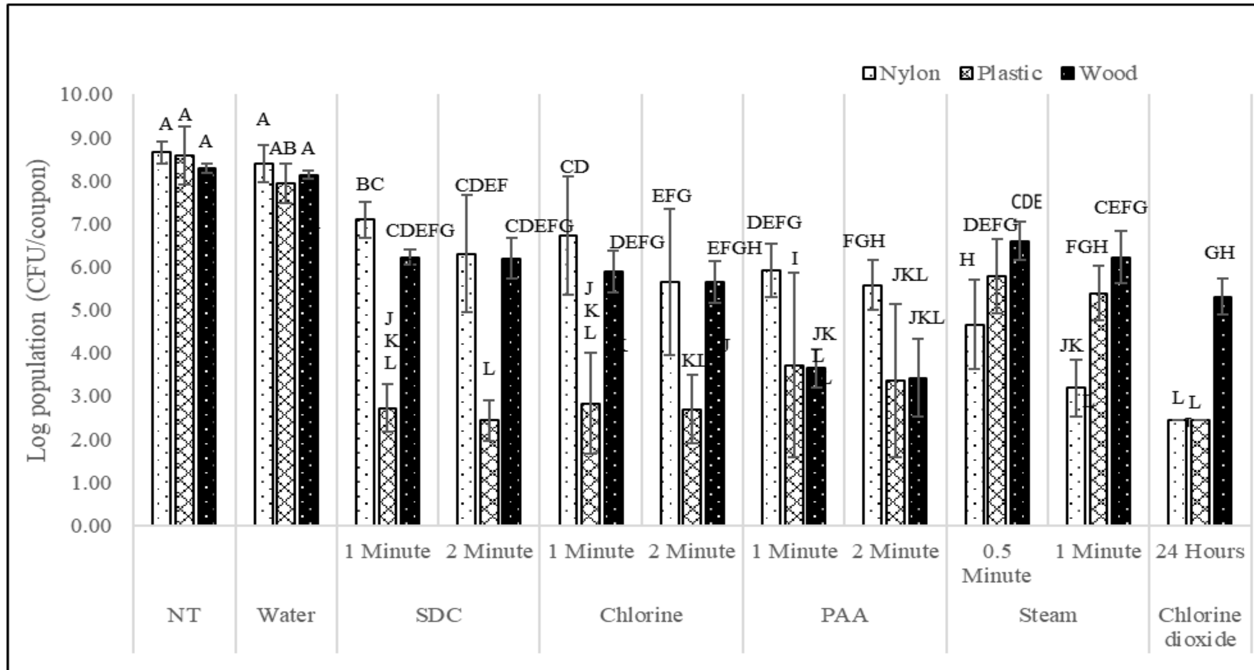

Figure 3. Shiga-toxigenic *Escherichia coli* Log population recovered from common food contact surfaces when treated with sanitizers at two different contact times (NT=no treatment control, control=water only, PAA=peracetic acid, SDC=silver dihydrogen citrate). Mean populations recovered with different letters indicate a significant difference $p \leq 0.05$.

CHAPTER 5

DISCUSSION

5.1. Comparison of efficacy for chemical and physical sanitizers

The concentration of liquid sanitizers PAA, SDC, and chlorine were selected based on the registration of the specific product label with the U.S. Environmental Protection Agency (EPA) under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (40 CFR Parts 150-189) [154].

According to previous study, PAA at 160 ppm and 200ppm was effective in reducing *L. monocytogenes* and *E. coli* O157:H7 to greater than 6.5 log CFU/ml reduction within 5 minutes on common food production facility surfaces (stainless steel, rubber, plastic, and glass) whereas only a 3-log reduction was observed for *Salmonella* [18]. *L. monocytogenes, and* STEC on plastic showed population values below limit of detection thereby had results consistent with the previous study which implied the concentration of PAA can be reduced to obtain similar results. *Salmonella* obtained higher reduction at 500 ppm concentration which showed at increased concentration might be beneficial for further improved efficacy of inactivation on plastic surfaces.

Past study of superheated steam at 150°C had 1.2-5 log inactivation for 5-30s on surfaces (polyvinyl chloride, and stainless steel) against *L. monocytogenes, Salmonella,* and STEC biofilms [23]. *L. monocytogenes, Salmonella, and* STEC steam treatment (80-90psi) at 100°C had similar log reductions at 0.5-1 minute for all the food contact surfaces in this study. Superheated steam had greater efficacy at lower time periods and can be implemented for temperature resistant materials like nylon and wood. Superheated steam had the ability to raise

temperature of surface above saturation temperature at constant pressure which was a limitation to its application on low heat resistant materials plastics like HDPE (<150°C) .

5.2. Surface attributes impact efficacy

L. monocytogenes, Salmonella, and STEC all showed variation in sanitizer application on surfaces evaluated. Hydrophobicity and hydration surfaces sanitizer effect was limited compared to hydrophilic surfaces. In comparison STEC showed maximum difference in sanitizers efficacy based on sanitizers tested. All the three microorganisms showed maximum log reduction on plastic surfaces.

When treated with SDC for 3 min, *L. monocytogenes* exhibited a >5 log CFU/coupon inactivation on stainless steel and cast iron coupon in previous study [128] consistent to plastic coupons of *L. monocytogenes, Salmonella,* and STEC at 1-2 minutes in this study. Microbial populations on plastic were also reduced below the limit of detection for the majority of treatments with the exception of steam treatment. The number cells attached to the surface varies according to the surface property of hydrophobicity or hydrophilicity. Plastic being hydrophilic with negative charged surfaces led to fewer attachment of bacterial cells. Thus, being non-porous provides less protection from sanitizer treatments providing maximum efficacy.

STEC displayed below limit of detection (1 CFU/coupon) on stainless steel, glass, plastic surfaces and 1.7±0 log CFU/coupon reduction on wooden surfaces by aqueous chlorine dioxide treatment at 200 ppm for 15 minutes and drying for 6 h resulted in additional 6.5 log CFU/coupon reduction [24]. The chlorine dioxide (70 ppm) application for 24 hours of STEC exhibited values below limit of detection (1 CFU) on plastic and nylon while showed reduction of approximately 3 log CFU on wooden surfaces. Wood is difficult to sanitize due to its rough

surface, porous nature, and low surface charge. Based on the outcome observed chlorine dioxide treatment can be recommended for decontamination of all the three major foodborne pathogens.

Cells on wooden surface were more resistant compared to other surfaces. They get trapped into the pores, and deep crevices which provides increased surface area and protection and results in large attachment of bacteria thereby limiting sanitizer application. Wood hydrophobicity, condition of surface during operations such as washing and scrubbing lead to cracks and scratches, which affects the bacterial attachment onto the surface. Low pressure might not fully reach the inner porous parts so high pressure (200-1000 psi) must be incorporated; however, this may further entrap bacteria deeper in the material if not inactivated. This emphasizes that food contact surface selection impacts the efficacy of the sanitizers, but even with less than ideal surfaces, microbial reductions can be achieved.

In a previous study, chlorine at 200 ppm at 5 min exposure resulted in 2.7-3.8 log CFU/coupon reduction of *L. monocytogenes* on stainless steel, low-density polyethylene, polyvinyl chloride, polyethylene terephthalate, and rubber surfaces [95] whereas chlorine at 500 ppm at 1-2 min showed similar reduction on wood against *L .monocytogenes, Salmonella, and* STEC. Nylon exhibited the least reduction among all the materials. The weave of nylon protected bacteria identical to the porous hydrophobic wooden surfaces. Repeated use results in abrasion which enhances the ability to entrap bacteria and increases resistance against cleaning and sanitation.

According to the EPA, sanitizers must demonstrate a 5-log reduction for target organisms on non-porous food contact surfaces. However, there are many porous surfaces utilized in the harvesting and packing of produce including nylon, wood, rubber, and foam. There was a

minimum of 1 log CFU/coupon reduction on the majority of treatments evaluated in this study on nylon and wood, and at least one treatment evaluated which resulted in at least a 3-log reduction of the target foodborne pathogen. The concentration of sanitizers in this study were used at maximum allowable limit based upon the product's EPA label, future research regarding decreased concentration for effective treatments should be studied to assess if lower concentrations would provide similar log inactivation. Steam showed limited efficacy against surfaces, thus one subject of research might be testing the efficacy by increasing the pressure of steam and replacement of steam with superheated steam for testing on porous surfaces at different contact times. Pre-rinse or soaking with water might be introduced prior to sanitizing treatment to check for further reduction of the microbial load. Expanding contact times of sanitizers can be evaluated on porous surfaces to verify increase in inactivation at present concentrations when limited efficacy was shown.

5.3. Conclusions

The appropriate sanitizing treatment should be chosen based on the type of material used in harvest and postharvest activities. Outcomes from this study can allow producers to select effective sanitation practices based on the target bacterial foodborne pathogen and surfaces encountered within the operation. Chemical or physical sanitizer treatments were identified which successfully reduced *L. monocytogenes,* STEC, and *Salmonella* beyond that of a no-treatment of water-only control in 1 to 2 min. Time played a limited role in providing significant differences across sanitizers and microorganisms. There was also a noticeable difference in the inactivation between porous and non-porous food contact surfaces. Cleaning surfaces before

sanitizing is also necessary for the efficacy of sanitizer treatment. A future prolonged investigation of combination sanitizer treatment could result in even more microbial reduction.

REFERENCES

1. Center for Disease Control and Prevention (CDC). *Listeria* Outbreak Linked to Enoki Mushrooms January 27, 2023; Available from: https://www.cdc.gov/*Listeria*/outbreaks/enoki-11-22/index.html.
2. Centers for Disease Control and Prevention, Outbreak of *Salmonella* Newport Infections Linked to Onions. October 8, 2020; Available from: https://www.cdc.gov/*Salmonella*/newport-07-20/index.html.
3. FoodSafety.gov 4 Steps to Food Safety. September 1, 2022; Available from: https://www.foodsafety.gov/keep-food-safe/4-steps-to-food-safety.
4. Outbreaks of *Escherichia coli* O157:H7 Infection and Cryptosporidiosis Associated with Drinking Unpasteurized Apple Cider -- Connecticut and New York, October 1996. Morbidity and Mortality Weekly Report
Centers for Disease Control and Prevention,
January 10, 1997.
5. Silver Dihydrogen Citrate - Agricultural Marketing Service. EFSA Journal 2006.
6. U.S. Food and Drug Administration, Outbreak Investigation of *Salmonella* Newport: Red Onions (July 2020). March 13, 2021.
7. Abbott, D.W. and A.B. Boraston, Structural biology of pectin degradation by Enterobacteriaceae. Microbiology and Molecular Biology Reviews, 2008. **72**(2): p. 301-316.
8. Ackers, M.-L., et al., An outbreak of *Escherichia coli* O157: H7 infections associated with leaf lettuce consumption. Journal of infectious diseases, 1998. **177**(6): p. 1588-1593.
9. Addis, M. and D. Sisay, A review on major food borne bacterial illnesses. Journal of Tropical Diseases & Public Health, 2015.
10. Akhtar, S., M.R. Sarker, and A. Hossain, Microbiological food safety: a dilemma of developing societies. Critical reviews in microbiology, 2014. **40**(4): p. 348-359.
11. Alegbeleye, O.O., I. Singleton, and A.S. Sant'Ana, Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. Food microbiology, 2018. **73**: p. 177-208.
12. Álvarez-Ordóñez, A., et al., Survival of acid adapted and non-acid adapted *Salmonella* Typhimurium in pasteurized orange juice and yogurt under different storage temperatures. Food science and technology international, 2013. **19**(5): p. 407-414.
13. Angelo, K., et al., Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. Epidemiology & Infection, 2017. **145**(5): p. 848-856.
14. Annous, B.A., et al., Efficacy of washing with a commercial flatbed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. Journal of Food Protection, 2001. **64**(2): p. 159-163.
15. Aoshi, T., et al., The cellular niche of *Listeria monocytogenes* infection changes rapidly in the spleen. European journal of immunology, 2009. **39**(2): p. 417-425.
16. Aragaw, K., et al., The characterization of *Salmonella* serovars isolated from apparently healthy slaughtered pigs at Addis Ababa abattoir, Ethiopia. Preventive veterinary medicine, 2007. **82**(3-4): p. 252-261.

17. Arnold, N., L. Yang, and R.R. Boyer, How is chlorine dioxide gas used to process foods? 2019.
18. Aryal, M. and P.M. Muriana, Efficacy of commercial sanitizers used in food processing facilities for inactivation of *Listeria monocytogenes*, *E. coli* O157: H7, and *Salmonella* biofilms. Foods, 2019. **8**(12): p. 639.
19. Aureli, P., et al., An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. New England Journal of Medicine, 2000. **342**(17): p. 1236-1241.
20. Babu, D.R., et al., Handling of apples during sorting-grading operation and measuring the mechanical properties firmness after controlled atmosphere storage. Int. J. Mech. Prod. Eng. Res. Dev, 2018. **8**: p. 617-634.
21. Balakrishnan, S., A. Sangeetha, and M. Dhanalakshmi, Prevalence of *Salmonella* in chicken meat and its slaughtering place from local markets in Orathanadu, Thanjavur district, Tamil Nadu. Journal of Entomology and Zoology studies, 2018. **6**(2): p. 2468-2471.
22. Balali, G.I., et al., Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today’s world. International Journal of Microbiology, 2020. **2020**.
23. Ban, G.-H., H. Yoon, and D.-H. Kang, A comparison of saturated steam and superheated steam for inactivation of *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* biofilms on polyvinyl chloride and stainless steel. Food Control, 2014. **40**: p. 344-350.
24. Bang, J., et al., Inactivation of *Escherichia coli* O157: H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. International journal of food microbiology, 2014. **191**: p. 129-134.
25. Barak, J.D. and B.K. Schroeder, Interrelationships of food safety and plant pathology: the life cycle of human pathogens on plants. Annual review of phytopathology, 2012. **50**: p. 241-266.
26. Bedaso, K.K., et al., Study on prevalence and distribution of *Salmonella* isolates from apparently healthy sheep and goats slaughtered at Addis Ababa Abattoir enterprise, Ethiopia. Journal of Veterinary Science and Technology, 2015. **6**(6).
27. Bennett, S., et al., Produce-associated foodborne disease outbreaks, USA, 1998–2013. Epidemiology & Infection, 2018. **146**(11): p. 1397-1406.
28. Berry, E.D., et al., Effect of proximity to a cattle feedlot on *Escherichia coli* O157: H7 contamination of leafy greens and evaluation of the potential for airborne transmission. Applied and Environmental Microbiology, 2015. **81**(3): p. 1101-1110.
29. Betts, R., Microbial update. International Food Hygiene, 2014. **19**(3).
30. Beuchat, L., Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. International journal of food microbiology, 1997. **34**(3): p. 329-333.
31. Beuchat, L.R., *Listeria monocytogenes*: incidence on vegetables. Food control, 1996. **7**(4-5): p. 223-228.

32. Beuchat, L.R., Pathogenic microorganisms associated with fresh produce. Journal of food protection, 1996. **59**(2): p. 204-216.
33. Bhat, S.A., et al., Isolation, molecular detection and antibiogram of *Listeria monocytogenes* from human clinical cases and fish of Kashmir, India. Comparative Clinical Pathology, 2013. **22**: p. 661-665.
34. Bhullar, M., et al., *Escherichia coli* survival on strawberries and unpacked romaine lettuce washed using contaminated water. Foods, 2021. **10**(6): p. 1390.
35. Bottichio, L., et al., Outbreak of *Salmonella* oslo infections linked to persian cucumbers—United States, 2016. Morbidity and Mortality Weekly Report, 2016. **65**(50 & 51): p. 1430-1433.
36. Brandl, M.T., Fitness of human enteric pathogens on plants and implications for food safety. Annu. Rev. Phytopathol., 2006. **44**: p. 367-392.
37. Brouwer, M.C., et al., Community-acquired *Listeria monocytogenes* meningitis in adults. Clinical Infectious Diseases, 2006. **43**(10): p. 1233-1238.
38. Buchholz, A.L., et al., Tracking an *Escherichia coli* O157: H7–contaminated batch of leafy greens through a pilot-scale fresh-cut processing line. Journal of food protection, 2014. **77**(9): p. 1487-1494.
39. Buchholz, U., et al., German outbreak of *Escherichia coli* O104: H4 associated with sprouts. New England Journal of Medicine, 2011. **365**(19): p. 1763-1770.
40. Cabral, J.P., Water microbiology. Bacterial pathogens and water. International journal of environmental research and public health, 2010. **7**(10): p. 3657-3703.
41. Camacho-Gonzalez, A., P.W. Spearman, and B.J. Stoll, Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatric Clinics, 2013. **60**(2): p. 367-389.
42. Carpentier, B. and O. Cerf, Biofilms and their consequences, with particular reference to hygiene in the food industry. Journal of applied bacteriology, 1993. **75**(6): p. 499-511.
43. Carstens, C.K., et al., Control of *Listeria monocytogenes* in caramel apples by use of sticks pretreated with potassium sorbate. Journal of Food Protection, 2018. **81**(12): p. 1921-1928.
44. Carstens, C.K., J.K. Salazar, and C. Darkoh, Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. Frontiers in microbiology, 2019. **10**: p. 2667.
45. Chapin, T.K., et al., Geographical and meteorological factors associated with isolation of *Listeria* species in New York State produce production and natural environments. Journal of Food Protection, 2014. **77**(11): p. 1919-1928.
46. Chen, Y., et al., Core genome multilocus sequence typing for identification of globally distributed clonal groups and differentiation of outbreak strains of *Listeria monocytogenes*. Applied and environmental microbiology, 2016. **82**(20): p. 6258-6272.
47. Chlebicz, A. and K. Śliżewska, Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. International journal of environmental research and public health, 2018. **15**(5): p. 863.
48. Cody, S.H., et al., An outbreak of *Escherichia coli* O157: H7 infection from unpasteurized commercial apple juice. Annals of Internal Medicine, 1999. **130**(3): p. 202-209.

49. Commichaux, S., et al., Assessment of plasmids for relating the 2020 *Salmonella* enterica serovar Newport onion outbreak to farms implicated by the outbreak investigation. BMC genomics, 2023. **24**(1): p. 1-18.
50. Cone, L.A., et al., Multiple cerebral abscesses because of *Listeria monocytogenes*: three case reports and a literature review of supratentorial *Listeria*l brain abscess (es). Surgical neurology, 2003. **59**(4): p. 320-328.
51. Control, C.f.D. and Prevention, Antibiotic resistance threats in the United States, 2019. 2019: US Department of Health and Human Services, Centres for Disease Control and ….
52. Control, C.f.D. and Prevention, Multistate outbreak of listeriosis associated with jensen farms cantaloupe--united states, august-september 2011. MMWR. Morbidity and mortality weekly report, 2011. **60**(39): p. 1357-1358.
53. Control, C.f.D. and Prevention, Multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe (final update). posted October, 2012. **5**: p. 2012.
54. Control, C.f.D., et al., Investigation update: multistate outbreak of human *Salmonella* I 4,[5], 12: i:-infections linked to alfalfa sprouts. 2012.
55. Control, U.C.f.D. and Prevention., Multistate outbreak of *Salmonella* infections linked to imported Maradol papayas (final update). 2017.
56. Cossart, P., J. Pizarro-Cerdá, and M. Lecuit, Invasion of mammalian cells by *Listeria monocytogenes*: functional mimicry to subvert cellular functions. Trends in cell biology, 2003. **13**(1): p. 23-31.
57. Critzer, F.J. and M.P. Doyle, Microbial ecology of foodborne pathogens associated with produce. Current opinion in biotechnology, 2010. **21**(2): p. 125-130.
58. Croxen, M.A., et al., Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical microbiology reviews, 2013. **26**(4): p. 822-880.
59. Crum-Cianflone, N.F., Salmonellosis and the gastrointestinal tract: more than just peanut butter. Current gastroenterology reports, 2008. **10**(4): p. 424-431.
60. Dai, Y., et al., Hygiene status of blueberry harvest containers cleaned and sanitized with various approaches. Food Bioscience, 2023. **52**: p. 102434.
61. Delannoy, S., L. Beutin, and P. Fach, Use of clustered regularly interspaced short palindromic repeat sequence polymorphisms for specific detection of enterohemorrhagic *Escherichia coli* strains of serotypes O26: H11, O45: H2, O103: H2, O111: H8, O121: H19, O145: H28, and O157: H7 by real-time PCR. Journal of clinical microbiology, 2012. **50**(12): p. 4035-4040.
62. DeSalvo, K.B., R. Olson, and K.O. Casavale, Dietary guidelines for Americans. Jama, 2016. **315**(5): p. 457-458.
63. Dhama, K., et al., Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. Pakistan journal of biological sciences: PJBS, 2013. **16**(20): p. 1076-1085.
64. Ding, S. and J. Yang, Germicidal efficacy of sanitizers on food-borne bacteria and effect of sanitizes in CIP and SIP simulation. European Food Research and Technology, 2013. **237**(2): p. 265-274.

65. Doyle, M.P., F. Diez-Gonzalez, and C. Hill, Food microbiology: fundamentals and frontiers. 2020: John Wiley & Sons.
66. Ejo, M., et al., Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. BioMed research international, 2016. **2016**.
67. Erhirhie, E.O., et al., Evaluation of microbial quality of vegetables and fruits in Nigeria: A review. International Journal of Nutrition Sciences, 2020. **5**(3): p. 99-108.
68. Ertaş, N., et al., Hayvansal Gıdalarda *Escherichia coli* O157: H7'nin Önemi. Journal of Faculty of Veterinary Medicine, Erciyes University/Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 2013. **10**(1).
69. Faille, C., et al., Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. Innovative Food Science & Emerging Technologies, 2018. **46**: p. 65-73.
70. Ferdous, M., et al., Is Shiga toxin-negative *Escherichia coli* O157: H7 enteropathogenic or enterohemorrhagic *Escherichia coli*? Comprehensive molecular analysis using whole-genome sequencing. Journal of Clinical Microbiology, 2015. **53**(11): p. 3530-3538.
71. Fierer, J. and D.G. Guiney, Diverse virulence traits underlying different clinical outcomes of *Salmonella* infection. The Journal of clinical investigation, 2001. **107**(7): p. 775-780.
72. Forshell, L.P. and M. Wierup, *Salmonella* contamination: a significant challenge to the global marketing of animal food products. Rev Sci Tech, 2006. **25**(2): p. 541-54.
73. Frank, D.L., Evaluation of fruit bagging as a pest management option for direct pests of apple. Insects, 2018. **9**(4): p. 178.
74. Gagliardi, J., et al., On-farm and postharvest processing sources of bacterial contamination to melon rinds. Journal of food protection, 2003. **66**(1): p. 82-87.
75. Gandhi, M. and M.L. Chikindas, *Listeria*: a foodborne pathogen that knows how to survive. International journal of food microbiology, 2007. **113**(1): p. 1-15.
76. Gaschignard, J., et al., Neonatal bacterial meningitis: 444 cases in 7 years. The Pediatric infectious disease journal, 2011. **30**(3): p. 212-217.
77. Gaul, L.K., et al., Hospital-acquired listeriosis outbreak caused by contaminated diced celery—Texas, 2010. Clinical infectious diseases, 2013. **56**(1): p. 20-26.
78. Gaulin, C., et al., Disinfectants and sanitizers for use on food contact surfaces. National Centre for Environmetal Health Canada. Available online at: http://www. ncceh. ca/sites/default/files/Food_Contact_Surface_Sanitizers_Aug_2011. pdf, 2011.
79. Geurtsen, J., et al., Genomics and pathotypes of the many faces of *Escherichia coli*. FEMS Microbiology Reviews, 2022. **46**(6): p. fuac031.
80. Gombas, D.E., et al., Survey of *Listeria monocytogenes* in ready-to-eat foods. Journal of food protection, 2003. **66**(4): p. 559-569.
81. Gordon, D., B.-A. Carruthers, and S. Theriault, Gaseous decontamination methods in high-containment laboratories. Applied Biosafety, 2012. **17**(1): p. 31-39.
82. Gottlieb, S.L., et al., Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. Clinical infectious diseases, 2006. **42**(1): p. 29-36.
83. Goudar, V. and N. Prasad, A Critical Review on *Listeria monocytogenes*. 2020.

84. Govindarajan, D.K., et al., Adherence patterns of *Escherichia coli* in the intestine and its role in pathogenesis. Medicine in Microecology, 2020. **5**: p. 100025.
85. Grad, Y.H., et al., Genomic epidemiology of the *Escherichia coli* O104: H4 outbreaks in Europe, 2011. Proceedings of the national academy of sciences, 2012. **109**(8): p. 3065-3070.
86. Guentzel, M.N., *Escherichia*, *klebsiella, enterobacter, serratia, citrobacter,* and *proteus*. Medical Microbiology. 4th edition, 1996.
87. Guibourdenche, M., et al., Supplement 2003–2007 (No. 47) to the white-Kauffmann-Le minor scheme. Research in microbiology, 2010. **161**(1): p. 26-29.
88. Guillet, C., et al., Human listeriosis caused by *Listeria* ivanovii. Emerging infectious diseases, 2010. **16**(1): p. 136.
89. Hanning, I.B., J. Nutt, and S.C. Ricke, Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne pathogens and disease, 2009. **6**(6): p. 635-648.
90. Hassan, R., et al., Multistate outbreaks of *Salmonella* infections linked to imported Maradol papayas–United States, December 2016–September 2017. Epidemiology & Infection, 2019. **147**: p. e265.
91. Heredia, N. and S. García, Animals as sources of food-borne pathogens: A review. Animal nutrition, 2018. **4**(3): p. 250-255.
92. Hoffmann, S., M.B. Batz, and J.G. Morris Jr, Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. Journal of food protection, 2012. **75**(7): p. 1292-1302.
93. Hood, S. and E. Zottola, Biofilms in food processing. Food control, 1995. **6**(1): p. 9-18.
94. Hsu, H., et al., Effect of high pressure treatment on the survival of Shiga toxin-producing *Escherichia coli* in strawberry puree. Food microbiology, 2014. **40**: p. 25-30.
95. Hua, Z., et al., Comparative evaluation of different sanitizers against *Listeria monocytogenes* biofilms on major food-contact surfaces. Frontiers in Microbiology, 2019. **10**: p. 2462.
96. Ishii, S. and M.J. Sadowsky, *Escherichia coli* in the environment: implications for water quality and human health. Microbes and environments, 2008. **23**(2): p. 101-108.
97. Jacob, C. and M. Melotto, Human pathogen colonization of lettuce dependent upon plant genotype and defense response activation. Frontiers in Plant Science, 2020. **10**: p. 1769.
98. Jay, M.T., et al., *Escherichia coli* O157: H7 in feral swine near spinach fields and cattle, central California coast. Emerging infectious diseases, 2007. **13**(12): p. 1908.
99. Jeyaletchumi, P., et al., Assessment of *Listeria monocytogenes* in salad vegetables through kitchen simulation study. J. Trop. Agric. Food Sci, 2012. **40**: p. 55-62.
100. John, J., W.-C. Joy, and K. Jovana, Prevalence of *Listeria* spp. in produce handling and processing facilities in the Pacific Northwest. Food microbiology, 2020. **90**: p. 103468.
101. Johnston, L.M., et al., A field study of the microbiological quality of fresh produce. Journal of food protection, 2005. **68**(9): p. 1840-1847.
102. Jones, S.L., et al., Swabbing the surface: critical factors in environmental monitoring and a path towards standardization and improvement. Critical reviews in food science and nutrition, 2020. **60**(2): p. 225-243.

103. Joshi, K., et al., Novel disinfectants for fresh produce. Trends in Food Science & Technology, 2013. **34**(1): p. 54-61.
104. Jung, Y., H. Jang, and K.R. Matthews, Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microbial Biotechnology, 2014. **7**(6): p. 517-527.
105. Karmali, M.A., Emerging public health challenges of Shiga toxin–producing *Escherichia coli* related to changes in the pathogen, the population, and the environment. Clinical Infectious Diseases, 2017. **64**(3): p. 371-376.
106. Karp, D.S., et al., Comanaging fresh produce for nature conservation and food safety. Proceedings of the National Academy of Sciences, 2015. **112**(35): p. 11126-11131.
107. Keller, S.E., Tree fruits and nuts: outbreaks, contamination sources, prevention, and remediation, in The produce contamination problem. 2014, Elsevier. p. 291-312.
108. Kemal, J., et al., Antimicrobial resistance patterns of *Salmonella* in Ethiopia: A review. African Journal of Microbiology Research, 2015. **9**(46): p. 2249-2256.
109. Kenney, S.J., S.L. Burnett, and L.R. Beuchat, Location of *Escherichia coli* O157: H7 on and in apples as affected by bruising, washing, and rubbing. Journal of food protection, 2001. **64**(9): p. 1328-1333.
110. Khoury, N.T., et al. Meningitis with a negative cerebrospinal fluid Gram stain in adults: risk classification for an adverse clinical outcome. in Mayo Clinic Proceedings. 2012. Elsevier.
111. Killinger, K. and A. Adhikari, "Assessment of sanitation techniques for tree fruit storage bins." CPS 2012 RFP Final Project Report, Center for Produce Safety. 2015.
112. Kohli, R., Microbial cleaning for removal of surface contamination, in Developments in Surface Contamination and Cleaning. 2013, Elsevier. p. 139-161.
113. Kumar, C.G. and S.K. Anand, Significance of microbial biofilms in food industry: a review. International journal of food microbiology, 1998. **42**(1-2): p. 9-27.
114. Kuo, J.-T., et al., Development of fluorescence in situ hybridization as a rapid, accurate method for detecting coliforms in water samples. Biosensors, 2020. **11**(1): p. 8.
115. Laughlin, M., et al., Multistate outbreak of *Salmonella* Poona infections associated with imported cucumbers, 2015–2016. Epidemiology & Infection, 2019. **147**: p. e270.
116. Lin, C.-M., et al., Cross-contamination between processing equipment and deli meats by *Listeria monocytogenes*. Journal of food protection, 2006. **69**(1): p. 71-79.
117. Linares-Morales, J.R., et al., Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as a sustainable option. Frontiers in Sustainable Food Systems, 2018. **2**: p. 50.
118. Luna-Guevara, J.J., et al., The role of pathogenic *E. coli* in fresh vegetables: Behavior, contamination factors, and preventive measures. International journal of microbiology, 2019. **2019**.
119. Lyautey, E., et al., Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. Applied and Environmental Microbiology, 2007. **73**(17): p. 5401-5410.
120. Lynch, M.F., R.V. Tauxe, and C.W. Hedberg, The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology & Infection, 2009. **137**(3): p. 307-315.

121. Macarisin, D., et al., Survival of outbreak, food, and environmental strains of *Listeria monocytogenes* on whole apples as affected by cultivar and wax coating. Scientific Reports, 2019. **9**(1): p. 12170.
122. Machado-Moreira, B., et al., Microbial contamination of fresh produce: what, where, and how? Comprehensive Reviews in Food Science and Food Safety, 2019. **18**(6): p. 1727-1750.
123. Mahon, B.E., et al., An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seeds. Journal of Infectious Diseases, 1997. **175**(4): p. 876-882.
124. Makinya, K.J., et al., The importance of store hygiene for reducing post-harvest losses in smallholder farmers' stores: Evidence from a maize-based farming system in Kenya. Journal of Stored Products Research, 2021. **90**: p. 101757.
125. Manuel, C., M. Moore, and L.A. Jaykus, Efficacy of a disinfectant containing silver dihydrogen citrate against GI. 6 and GII. 4 human norovirus. Journal of applied microbiology, 2017. **122**(1): p. 78-86.
126. Manville, E., et al., Evaluation of *Listeria monocytogenes* biofilms attachment and formation on different surfaces using a CDC biofilm reactor. International Journal of Food Microbiology, 2023: p. 110251.
127. Marus, J.R., Notes from the field: outbreak of listeriosis likely associated with prepackaged caramel apples—United States, 2017. MMWR. Morbidity and mortality weekly report, 2019. **68**.
128. Masuku, S., et al., Lethality of moist heat and silver dihydrogen citrate sanitizer combinations on *Listeria* spp. adhered to components of a deli meat slicer. Food control, 2014. **44**: p. 227-232.
129. Mazaheri, T., et al., *Listeria monocytogenes* biofilms in the food industry: is the current hygiene program sufficient to combat the persistence of the pathogen? Microorganisms, 2021. **9**(1): p. 181.
130. McCollum, J.T., et al., Multistate outbreak of listeriosis associated with cantaloupe. New England Journal of Medicine, 2013. **369**(10): p. 944-953.
131. McCormic, Z., et al., Bi-national outbreak of *Salmonella* Newport infections linked to onions: the United States experience. Epidemiology & Infection, 2022. **150**: p. e199.
132. McDaniel, C. and R. Jadeja, A review of fresh produce outbreaks, current interventions, food safety concerns and potential benefits of novel antimicrobial sodium acid sulfate. MOJ Food Processing & Technology, 2019. **7**(3): p. 59-67.
133. Michino, H., et al., Massive outbreak of *Escherichia coli* O157: H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. American journal of epidemiology, 1999. **150**(8): p. 787-796.
134. Mohle-Boetani, J., et al., *Salmonella* infections associated with mung bean sprouts: epidemiological and environmental investigations. Epidemiology & Infection, 2009. **137**(3): p. 357-366.
135. Musa, J.A., et al., Prevalence and antibiotic sensitivity pattern of *Salmonella* isolates from milk products and water reservoirs in maiduguri, north-eastern Nigeria. IOSR Journal of Agriculture and Veterinary Science, 2017. **10**(2): p. 87-92.

136. Nsubuga, J., et al., Multistate outbreak investigation of *Salmonella* infections linked to Kratom: a focus on traceback, laboratory, and regulatory activities. Journal of Food Protection, 2022. **85**(5): p. 747-754.
137. O'Ryan, M., et al., Vaccines for viral and bacterial pathogens causing acute gastroenteritis: Part II: Vaccines for Shigella, *Salmonella*, enterotoxigenic *E. coli* (ETEC) enterohemorragic *E. coli* (EHEC) and Campylobacter jejuni. Human vaccines & immunotherapeutics, 2015. **11**(3): p. 601-619.
138. Okike, I.O., R.F. Lamont, and P.T. Heath, Do we really need to worry about *Listeria* in newborn infants? The Pediatric infectious disease journal, 2013. **32**(4): p. 405-406.
139. Orsi, R.H. and M. Wiedmann, Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. Applied microbiology and biotechnology, 2016. **100**: p. 5273-5287.
140. Patange, A., Atmospheric cold plasma interactions with microbiological risks in fresh food processing. 2019.
141. Paulus, W., Oxidizing Agents, in Microbicides for the Protection of Materials: A Handbook. 1993, Springer. p. 430-443.
142. Pérez-Trallero, E., et al., Two outbreaks of *Listeria monocytogenes* infection, Northern Spain. Emerging infectious diseases, 2014. **20**(12): p. 2155.
143. Perulli, G.D., et al., Treated wastewater as irrigation source: A microbiological and chemical evaluation in apple and nectarine trees. Agricultural Water Management, 2021. **244**: p. 106403.
144. Pires, S.M., et al., Global and regional source attribution of Shiga toxin-producing *Escherichia coli* infections using analysis of outbreak surveillance data. Epidemiology & Infection, 2019. **147**.
145. Proctor, M.E., et al., Use of pulsed-field gel electrophoresis to link sporadic cases of invasive listeriosis with recalled chocolate milk. Applied and Environmental Microbiology, 1995. **61**(8): p. 3177-3179.
146. Rai, P.K. and B. Tripathi, Microbial contamination in vegetables due to irrigation with partially treated municipal wastewater in a tropical city. International Journal of Environmental Health Research, 2007. **17**(5): p. 389-395.
147. Ramaswamy, V., et al., *Listeria*-review of epidemiology and pathogenesis. Journal of Microbiology Immunology and Infection, 2007. **40**(1): p. 4.
148. Rørvik, L.M., *Listeria monocytogenes* in the smoked salmon industry. International journal of food microbiology, 2000. **62**(3): p. 183-190.
149. Salazar, J.K., et al., Roles of a novel Crp/Fnr family transcription factor Lmo0753 in soil survival, biofilm production and surface attachment to fresh produce of *Listeria monocytogenes*. PLoS One, 2013. **8**(9): p. e75736.
150. Sanchez, S., et al., Animal sources of salmonellosis in humans. Journal of the American Veterinary Medical Association, 2002. **221**(4): p. 492-497.
151. Scallan, E., et al., Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis, 2011. **17**(1): p. 7-15.
152. Schwensohn, C., et al., A Multiple-Serotype Outbreak of *Salmonella* Infections Linked to Kratom, United States, 2017–2018. Foodborne Pathogens and Disease, 2022. **19**(9): p. 648-653.

153. Self, J.L., et al., Multistate outbreak of listeriosis associated with packaged leafy green salads, United States and Canada, 2015–2016. Emerging infectious diseases, 2019. **25**(8): p. 1461.
154. Service, U.A.a.P.H.I. and U.S.D.o. Agriculture. EPA Regulation of Disinfectants Available from: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/NVAP-Reference-Guide/Cleaning-and-Disinfection/Regulation-of-Disinfectants#:~:text=Chemical%20disinfectants%20in%20the%20United,CFR%20Parts%20150%2D189).
155. Shaffer, J. and G. Miller, FDA form 483 inspectional observations. Baltimore: Food and Drug Administration, September, 2001. **7**.
156. Sivapalasingam, S., et al., Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of food protection, 2004. **67**(10): p. 2342-2353.
157. Solomon, E.B., S. Yaron, and K.R. Matthews, Transmission of *Escherichia coli* O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Applied and environmental microbiology, 2002. **68**(1): p. 397-400.
158. Sperber, W.H., Introduction to the microbiological spoilage of foods and beverages. Compendium of the microbiological spoilage of foods and beverages, 2009: p. 1-40.
159. Stea, E.C., et al., Comparison of the prevalences and diversities of *Listeria* species and *Listeria monocytogenes* in an urban and a rural agricultural watershed. Applied and Environmental Microbiology, 2015. **81**(11): p. 3812-3822.
160. Stoica, C., et al., Sensitivity of Pathogenic Bacteria Strains to Treated Mine Water. International Journal of Environmental Research and Public Health, 2022. **19**(23): p. 15535.
161. Streif, J., et al. Pre-and post-harvest management of fruit quality, ripening and senescence. in VI International Postharvest Symposium 877. 2009.
162. Taddese, D., et al., Antibiograms and risk factors of *Salmonella* isolates from laying hens and eggs in Jimma Town, South Western Ethiopia. BMC Research Notes, 2019. **12**: p. 1-7.
163. Tarr, P.I., C.A. Gordon, and W.L. Chandler, Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. The lancet, 2005. **365**(9464): p. 1073-1086.
164. Tegegne, F., Epidemiology of *Salmonella* and its serotypes in human, food animals, foods of animal origin, animal feed and environment. J Food Nutr Health, 2019. **2**(1): p. 7-14.
165. Thakur, K., D. Singh, and S. Kaushal, Food threats in organic produce through biological contaminants.
166. Tham, W., et al., Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. International journal of food microbiology, 2000. **62**(3): p. 173-175.
167. Todd, E. and S. Notermans, Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. Food control, 2011. **22**(9): p. 1484-1490.
168. Uçar, G., N. Yörük, and A. Güner, *Escherichia coli* infections. Turkiye Klinikleri Journals Food Hygiene Technology, 2015. **1**(3): p. 22-29.

169. Vázquez-Boland, J.A., et al., *Listeria* pathogenesis and molecular virulence determinants. Clinical microbiology reviews, 2001. **14**(3): p. 584-640.
170. Venable, M.E. and M.R. Podbielski, Impact of substrate material on algal biofilm biomass growth. Environmental Science and Pollution Research, 2019. **26**: p. 7256-7262.
171. Vigneault, C., J. Thompson, and S. Wu, Designing container for handling fresh horticultural produce. Postharvest technologies for horticultural crops, 2009. **2**(2): p. 25-47.
172. Waltenburg, M.A., et al., Two multistate outbreaks of a reoccurring Shiga toxin-producing *Escherichia coli* strain associated with romaine lettuce: USA, 2018–2019. Epidemiology & Infection, 2022. **150**: p. e16.
173. Walters, D., Fortress plant: how to survive when everything wants to eat you. 2017: Oxford University Press.
174. Ward, S., W. Bedale, and K.A. Glass, *Listeria monocytogenes* Outbreaks Related to Commercially Produced Caramel Apples: Developments in Sanitation, Product Formulation, and Packaging: A Review. Journal of Food Protection, 2022. **85**(9): p. 1287-1299.
175. Williamson, K., Evaluation of naturally occurring and inoculated microorganisms on stone fruit carriers. 2018, California State University, Fresno.
176. Wing, E.J. and S.H. Gregory, *Listeria monocytogenes*: clinical and experimental update. The Journal of infectious diseases, 2002. **185**(Supplement_1): p. S18-S24.
177. Wright, K.M., et al., Differences in internalization and growth of E scherichia coli O157: H7 within the apoplast of edible plants, spinach and lettuce, compared with the model species N icotiana benthamiana. Microbial biotechnology, 2017. **10**(3): p. 555-569.
178. Yang, S.-C., et al., Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. Archives of microbiology, 2017. **199**: p. 811-825.
179. Zhu, Q., R. Gooneratne, and M.A. Hussain, *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. Foods, 2017. **6**(3): p. 21.

APPENDIX

Table 2: Report of Tukey HSD test of *L.monocytogenes* treated with sanitizers at different contact times.

Level		Least Sq Mean
NT,Nylon	A	7.1702172
NT,Plastic	A	7.1001666
Water,Nylon	A	7.0824475
ClO2,Nylon	A B	6.7478718
NT,Wood	B C	6.3221097
Water,Plastic	B C D	6.2410481
Water,Wood	B C D E	6.0083809
Steam 30 s,Wood	C D E F	5.8047331
Steam 1 min,Wood	D E F G	5.6081596
Steam 30 s,Plastic	E F G	5.3155186
Cl 2 min,Nylon	E F G	5.3120547
Cl 1 min,Nylon	F G H	5.0486060
Steam 30 s,Nylon	G H	4.9242083
Steam 1 min,Plastic	G H	4.9197978
Steam 1 min,Nylon	H I	4.4690299
SDC 1 min,Wood	H I J	4.3637589
Cl 2 min,Wood	H I J	4.3495253
Cl 1 min,Wood	H I J	4.3445435
SDC 2 min,Wood	I J K	3.8069904
PAA 1 min,Wood	J K L	3.6160746
ClO2,Wood	K L M	3.5331405
PAA 2 min,Wood	K L M	3.4228719
PAA 1 min,Nylon	K L M N	3.1344463
SDC 2 min,Nylon	K L M N	3.1075902
SDC 1 min,Nylon	K L M N	3.0811501
SDC 1 min,Plastic	L M N	2.9134354
ClO2,Plastic	M N	2.7797671
PAA 2 min,Nylon	N	2.4300000
PAA 1 min,Plastic	N	2.4300000
Cl 1 min,Plastic	N	2.4300000
SDC 2 min,Plastic	N	2.4300000
Cl 2 min,Plastic	N	2.4300000
PAA 2 min,Plastic	N	2.4300000

Levels not connected by same letter are significantly different.

Table 3: Report of Tukey HSD test of *Salmonella* treated with sanitizers at different contact times.

Level														Least Sq Mean
NT,Plastic	A													8.5919689
Water,Nylon	A	B	C											8.3585987
NT,Nylon	A		C											8.2939601
NT,Wood		B												7.7156464
Water,Plastic		B	C	D										7.6941831
Water,Wood		B		D										7.5769223
Cl 2 min,Nylon				D	E									6.8537925
Cl 1 min,Nylon					E	F								6.4867296
Steam 30 s,Wood						F	G							5.9091873
Steam 30 s,Plastic						F	G	H						5.7147931
Steam 1 min,Plastic							G	H						5.5975368
SDC 1 min,Nylon							G	H						5.5341472
Cl 1 min,Wood							G	H						5.5246214
Steam 1 min,Wood							G	H						5.5216780
SDC 1 min,Wood							G	H	I					5.2556413
Cl 2 min,Wood							G	H	I					5.2146550
SDC 2 min,Wood							G	H	I					5.1559951
PAA 1 min,Nylon							G	H	I					5.1385770
SDC 2 min,Nylon								H	I					4.9285427
PAA 2 min,Nylon									I	J				4.5838257
PAA 1 min,Wood									I	J				4.5226233
Steam 1 min,Nylon										J	K			3.7282527
Steam 30 s,Nylon											K	L		3.5405376
PAA 2 min,Wood											K	L		3.4772444
Cl 1 min,Plastic											K	L		3.3709960
ClO2,Nylon											K	L	M	3.0191003
ClO2,Wood											K	L	M	2.9525885
Cl 2 min,Plastic												L	M	2.7739808
ClO2,Plastic												L	M	2.7259212
PAA 1 min,Plastic												L	M	2.6648616
PAA 2 min,Plastic													M	2.4300000
SDC 1 min,Plastic													M	2.4300000
SDC 2 min,Plastic													M	2.4300000

Levels not connected by same letter are significantly different.

Table 4: Report of Tukey HSD test of *Escherichia coli* treated with sanitizers at different contact times.

Level													Least Sq Mean
NT,Nylon	A												8.6498761
NT,Plastic	A												8.5719948
Water,Nylon	A												8.3822440
NT,Wood	A												8.2817863
Water,Wood	A												8.1276035
Water,Plastic	A	B											7.9309967
SDC 1 min,Nylon		B	C										7.0898363
Cl 1 min,Nylon			C	D									6.7287470
Steam 30 s,Wood			C	D	E								6.5940049
SDC 2 min,Nylon			C	D	E	F							6.3006509
Steam 1 min,Wood			C	D	E	F	G						6.2179614
SDC 1 min,Wood			C	D	E	F	G						6.2141096
SDC 2 min,Wood			C	D	E	F	G						6.1954428
PAA 1 min,Nylon				D	E	F	G						5.9123345
Cl 1 min,Wood				D	E	F	G						5.8805653
Steam 30 s,Plastic				D	E	F	G						5.7691255
Cl 2 min,Wood					E	F	G	H					5.6539406
Cl 2 min,Nylon					E	F	G	H					5.6462430
PAA 2 min,Nylon						F	G	H					5.5768588
Steam 1 min,Plastic						F	G	H					5.3799446
ClO2,Wood							G	H					5.3025228
Steam 30 s,Nylon								H	I				4.6612533
PAA 1 min,Plastic									I	J			3.7133658
PAA 1 min,Wood										J	K		3.6398847
PAA 2 min,Wood										J	K	L	3.4195025
PAA 2 min,Plastic										J	K	L	3.3505342
Steam 1 min,Nylon										J	K	L	3.1817382
Cl 1 min,Plastic										J	K	L	2.8312749
SDC 1 min,Plastic										J	K	L	2.7182640
Cl 2 min,Plastic											K	L	2.6942265
SDC 2 min,Plastic												L	2.4313638
ClO2,Plastic												L	2.4313638
ClO2,Nylon												L	2.4313638

Levels not connected by same letter are significantly different.