# Exploring Innovative Food Safety Practices for Harvest and Postharvest Handling of Fresh Produce

by

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Postharvest, Harvest, Nano-Textured Coating, *Salmonella enterica*, *E. coli* O157:H7, Living Lettuce, Blueberry, Food Safety



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## Abstract

Fresh produce has been associated with foodborne outbreaks and recalls due to pathogens such as *E. coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes*. To minimize cross-contamination and reduce microbial loads in the final product, it is crucial to improve food safety practices during the harvest and postharvest stages. This project evaluates two innovative approaches to enhance microbial safety in fresh produce handling: the use of nano-textured coatings on harvesting equipment and the application of sanitizers during postharvest washing. The first approach investigates the effectiveness of hydrophobic and superhydrophobic coatings in reducing the transfer of *E. coli* O157:H7 during a simulated blueberry harvest. The second practice focuses on living lettuce, a popular product known for its intact roots and extended shelf life, by assessing the effectiveness of root washing with sanitizers to control *Salmonella enterica*. Together, these studies highlight practical interventions that can significantly enhance produce safety, offering valuable guidance for local growers to reduce contamination risks throughout the supply chain.



**Keywords:** Postharvest, Harvest, Nano-Textured Coating, *Salmonella enterica*, *E. coli* O157:H7, Living Lettuce, Blueberry, Food Safety.

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(g) and without (h) media plugs; Hue angle (°) values of living lettuce leaves with (i) and without (j) media plugs. Data represents means and error bars represent standard errors. Lowercase letters indicate no significant differences ($p > 0.05$) among treatments within the same time point. Uppercase letters indicate no significant differences ($p > 0.05$) within each treatment across different time points, based on Tukey's test. Abbreviations: c*: chroma (saturation); H2O2: hydrogen peroxide; PAA: peroxyacetic acid; ..... 115

List of Abbreviation

| | |
|---|---|
| a* | Redness/greenness values |
| ANOVA | Analysis of variance |
| ATCC | American Type Culture Collection |
| b* | Yellowness/blueness values |
| BI | Brightness index |
| BPW | Buffered Peptone Water |
| c* | Saturation values |
| CA | Contact angle |
| CDC | Centers for Disease Control and Prevention |
| CEA | Controlled Environment Agriculture |
| CFU | Colonies-forming units |
| ΔE | Color change |
| DNA | Deoxyribonucleic acid |
| EHEC | Enterohemorrhagic *E. coli* |
| FAO | Food and Agriculture Organization of the United Nations |
| FDA | Food and Drug Administration |
| GAPs | Good Agricultural Practice |
| GLM | Generalized Linear Model |

| | |
|---|---|
| GRAS | Generally Recognized As Safe |
| $H_2O_2$ | Hydrogen peroxide |
| HDPE | High-Density Polyethylene |
| L* | Lightness values |
| LOD | Limit of detection |
| NFT | Nutrient Film Technology |
| NPs | Nanoparticles |
| NR | No-rinse |
| PAA | Peroxyacetic acid |
| PAL | Phenylalanine ammonia-lyase |
| PDA | Potato Dextrose Agar |
| PBS | Phosphate Buffered Saline |
| TSB | Tryptic Soy Broth |
| TSBRN | Tryptic Soy Broth with Nalidixic and Rifampicin resistance |
| TSI | Triple Iron Sugar |
| US | United States of America |
| USDA | United States Department of Agriculture |
| USDA-NASS | USDA National Agriculture Statistics Services |
| UV | Ultraviolet |

| | |
|---|---|
| WHO | World Health Organization |
| WI | Whiteness index |
| XLD | Xylose Lysine Decarboxylase |

# Chapter 1

## 1.0 Literature review

### *1.1 Food Safety*

As globalization expands, food safety issues have become a significant challenge for the food industry. Food safety programs can be implemented for the preparation, handling, and storage of foods to prevent foodborne diseases/illnesses, including many routes that have to be followed to prevent possible health hazards (Awuchi, 2023). The main cause of food contamination is due to improper food safety programs during production and handling (WHO, 2016). Additionally, a food safety incident not only impacts public health but also can be harmful to a business's reputation, causing economic losses and more (Murray et al., 2017).

Food safety issues in the United States (US) have a significant economic impact, costing an estimated $75 billion annually in healthcare expenses, lost productivity, and premature deaths (Hoffmann et al., 2024) Food loss and waste happens at every stage across the food supply chain. The percentages of food loss in production, postharvest and consumption stages are 24, 24 and 35 per cent, respectively (Chauhan et al., 2021) Microbial spoilage accounts for 25% of postharvest food loss globally (Bassey et al., 2021)

A significant portion of these food losses in fresh produce can be traced back to contamination risks at the farm level. Several factors at the farm level can compromise the safety of fresh produce, including manure, water, and soil (Black et al., 2021). Fields that contain animal activities are at a higher risk of contamination with enteric pathogens.

Additionally, the microbial safety of fresh produce is influenced by the quality of water used for irrigation (Olaimat & Holley, 2012) as fruit and vegetable production typically occurs in exposed environments making the crops highly susceptible to contamination from various sources (Balali et al., 2020).

To minimize the risk of contamination, growers must adhere to stringent food safety practices during the production, harvest, and postharvest stages, which are essential for safeguarding public health and maintaining the integrity of the food supply chain (Murray et al., 2017). By implementing these safety protocols, growers not only protect consumers but also build and sustain trust in their products. Furthermore, compliance with regulatory standards ensures long-term business viability while strengthening the overall food system (Chen et al., 2023).

### *1.2 Fruit and Vegetable Production*

Fruits and vegetables are widely recognized as healthy options in the human diet, and their consumption is strongly recommended by organizations such as the World Health Organization (WHO) (WHO, 2019). In 2022, the US produced a total of 42.92 billion pounds of fresh vegetables and 19.11 billion pounds of fresh fruit (Ribera & Young, 2024). According to the US Department of Agriculture (USDA) summary report for 2024, the top five vegetables produced were tomatoes, sweet corn, onions, carrots, and watermelons. The report also indicates a general decline in production for most leafy greens compared to 2023, with significant decreases noted for spinach and romaine lettuce (USDA, 2025). Recently, climate change has been affecting agricultural activities, especially the consistent supply of horticultural crops. In this context, there is an urgent need for sustainable technologies, such

as vertical farming, hydroponics and aquaponics, to efficiently utilize terrestrial resources, particularly in urban areas (Carrasco et al., 2024). Thus, it is imperative to develop and implement sustainable technologies that optimize terrestrial water resources, especially in urban settings.

Small fruits, including strawberries, blueberries, and raspberries, are recognized as excellent sources of essential nutrients and bioactive compounds. They are associated with various biological benefits, such as antioxidant, anti-inflammatory, anti-cancer, and antiproliferative properties (Neri-Numa et al., 2018). In Alabama, the acreage dedicated to berry production increased by 30% from 2017 to 2022. By 2022, berry sales reached $12.39 million, showing significant growth compared to 2017 (Sawadgo, 2024). Among the varieties of blueberries, both rabbiteye (*Vaccinium virgatum*) and southern highbush (*Vaccinium corymbosum* hybrids) are commonly cultivated in the state due to their adaptability to Alabama's warm climate and soil conditions (Coneva & Singh, 2025; Hollis, 2024)

Leafy greens, such as lettuce and arugula, provide several advantages over other types of produce due to their shorter growth cycles. Lettuce production in the United States is primarily concentrated in California and Arizona. However, since 2022, southeastern states like Florida have increased their lettuce production, reaching a total of 800,000 pounds by 2023 (USDA, 2023). In Alabama, the main challenge for leafy greens production is the summer heat. As a solution, controlled environment agriculture (CEA) has emerged, allowing for year-round lettuce cultivation with an average crop cycle of 30 days from transplant to harvest (Pickens et al., 2022).

CEA production involves the production of agricultural commodities under protected environments by optimizing climate and inputs, such as, water, nutrients, labor and energy (Baghalian et al., 2023). CEA systems often use a broad range of agricultural production methods, such as, hydroponics, aquaponics, vertical farming and aeroponics (Cowan et al., 2022). One notable product of these systems is living lettuce, which is typically grown hydroponically and sold with its roots still attached, allowing extended freshness and shelf life (Waitt et al., 2014). Vertical farming is a significant advancement in agricultural technology, yielding higher production per square meter. It optimizes space by stacking layers of crops and utilizes a controlled environment and soil-less farming methods (Carrasco et al., 2024).

### *1.3 Foodborne outbreaks on fresh produce*

Since the 1980s, fresh produce consumption has significantly increased, constituting an important portion of a healthy human diet (Melo & Quintas, 2023). As a result of globalization and the emergence of new pathogens, the number of foodborne outbreaks associated with contaminated produce has increased rapidly. An outbreak of foodborne disease is defined as the occurrence of two or more cases of a similar illness (except for *Clostridium*, which requires only one case) resulting from the ingestion of contaminated food (Yan et al., 2023). Fresh produce accounts for nearly 46% of foodborne illnesses in the US. In the past few years, the US. experienced two large multistate outbreaks of *Escherichia coli* O157:H7 associated with the consumption of romaine lettuce in 2018, three outbreaks in 2019, and six outbreaks in 2020 (Bottichio et al., 2020). In 2024, outbreaks of foodborne illnesses were attributed to *Salmonella*, *E. coli*, and *Listeria monocytogenes* (FDA, 2025).

The most common ready-to-eat foods related to foodborne illness outbreaks in the last years are leafy greens, sprouts, tomatoes, and cantaloupes. Foodborne outbreaks associated with fresh produce in the last five years are summarized in Table 1.1.

**Table** 1.1**.** Foodborne outbreaks linked to fresh produce from 2019 to 2024 within the US.

| Year | Pathogen | Agriculture Commodity | Number of Cases | Reference |
|---|---|---|---|---|
| 2019 | *Salmonella* Carrau | Pre-cut melon | 137 | (CDC, 2019a) |
| 2019 | *Salmonella* Uganda | Papaya | 81 | (CDC, 2019b) |
| 2019 | *Cyclospora cayetanensis* | Fresh basil | 241 | (CDC, 2019c) |
| 2019 | *E. coli* O157:H7 | Romaine lettuce | 167 | (CDC, 2020) |
| 2020 | *E. coli* O157:H7 | Leafy greens | 40 | (CDC, 2024e) |
| 2021 | *E. coli* O157:H7 | Baby spinach | 15 | (FAOSTAT, 2021) |
| 2022 | *Salmonella* Typhimurium | Alfalfa sprouts | 63 | (CDC, 2023a) |

| | | | | |
|---|---|---|---|---|
| 2023 | *Listeria monocytogenes* | Leafy greens | 19 | (CDC, 2023b) |
| 2024 | *Salmonella* Typhimurium | Fresh Basil | 36 | (CDC, 2024d) |
| 2024 | *Salmonella* Africana and *Salmonella* Braenderup | Cucumber | 449 (38 new Africana, 215 Braenderup) | (CDC, 2024e) |

Particularly, *Listeria monocytogenes* is responsible for approximately 1,600 illnesses annually in the US, of which 1,400 result in hospitalizations and 250 lead to fatalities (Angelo et al., 2017). Although outbreaks of *L. monocytogenes* are less frequent compared to those caused by *Salmonella* and *E. coli*, they still have a significant economic impact (Angelo et al., 2017).

### *1.4 Salmonella enterica*

*Salmonella enterica* is a member of the *Enterobacteriaceae,* belonging to the genus *Salmonella,* comprised of six subspecies: *Salmonella enterica* subsp. *enterica* (I), *salamae* (II), *arizonae* (III), *diarizona* (IIIb), *houtenae* (IV), and *indica* (VI) (References?). Among these, *Salmonella enterica* subsp. *enterica* is particularly significant for food safety and public health, as it is subdivided into over 1,500 serovars based on surface antigens (Micallef, 2023). These species can be divided into typhoidal (*Salmonella enterica* serovar Typhi) and

non-typhoidal (*Salmonella enterica* serovar Typhimurium) types, which differ in regard to disease manifestation and host tropism (Wang et al., 2023). Typhoidal *Salmonella* is limited to humans and causes typhoid fever. Although its incidence has decreased globally, it remains a significant issue in parts of Asia, Africa, and Oceania. In contrast, non-typhoidal *Salmonella* causes gastroenteritis in multiple hosts and poses serious food safety risks due to its persistence in food production areas (Als et al., 2018; Koutsoumanis et al., 2024; Micallef, 2023).

In recent years, *Salmonella* has been one of the major pathogens of concern for the fresh produce industry (Table 1). According to the WHO, microbial contamination within the food production system occurs due to several factors, including animals, contaminated water, land use, fertilizers, post-harvest practices, workers' poor health, and hygiene practices (Wadamori et al., 2017). To reduce the risk of cross-contamination, it is crucial to implement Good Agricultural Practices (GAPs) and postharvest food safety plans within farming operations. These practices help to prevent harmful microorganisms from being introduced or spreading in a food supply chain (American Society for Microbiology, 2010).

### *1.5 Escherichia coli O157:H7*

*Escherichia coli* (*E. coli*) is a gram-negative, rod-shaped, and facultative anaerobic bacterium that typically resides harmlessly in the human gut (El-Saadony et al., 2025). Although there are some strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands (Lim et al., 2010). This pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors (Kaper et al., 2004). Only

the most successful combinations of virulence factors have persisted, giving rise to specific *E. coli* 'pathotypes' capable of causing disease in healthy individuals.

Three general clinical syndromes can result from infection with one of these pathotypes including enteric/diarrhoeal disease, urinary tract infections (UTIs), and sepsis/meningitis. Among the intestinal pathogens, there are six well-described categories: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (Kaper et al., 2004). *E. coli* STEC/EHEC, with over 400 serotypes, is named for its ability to produce verocytoxin/Stx. This toxin targets vero cells in its host, disrupting protein synthesis (Alhadlaq et al., 2024).

Shiga toxin-producing *E. coli* O157:H7 is a causative agent of hemorrhagic diarrhea, which can lead to fatal hemolytic uremic syndrome (HUS), which is characterized by hemolytic anemia, acute renal failure, and thrombocytopenia (Griffin & Tauxe, 1991). According to the Centers for Disease Control and Prevention (CDC), it estimates that 265,000 STEC infections (36% O157:H7 serogroup-related) occur each year in the US (Schaut et al., 2019). Transmission of STECs to humans occurs through consumption of contaminated foods, such as raw or undercooked ground meat and raw vegetables, or direct contact with an infected person (Baazize-Ammi et al., 2015)

### *1.6 Food Safety on harvest and postharvest production*

#### *1.6.1 Harvest*

Recent foodborne illness outbreaks and recalls have brought increased attention to leafy green vegetables as potential vehicles for contamination. Root cause analyses have revealed fresh produce can become contaminated with pathogens and parasites at various stages such as field cultivation, harvesting and postharvest handling, processing and distribution (Van Boxstael et al., 2013). Fresh produce is cultivated in several climates and locations each employing diverse agricultural techniques. The microbial food safety hazards and contamination sources can differ based on the crop type and production practices, even for the same crop in different settings (FAO/WHO, 2008).

During the harvest season, crops can become contaminated with pathogens that are associated with harvesting equipment or farm workers. If workers do not clean and sanitize their harvesting knives regularly, they can transfer pathogens from a single contaminated crop to an entire container. Furthermore, automated equipment can also spread pathogens from one field to another if it is not cleaned and sanitized before moving between fields (Luna-Guevara et al., 2019; Machado-Moreira et al., 2019). Washing harvest equipment, such as knives, containers, and bins, can remove visible debris but is unlikely to significantly reduce microbial contamination (Alegbeleye et al., 2018; Balali et al., 2020). To effectively manage this risk, it is essential to establish standard, enforceable policies and provide sanitation training for all employees involved in primary production.

### *1.6.1 Postharvest*

The main goal of the postharvest is to ensure the fresh produce obtains new markets and maintains the quality, minimizes the postharvest losses, due to the short shelf-life, and ensures the safety of the produce. Food safety programs, including GAPs, Good Handling Practices (GHPs), and Good Manufacturing Practices (GMPs), play an important role in preventing the risk of contamination with food safety hazards during harvest and postharvest/packing (Lepper et al., 2021)

Postharvest handling can be categorized into primary preparation and storage/transportation (Gil et al., 2015). The preparation process involves cleaning, trimming, and coring raw materials. For example, lettuce can become cross-contaminated through contact with workers' hands, knives, automated equipment, and wash water. Additionally, the cut end of the lettuce is rich in nutrients that promote bacterial growth (Brandl, 2008; Stein, 2024). This creates a potential risk for microbial contamination due to unsanitary handling conditions. Therefore, it is crucial to ensure proper cleaning and sanitation as preventive measures and to confirm that re-circulated antimicrobial solutions do not become a source of contamination.

During storage and transportation, if a product is contaminated with a bacterial pathogen like *L. monocytogenes*, refrigeration may prolong its survival and allow for slow growth, particularly at temperatures above 4 °C (Osek et al., 2022; G. M. Sapers et al., 2005) Refrigeration units are believed to spread bacteria and mold throughout warehouses, which is why routine servicing of air filters and refrigeration systems is essential (Chawla et al., 2023; James, 2006). To ensure the safety of produce, it is important to implement preventive

measures, including good hygiene and cleaning practices during storage and transportation. Additionally, disinfection technologies, such as ozone, have been employed as intervention strategies to reduce contamination in cooling and storage facilities (Gil et al., 2015)

*1.7 Food contact surfaces*

Pathogenic microorganisms are known to transfer to food via contact with contaminated surfaces. For instance, these microorganisms can attach to processing equipment, potentially invalidating cleaning and sanitizing measures, which can lead to the contamination of processed products. This attachment is the initial step in microbial infections; if a cell does not adhere, it will be carried away from its potential host (Frank, 2001; Pierrat et al., 2021). *L. monocytogenes* has the ability to colonize and survive on food processing surfaces and is able to adhere and form biofilms on various surfaces, such as stainless steel, glass, and rubber (Lake et al., 2024).

In the formation of a biofilm, several factors play a crucial role. These include the characteristics of the bacteria, such as cell surface properties and surface charge. Additionally, the nature of the surface to which the bacteria attach, such as the material it is made of and its wettability, also influences biofilm formation. Finally, environmental conditions like pH and temperature are significant contributors to the development of biofilms (García-Gonzalo & Pagán, 2015).

One of the important factors of interest that can affect the adhesion of microorganisms to surfaces is the hydrophobicity of the material and the cell surface. The hydrophobic/hydrophilic interactions have been responsible for a wide range of adherence

processes (Czaczyk et al., 2008; Oh et al., 2018). Materials with a hydrophobic coating are more resistant to bacterial adhesion than hydrophilic materials. For those materials with hydrophobicity coating, such as metal, and anti-biofouling and anti-adhesive materials, have been determined mainly by contact angle (CA) measurement. These types of surfaces rely on a superhydrophobic effect, in which it has to achieve a CA of water greater than 150°. A superhydrophobic surface can effectively repel aqueous residues, spills, and droplets carrying bacterial suspensions (Myszka & Czaczyk, 2011; Oh et al., 2019).

*1.8 Harvest and postharvest technologies*

New technologies, in combination with sanitizers, are being evaluated to reduce pathogens within biofilms and prevent their internalization in the tissues of produce. These methods include the use of irradiation, cold plasma, controlled atmosphere storage, edible films, nano-textured coatings, and biological control agents like bacteriophages (Mendez et al., 2022; Mickos et al., 2025).

In the food industry, companies have recently implemented antimicrobial coatings on food-contact surfaces as a strategy for controlling biofilms and reducing cross-contamination. DeFlorio et al. (2021) classified these coatings into four main categories: release-based and contact-based coatings, which release antimicrobial agents into bacterial suspensions, effectively inactivating pathogenic microorganisms that adhere to the surfaces; and repulsion-based and superhydrophobic antifouling surfaces, which do not inactivate bacteria but instead prevent or significantly reduce bacterial attachment and biofilm formation.

*1.8.1 Harvest: nano-textured coatings*

Coatings, also known as thin films, are layers of material applied to a surface to change its properties of appearance. Advances in nanotechnology have allowed for the creation of thin coatings with specific properties, which have opened up new possibilities in the food safety field (Cheng et al., 2023). Superhydrophobic coatings are extremely repellent to liquids, and thus, the solid-liquid contact area is minimal, and adhesion is weak. Initially developed to impact surfaces with special functionalities such as self-cleaning, anti-adhesion/anti-fouling, and anti-icing properties (Ruzi et al., 2022). For a coating to be considered superhydrophobic, it must achieve a water droplet CA greater than 150°. This phenomenon is described by the Cassie-Baxter equation (Bayer, 2020).

Cassie-Baxter are credited with first reporting the basis of superhydrobicity in 1944, expanding on the work by Wenzel in 1936. The equation explains how a liquid droplet behaves on a rough surface made of two different phases, such as solid and trapped air (Lai et al., 2025). For superhydrophobic surfaces, where the second phase is air, the equation is:

$$cos\theta^* = f(1 + \cos\theta) - 1$$

Where the $f$ is the fraction of solid under the droplet and $\theta$ is referred to CA of water on a surface. The CA, which refers to the angle where the liquid-air interface meets the solid-liquid interface, and is commonly used to determine the wettability of a flat surface (Chan et al., 2021). Flat materials with CA > 90° in contact with water are referred to as hydrophobic, whereas materials with CA < 90° are referred to as hydrophilic (Lai et al., 2025).

Superhydrophobic surfaces are generally created by a micro-nanostructured rough surface and low-surface-energy material modification (Gong & He, 2020). The use of nanoparticles (NPs) on superhydrophobic coatings demonstrated an excellent inhibition of bacteria and antibiofilm efficacy. For example, the use of copper oxide NPs can cause DNA damage to bacteria by forming reactive oxygen species, and interfere the ability to form biofilms (Dawan et al., 2025). Another NPs is silica, in which, Oh et al. (2019) demonstrated that aluminum surfaces coated with silica NPs exhibit excellent mud-repelling activity, and the attachment of *S.* Typhimurium LT2 and *L. innocua* on the coated surface was reduced by > 99.0%. Additionally, fluoroalkoxysilane-coated structures involving silica colloids were found to reduce adhesion of *S. aureus* and *P. aeruginosa* by 2.08 and 1.76 log CFU/g, respectively (Privett et al., 2011). Similarly, Liu et al. (2020) a dual-functional coating composed of silica NPs applied to aluminum surfaces was developed, which resulted in a 6.5 log-cycle reduction in bacterial colonization for gram-negative *S.* Typhimurium LT2 and a 4 log-cycle reduction for gram-positive *L. innocua.*

Research has been exploring nano-coatings as alternatives to prevent microbial contamination due to inhibition properties, such as *Salmonella* and *E. coli* O157:H7 (DeFlorio et al., 2023). Since foodborne pathogens like *Salmonella* can adhere to various surfaces used in food processing (e.g., stainless steel), applying nanoscale coatings may help reduce cross-contamination during food handling and slaughtering (Schumann-Muck et al., 2023).

### *1.8.2 Postharvest: washing treatments*

The majority of fresh produce undergoes washing after harvest, typically carried out by processors using flume systems, batch tanks, or water sprays. This washing step is crucial for removing soil and debris, as it aims to eliminate field contamination (Bornhorst et al., 2018; Murray et al., 2017), improve produce quality and marketability, and lower the temperature of the produce. Antimicrobial agents, such as sanitizers, are commonly used in produce washing operations to prevent the cross-contamination of fresh produce through wash water (Dharmarha et al., 2020).

The effectiveness of antimicrobial treatments can vary based on several factors, including concentration, exposure time, temperature, pH, washing dynamics, the level of soil on the produce, and the way microorganisms adhere to the produce. Common antimicrobial agents used in the food industry to reduce or eliminate potential pathogens on produce include chlorine-based products, peroxyacetic acid (PAA), and hydrogen peroxide (Fan et al., 2009; Mendoza et al., 2022).

#### *1.8.2.1 Chlorine*

Chlorine is commonly used as a sanitizer to wash produce due to its antibacterial effect and low cost (Chaidez et al., 2012; Martinez-Ramos et al., 2022). In commercial applications, chlorinated water is utilized in dump tanks, flume washes, and sprays to clean various types of fresh fruits and vegetables after harvest and has been reported to prevent microbial contamination in produce-processing lines (Allende et al., 2025; Ukuku et al., 2012). The recommended contact time for produce with chlorinated water is 1 – 2 minutes,

with a chlorine concentration ranging from 50 to 200 ppm, which can result in an average of 1 – 2 log 10 (90 – 99%) bacterial inactivation (Chaidez et al., 2012; Dev Kumar et al., 2017). Chlorine is efficient against bacteria, molds, yeast, and viruses, but not spores (Artasensi et al., 2021; G. Sapers, 2005). For example, Fishburn et al. (2012) evaluated different sanitizers and found that rinsing with a bleach solution of 70 ppm for 2 min was the most effective treatment for reducing *S. enterica* lettuce and green onions, with reductions ranging from 2.05 to 3.89 log CFU/g. Additionally, Mokhtari et al. (2022) treated lettuce inoculated with *S. enterica* using a 100 ppm chlorine solution for 2 min, resulting in a reduction of 3 log CFU/g.

On the other hand, the effectiveness of chlorine is affected by pH, temperature, fresh product type, and microflora (Chinchkar et al., 2022). If pH gets lowered below 4, it means free chlorine in the form of gas emerges from the solution, which is hazardous to health (Block & Rowan, 2020; Erkan & Yıldırım, 2017). Organic matter in the sanitizing solution significantly reduces the effectiveness of chlorine because it reacts rapidly with free chlorine (Stopforth et al., 2004; Teng et al., 2018). For example, Mina et al. (2025) demonstrate that 1% organic load can decrease the available chlorine from 100 ppm to less than 2 ppm.

#### *1.8.2.2 Hydrogen Peroxide ($H_2O_2$)*

$H_2O_2$ is an antimicrobial compound commonly used in the produce industry for washing due to its Generally Recognized As Safe (GRAS) status and its ability to break down into hydrogen and oxygen without residual toxicity. Due to this, $H_2O_2$ is an environmentally friendly, odorless, colorless, and non-corrosive agent (Hall et al., 2008; Marriott et al., 2018). Consequently, this sanitizer has a mild effect on the water recovery system and can be used

as a suitable alternative to other sanitizers (Abdelshafy et al., 2024). Additionally, it is effective against a wide range of microorganisms, including bacteria, yeast, fungi, viruses, and spores (Bimal Sheth et al., 2025).

$H_2O_2$ is as effective as 200 ppm of chlorine in concentrations ranging from 1% to 5%. It acts on foodborne bacteria by producing hydroxyl free radicals that target membrane lipids, DNA, and other essential cellular components (Bimal Sheth et al., 2025; Ukuku et al., 2012). Using a 3% $H_2O_2$ solution for 1 min effectively reduced the levels of *E. coli* O157:H7 on the surface of strawberries by 2.2 log CFU/g. This reduction was significantly greater than that achieved with other chemical treatments tested (Fan et al., 2009; Zoellner et al., 2018). Applying a 5% $H_2O_2$ solution exhibited the most effective antimicrobial activity, achieving reductions of L. monocytogenes and S. enterica by 4.9 and 5.4 log CFU/g, respectively (Abdelshafy et al., 2024). Treating fresh-cut lettuce leaves with 4% $H_2O_2$ solution for 2 min reduced *L. innocua* and *E. coli* O157:H7 by 5.1 and 3.7 log CFU/g, respectively (Cossu et al., 2017).

*1.8.2.3 Peroxyacetic Acid (PAA)*

PAA is produced by the reaction of acetic acid with hydrogen peroxide. The advantages of PAA are that it is effective at varying pH levels, is not corrosive to postharvest equipment, such as stainless steel and aluminum, and is not sensitive to organic matter like chlorine (Long et al., 2024). Additionally, PAA has a stronger oxidizing potential than chlorine, chlorine dioxide, chlorous acid, and hydrogen peroxide, but less than ozone (Fan et al., 2009; Sciscenko et al., 2024). According to the US Food and Drug Administration (FDA), PAA can be used for washing fruits and vegetables, but should not exceed 80 ppm, as higher

levels can lead to unacceptable residues on fresh produce (González-Aguilar et al., 2012; Pabst et al., 2024).

PAA has a disinfection efficiency against different organisms ranked on a general basis: bacteria > viruses > bacterial spores > protozoan cysts (Alvaro et al., 2009; McCaughan et al., 2025). A characteristic of PAA is that it is produced from the reaction of acetic acid, which is based on the release of active oxygen (Figure 1.1). Sensitive sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites are oxidized, and double bonds are reacted. Additionally, it is suggested that PAA can disrupt the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport through the dislocation or rupture of cell walls (González-Aguilar et al., 2012; Stadler & Fischer, 2020).

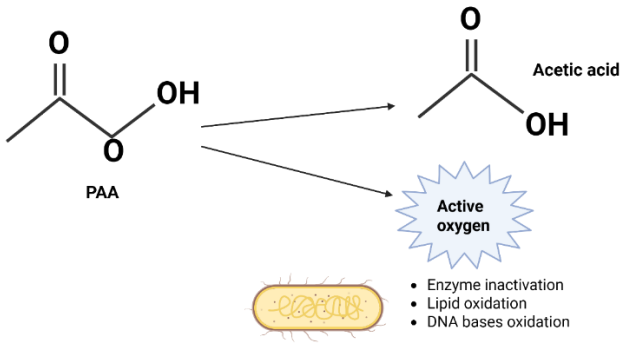


**Figure 1.1.** Antimicrobial mechanism of PAA based on active oxygen production and oxidation of biomolecules of vital importance for pathogenic bacteria.

In summary, there has been a notable increase in foodborne outbreaks associated with fresh produce in recent years. This trend has led to the development of various technologies for harvest and postharvest handling. One promising technique during the harvest phase is

the application of superhydrophobic coatings, which can repel bacteria. In the postharvest stage, using effective sanitizers is crucial to minimize cross-contamination during the washing process. Together, these practices are essential for ensuring food safety and quality without compromising either aspect.

## 1.9 References


Abdelshafy, A. M., Hu, Q., Luo, Z., Ban, Z., & Li, L. (2024). Hydrogen Peroxide from Traditional Sanitizer to Promising Disinfection Agent in Food Industry. *Food Reviews International*, *40*(2), 658–690. https://doi.org/10.1080/87559129.2023.2191690

Alegbeleye, O. O., Singleton, I., & Sant'Ana, A. S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiology*, *73*, 177–208. https://doi.org/10.1016/j.fm.2018.01.003

Alexandre, E. M. C., Brandão, T. R. S., & Silva, C. L. M. (2012). Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. *Food Control*, *27*(2), 362–368. https://doi.org/10.1016/j.foodcont.2012.04.012

Alhadlaq, M. A., Aljurayyad, O. I., Almansour, A., Al-Akeel, S. I., Alzahrani, K. O., Alsalman, S. A., Yahya, R., Al-Hindi, R. R., Hakami, M. A., Alshahrani, S. D., Alhumeed, N. A., Al Moneea, A. M., Al-Seghayer, M. S., AlHarbi, A. L., AL-Reshoodi, F. M., & Alajel, S. (2024). Overview of pathogenic Escherichia coli, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982–2024) and food

safety criteria. *Gut Pathogens*, *16*(1), 57. https://doi.org/10.1186/s13099-024-00641-9

Allende, A., Alvarez-Ordóñez, A., Bortolaia, V., Bover-Cid, S., De Cesare, A., Dohmen, W., Guillier, L., Herman, L., Jacxsens, L., Mughini-Gras, L., Nauta, M., Ottoson, J., Peixe, L., Perez-Rodriguez, F., Skandamis, P., Suffredini, E., Banach, J., Zhou, B., da Silva Felício, M. T., … Botteon, A. (2025). Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH). Part 3 (Fresh-whole FVH process water management plan). *EFSA Journal*, *23*(1). https://doi.org/10.2903/j.efsa.2025.9170

Als, D., Radhakrishnan, A., Arora, P., Gaffey, M. F., Campisi, S., Velummailum, R., Zareef, F., & Bhutta, Z. A. (2018). Global Trends in Typhoidal Salmonellosis: A Systematic Review. *The American Journal of Tropical Medicine and Hygiene*, *99*(3_Suppl), 10–19. https://doi.org/10.4269/ajtmh.18-0034

Alvaro, J. E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., & Urrestarazu, M. (2009). Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *Journal of Food Engineering*, *95*(1), 11–15. https://doi.org/10.1016/j.jfoodeng.2009.05.003

American Society for Microbiology. (2010). *Global Food Safety: Keeping Food Safe from Farm to Table*.

Amodu, A., Oliver, J. E., Lawrence, K., Patel, S., Koebernick, J., Patel, J., Coneva, E., & Ru, S. (2025). Identifying the Distribution and Causal Pathogens of Blueberry Stem Blight Disease in Alabama and Nearby States. *Plant Disease*, PDIS-07-24-1404-SR. https://doi.org/10.1094/PDIS-07-24-1404-SR

Angelo, K. M., Conrad, A. R., Saupe, A., Dragoo, H., West, N., Sorenson, A., Barnes, A., Doyle, M., Beal, J., Jackson, K. A., Stroika, S., Tarr, C., Kucerova, Z., Lance, S., Gould, L. H., Wise, M., & Jackson, B. R. (2017). Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. *Epidemiology and Infection*, *145*(5), 848–856. https://doi.org/10.1017/S0950268816003083

Artasensi, A., Mazzotta, S., & Fumagalli, L. (2021). Back to Basics: Choosing the Appropriate Surface Disinfectant. *Antibiotics*, *10*(6), 613. https://doi.org/10.3390/antibiotics10060613

Awuchi, C. G. (2023). HACCP, quality, and food safety management in food and agricultural systems. *Cogent Food & Agriculture*, *9*(1), 2176280. https://doi.org/10.1080/23311932.2023.2176280

Baazize-Ammi, D., Gassem, O., Derrar, F., Izri, K., Brahim-Errahmani, M., Gagnon, J., Guetarni, D., & Chebloune, Y. (2015). Prevalence of Asymptomatic Carriers of Shiga Toxin-Producing *Escherichia Coli (STEC)* in Dairy Cattle Farms in the Governorate of Blida (Algeria). *Bulletin of the Veterinary Institute in Pulawy*, *59*(1), 23–28. https://doi.org/10.1515/bvip-2015-0004

Baghalian, K., Hajirezaei, M.-R., & Lawson, T. (2023). Editorial: Current and future perspectives for controlled environment agriculture (CEA) in the 21st century. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1334641

Balali, G. I., Yar, D. D., Afua Dela, V. G., & Adjei-Kusi, P. (2020). Microbial Contamination, an Increasing Threat to the Consumption of Fresh Fruits and Vegetables in Today's World. *International Journal of Microbiology*, *2020*, 1–13. https://doi.org/10.1155/2020/3029295

Balliu, A., Zheng, Y., Sallaku, G., Fernández, J. A., Gruda, N. S., & Tuzel, Y. (2021). Environmental and Cultivation Factors Affect the Morphology, Architecture and Performance of Root Systems in Soilless Grown Plants. *Horticulturae*, *7*(8), 243. https://doi.org/10.3390/horticulturae7080243

Bassey, A. P., Ye, K., Li, C., & Zhou, G. (2021). Transcriptomic-proteomic integration: A powerful synergy to elucidate the mechanisms of meat spoilage in the cold chain. *Trends in Food Science & Technology*, *113*, 12–25. https://doi.org/10.1016/j.tifs.2021.02.051

Bayer, I. S. (2020). Superhydrophobic Coatings from Ecofriendly Materials and Processes: A Review. *Advanced Materials Interfaces*, *7*(13), 2000095. https://doi.org/10.1002/admi.202000095

Behrsing, J., Winkler, S., Franz, P., & Premier, R. (2000). Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. *Postharvest Biology and Technology*, *19*(2), 187–192. https://doi.org/10.1016/S0925-5214(00)00092-2

Beuchat, L. R. (2006). Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *British Food Journal*, *108*(1), 38–53. https://doi.org/10.1108/00070700610637625

Bimal Sheth, U., Haque, M. A., Jang, M. J., Haruna, S., Johnston, T. V., Choe, D., Gao, Y., & Ku, S. (2025). From Soil to Salad: Strategies for Reducing Foodborne Illness Outbreaks. *Food Science & Nutrition*, *13*(1), e4521. https://doi.org/10.1002/fsn3.4521

Black, Z., Balta, I., Black, L., Naughton, P. J., Dooley, J. S. G., & Corcionivoschi, N. (2021). The Fate of Foodborne Pathogens in Manure Treated Soil. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.781357

Block, M. S., & Rowan, B. G. (2020). Hypochlorous Acid: A Review. *Journal of Oral and Maxillofacial Surgery*. https://www.sciencedirect.com/science/article/pii/S0278239120306728

Bornhorst, E. R., Luo, Y., Park, E., Vinyard, B. T., Nou, X., Zhou, B., Turner, E., & Millner, P. D. (2018). Immersion-free, single-pass, commercial fresh-cut produce washing system: An alternative to flume processing. *Postharvest Biology and Technology*, *146*, 124–133. https://doi.org/10.1016/j.postharvbio.2018.08.008

Bottichio, L., Keaton, A., Thomas, D., Fulton, T., Tiffany, A., Frick, A., Mattioli, M., Kahler, A., Murphy, J., Otto, M., Tesfai, A., Fields, A., Kline, K., Fiddner, J., Higa, J., Barnes, A., Arroyo, F., Salvatierra, A., Holland, A., … Gieraltowski, L. (2020). Shiga Toxin–Producing Escherichia coli Infections Associated With Romaine Lettuce—United

States, 2018. *Clinical Infectious Diseases*, *71*(8), e323–e330. https://doi.org/10.1093/cid/ciz1182

Brandl, M. T. (2008). Plant Lesions Promote the Rapid Multiplication of *Escherichia coli* O157:H7 on Postharvest Lettuce. *Applied and Environmental Microbiology*, *74*(17), 5285–5289. https://doi.org/10.1128/AEM.01073-08

Brown, JamesF., & Schneider, R. A. (2000). *Hydrophobic coating compositions, articles coated with said compositions, and processes for manufacturing same* (Patent US6156389A). https://patents.google.com/patent/US6156389A/en

Carrasco, G., Fuentes-Peñailillo, F., Manríquez, P., Rebolledo, P., Vega, R., Gutter, K., & Urrestarazu, M. (2024). Enhancing Leafy Greens' Production: Nutrient Film Technique Systems and Automation in Container-Based Vertical Farming. *Agronomy*, *14*(9), 1932. https://doi.org/10.3390/agronomy14091932

CDC. (2019a, May 24). *Outbreak of Salmonella Infections Linked to Pre-Cut Melons | Outbreak of Salmonella Infections Linked to Pre-Cut Melon | April 2019 | Salmonella | CDC*. https://www.cdc.gov/salmonella/carrau-04-19/index.html

CDC. (2019b, October 11). *Outbreak of Salmonella Infections Linked to Cavi Brand Whole, Fresh Papayas | Outbreak of Salmonella Infections Linked to Whole, Fresh Papayas Imported from Mexico | June 2019 | Salmonella | CDC*. https://www.cdc.gov/salmonella/uganda-06-19/index.html

CDC. (2019c, December 2). *Cyclosporiasis Outbreak Investigations—United States, 2019*. https://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2019/weekly/index.html

CDC. (2020, January 15). *Outbreak of E. coli Infections Linked to Romaine Lettuce | E.coli Infections | November 2019 | E. coli | CDC*. https://www.cdc.gov/ecoli/2019/o157h7-11-19/index.html

CDC. (2023a, February 28). *CDC: Salmonella Outbreak Linked to Alfalfa Sprouts*. Centers for Disease Control and Prevention. https://www.cdc.gov/salmonella/typhimurium-12-22/index.html

CDC. (2023b, June 13). *CDC: Listeria Outbreak Linked to Leafy Greens*. Centers for Disease Control and Prevention. https://www.cdc.gov/listeria/outbreaks/monocytogenes-02-23/index.html

CDC. (2024a). *E. coli Outbreak Linked to Organic Carrots*. https://www.cdc.gov/ecoli/outbreaks/e-coli-o121.html

CDC. (2024b). *Salmonella Outbreak Linked to Cucumbers*. https://www.cdc.gov/salmonella/outbreaks/cucumbers-11-24/index.html

CDC. (2024c, April 24). *How Food Gets Contaminated: The Food Production Chain*. https://www.cdc.gov/foodborne-outbreaks/foodproductionchain/index.html

CDC. (2024d, June 18). *Salmonella Outbreak Linked to Fresh Basil | CDC*. https://www.cdc.gov/salmonella/basil-04-24/index.html

CDC. (2024e, July 2). *Salmonella Outbreak Linked to Cucumbers | CDC*. https://www.cdc.gov/salmonella/africana-06-24/index.html

Chaidez, C., Campo, N. C., Heredia, J. B., Contreras-Angulo, L., González–Aguilar, G., & Ayala–Zavala, J. F. (2012). Chlorine. In V. M. Gómez-López (Ed.), *Decontamination of Fresh and Minimally Processed Produce* (1st ed., pp. 121–133). Wiley. https://doi.org/10.1002/9781118229187.ch7

Chan, Y., Wu, X. H., Chieng, B. W., Ibrahim, N. A., & Then, Y. Y. (2021). Superhydrophobic Nanocoatings as Intervention against Biofilm-Associated Bacterial Infections. *Nanomaterials*, *11*(4), 1046. https://doi.org/10.3390/nano11041046

Chauhan, C., Dhir, A., Akram, M. U., & Salo, J. (2021). Food loss and waste in food supply chains. A systematic literature review and framework development approach. *Journal of Cleaner Production*, *295*, 126438. https://doi.org/10.1016/j.jclepro.2021.126438

Chawla, H., Anand, P., Garg, K., Bhagat, N., Varmani, S. G., Bansal, T., McBain, A. J., & Marwah, R. G. (2023). A comprehensive review of microbial contamination in the indoor environment: Sources, sampling, health risks, and mitigation strategies. *Frontiers in Public Health*, *11*. https://doi.org/10.3389/fpubh.2023.1285393

Chazotte, B. (2012). Labeling Golgi with Fluorescent Ceramides. *Cold Spring Harbor Protocols*, *2012*(8), pdb.prot070599. https://doi.org/10.1101/pdb.prot070599

Chen, Y., Chen, A., & Zhang, H. (2023). The Application of Food Safety Standards in Food Safety Management Practices. *International Journal of Food Science and Agriculture*, *7*(2), 242–246. https://doi.org/10.26855/ijfsa.2023.06.011

Cheng, W., Wang, J., Ma, X., Liu, P., Liaw, P. K., & Li, W. (2023). A review on microstructures and mechanical properties of protective nano-multilayered films or coatings. *Journal of Materials Research and Technology*, *27*, 2413–2442. https://doi.org/10.1016/j.jmrt.2023.10.012

Chevez, Z. R., Dunn, L. L., Da Silva, A. L. B. R., & Rodrigues, C. (2024). Prevalence of STEC virulence markers and Salmonella as a function of abiotic factors in agricultural water in the southeastern United States. *Frontiers in Microbiology*, *15*, 1320168. https://doi.org/10.3389/fmicb.2024.1320168

Chinchkar, A. V., Singh, A., Singh, S. V., Acharya, A. M., & Kamble, M. G. (2022). Potential sanitizers and disinfectants for fresh fruits and vegetables: A comprehensive review. *Journal of Food Processing and Preservation*, *46*(10). https://doi.org/10.1111/jfpp.16495

Cimowsky, S., Kumar, G. D., Biscaia Ribeiro Da Silva, A. L., White, E., Kerr, W. L., Rodrigues, C., Juneja, V. K., & Dunn, L. L. (2022). Postharvest control of Escherichia coli O157:H7 on romaine lettuce using a novel pelargonic acid sanitizer. *LWT*, *154*, 112168. https://doi.org/10.1016/j.lwt.2021.112168

Coneva, E., & Singh, J. (2025). *Research Update: Assessment of Rabbiteye Blueberry Cultivars*. Alabama Extension. Research Update: Assessment of Rabbiteye Blueberry Cultivars

Cornell CALS. (2015). *Postharvest Water*. https://cals.cornell.edu/national-good-agricultural-practices-program/resources/educational-materials/decision-

trees/postharvest-water#:~:text=A%20number%20of%20chemical%20sanitizers,or%20if%20you%20have%20questions.

Cossu, A., Dou, F., Young, G. M., & Nitin, N. (2017). Biomarkers of oxidative damage in bacteria for the assessment of sanitation efficacy in lettuce wash water. *Applied Microbiology and Biotechnology*, *101*(13), 5365–5375. https://doi.org/10.1007/s00253-017-8314-5

Cowan, N., Ferrier, L., Spears, B., Drewer, J., Reay, D., & Skiba, U. (2022). CEA Systems: The Means to Achieve Future Food Security and Environmental Sustainability? *Frontiers in Sustainable Food Systems*, *6*. https://doi.org/10.3389/fsufs.2022.891256

CSH Protocols. (2006). Phosphate-buffered saline (PBS). *Cold Spring Harbor Protocols*, *2006*(1), pdb.rec8247. https://doi.org/10.1101/pdb.rec8247

Czaczyk, K., Białas, W., & Myszka, K. (2008). Cell surface hydrophobicity of Bacillus spp. As a function of nutrient supply and lipopeptides biosynthesis and its role in adhesion. *Polish Journal of Microbiology*, *57*(4), 313–319.

Dagnas, S., & Membré, J.-M. (2013). Predicting and Preventing Mold Spoilage of Food Products. *Journal of Food Protection*, *76*(3), 538–551. https://doi.org/10.4315/0362-028x.jfp-12-349

Dai, Y., Holland, R., Doane, S., Yang, W., & Chen, J. (2025). Hygiene Status of Over-the-Row Blueberry Machine Harvesters Cleaned and Sanitized Using Various

Approaches. *Horticulturae*, *11*(1), 103. https://doi.org/10.3390/horticulturae11010103

Dankwa, A. S., Machado, R. M., & Perry, J. J. (2021). Sanitizer efficacy in reducing microbial load on commercially grown hydroponic lettuce. *Journal of the Science of Food and Agriculture*, *101*(4), 1403–1410. https://doi.org/10.1002/jsfa.10753

Dawan, J., Zhang, S., & Ahn, J. (2025). Recent Advances in Biofilm Control Technologies for the Food Industry. *Antibiotics*, *14*(3), Article 3. https://doi.org/10.3390/antibiotics14030254

De Corato, U. (2020). Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: A comprehensive critical review from the traditional technologies into the most promising advancements. *Critical Reviews in Food Science and Nutrition*, *60*(6), 940–975. https://doi.org/10.1080/10408398.2018.1553025

De Siqueira Oliveira, L., Eça, K. S., De Aquino, A. C., & Vasconcelos, L. B. (2018). Hydrogen Peroxide (H2O2) for Postharvest Fruit and Vegetable Disinfection. In *Postharvest Disinfection of Fruits and Vegetables* (pp. 91–99). Elsevier. https://doi.org/10.1016/B978-0-12-812698-1.00004-2

DeFlorio, W., Liu, S., Arcot, Y., Ulugun, B., Wang, X., Min, Y., Cisneros-Zevallos, L., & Akbulut, M. (2023). Durable superhydrophobic coatings for stainless-steel: An effective defense against Escherichia coli and Listeria fouling in the post-harvest

environment. *Food Research International*, *173*, 113227. https://doi.org/10.1016/j.foodres.2023.113227

DeFlorio, W., Liu, S., White, A. R., Taylor, T. M., Cisneros-Zevallos, L., Min, Y., & Scholar, E. M. A. (2021). Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 3093–3134. https://doi.org/10.1111/1541-4337.12750

Defrizal, M., Kemala, T., & Khotib, M. (2022). Synthesis and characterization of superhydrophobic coating from silica-HDPE composite and its potential application for windshield. *AIP Conference Proceedings*, *2638*, 020013. https://doi.org/10.1063/5.0104485

Dev Kumar, G., Ravishankar, S., & Juneja, V. K. (2017). Interventions for Fresh Produce. In V. K. Juneja, H. P. Dwivedi, & J. N. Sofos (Eds.), *Microbial Control and Food Preservation* (pp. 199–223). Springer New York. https://doi.org/10.1007/978-1-4939-7556-3_10

Dhaliwal, H. K., Sonkar, S., V, P., Puente, L., & Roopesh, M. S. (2025). Process Technologies for Disinfection of Food-Contact Surfaces in the Dry Food Industry: A Review. *Microorganisms*, *13*(3), 648. https://doi.org/10.3390/microorganisms13030648

Dharmarha, V., Boyer, R., Strawn, L., Drape, T. A., Eifert, J., Vallotton, A., Pruden, A., & Ponder, M. A. (2020). An Assessment of Produce Growers' Sanitizer Knowledge and Practices on the Correct Use of Sanitizers. *Food Protection Trends*, *40*(3), 140–146.

Dunn, L. L., Harness, M. L., Smith, D. M., Gorman, S. J., Zhong, Q., Davidson, P. M., & Critzer, F. J. (2019). Essential Oil Emulsions as Postharvest Sanitizers To Mitigate Salmonella Cross-Contamination on Peppers. *Journal of Food Protection*, *82*(1), 159–163. https://doi.org/10.4315/0362-028X.JFP-18-190

Ells, T., Tregunno, N., Fan, L., Elliot, M., Doucette, C., Lyu, H., & Jollimore, A. (2024). Microbiological Analysis of Wild Lowbush Blueberries Harvested in Nova Scotia, Canada for the Fresh Produce Market. *Microorganisms*, *12*(11), 2251. https://doi.org/10.3390/microorganisms12112251

El-Saadony, M. T., Saad, A. M., Mohammed, D. M., Korma, S. A., Alshahrani, M. Y., Ahmed, A. E., Ibrahim, E. H., Salem, H. M., Alkafaas, S. S., Saif, A. M., Elkafas, S. S., Fahmy, M. A., Abd El-Mageed, T. A., Abady, M. M., Assal, H. Y., El-Tarabily, M. K., Mathew, B. T., AbuQamar, S. F., El-Tarabily, K. A., & Ibrahim, S. A. (2025). Medicinal plants: Bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. *Frontiers in Immunology*, *16*. https://doi.org/10.3389/fimmu.2025.1491777

Erkan, M., & Yıldırım, I. (2017). Postharvest Quality and Safety of Fresh-Cut Vegetables. In F. Yildiz & R. C. Wiley (Eds.), *Minimally Processed Refrigerated Fruits and Vegetables* (pp. 271–326). Springer US. https://doi.org/10.1007/978-1-4939-7018-6_8

Fan, X., Niemira, B. A., Doona, C. J., Feeherry, F. E., & Gravani, R. B. (Eds.). (2009). *Microbial Safety of Fresh Produce* (1st ed.). Wiley. https://doi.org/10.1002/9781444319347

FAOSTAT. (2021). *Food and Agriculture Organisation of the United Nations Statistical Database; Statistical Division; FAO: Rome, Italy*. Food and Agriculture Organization of the United Nations. http://www.fao.org/statistics/en/

FAO/WHO. (2008). *Microbiological hazards in fresh leafy vegetables and herbs*. https://openknowledge.fao.org/server/api/core/bitstreams/edec0655-cff2-438a-887c-71e15d3cfa91/content

FDA. (1998). *Guidance for industry: Guide to minimize microbial food safety hazards for fresh fruits and vegetables*. https:// www.fda.gov/regulatory-information/ search-fda-guidance-documents/guid ance-industry-guide-minimize-microbi al-food-safety-hazards-fresh-fruits-and-veg etables

FDA. (2018). *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-fruits-and-vegetables

FDA. (2021). *Factors Potentially Contributing to the Contamination of Packaged Leafy Greens Implicated in the Outbreak of Salmonella Typhimurium During the Summer of 2021*. https://www.fda.gov/food/outbreaks-foodborne-illness/factors-potentially-contributing-contamination-packaged-leafy-greens-implicated-outbreak-salmonella

FDA. (2024, September). *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption: What You Need to Know About the FDA Regulation: Guidance for Industry Small Entity Compliance Guide*. https://www.fda.gov/media/107298/download

FDA. (2025, February 26). *Investigations of Foodborne Illness Outbreaks*. https://www.fda.gov/food/outbreaks-foodborne-illness/investigations-foodborne-illness-outbreaks

Fedel, M., Olivier, M., Poelman, M., Deflorian, F., Rossi, S., & Druart, M.-E. (2009). Corrosion protection properties of silane pre-treated powder coated galvanized steel. *Progress in Organic Coatings*, *66*(2), 118–128. https://doi.org/10.1016/j.porgcoat.2009.06.011

Ferris, W., Tate, S., & Ulaky, A. (2023). *Controlled Environment Agriculture Strategy and Roadmap in GO Virginia Region 3*. https://cece.vt.edu/content/dam/cece_vt_edu/projects/CEA%20Strategy%20and%20Road%20Map%20Final%20Report.pdf

Fishburn, J. D., Tang, Y., & Frank, J. F. (2012). Efficacy of Various Consumer-Friendly Produce Washing Technologies in Reducing Pathogens on Fresh Produce. *Food Protection Trends*. https://www.foodprotection.org/files/food-protection-trends/Aug-12-Fishburn.pdf

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors Affecting Quality and Safety of Fresh-Cut Produce. *Critical*

*Reviews in Food Science and Nutrition*, *52*(7), 595–610. https://doi.org/10.1080/10408398.2010.503685

Frank, J. F. (2001). Microbial attachment to food and food contact surfaces. In *Advances in Food and Nutrition Research* (Vol. 43, pp. 319–370). Elsevier. https://doi.org/10.1016/S1043-4526(01)43008-7

García-Gonzalo, D., & Pagán, R. (2015). Influence of Environmental Factors on Bacterial Biofilm Formation in the Food Industry: A Review. *Postdoc Journal*, *3*(6). https://doi.org/10.14304/SURYA.JPR.V3N6.2

Gil, M. I., Selma, M. V., Suslow, T., Jacxsens, L., Uyttendaele, M., & Allende, A. (2015). Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables. *Critical Reviews in Food Science and Nutrition*, *55*(4), 453–468. https://doi.org/10.1080/10408398.2012.657808

Gómez-López, V. M. (Ed.). (2012). *Decontamination of fresh and minimally processed produce*. Blackwell Pub. 10.1002/9781118229187

Gong, X., & He, S. (2020). Highly Durable Superhydrophobic Polydimethylsiloxane/Silica Nanocomposite Surfaces with Good Self-Cleaning Ability. *ACS Omega*, *5*(8), 4100–4108. https://doi.org/10.1021/acsomega.9b03775

González-Aguilar, G., Ayala-Zavala, J. F., Chaidez-Quiroz, C., Heredia, J. B., & Campo, N. C. (2012). Peroxyacetic Acid. In V. M. Gómez-López (Ed.), *Decontamination of*

*Fresh and Minimally Processed Produce* (1st ed., pp. 215–223). Wiley. https://doi.org/10.1002/9781118229187.ch12

Griffin, P. M., & Tauxe, R. V. (1991). The Epidemiology of Infections Caused by Escherichia coli O157: H7, Other Enterohemorrhagic E. coli, and the Associated Hemolytic Uremic Syndrome. *Epidemiologic Reviews*, *13*(1), 60–98. https://doi.org/10.1093/oxfordjournals.epirev.a036079

Hall, L., Otter, J. A., Chewins, J., & Wengenack, N. L. (2008). Deactivation of the dimorphic fungi *Histoplasma capsulatum, Blastomyces dermatitidis* and *Coccidioides immitis* using hydrogen peroxide vapor1. *Medical Mycology*, *46*(2), 189–191. https://doi.org/10.1080/13693780701744809

Hoffmann, S., White, A. E., McQueen, R. B., Ahn, J.-W., Gunn-Sandell, L. B., & Scallan Walter, E. J. (2024). Economic Burden of Foodborne Illnesses Acquired in the United States. *Foodborne Pathogens and Disease*. https://doi.org/10.1089/fpd.2023.0157

Hollis, P. (2024). *Researchers work to promote "superfood" production in Alabama*. https://wire.auburn.edu/content/agriculture/2024/07/20240725-JaySpiers-blueberries.php#:~:text=According%20to%20the%20latest%20U.S.%20Census%20of,2017%20to%20approximately%201%2C000%20acres%20in%202022.

Hoorfar, J. (Ed.). (2014). *Global safety of fresh produce: A handbook of best practice, innovative commercial solutions and case studies*. Woodhead Publishing.

James, J. (2006). Overview of Microbial Hazards in Fresh Fruit and Vegetables Operations. In J. James (Ed.), *Microbial Hazard Identification in Fresh Fruit and Vegetables* (1st ed., pp. 1–36). Wiley. https://doi.org/10.1002/0470007761.ch1

Jechalke, S., Schierstaedt, J., Becker, M., Flemer, B., Grosch, R., Smalla, K., & Schikora, A. (2019). Salmonella Establishment in Agricultural Soil and Colonization of Crop Plants Depend on Soil Type and Plant Species. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00967

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology*, *2*(2), 123–140. https://doi.org/10.1038/nrmicro818

Karl R Matthews. (2013). Sources of enteric pathogen contamination of fruits and vegetables: Future directions of research. *Stewart Postharvest Review*, *9*(1), 1–5. https://doi.org/10.2212/spr.2013.1.2

Kilonzo-Nthenge, A., Chen, F.-C., & Godwin, S. L. (2006). Efficacy of Home Washing Methods in Controlling Surface Microbial Contamination on Fresh Produce. *Journal of Food Protection*, *69*(2), 330–334. https://doi.org/10.4315/0362-028x-69.2.330

Korber, D. R., Lawrence, J. R., Lappin-Scott, H. M., & Costerton, J. W. (1995). Growth of Microorganisms on Surfaces. In H. M. Lappin-Scott & J. W. Costerton (Eds.), *Microbial Biofilms* (1st ed., pp. 15–45). Cambridge University Press. https://doi.org/10.1017/CBO9780511525353.003

Koutsoumanis, K., Allende, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G.,

Simmons, M., Skandamis, P., Suffredini, E., Fox, E., Gosling, R. (Becky), Gil, B. M., … Alvarez-Ordóñez, A. (2024). Persistence of microbiological hazards in food and feed production and processing environments. *EFSA Journal*, *22*(1). https://doi.org/10.2903/j.efsa.2024.8521

Kuda, T., Shibata, G., Takahashi, H., & Kimura, B. (2015). Effect of quantity of food residues on resistance to desiccation of food-related pathogens adhered to a stainless steel surface. *Food Microbiology*, *46*, 234–238. https://doi.org/10.1016/j.fm.2014.08.014

Kuda, T., Yano, T., & Kuda, M. T. (2008). Resistances to benzalkonium chloride of bacteria dried with food elements on stainless steel surface. *LWT - Food Science and Technology*, *41*(6), 988–993. https://doi.org/10.1016/j.lwt.2007.06.016

Lai, Y. J., Oh, P. C., Chew, T. L., & Ahmad, A. L. (2025). Surface Repellency beyond Hydrophobicity: A Review on the Latest Innovations in Superomniphobic Surfaces. *ACS Omega*, *10*(6), 5172–5192. https://doi.org/10.1021/acsomega.4c08269

Lake, F. B., Chen, J., Van Overbeek, L. S., Baars, J. J. P., Abee, T., & Den Besten, H. M. W. (2024). Biofilm formation and desiccation survival of Listeria monocytogenes with microbiota on mushroom processing surfaces and the effect of cleaning and disinfection. *International Journal of Food Microbiology*, *411*, 110509. https://doi.org/10.1016/j.ijfoodmicro.2023.110509

Le, T., Eifert, J. D., Etaka, C. A., & Strawn, L. K. (2024). Recovery and Survival of Aerosolized *ESCHERICHIA COLI* and *ENTEROCOCCUS FAECIUM* on Food-Grade

Rubber, HDPE Plastic, Stainless Steel, and Waxed Cardboard. *Journal of Food Safety*, *44*(6), e70002. https://doi.org/10.1111/jfs.70002

Leaman, S., Kerr, J., Salas, S., Malik, A., Suslow, T., Wiedmann, M., & Davis, D. A. (2023). Fresh Produce Harvesting Equipment – A Review of Cleaning and Sanitizing Practices and Related Science. *Food Protection Trends*, 126–143. https://doi.org/10.4315/FPT-22-023

Lepper, J. A., Schneider, K. R., Goodrich-Schneider, R. M., & Sreedharan, A. (2021). Food Safety on the Farm: Good Agricultural Practices and Good Handling Practices – Worker Health and Hygiene. *UF/IFAS*. https://edis.ifas.ufl.edu/publication/FS158

Lim, J. Y., Yoon, J., & Hovde, C. J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, *20*(1), 5–14.

Liu, S., Zheng, J., Hao, L., Yegin, Y., Bae, M., Ulugun, B., Taylor, T. M., Scholar, E. A., Cisneros-Zevallos, L., Oh, J. K., & Akbulut, M. (2020). Dual-Functional, Superhydrophobic Coatings with Bacterial Anticontact and Antimicrobial Characteristics. *ACS Applied Materials & Interfaces*, *12*(19), 21311–21321. https://doi.org/10.1021/acsami.9b18928

Long, F., Strawn, L., Schonberger, L., & Boyer, R. (2024, July). *Sanitizers for Vegetables in Harvest and Post-Harvest Water for Small Farmers*. *FST-479NP*. https://www.pubs.ext.vt.edu/FST/fst-479/fst-479.html

López-Gálvez, F., Allende, A., & Gil, M. I. (2021). Recent progress on the management of the industrial washing of fresh produce with a focus on microbiological risks. *Current Opinion in Food Science*, *38*, 46–51. https://doi.org/10.1016/j.cofs.2020.10.026

Losasso, C., Cibin, V., Cappa, V., Roccato, A., Vanzo, A., Andrighetto, I., & Ricci, A. (2012). Food safety and nutrition: Improving consumer behaviour. *Food Control*, *26*(2), 252–258. https://doi.org/10.1016/j.foodcont.2012.01.038

Luna-Guevara, J. J., Arenas-Hernandez, M. M. P., Martínez De La Peña, C., Silva, J. L., & Luna-Guevara, M. L. (2019). The Role of Pathogenic*E. coli*in Fresh Vegetables: Behavior, Contamination Factors, and Preventive Measures. *International Journal of Microbiology*, *2019*, 1–10. https://doi.org/10.1155/2019/2894328

Machado-Moreira, B., Richards, K., Brennan, F., Abram, F., & Burgess, C. M. (2019). Microbial Contamination of Fresh Produce: What, Where, and How? *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 1727–1750. https://doi.org/10.1111/1541-4337.12487

Malka, S. K., & Park, M.-H. (2022). Fresh Produce Safety and Quality: Chlorine Dioxide's Role. *Frontiers in Plant Science*, *12*, 775629. https://doi.org/10.3389/fpls.2021.775629

Mao, Z., Qiu, H., Shih, C., & Kang, Z. (2024). P-13.12: The Delta E Color Dissimilarity Analysis of LCD Panels. *SID Symposium Digest of Technical Papers*, *55*(S1), 1404–1414. https://doi.org/10.1002/sdtp.17382

Marriott, N. G., Schilling, M. W., & Gravani, R. B. (2018). Sanitizers. In *Food Science Text Series* (pp. 175–198). Springer International Publishing. https://doi.org/10.1007/978-3-319-67166-6_10

Martinez-Ramos, P., Corradini, M. G., Stoufer, S., Moore, M. D., Autio, W., & Kinchla, A. J. (2022). Assessment of Preparation Methods to Produce a Postharvest Spinach Wash Water Model for Sanitizer Validation Studies and Comparison of Sanitizer Quantitation Methods. *ACS Food Science & Technology*, *2*(1), 57–65. https://doi.org/10.1021/acsfoodscitech.1c00295

McCaughan, K. J., Scott, Z., Rock, C., & Kniel, K. E. (2025). Evaluation of aqueous chlorine and peracetic acid sanitizers to inactivate protozoa and bacteria of concern in agricultural water. *Applied and Environmental Microbiology*, *91*(1). https://doi.org/10.1128/aem.01653-24

Melo, J., & Quintas, C. (2023). Minimally processed fruits as vehicles for foodborne pathogens. *AIMS Microbiology*, *9*(1), 1–19. https://doi.org/10.3934/microbiol.2023001

Mendez, E. A., Tande, B., Vipham, J., & Trinetta, V. (2022). Preliminary Investigation of the Effect of Chemical Sanitizers and UV-C Light on Listeria monocytogenes Biofilm Survivability. *Food Protection Trends*, 278–283. https://doi.org/10.4315/fpt-21-025

Mendoza, I. C., Luna, E. O., Pozo, M. D., Vásquez, M. V., Montoya, D. C., Moran, G. C., Romero, L. G., Yépez, X., Salazar, R., Romero-Peña, M., & León, J. C. (2022).

Conventional and non-conventional disinfection methods to prevent microbial contamination in minimally processed fruits and vegetables. *LWT*, *165*, 113714. https://doi.org/10.1016/j.lwt.2022.113714

Mensah, A. A., Lewis Ivey, M. L., Moodispaw, M. R., & Ilic, S. (2024). Effectiveness of Chemical Sanitizers against Salmonella Typhimurium in Nutrient Film Technique (NFT) Hydroponic Systems: Implications for Food Safety, Crop Quality, and Nutrient Content in Leafy Greens. *Foods*, *13*(12), 1929. https://doi.org/10.3390/foods13121929

Micallef, S. A. (2023). Advances in understanding contamination of fresh produce by Salmonella. In Rutgers University, USA & M. Karl R. (Eds.), *Burleigh Dodds Series in Agricultural Science* (pp. 3–32). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2023.0121.01

Mickos, V. P., Blanchard, C., Pizzo, J. S., Kitchens, S., Price, S., Wells, D., & Rodrigues, C. (2025). Controlling Salmonella enterica in Water-recirculating Systems for Lettuce Production Using a Bacteriophage Cocktail. *HortScience*, *60*(8), 1319–1325. https://doi.org/10.21273/hortsci18643-25

Min, S. C., Roh, S. H., Boyd, G., Sites, J. E., Uknalis, J., Fan, X., & Niemira, B. A. (2017). Inactivation of Escherichia coli O157:H7 and Aerobic Microorganisms in Romaine Lettuce Packaged in a Commercial Polyethylene Terephthalate Container Using Atmospheric Cold Plasma. *Journal of Food Protection*, *80*(1), 35–43. https://doi.org/10.4315/0362-028x.jfp-16-148

Mina, H., Sarria, D., & Deering, A. (2025). Understanding the Impact of Organic Matter on Free Available Chlorine (FAC) Concentration in Postharvest Water. *Facts for Fancy Fruit*. https://fff.hort.purdue.edu/article/understanding-the-impact-of-organic-matter-on-free-available-chlorine-fac-concentration-in-postharvest-water/

Mokhtari, A., Pang, H., Santillana Farakos, S., Davidson, G. R., Williams, E. N., & Van Doren, J. M. (2022). Evaluation of Potential Impacts of Free Chlorine during Washing of Fresh-Cut Leafy Greens on *Escherichia coli* O157:H7 Cross-Contamination and Risk of Illness. *Risk Analysis*, *42*(5), 966–988. https://doi.org/10.1111/risa.13818

Moore, G., & Griffith, C. (2002). A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiology*, *19*(1), 65–73. https://doi.org/10.1006/fmic.2001.0464

Møretrø, T., & Langsrud, S. (2017). Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1022–1041. https://doi.org/10.1111/1541-4337.12283

Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety*, *1*(4), 289–301. https://doi.org/10.1093/fqsafe/fyx027

Myszka, K., & Czaczyk, K. (2011). Bacterial Biofilms on Food Contact Surfaces—A Review. *Polish Journal of Food and Nutrition Sciences*, *61*(3), 173–180. https://doi.org/10.2478/v10222-011-0018-4

Neri-Numa, I. A., Soriano Sancho, R. A., Pereira, A. P. A., & Pastore, G. M. (2018). Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. *Food Research International*, *103*, 345–360. https://doi.org/10.1016/j.foodres.2017.10.053

Nourzad, S., Naghdi Badi, H., Kalateh Jari, S., Mehrafarin, A., & Saeidi-Sar, S. (2024). Investigation of the qualitative and appearance characteristics of *Eryngium caeruleum* L. based on colorimetric and browning indices in storage conditions. *Food Science & Nutrition*, *12*(9), 6690–6698. https://doi.org/10.1002/fsn3.4243

Oh, J. K., Kohli, N., Zhang, Y., Min, Y., Jayaraman, A., Cisneros-Zevallos, L., & Akbulut, M. (2016). Nanoporous aerogel as a bacteria repelling hygienic material for healthcare environment. *Nanotechnology*, *27*(8), 085705. https://doi.org/10.1088/0957-4484/27/8/085705

Oh, J. K., Liu, S., Jones, M., Yegin, Y., Hao, L., Tolen, T. N., Nagabandi, N., Scholar, E. A., Castillo, A., Taylor, T. M., Cisneros-Zevallos, L., & Akbulut, M. (2019). Modification of aluminum surfaces with superhydrophobic nanotextures for enhanced food safety and hygiene. *Food Control*, *96*, 463–469. https://doi.org/10.1016/j.foodcont.2018.10.005

Oh, J. K., Rapisand, W., Zhang, M., Yegin, Y., Min, Y., Castillo, A., Cisneros-Zevallos, L., & Akbulut, M. (2016). Surface modification of food processing and handling gloves for enhanced food safety and hygiene. *Journal of Food Engineering*, *187*, 82–91. https://doi.org/10.1016/j.jfoodeng.2016.04.018

Oh, J. K., Yegin, Y., Yang, F., Zhang, M., Li, J., Huang, S., Verkhoturov, S. V., Schweikert, E. A., Perez-Lewis, K., Scholar, E. A., Taylor, T. M., Castillo, A., Cisneros-Zevallos, L., Min, Y., & Akbulut, M. (2018). The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-35343-1

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, *32*(1), 1–19. https://doi.org/10.1016/j.fm.2012.04.016

Osek, J., Lachtara, B., & Wieczorek, K. (2022). Listeria monocytogenes – How This Pathogen Survives in Food-Production Environments? *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.866462

Pabst, C. R., Kharel, K., De, J., Bardsley, C. A., Bertoldi, B., & Schneider, K. R. (2024). Evaluating the efficacy of peroxyacetic acid in preventing Salmonella cross-contamination on tomatoes in a model flume system. *Heliyon*, *10*(10), e31521. https://doi.org/10.1016/j.heliyon.2024.e31521

Pahariya, P., Choudhary, R., & Fisher, D. J. (2019). Antimicrobial Effect of Sanitizing Solutions on Fresh Romaine Lettuce. *2019 Boston, Massachusetts July 7- July 10,*

*2019*. 2019 Boston, Massachusetts July 7- July 10, 2019. https://doi.org/10.13031/aim.201901623

Palumbo, M., Harris, L. J., & Danyluk, M. D. (2013). Outbreaks of Foodborne Illness Associated with Common Berries, 1983 through May 2013. *EDIS*, *2013*(11). https://doi.org/10.32473/edis-fs232-2013

Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anciso, J., & Ivanek, R. (2012). Risk Factors for Microbial Contamination in Fruits and Vegetables at the Preharvest Level: A Systematic Review. *Journal of Food Protection*, *75*(11), 2055–2081. https://doi.org/10.4315/0362-028x.jfp-12-160

Pickens, J., Wells, D., & Blanchard, C. (2022, August 5). *Greenhouse Lettuce Production*. Alabama Extension. https://www.aces.edu/blog/topics/crop-production/greenhouse-lettuce-production/?utm_source=chatgpt.com

Pierrat, X., Wong, J. P. H., Al-Mayyah, Z., & Persat, A. (2021). The Mammalian Membrane Microenvironment Regulates the Sequential Attachment of Bacteria to Host Cells. *mBio*, *12*(4). https://doi.org/10.1128/mbio.01392-21

Pironti, C., Dell'Annunziata, F., Giugliano, R., Folliero, V., Galdiero, M., Ricciardi, M., Motta, O., Proto, A., & Franci, G. (2021). Comparative analysis of peracetic acid (PAA) and permaleic acid (PMA) in disinfection processes. *Science of The Total Environment*, *797*, 149206. https://doi.org/10.1016/j.scitotenv.2021.149206

Pizzo, J. S., Pelvine, R. A., Da Silva, A. L. B. R., Mikcha, J. M. G., Visentainer, J. V., & Rodrigues, C. (2023). Use of Essential Oil Emulsions to Control Escherichia coli

O157:H7 in the Postharvest Washing of Lettuce. *Foods*, *12*(13), 2571. https://doi.org/10.3390/foods12132571

Poimenidou, S. V., Bikouli, V. C., Gardeli, C., Mitsi, C., Tarantilis, P. A., Nychas, G.-J., & Skandamis, P. N. (2016). Effect of single or combined chemical and natural antimicrobial interventions on Escherichia coli O157:H7, total microbiota and color of packaged spinach and lettuce. *International Journal of Food Microbiology*, *220*, 6–18. https://doi.org/10.1016/j.ijfoodmicro.2015.12.013

Privett, B. J., Youn, J., Hong, S. A., Lee, J., Han, J., Shin, J. H., & Schoenfisch, M. H. (2011). Antibacterial Fluorinated Silica Colloid Superhydrophobic Surfaces. *Langmuir*, *27*(15), 9597–9601. https://doi.org/10.1021/la201801e

Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-Silleras, B., & Redondo-Del-Río, M. P. (2019). Food Safety through Natural Antimicrobials. *Antibiotics*, *8*(4), 208. https://doi.org/10.3390/antibiotics8040208

Ren, T., Yang, M., Wang, K., Zhang, Y., & He, J. (2018). CuO Nanoparticles-Containing Highly Transparent and Superhydrophobic Coatings with Extremely Low Bacterial Adhesion and Excellent Bactericidal Property. *ACS Applied Materials & Interfaces*, *10*(30), 25717–25725. https://doi.org/10.1021/acsami.8b09945

Ribera, L. A., & Young, L. K. (2024). *Outlook of Fresh Fruits and Vegetables in the United States* (CNAS Report 2024-01). https://www.freshproduce.com/siteassets/files/advocacy/2024.01.outlook-of-fresh-fruits-and-vegetables-in-the-united-states-luis-final.pdf

Riemenschneider, P. (2017, January 24). Living lettuce takes root. *Produce Market Guide*. https://www.producemarketguide.com/news/living-lettuce-takes-root

Rosli, S. Z., Noranizan, M. A., Radu, S., Karim, R., Mohd Adzahan, N., Aadil, R. M., & Koh, P. C. (2022). Impact of sanitizer solutions on microbial reduction and quality of fresh-cut pennywort (Centella asiatica) leaves. *Journal of Food Science and Technology*, *59*(3), 1211–1220. https://doi.org/10.1007/s13197-021-05131-3

Rouphael, Y., & Colla, G. (2005). Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and growing seasons. *Scientia Horticulturae*, *105*(2), 177–195. https://doi.org/10.1016/j.scienta.2005.01.025

Ru, S., Coneva, E., Munoz, P., Ashrafi, H., Spencer, J., Bao, Y., & Babiker, E. (2023). *EXPANDING SOUTHERN HIGHBUSH BLUEBERRIES TO UNDERSERVED REGIONS OF SOUTHEASTERN U.S.* United States Department of Agriculture, National Agricultural Library. https://www.nal.usda.gov/research-tools/food-safety-research-projects/expanding-southern-highbush-blueberries-underserved-regions-southeastern-us

Ruzi, M., Celik, N., & Onses, M. S. (2022). Superhydrophobic coatings for food packaging applications: A review. *Food Packaging and Shelf Life*, *32*, 100823. https://doi.org/10.1016/j.fpsl.2022.100823

Sapers, G. (2005). Washing and Sanitizing Treatments for Fruits and Vegetables. In J. Gorny, A. Yousef, & G. Sapers (Eds.), *Microbiology of Fruits and Vegetables* (pp. 375–400). CRC Press. https://doi.org/10.1201/9781420038934.ch17

Sapers, G. M., Gorny, J. R., & Yousef, A. E. (Eds.). (2005). *Microbiology of Fruits and Vegetables* (0 ed.). CRC Press. https://doi.org/10.1201/9781420038934

Sawadgo, W. (2024). *Southern Berry Farms Continue to Grow*. Souther Region Small Fruit Consortium. https://smallfruits.org/2024/07/southern-berry-farms-continue-to-grow/

Schaut, R. G., Boggiatto, P. M., Loving, C. L., & Sharma, V. K. (2019). Cellular and Mucosal Immune Responses Following Vaccination with Inactivated Mutant of Escherichia coli O157:H7. *Scientific Reports*, *9*(1), 6401. https://doi.org/10.1038/s41598-019-42861-z

Schifferstein, H. N. J., Wehrle, T., & Carbon, C.-C. (2019). Consumer expectations for vegetables with typical and atypical colors: The case of carrots. *Food Quality and Preference*, *72*, 98–108. https://doi.org/10.1016/j.foodqual.2018.10.002

Schumann-Muck, F. M., Hillig, N., Braun, P. G., Griebel, J., & Koethe, M. (2023). Impact of nanoscale coating of stainless steel on *Salmonella* Enteritidis and *Escherichia coli*. *Journal of Food Safety*, *43*(5), e13075. https://doi.org/10.1111/jfs.13075

Sciscenko, I., Vione, D., & Minella, M. (2024). Infancy of peracetic acid activation by iron, a new Fenton-based process: A review. *Heliyon*, *10*(5), e27036. https://doi.org/10.1016/j.heliyon.2024.e27036

Sela Saldinger, S., Rodov, V., Kenigsbuch, D., & Bar-Tal, A. (2023). Hydroponic Agriculture and Microbial Safety of Vegetables: Promises, Challenges, and Solutions. *Horticulturae*, *9*(1), 51. https://doi.org/10.3390/horticulturae9010051

Singh, P., Hung, Y., & Qi, H. (2018). Efficacy of Peracetic Acid in Inactivating Foodborne Pathogens on Fresh Produce Surface. *Journal of Food Science*, *83*(2), 432–439. https://doi.org/10.1111/1750-3841.14028

Stadler, E., & Fischer, U. (2020). Sanitization of Oak Barrels for Wine—A Review. *Journal of Agricultural and Food Chemistry*, *68*(19), 5283–5295. https://doi.org/10.1021/acs.jafc.0c00816

Stein, M. (2024, February 29). Refrigerate lettuce to reduce risk of E. coli contamination, researchers say. *College of Agricultural, Consumer & Environmental Science - UIUC*. https://aces.illinois.edu/news/refrigerate-lettuce-reduce-risk-e-coli-contamination-researchers-say#:~:text=coli%20O157:H7%20to%20compare,bacterial%20growth%2C%E2%80%9D%20Dong%20explained.

Stopforth, J. D., Ikeda, J. S., Kendall, P. A., & Sofos, J. N. (2004). Survival of acid-adapted or nonadapted Escherichia coli O157:H7 in apple wounds and surrounding tissue following chemical treatments and storage. *International Journal of Food Microbiology*, *90*(1), 51–61. https://doi.org/10.1016/S0168-1605(03)00171-5

Strawn, L. K., Fortes, E. D., Bihn, E. A., Nightingale, K. K., Gröhn, Y. T., Worobo, R. W., Wiedmann, M., & Bergholz, P. W. (2013). Landscape and Meteorological Factors

Affecting Prevalence of Three Food-Borne Pathogens in Fruit and Vegetable Farms. *Applied and Environmental Microbiology*, *79*(2), 588–600. https://doi.org/10.1128/AEM.02491-12

Takeda, F., Li, C., DeVetter, L. W., Williamson, J., Sargent, S., & Yang, W. Q. (2021, May 11). *Transitioning from Hand to Machine Harvesting of Blueberries for Fresh Market | Progressive Crop Consultant*. https://progressivecrop.com/2021/05/11/transitioning-from-hand-to-machine-harvesting-of-blueberries-for-fresh-market/

Teng, Z., Luo, Y., Alborzi, S., Zhou, B., Chen, L., Zhang, J., Zhang, B., Millner, P., & Wang, Q. (2018). Investigation on chlorine-based sanitization under stabilized conditions in the presence of organic load. *International Journal of Food Microbiology*, *266*, 150–157. https://doi.org/10.1016/j.ijfoodmicro.2017.11.027

Topalcengiz, Z., Chandran, S., & Gibson, K. E. (2024). A comprehensive examination of microbial hazards and risks during indoor soilless leafy green production. *International Journal of Food Microbiology*, *411*, 110546. https://doi.org/10.1016/j.ijfoodmicro.2023.110546

Ukuku, D. O., Bari, L., & Kawamoto, S. (2012). Hydrogen Peroxide. In V. M. Gómez-López (Ed.), *Decontamination of Fresh and Minimally Processed Produce* (1st ed., pp. 197–214). Wiley. https://doi.org/10.1002/9781118229187.ch11

Ukuku, D. O., Bari, M. L., Kawamoto, S., & Isshiki, K. (2005). Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of

bacterial pathogens from whole melon surfaces to fresh-cut pieces. *International Journal of Food Microbiology*, *104*(2), 225–233. https://doi.org/10.1016/j.ijfoodmicro.2005.01.016

Unruh, D. A., Stull, K. J., Pliakoni, E. D., & Gragg, S. E. (2021). A Bisulfate of Soda and Peroxyacetic Acid Solution Reduces Salmonella on Fresh-Cut Spinach. *Food Protection Trends*, *41*(4), 409–415.

USDA. (2017). *Microbial quality of blueberries and hygiene conditions of fresh blueberry packing lines—UNIVERSITY OF GEORGIA*. National Institue of Food and Agriculture. https://portal.nifa.usda.gov/web/crisprojectpages/1014538-microbial-quality-of-blueberries-and-hygiene-conditions-of-fresh-blueberry-packing-lines.html

USDA. (2023). *U.S. lettuce production shifts regionally by season*. https://www.ers.usda.gov/data-products/charts-of-note/chart-detail?chartId=106516#:~:text=Lettuce%E2%80%94the%20main%20ingredient%20in,%2C%20and%20community%2Dsupported%20agriculture.

USDA. (2025). *Vegetables 2024 Summary*. https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v86p/v405v633x/tt44rh680/vegean25.pdf

USDA FSIS. (2015). *RESPONSE TO QUESTIONS POSED BY THE DEPARTMENT OF DEFENSE REGARDING MICROBIOLOGICAL CRITERIA AS INDICATORS OF PROCESS CONTROL OR INSANITARY CONDITIONS*. https://www.fsis.usda.gov/sites/default/files/media_file/2020-

12/Response%20to%20Questions%20Posed%20By%20the%20Department%20of%20Defense%20Regarding%20Microbiological%20Criteria%20As%20Indicators%20of%20Process%20Control%20or%20Insanitary%20Conditions%20.pdf

USDA-NASS. (2024). *Census of Agriculture*. United States Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/Publications/AgCensus/2022/Full_Report/Volume_1,_Chapter_2_US_State_Level/

Valloton, A., Bardsley, C., Edwards, A., & Strawn, L. (2021). *Assessing On-Farm Produce Safety Risks: Production Stage*. https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/FST/fst-403/FST-403.pdf

Valloton, A., Bardsley, C., Edwards, A., & Strawn, L. K. (2022, January 20). *Assessing On-Farm Produce Safety Risks: Post-Harvest Handling Stage*. https://pubs.ext.vt.edu/content/pubs_ext_vt_edu/en/FST/FST-405/FST-405.html

Van Boxstael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van De Perre, E., Rajkovic, A., Lopez-Galvez, F., Sampers, I., Spanoghe, P., De Meulenaer, B., & Uyttendaele, M. (2013). Food safety issues in fresh produce: Bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control*, *32*(1), 190–197. https://doi.org/10.1016/j.foodcont.2012.11.038

Wadamori, Y., Gooneratne, R., & Hussain, M. A. (2017). Outbreaks and factors influencing microbiological contamination of fresh produce. *Journal of the Science of Food and Agriculture*, *97*(5), 1396–1403. https://doi.org/10.1002/jsfa.8125

Waitt, J. A., Kuhn, D. D., Welbaum, G. E., & Ponder, M. A. (2014). Postharvest transfer and survival of *Salmonella enterica* serotype enteritidis on living lettuce. *Letters in Applied Microbiology*, *58*(2), 95–101. https://doi.org/10.1111/lam.12170

Wan Ikhsan, S. N., Yusof, N., Aziz, F., Ismail, A. F., Jaafar, J., Wan Salleh, W. N., & Misdan, N. (2021). Superwetting materials for hydrophilic-oleophobic membrane in oily wastewater treatment. *Journal of Environmental Management*, *290*, 112565. https://doi.org/10.1016/j.jenvman.2021.112565

Wang, B. X., Butler, D. S., Hamblin, M., & Monack, D. M. (2023). One species, different diseases: The unique molecular mechanisms that underlie the pathogenesis of typhoidal Salmonella infections. *Current Opinion in Microbiology*, *72*, 102262. https://doi.org/10.1016/j.mib.2022.102262

WHO. (2019). *Healthy diet WHO-EM/NUT/282/E*. https://iris.who.int/bitstream/handle/10665/325828/EMROPUB_2019_en_23536.pdf

WHO. (2025). *Healthy diet*. https://www.who.int/initiatives/behealthy/healthy-diet

Xi, F., Huang, Y., Zhao, Y., Liu, Y., Dai, W., & Tian, Y. (2022). Effects of Substrate Roughness on Microstructure and Fatigue Behavior of Plasma Electrolytic

Oxidation-Coated Ti-6Al-4V Alloy. *Materials (Basel, Switzerland)*, *15*(12), 4256. https://doi.org/10.3390/ma15124256

Xu, Q. F., Liu, Y., Lin, F.-J., Mondal, B., & Lyons, A. M. (2013). Superhydrophobic $TiO_2$ – Polymer Nanocomposite Surface with UV-Induced Reversible Wettability and Self-Cleaning Properties. *ACS Applied Materials & Interfaces*, *5*(18), 8915–8924. https://doi.org/10.1021/am401668y

Yan, M., Ge, H., & Gomez, M. (2023). Risk Management Strategy of Food Safety: The Case of the US Fresh Produce Supply Chain. *Agricultural & Applied Economics Association*, *335580*. https://ideas.repec.org/p/ags/aaea22/335580.html#download

Yemmireddy, V., Adhikari, A., & Moreira, J. (2022). Effect of ultraviolet light treatment on microbiological safety and quality of fresh produce: An overview. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.871243

Yoon, J.-H., & Lee, S.-Y. (2018). Review: Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. *Critical Reviews in Food Science and Nutrition*, *58*(18), 3189–3208. https://doi.org/10.1080/10408398.2017.1354813

Zhang, Z.-X., Li, Y., Ye, M., Boonkerd, K., Xin, Z., Vollmer, D., Kim, J. K., & Deng, X. (2014). Fabrication of superhydrophobic surface by a laminating exfoliation method. *J. Mater. Chem. A*, *2*(5), 1268–1271. https://doi.org/10.1039/C3TA14204C

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J., & Hwang, G. (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions

on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.643722

Zoellner, C., Aguayo-Acosta, A., Siddiqui, M. W., & Dávila-Aviña, J. E. (2018). Peracetic Acid in Disinfection of Fruits and Vegetables. In *Postharvest Disinfection of Fruits and Vegetables* (pp. 53–66). Elsevier. https://doi.org/10.1016/b978-0-12-812698-1.00002-9

# Chapter 2

## 2.0 Research Justification

This research aims to establish food safety practices for the harvest and postharvest handling of fresh produce. Fresh produce faces various challenges during processing. The most significant concern is that many fruits and vegetables are consumed raw, meaning there is no effective step to eliminate foodborne pathogens that may be present. On the farm, contamination can originate from multiple sources, including soil, water, animals, humans, and equipment (Strawn et al., 2013), which can lead to cross-contamination of the final product.

In 1998, the FDA named harvesting machinery, knives, containers, tables, baskets, packaging material, buckets, etc., as field equipment that can easily spread microorganisms to fresh produce, and recommends keeping harvesting and packing equipment as clean as practicable (FDA, 1998). For small/medium operations, many commodities are harvested with hand-held tools and placed by hand into containers or onto mechanized harvest-aide equipment, surface, or conveyor belts. As a result, contaminated or insufficiently sanitized food-contact surfaces are one of the main pathways for bacterial contamination and cross-contamination, leading to foodborne illnesses (Oh et al., 2019).

For instance, one proposed practice at the harvest level is the application of superhydrophobic coatings on food-contact surfaces. These coatings are primarily known for their anti-biofouling and anti-adhesive properties, which help repel water-based residues, spills, and both gram-positive and gram-negative bacteria. Additionally, these coatings can

help reduce water consumption during the cleaning process of these surfaces. (DeFlorio et al., 2021).

Safety practices must be maintained throughout the supply chain, especially during postharvest handling. Most produce is washed after harvest, but it is crucial to note that postharvest water can become contaminated by the produce that comes into contact with it, even if the water is clean at the start (Valloton et al., 2022). One effective measure is to add a specific amount of an antimicrobial agent to all batch or bulk water. While adding a sanitizer does not clean each individual piece of produce, it helps prevent cross-contamination from the water to the produce by minimizing the buildup of pathogens and other microorganisms in the water (Cornell CALS, 2015). However, there is still a gap in knowledge regarding how these antimicrobial agents may affect the harvest and postharvest quality of crops. However, there is a growing consumer trend toward purchasing local produce, such as living lettuce, which is sold with its roots attached to extend shelf life. Although this practice is believed to offer a longer shelf life and promote sustainability, it raises food safety concerns regarding the potential for cross-contamination.

These approaches offers both valuable and sustainable solutions for growers to prevent foodborne illnesses associated with fresh produce. The use of superhydrophobic coatings is expected to reduce the microbial load that attaches to the surface of crops, like blueberry and other small fruits. These coatings can be marketed to growers, allowing them to optimize both their time and budget. While the use of antimicrobial agents (chlorine, PAA and $H_2O_2$) are a promising strategy to reduce the cross-contamination risks on the roots of living lettuce. Incorporating a sanitizing step in the postharvest washing process could

become a standard practice for this type of produce. However, research on the use of sanitizers specifically for the roots of living lettuce is still limited. This initiative not only aims to fill this knowledge gap but also supports the efforts to ensure the microbial safety of living lettuce for consumers.

## 2.1 References


Cornell CALS. (2015). *Postharvest Water*. https://cals.cornell.edu/national-good-agricultural-practices-program/resources/educational-materials/decision-trees/postharvestwater#:~:text=A%20number%20of%20chemical%20sanitizers,or%20if%20you%20have%20questions.

DeFlorio, W., Liu, S., White, A. R., Taylor, T. M., Cisneros-Zevallos, L., Min, Y., & Scholar, E. M. A. (2021). Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 3093–3134. https://doi.org/10.1111/1541-4337.12750

FDA. (1998). *Guidance for industry: Guide to minimize microbial food safety hazards for fresh fruits and vegetables*. https:// www.fda.gov/regulatory-information/ search-fda-guidance-documents/guid ance-industry-guide-minimize-microbi al-food-safety-hazards-fresh-fruits-and-veg etables

Oh, J. K., Liu, S., Jones, M., Yegin, Y., Hao, L., Tolen, T. N., Nagabandi, N., Scholar, E. A., Castillo, A., Taylor, T. M., Cisneros-Zevallos, L., & Akbulut, M. (2019). Modification of aluminum surfaces with superhydrophobic nanotextures for

enhanced food safety and hygiene. *Food Control*, *96*, 463–469. https://doi.org/10.1016/j.foodcont.2018.10.005

Strawn, L. K., Fortes, E. D., Bihn, E. A., Nightingale, K. K., Gröhn, Y. T., Worobo, R. W., Wiedmann, M., & Bergholz, P. W. (2013). Landscape and Meteorological Factors Affecting Prevalence of Three Food-Borne Pathogens in Fruit and Vegetable Farms. *Applied and Environmental Microbiology*, *79*(2), 588–600. https://doi.org/10.1128/AEM.02491-12

Valloton, A., Bardsley, C., Edwards, A., & Strawn, L. K. (2022, January 20). *Assessing On-Farm Produce Safety Risks: Post-Harvest Handling Stage*. https://pubs.ext.vt.edu/content/pubs_ext_vt_edu/en/FST/FST-405/FST-405.html

# Chapter 3

## 3.0 Objectives

The main objective of this research is to investigate the use of superhydrophobic coatings and sanitizers as a control for foodborne pathogens in a simulated harvest and postharvest washing step, respectively. The specific objectives of this project are:

**Objective 1** – Evaluate the efficacy of two nano-textured coatings, one hydrophobic and one superhydrophobic, in reducing *E. coli* O157:H7, total coliforms, aerobic bacteria, and yeasts and molds on HDPE food-contact surfaces during a simulated blueberry harvest.

**Research hypothesis –** The superhydrophobic coating will significantly reduce microbial attachment and survival (*E. coli* O157:H7, total coliforms, aerobic bacteria, and yeasts and molds) on HDPE food-contact surfaces, compared to the hydrophobic coating and untreated control during a simulated blueberry harvest. This is due to its higher water repellency and lower surface energy, which limit microbial adhesion more effectively.

**Objective 2** – Evaluate the efficacy of chemical sanitizers (chlorine, PAA and $H_2O_2$) in reducing *Salmonella enterica* from living lettuce roots with and without media plugs and their impact on the leaf physiology and weight loss

**Research hypothesis –** PAA and chlorine are expected to be the most effective sanitizers for reducing *S. enterica* on living lettuce roots, both with and without a media plug. PAA is likely to perform slightly better due to its stability in organic matter. Additionally, minimal effects on leaf physiology and weight loss are anticipated across all treatments.

# Chapter 4

## 4.0 Evaluation of the Efficacy of Hydrophobic and Superhydrophobic Coatings in Reducing E. coli O157:H7 Attachment in Simulated Blueberry Harvest Contamination

### 4.1 Abstract

Blueberry production has increased across both large- and small-scale operations. Unlike many other crops, blueberries are typically not subjected to conventional washing, increasing the risk of microbial contamination during harvest. Hydrophobic coatings have recently gained attention for their ability to repel water, soil, and microorganisms, offering potential applications in food safety. This study evaluated the efficiency of two nano-textured coatings (a commercial fluorinated formulation and an experimental silica-based coating) in reducing microbial adhesion and transfer on high-density polyethylene (HDPE) bucket surfaces during simulated blueberry harvest. Inoculated blueberries were placed into coated and uncoated buckets, and microbial populations were assessed at four sampling points: pre-wash bucket surface, wash water, post-washed blueberries, and post-wash bucket surface. The post-wash bucket surfaces were further evaluated at 0, 6, 9, 12, and 24 h to assess microbial persistence over time. The commercial coating significantly reduced *Escherichia coli* O157:H7 on post-washed blueberries to 4.62 log CFU/g, and achieved levels below the limit of detection (LOD) on post-wash bucket surfaces within 6 h (4 log CFU/g reduction). Similar reductions were observed for total coliforms and aerobic bacteria by 24 h. In contrast, the experimental coating showed limited and inconsistent antimicrobial activity. Yeast and mold populations were also higher on surfaces treated with experimental than commercial coating, suggesting structural imperfections that may have supported yeast and mold growth.

These findings demonstrate the potential of nano-textured hydrophobic coatings to reduce bacterial cross-contamination on food-contact surfaces during harvest. Further research is needed to optimize formulation performance and validate efficacy under commercial field conditions.

**Keywords:** food safety, harvest, blueberries, *E. coli* O157:H7*,* nano-textured coatings, food-contact surface.

## 4.2 Introduction

Blueberries (*Vaccinium corymbosum*) are increasingly cultivated worldwide due to their recognized nutritional value, antioxidant content, and consumer appeal (Amodu et al., 2025). Global production has tripled over the past decade, increasing from 327,8666 metric tons in 2010 to 1,113,260 metric tons in 2021, driven by rising demand and improvements in postharvest quality management (Amodu et al., 2025). In the United States (U.S.), blueberry cultivation continues to expand, including in southeastern states (Ru et al., 2023). In Alabama, blueberries are the second-largest fruit crop by planted area and play an important economic role for local growers, despite the relatively small acreage dedicated to their production (USDA-NASS, 2024).

Harvest methods vary depending on the scale of the operation with large commercial farms often utilizing mechanical harvesters (Takeda et al., 2021).While medium to small size operations continue to rely on hand-picking, which involves direct contact and friction with fruit clusters (Dai et al., 2025). This variability in handling practices underscores the importance of addressing food safety risks associated with harvest-related cross-

contamination. Unlike many other fruits, fresh blueberries are typically packaged without undergoing a water-based washing step. Excess moisture can promote mold growth and reduce shelf life (Dagnas & Membré, 2013). Instead, blueberries are often cleaned using air blowers that remove debris such as leaves and stems (Ells et al., 2024). Therefore, ensuring hygienic condition of harvest and postharvest handling equipment is essential for minimizing risks of microbial contamination (FDA, 2018; Leaman et al., 2023). In recent years, foodborne outbreaks and recalls associated with blueberries have become more frequent. In 2009 and 2010 was reported *Salmonella* Muenchen and Newport (USDA, 2017) (Palumbo et al., 2013). Such findings reinforce the need for preventive strategies targeting contamination at the point of harvest.

Contact surfaces such as harvest buckets, bins, and tools play a critical role in microbial transfer. To mitigate this risk, interest has grown in applying protective coatings to food-contact surfaces using anti-biofouling and anti-adhesive materials (Oh et al., 2019). These coatings often rely on superhydrophobic properties, defined by water droplet contact angles greater than 150° to repel water, dirt, and microorganisms, in which, exhibits Cassie-Baxter wetting behavior (Bayer, 2020). The Cassie-Baxter model is characterized by a layer of air trapped at the interface between the solid substrate surface and the aqueous phase, where planktonic bacteria reside, creating a physical barrier to cell absorption (DeFlorio et al., 2023).

Nano-textured hydrophobic coatings, in particular, have demonstrated potential to reduce microbial loads and prolong shelf life by limiting the adhesion and survival of spoilage organisms and foodborne pathogens (DeFlorio et al., 2021; Oh et al., 2019; Quinto

et al., 2019). However, there is limited research evaluating their performance on high-density polyethylene (HDPE), a common material used in harvest equipment, under practical, produce-harvesting conditions.

The objective of this study was to evaluate the efficacy of two nano-textured hydrophobic coatings, one commercial (fluorinated) and one experimental (silica-based), in reducing *E. coli* O157:H7, total coliforms, aerobic bacteria, and yeasts and molds on HDPE food-contact surfaces during a simulated blueberry harvest contamination. We hypothesize that superhydrophobic coating will significantly reduce microbial attachment and survival on food-contact surfaces, compared to the hydrophobic coating and untreated control. The results aim to inform practical intervention strategies for minimizing microbial contamination during the harvest of minimally processed fruits.

## 4.3 Material and methods

### *4.3.1 Preparation of coatings*

#### *4.3.1.1 Commercial coating*

The commercial superhydrophobic coating WX2100 (CYTONIX, Silver Spring, MD, USA) was applied to 3.785 L HDPE buckets (ULINE, Pleasant Prairie, WI, USA) following the manufacturer's instructions. Prior to application, the spray can was shaken vigorously for 2 min. During application, it was held vertically approximately 20 cm from the bucket's internal surface and shaken every 15 s to ensure consistent mixing. The internal surface was evenly coated and allowed to cure at room temperature (25 ± 1 °C) for 10 days before hydrophobicity assessment.

*4.3.1.2 Experimental coating*

The experimental coating was developed by the Artie McFerrin Department of Chemical Engineering at Texas A&M University (College Station, TX, USA). Silica nanoparticles were synthesized by dissolving 1 g of fused silica (Sigma-Aldrich, St. Louis, MO, USA) and 1.3 g of octadecyltrichlorosilane (95%; Thermo Fisher Scientific Inc, Waltham, MA, USA) in 200 mL of hexane (VWR International, Radnor, PA, USA). The mixture was magnetically stirred at 500 RPM for 24 h at room temperature (25 ± 1 °C) using a hot plate stirrer (Thermolyne Cimarec Model #S131435, American Laboratory Trading, East Lyme, CT, USA). After stirring, the solution was transferred to 50 mL conical tubes (VWR International, Radnor, PA, USA) and centrifuged at 12,298 RCF for 30 min to precipitate the silica particles. The precipitate was isolated and washed three times with hexane by vortexing for 1 min and centrifuging at 10,000 RPM for 8 min each time. The cleaned nanoparticles were transferred to a 200 mL beaker and dried overnight at 50 °C. Then, the dried nanoparticles were stored in a 60 mL HDPE bottle (VWR International, Radnor, PA, USA) until further use.

To prepare the coating solution, 10 g of a 1:1 (w/w) resin:hardener mixture (EasyPour Epoxy, Lexington, TN, USA) was dissolved in 10 mL of acetone (99.5%; Sigma-Aldrich, St. Louis, MO, USA) and sonicated for 1 h in an ultrasonic cleaner (Morantz Ultrasonics, Philadelphia, PA, USA) to fully dissolve the resin. Afterward, 2 g of 1-octadecylamine (97%; Thermo Fisher Scientific Inc., Waltham, MA, USA) was added, and the mixture was sonicated for an additional hour. Finally, 0.3 g of the dried hydrophobic silica nanoparticles

were added to the mixture, and the suspension was sonicated for another 30 min to ensure uniform dispersion.

The experimental coating was applied to the internal surface of the bucket using a paint brush (Linzer Products Corp.; West Babylon, NY, USA). After the first layer was applied, it was cured for 24 h. A second layer was then applied and cured for 36 h prior to testing.

*4.3.2 Contact angle measurements*

To evaluate the surface wetting characteristics of the coated bucket surfaces, static water contact angles were measured using the sessile drop technique, following the method described by Oh et al. (2019). A 5 µL water droplet was placed on the coated bucket surface using a micropipette, and droplet images were captured with a digital camera. Contact angles were analyzed using Adobe Photoshop 2023 software (version 24.6.0). Surfaces with contact angles ≥ 150° were classified as superhydrophobic, while those < 150° were considered hydrophobic. Results were reported as the average of three independent measurements.

*4.3.3 Preparation of bacterial culture*

*E. coli* O157:H7 (American Type Culture Collection, ATCC 43895) was obtained from the Department of Horticulture at Auburn University (Auburn, AL, USA). To confer resistance, the strain was adapted to 50 ppm nalidixic acid (Sigma-Aldrich, Saint Louis, MO, USA) and 50 ppm rifampicin (Sigma-Aldrich, Saint Louis, MO, USA) through a stepwise adaptation procedure. Initially, a loopful of the culture was inoculated into tryptic soy broth (TSB; BD Difco, Sparks, MD, USA) with nalidixic acid concentrations incrementally

increased by 10 ppm at each transfer. Transfers were performed every 24 h in fresh TSB until resistance was achieved at 50 ppm. The same procedure was repeated for rifampicin adaptation, resulting in a strain resistant to both 50 ppm nalidixic acid and 50 ppm rifampicin in TSB (TSBRN). Subsequently, a 10 µL loopful of the antibiotic-adapted culture was transferred into fresh TSBRN and incubated for 24 h at 37 °C. This transfer step was repeated twice prior to use (Pizzo et al., 2023). The bacterial culture was adjusted to a McFarland scale of 0.5 using approximately 300 mL of phosphate-buffered solution (PBS) (Chazotte, 2012), resulting in a final *E. coli* O157:H7 concentration of approximately 1.5 x $10^8$ CFU/mL.

*4.3.4 Blueberry inoculation*

Fresh blueberries (*Vaccinium corymbosum*) were purchased from a local grocery store, rinsed with deionized water to remove dust and surface debris, and air-dried at room temperature (25 ± 1 °C) for 2 h. The blueberries were then placed on sterilized aluminum foil trays and exposed to ultraviolet (UV) light (254 nm) for 10 min on each side to reduce naturally occurring microorganisms. Following UV treatment, 18 oz of blueberries per treatment per replicate were transferred to sterile Whirl-Pak® bags (Nasco, Madison, WI, USA) and stored at 4 °C until inoculation. For inoculation, blueberries were submerged in the prepared *E. coli* O157:H7 inoculum for 2 min without agitation, using sterile tweezers to ensure complete surface coverage. Excess inoculum was removed using a sanitized salad spinner, and the inoculated blueberries were air-dried in a biosafety cabinet for 2 h. A 15 g sample was transferred to an uncoated bucket to serve as a control. All control samples were prepared in triplicate.

*4.3.5 Experimental design and bacterial adhesion assay*

The experiment was designed as a completely randomized design (CRD) with three treatments: a control (no coating), a commercial coating, and an experimental coating. Each treatment was replicated three times. Fresh blueberries were inoculated with *E. coli* O157:H7 and held for 2 h before being transferred to buckets for treatment, where the effectiveness of each coating was evaluated. At the start (0 h), samples were collected from four sources to assess initial bacterial adhesion: pre-wash surface swabs, wash water, berry surfaces (post-wash), and the post-wash bucket surface. From 6 to 24 h, only samples from the post-wash bucket surface were collected to monitor bacterial survival over time. Sampling occurred at five time points: 0, 6, 9, 12, and 24 h post-treatment. In total, 72 samples were analyzed: 36 samples at 0 h (4 sample types × 3 treatments × 3 replicates) and an additional 36 samples collected at 6, 9, 12, and 24 h (1 sample type × 3 treatments × 3 replicates × 4 time points). This design allowed for the evaluation of the coatings' effectiveness in reducing microbial contamination on blueberries and food-contact surfaces during harvest handling.

Six 25 $cm^2$ areas were marked on the bottom surface of each bucket and pre-rinsed with deionized water. The inoculated blueberries were then transferred into the buckets, positioned directly over the marked areas. The buckets were placed inside a biosafety cabinet for 2 h to allow bacterial interaction with the bucket surface. Following this period (defined as time point 0 h), four analyses were performed across different sample types: (1) one marked area in each bucket was swabbed using a sterile sponge stick pre-soaked in neutralizing buffer (NEOGEN®, Lansing, MI, USA) to assess surface *E. coli* O157:H7 populations pre-washing. (2) The buckets containing the inoculated blueberries were washed

with 1.89 L of deionized water. From this, 300 mL of the rinse water was collected and filtered using a membrane filtration method through a 47 mm diameter, 0.45 μm pore-size sterile filter (Pall Corporation, Ann Arbor, Michigan, USA) as described by Chevez et al. (2024). The filter was then plated on MaConkey Sorbitol Agar (Thermo Fisher Scientific Inc., Waltham, MA, USA) for microbial enumeration. (3) All blueberries were then transferred to sterile Whirl-Pak® bags, diluted 1:5 (w/v) in PBS containing 0.2% Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA) and 0.1% sodium thiosulfate (Fisher Scientific, Fair Lawn, NJ, USA), and processed using a stomacher (Stomacher® 400CIRCULATOR, Seward Inc, Bohemia, NY, USA) at 300 RPM for 30 s to detach bacterial cells from the fruit surface. (4) Another marked area in each bucket was swabbed post-washing using a sterile sponge stick to evaluate residual microorganisms.

All swab (bucket surfaces) and post-washed blueberry samples were serially diluted in buffered peptone water (BPW; Oxoid, CM 509; Fisher Scientific, Fair Lawn, NJ, USA) prior to microbiological analysis. Analysis at 0 h included *E. coli* O157:H7, total aerobic bacteria, total coliforms, yeast, and mold for both post-washed blueberries and post-wash bucket surfaces. Post-wash bucket surfaces were also analyzed for the same microorganisms at 6 h, 9 h, 12 h, and 24 h.

For *E. coli* O157:H7 enumeration, 100 μL of each dilution was spread plated onto MacConkey Sorbitol Agar (Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with 50 ppm nalidixic acid and 50 ppm rifampicin, using a sterile L-spreader. For yeast and mold, 100 μL of diluted samples were also spread plated onto Potato Dextrose Agar (PDA; Thermo Fisher Scientific Inc., Waltham, MA, USA). For aerobic and total

coliforms enumeration, 1 mL of each diluted sample was poured onto Petrifilm® (NEOGEN®, Lansing, MI, USA).

The MacConkey agar plates and Petrifilm® were incubated at 37 °C for 24 h, while PDA plates were incubated at 25 °C for 5 days. If no growth was observed on PDA after 5 days, incubation was extended for an additional 48 h. All treatments were conducted in triplicate, and results were expressed as log CFU/g.

*4.3.6 Statistical analysis*

SAS Studio 5.2 was used to perform all the statistical analyses. Specifically, a generalized linear model (GLM) was performed to address non-normal distributed data. For the time point 0 h, the microbial population data (CFU/g) were analyzed using treatment (control, commercial, and experimental) and sampling types (pre-wash bucket surfaces, wash water, and post-washed blueberries), and their interaction as fixed effects. For the post-wash bucket surfaces, the microbial population data (CFU/g) were analyzed using treatment (control, commercial, and experimental), time of storage (0 h, 6 h, 9 h, 12 h, and 24 h), and their interaction as fixed effects. For all analyses, least squares means comparisons were performed using Tukey's HSD post-hoc with a significance level of 0.05.

## 4.4 Results

### *4.4.1. Contact angle*

The static contact angle on an HDPE surface with the experimental coating was θ = 130° ± 1.67, indicating that this treatment demonstrated hydrophobic properties (Figure 4.1). In comparison, the water contact angle for the commercial coating was θ = 152.3° ± 0.84, which showed superhydrophobic properties on the surface of the bucket.

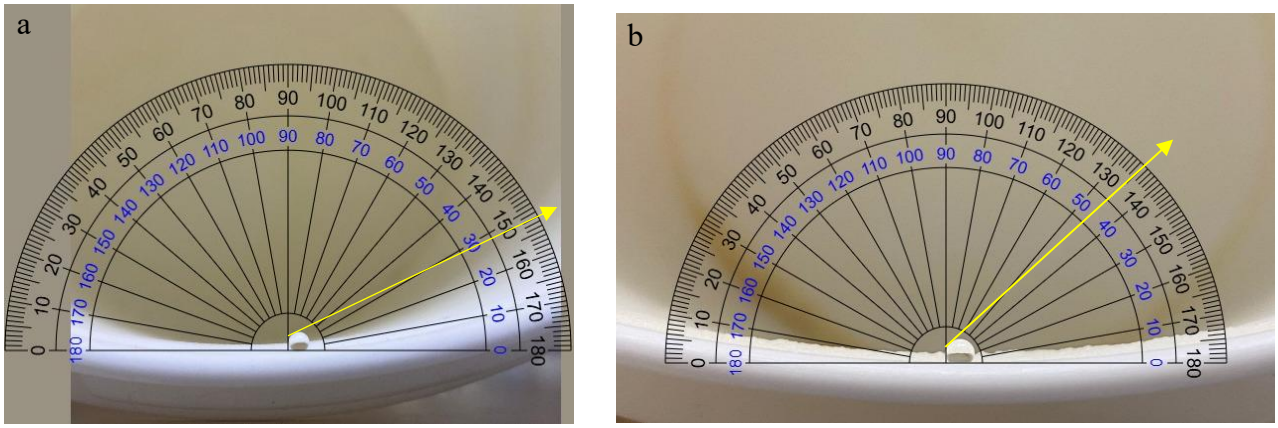


**Figure 4.1**. Measurement of contact angles for (a) the commercial coating and (b) the experimental coating.

### *4.4.2. Microbial populations on pre-wash bucket surfaces, wash water, and post-washed blueberries*

The microorganism populations recovered at 0 h from wash water, post-washed blueberries, and pre-wash bucket surfaces under different coating treatments are presented in Table 4.1. No statistically significant treatment ($p > 0.05$) was observed for blueberries, post-washed blueberries, or the wash water. However, the coatings treatments had a statistically significant effect ($p = 0.025$) on the *E. coli* O157:H7 population on the pre-wash surface (Figure 4.2).

At 0 h, *E. coli* O157:H7 populations on the pre-wash bucket surfaces ranged from 5.46 to 6.23 log CFU/cm². The experimental coating demonstrated the lowest initial surface contamination (5.46 log CFU/cm²), significantly lower than the control (6.23 ± 0.16 log CFU/cm²) and commercial (6.15 ± 0.16 log CFU/cm²) treatments ($p \leq 0.05$).

The *E. coli* O157:H7 populations on the wash water ranged from 5.54 to 5.68 log CFU/mL, with no significant differences ($p > 0.05$) observed between the treatments. In contrast, aerobic bacteria, total coliforms, and yeast and mold populations on the pre-wash bucket surfaces and wash water were below the limit of detection (LOD).

Following the washing step, *E. coli* O157:H7 populations on blueberries ranged from 4.62 to 5.13 log CFU/g. The commercial coating significantly reduced *E. coli* O157:H7 counts to 4.62 log CFU/g, compared to 5.13 log CFU/g in the experimental coating and 4.92 log CFU/g in the control treatment ($p \leq 0.05$). In contrast, populations of aerobic bacteria, total coliforms, and yeast and molds on post-washed blueberries ranged from 5.22 to 5.64 log CFU/g, with no statistically significant differences observed among treatments ($p > 0.05$).

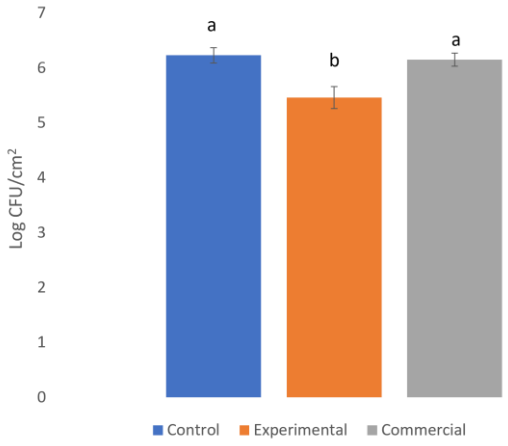

**Figure 4.2.** *E. coli* O157:H7 recovered at 0h from pre-wash surface bucket under different coating treatments. Similar lowercase letters indicate no statistically significant differences among treatments at the same time point ($p > 0.05$)

**Table 4.1**.Microorganism populations recovered at 0 h from wash water, post-washed blueberries, and pre-wash bucket surfaces under different coating treatments.

| Microorganism | Wash water (log CFU/mL) | Post-wash blueberry (log CFU/g) |
|---|---|---|
| *E. coli* O157:H7 | 5.61 ± 0.15 | 4.88 ± 0.08 |
| Aerobic bacteria | < LOD | 5.41 ± 0.03 |
| Total coliforms | < LOD | 5.35 ± 0.07 |
| Yeast and molds | < LOD | 5.50 ± 0.07 |

Values represent means ± standard error. No statistically significant differences among treatments for the same microorganism and sampling type ($p > 0.05$). Abbreviations: LOD - Limit of detection = < 1 log CFU/mL.

*4.4.3. Microbial populations on post-wash bucket surfaces over time*

Microbial populations on post-wash bucket surfaces were monitored at 0 h, 6 h, 9 h, 12 h, and 24 h to assess the persistence or reduction of microbial populations across treatments over time (Figure 4.3).

Aerobic bacteria populations varied significantly across time and treatments ($p \leq 0.05$; Figure 4.3a). At 0 h, bacteria loads ranged from 3.57 log CFU/cm² (commercial coating) to 5.78 log CFU/cm² (experimental coating), with no significant differences observed between the control and experimental treatments ($p > 0.05$). By 9 h, the commercial coating achieved a reduction of 1.08 log CFU/cm² compared to the control. This trend continued, with the commercial coating maintaining the lowest bacterial loads at each

subsequent time point and reaching no detectable levels at 24 h. In contrast, the experimental coating exhibited variable performance, with populations fluctuating between 3.54 log CFU/cm² at 6 h and 3.38 log CFU/cm² at 12 h, ultimately reaching undetectable levels by 24 h, similar to the commercial treatment. However, no significant differences were observed between the experimental and control treatments at any time point, except at 24 h ($p > 0.05$).

Total coliform populations were also significantly affected by both time and treatment ($p \leq 0.05$; Figure 4.3b). At 0 h, populations ranged from 3.42 log CFU/cm² for the commercial coating to 5.88 log CFU/cm² for the experimental coating. The commercial coating showed the highest reductions over time, achieving 1 to 2 log CFU/cm² decreases within the first 12 h compared to the control, and reaching no detectable levels by 24 h. In contrast, the experimental coating reduced coliform counts to 0.25 log CFU/cm² by 12 h compared to the control, but these reductions were not statistically different from the control at this time point ($p > 0.05$).

Yeast and mold populations were significantly influenced by both time and treatment ($p \leq 0.05$; Figure 4.3c). At 0h, counts ranged from 3.66 log CFU/cm² (commercial coating) to 6.38 log CFU/cm² (experimental coating). The commercial coating demonstrated the highest reductions over time, resulting in a 1.57 log CFU/cm² decrease by 24 h compared to the control ($p \leq 0.05$). In contrast, the experimental coating showed fluctuations and did not significantly differ from the control at 6 h and 9 h ($p > 0.05$), suggesting inconsistent antifungal activity.

The *E. coli* O157:H7 population was significantly reduced by the commercial coating, reaching undetectable levels after 6 h, corresponding to a reduction of 4 to 5 log CFU/g compared to the control ($p \leq 0.05$; Figure 4.3d). The experimental coating showed a more gradual *E. coli* O157:H7 reduction, achieving a 3.04 log CFU/cm² decrease by 12 h. After this point, no additional bacterial growth was observed. By 24 h, *E. coli* O157:H7 levels were undetectable across all treatments (control, commercial, and experimental).

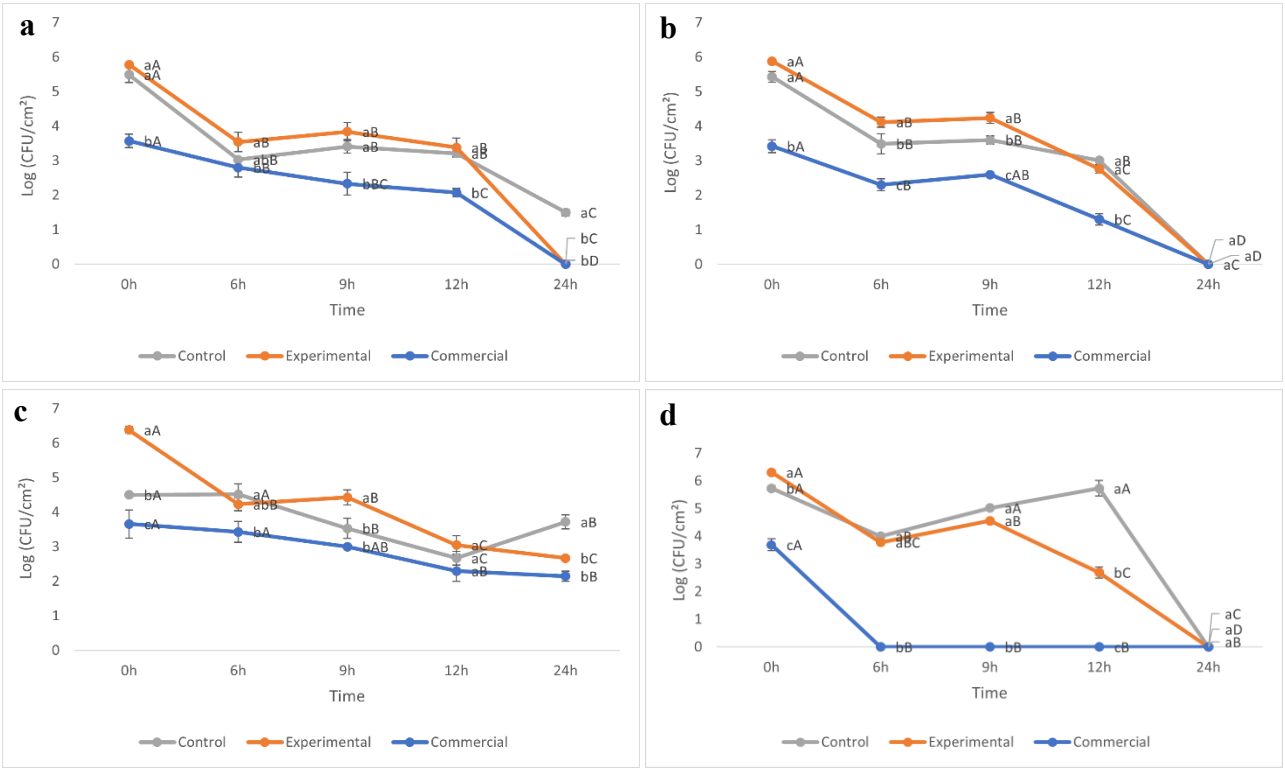


**Figure 4.3**. Microorganism population on post-wash bucket surfaces over 24 h: (a) Aerobic bacteria, (b) total coliforms, (c) yeast and molds, and (d) *E. coli* O157:H7. Bars represent mean values ± standard error. Similar lowercase letters indicate no statistically significant differences among treatments at the same time points ($p > 0.05$). Similar uppercase letters indicate no statistically significant differences across time points within the same treatment ($p > 0.05$).

## 4.5 Discussion

Fresh blueberries are particularly vulnerable to microbial contamination from a variety of on-farm sources, including humans, soil, wildlife animals, irrigation water, and harvest equipment (Valloton et al., 2021). Therefore, reducing microbial contamination during harvest is essential for improving the microbial safety of this high-value fruit (Yemmireddy et al., 2022). Given that fresh blueberries are often harvested into reusable containers and are not subjected to postharvest washing, contaminated surfaces can serve as critical vectors for microbial transfer and cross-contamination (Karl R Matthews, 2013).

In this study, two nano-textured hydrophobic coatings, one commercial (fluorinated) and one experimental (silica-based), were applied to HDPE bucket surfaces and evaluated for their effectiveness in reducing microbial adhesion and cross-contamination in a simulated blueberry harvest scenario. The results demonstrate the potential of hydrophobic coatings, particularly fluorinated formulations, as a practical intervention to mitigate microbial risks during the harvest of minimally processed fruits. Overall, the commercial coating demonstrated greater and more consistent antimicrobial performance across multiple microbial groups and sampling types, supporting its potential application for improving food safety during harvest operations. The active ingredient in this treatment is FluoroThane-MV™ which contains fluorinated groups, like $-CF_2$ and $-CF_3$ (Brown & Schneider, 2000). The $-CF_3$ group can create a low surface free energy, causing water droplets to bead up instead of spreading, thereby reducing the interactions between water and the surface (Wan Ikhsan et al., 2021).

At the pre-wash stage, the experimental coating exhibited the lowest *E. coli* O157:H7 levels on bucket surfaces, indicating a possible initial anti-adhesive effect. However, this benefit was not sustained post-washing or during extended storage, indicating that the coating's hydrophobic or antimicrobial properties may have been insufficient to provide long-term protection. In contrast, while the commercial coating did not have the lowest pre-wash *E. coli* O157:H7 counts, it significantly reduced *E. coli* O157:H7 on post-washed blueberries, suggesting a protective antimicrobial effect that limited reattachment or survival of the pathogen following contact with the coated surface.

Microbial populations in wash water did not significantly differ among treatments, and most background organisms (aerobic bacteria, total coliforms, yeasts, and molds) remained below the LOD on both pre-wash surfaces and in wash water samples. These findings suggest that microbial transfer to wash water was minimal or that the brief washing step was insufficient to dislodge surface-bound organisms (Kilonzo-Nthenge et al., 2006)

More pronounced treatment effects emerged during the 24 h storage assessment of post-wash bucket surfaces. The commercial coating reduced *E. coli* O157:H7 to undetectable levels by 6 h and maintained these reductions through 24 h. This pattern was also observed for total coliforms and aerobic bacteria, with undetectable levels achieved by 24 h. These results are consistent with previous research demonstrating the antimicrobial potential of fluorinated coatings, which reduce microbial adhesion by minimizing surface energy and enhancing water repellency (Wan Ikhsan et al., 2021). The commercial product used in this study is marketed for its superhydrophobicity across various substrates, including wood, metal, plastics, rubber, and paper-based surfaces. On the HDPE surfaces, it achieved

reductions of *E. coli* O157:H7 4.00, 5.02, and 5.73 log CFU/cm² at 6 h, 9 h, and 12 h of storage, respectively. Similar results were reported by DeFlorio et al. (2023), who evaluated a superhydrophobic coating on a stainless-steel surface by submersion of gram-negative *E. coli* O157:H7, and demonstrated a reduction greater than 99.5% in the density of cells adhered to the surface after 24h.

In contrast, the experimental coating demonstrated inconsistent antimicrobial activity. Although it achieved a 3.04 log CFU/cm² reduction in *E. coli* O157:H7 by 12 h, its performance against total coliforms and aerobic bacteria was limited and was not significantly different from the control ($p > 0.05$). Visual inspection of the coated surface revealed cracking during the curing process, potentially providing niches for microbial colonization (Korber et al., 1995). These microstructural defects may have increased surface roughness, reducing coating efficiency (Xi et al., 2022).

In addition, the experimental coating did not demonstrate the superhydrophobic performance expected of a silica nanoparticle-based system. This may be due to inadequate surface interaction with the HDPE substrate (Defrizal et al., 2022). Previous studies have shown that effective bonding of silica nanoparticles to polyethylene often requires surface activation or thermal integration to ensure durability and hydrophobicity (Xu et al., 2013; Zhang et al., 2014). For example, Xu et al. (2013) created a polymer surface by physically integrating titanium dioxide ($TiO_2$) nanoparticles into an HDPE sheet under high temperature, which exhibited a static contact angle of 158°, achieving superhydrophobicity. Similarly, Zhang et al. (2014) developed a polymer film involving HDPE and polypropylene

through thermal compression, successfully creating a superhydrophobic “peel and use” film without the need for chemical treatment (Fedel et al., 2009).

The number of aerobic bacteria and total coliforms decreased over the first 24 hours. This decline in microorganisms on a dry surface that is free of produce can be attributed to the varying sensitivity of different bacteria to desiccation stress. It is important to recognize that the recovery of microorganisms depends on several factors, including the properties of the target bacteria (such as attachment strength, stress injuries, and growth requirements) and the characteristics of the sampling site (like humidity and surface material) (Møretrø & Langsrud, 2017). Total coliforms, which are primarily gram-negative bacteria, are particularly vulnerable to low humidity levels. Le et al. (2024) demonstrated that gram-negative bacteria, such as *E. coli*, are more susceptible to drying; they exhibit increased cell wall roughness and stiffness when relative humidity is at or below 84%. Similarly, aerobic bacteria, which include both gram-positive and gram-negative bacteria, tend to have improved survival on surfaces when water and nutrients, such as those found in produce, are present (Moore & Griffith, 2002). Conversely, spore-forming bacteria, including yeast and molds, are more resilient and can persist for longer periods under dry conditions (Møretrø & Langsrud, 2017).

The presence of *E. coli* O157:H7 on food contact surfaces decreased over the first 24 hours of inoculation. This decline can be attributed to desiccation stress. The attachment of bacteria to surfaces, especially in the presence of organic residues, offers significant protection against desiccation (Kuda et al., 2008, 2015). Furthermore, the degree of microbial

adhesion is influenced by the amount of organic residues present on the food contact surface (Dhaliwal et al., 2025).

Interestingly, yeast and mold populations on surfaces treated with the experimental coating occasionally exceeded those observed in the control. This further suggests that surface imperfections in the coating may have facilitated fungal persistence or growth (Zheng et al., 2021). In contrast, the commercial coating reduced yeast and mold counts over time, reaching a 1.57 log CFU/cm² reduction by 24 h, highlighting its broader antimicrobial activity.

These findings support the use of commercial superhydrophobic coatings as a practical and effective strategy to reduce microbial contamination of food-contact surfaces during harvest. Their application may be particularly valuable in small-scale or U-Pick operations where sanitization is limited or absent. However, the variability observed with the experimental coating underscores the need for optimized formulations and appropriate surface compatibility. Although silica nanoparticle-based coatings have shown efficacy on glass (Ren et al., 2018), quartz (Oh, Kohli, et al., 2016), and polyethylene gloves (Oh, Rapisand, et al., 2016), their performance on HDPE requires further development.

Future work should focus on field-scale validation of coating efficacy, longevity under operational conditions, and assessment across various produce types and contact surfaces. In addition, regulatory, economic, and environmental considerations will be critical to inform real-world adoption.

## 4.6 Conclusion

This study demonstrated that nano-textured hydrophobic coatings can reduce microbial contamination on food-contact surfaces in a simulated blueberry harvest. The commercial coating significantly reduced *E. coli* O157:H7 populations on both bucket surfaces and post-washed blueberries, reaching undetectable levels within 6 h and achieving reductions of up to 5.73 log CFU/g. It also consistently reduced total coliforms, aerobic bacteria, and yeast and mold populations on bucket surfaces over 24 h. In contrast, the experimental coating exhibited inconsistent performance, with limited reductions and surface defects that may have promoted yeast and mold persistence. While both coatings showed some potential, only the commercial treatment demonstrated consistent, broad-spectrum antimicrobial efficacy. These findings support the application of commercial superhydrophobic coatings as a practical intervention to reduce cross-contamination during harvest, particularly for minimally processed fruits, such as blueberries. Further research is needed to validate these outcomes under commercial field conditions, optimize formulations for different surfaces, and assess long-term durability and regulatory feasibility for widespread adoption in the produce industry.

## 4.7 References

Amodu, A., Oliver, J. E., Lawrence, K., Patel, S., Koebernick, J., Patel, J., Coneva, E., & Ru, S. (2025). Identifying the Distribution and Causal Pathogens of Blueberry Stem Blight Disease in Alabama and Nearby States. *Plant Disease*, PDIS-07-24-1404-SR. https://doi.org/10.1094/PDIS-07-24-1404-SR

Bayer, I. S. (2020). Superhydrophobic Coatings from Ecofriendly Materials and Processes: A Review. *Advanced Materials Interfaces*, *7*(13), 2000095. https://doi.org/10.1002/admi.202000095

Brown, JamesF., & Schneider, R. A. (2000). *Hydrophobic coating compositions, articles coated with said compositions, and processes for manufacturing same* (Patent US6156389A). https://patents.google.com/patent/US6156389A/en

Chazotte, B. (2012). Labeling Golgi with Fluorescent Ceramides. *Cold Spring Harbor Protocols*, *2012*(8), pdb.prot070599. https://doi.org/10.1101/pdb.prot070599

Chevez, Z. R., Dunn, L. L., Da Silva, A. L. B. R., & Rodrigues, C. (2024). Prevalence of STEC virulence markers and Salmonella as a function of abiotic factors in agricultural water in the southeastern United States. *Frontiers in Microbiology*, *15*, 1320168. https://doi.org/10.3389/fmicb.2024.1320168

Dagnas, S., & Membré, J.-M. (2013). Predicting and Preventing Mold Spoilage of Food Products. *Journal of Food Protection*, *76*(3), 538–551. https://doi.org/10.4315/0362-028x.jfp-12-349

Dai, Y., Holland, R., Doane, S., Yang, W., & Chen, J. (2025). Hygiene Status of Over-the-Row Blueberry Machine Harvesters Cleaned and Sanitized Using Various Approaches. *Horticulturae*, *11*(1), 103. https://doi.org/10.3390/horticulturae11010103

DeFlorio, W., Liu, S., Arcot, Y., Ulugun, B., Wang, X., Min, Y., Cisneros-Zevallos, L., & Akbulut, M. (2023). Durable superhydrophobic coatings for stainless-steel: An

effective defense against Escherichia coli and Listeria fouling in the post-harvest environment. *Food Research International*, *173*, 113227. https://doi.org/10.1016/j.foodres.2023.113227

DeFlorio, W., Liu, S., White, A. R., Taylor, T. M., Cisneros-Zevallos, L., Min, Y., & Scholar, E. M. A. (2021). Recent developments in antimicrobial and antifouling coatings to reduce or prevent contamination and cross-contamination of food contact surfaces by bacteria. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 3093–3134. https://doi.org/10.1111/1541-4337.12750

Defrizal, M., Kemala, T., & Khotib, M. (2022). Synthesis and characterization of superhydrophobic coating from silica-HDPE composite and its potential application for windshield. *AIP Conference Proceedings*, *2638*, 020013. https://doi.org/10.1063/5.0104485

Dhaliwal, H. K., Sonkar, S., V, P., Puente, L., & Roopesh, M. S. (2025). Process Technologies for Disinfection of Food-Contact Surfaces in the Dry Food Industry: A Review. *Microorganisms*, *13*(3), 648. https://doi.org/10.3390/microorganisms13030648

Ells, T., Tregunno, N., Fan, L., Elliot, M., Doucette, C., Lyu, H., & Jollimore, A. (2024). Microbiological Analysis of Wild Lowbush Blueberries Harvested in Nova Scotia, Canada for the Fresh Produce Market. *Microorganisms*, *12*(11), 2251. https://doi.org/10.3390/microorganisms12112251

FDA. (2018). *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-fruits-and-vegetables

Fedel, M., Olivier, M., Poelman, M., Deflorian, F., Rossi, S., & Druart, M.-E. (2009). Corrosion protection properties of silane pre-treated powder coated galvanized steel. *Progress in Organic Coatings*, *66*(2), 118–128. https://doi.org/10.1016/j.porgcoat.2009.06.011

Karl R Matthews. (2013). Sources of enteric pathogen contamination of fruits and vegetables: Future directions of research. *Stewart Postharvest Review*, *9*(1), 1–5. https://doi.org/10.2212/spr.2013.1.2

Kilonzo-Nthenge, A., Chen, F.-C., & Godwin, S. L. (2006). Efficacy of Home Washing Methods in Controlling Surface Microbial Contamination on Fresh Produce. *Journal of Food Protection*, *69*(2), 330–334. https://doi.org/10.4315/0362-028x-69.2.330

Korber, D. R., Lawrence, J. R., Lappin-Scott, H. M., & Costerton, J. W. (1995). Growth of Microorganisms on Surfaces. In H. M. Lappin-Scott & J. W. Costerton (Eds.), *Microbial Biofilms* (1st ed., pp. 15–45). Cambridge University Press. https://doi.org/10.1017/CBO9780511525353.003

Kuda, T., Shibata, G., Takahashi, H., & Kimura, B. (2015). Effect of quantity of food residues on resistance to desiccation of food-related pathogens adhered to a stainless steel surface. *Food Microbiology*, *46*, 234–238. https://doi.org/10.1016/j.fm.2014.08.014

Kuda, T., Yano, T., & Kuda, M. T. (2008). Resistances to benzalkonium chloride of bacteria dried with food elements on stainless steel surface. *LWT - Food Science and Technology*, *41*(6), 988–993. https://doi.org/10.1016/j.lwt.2007.06.016

Le, T., Eifert, J. D., Etaka, C. A., & Strawn, L. K. (2024). Recovery and Survival of Aerosolized *ESCHERICHIA COLI* and *ENTEROCOCCUS FAECIUM* on Food-Grade Rubber, HDPE Plastic, Stainless Steel, and Waxed Cardboard. *Journal of Food Safety*, *44*(6), e70002. https://doi.org/10.1111/jfs.70002

Leaman, S., Kerr, J., Salas, S., Malik, A., Suslow, T., Wiedmann, M., & Davis, D. A. (2023). Fresh Produce Harvesting Equipment – A Review of Cleaning and Sanitizing Practices and Related Science. *Food Protection Trends*, 126–143. https://doi.org/10.4315/FPT-22-023

Moore, G., & Griffith, C. (2002). A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiology*, *19*(1), 65–73. https://doi.org/10.1006/fmic.2001.0464

Møretrø, T., & Langsrud, S. (2017). Residential Bacteria on Surfaces in the Food Industry and Their Implications for Food Safety and Quality. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1022–1041. https://doi.org/10.1111/1541-4337.12283

Oh, J. K., Kohli, N., Zhang, Y., Min, Y., Jayaraman, A., Cisneros-Zevallos, L., & Akbulut, M. (2016). Nanoporous aerogel as a bacteria repelling hygienic material for

healthcare environment. *Nanotechnology*, *27*(8), 085705. https://doi.org/10.1088/0957-4484/27/8/085705

Oh, J. K., Liu, S., Jones, M., Yegin, Y., Hao, L., Tolen, T. N., Nagabandi, N., Scholar, E. A., Castillo, A., Taylor, T. M., Cisneros-Zevallos, L., & Akbulut, M. (2019). Modification of aluminum surfaces with superhydrophobic nanotextures for enhanced food safety and hygiene. *Food Control*, *96*, 463–469. https://doi.org/10.1016/j.foodcont.2018.10.005

Oh, J. K., Rapisand, W., Zhang, M., Yegin, Y., Min, Y., Castillo, A., Cisneros-Zevallos, L., & Akbulut, M. (2016). Surface modification of food processing and handling gloves for enhanced food safety and hygiene. *Journal of Food Engineering*, *187*, 82–91. https://doi.org/10.1016/j.jfoodeng.2016.04.018

Palumbo, M., Harris, L. J., & Danyluk, M. D. (2013). Outbreaks of Foodborne Illness Associated with Common Berries, 1983 through May 2013. *EDIS*, *2013*(11). https://doi.org/10.32473/edis-fs232-2013

Pizzo, J. S., Pelvine, R. A., Da Silva, A. L. B. R., Mikcha, J. M. G., Visentainer, J. V., & Rodrigues, C. (2023). Use of Essential Oil Emulsions to Control Escherichia coli O157:H7 in the Postharvest Washing of Lettuce. *Foods*, *12*(13), 2571. https://doi.org/10.3390/foods12132571

Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-Silleras, B., & Redondo-Del-Río, M. P. (2019). Food Safety through Natural Antimicrobials. *Antibiotics*, *8*(4), 208. https://doi.org/10.3390/antibiotics8040208

Ren, T., Yang, M., Wang, K., Zhang, Y., & He, J. (2018). CuO Nanoparticles-Containing Highly Transparent and Superhydrophobic Coatings with Extremely Low Bacterial Adhesion and Excellent Bactericidal Property. *ACS Applied Materials & Interfaces*, *10*(30), 25717–25725. https://doi.org/10.1021/acsami.8b09945

Ru, S., Coneva, E., Munoz, P., Ashrafi, H., Spencer, J., Bao, Y., & Babiker, E. (2023). *EXPANDING SOUTHERN HIGHBUSH BLUEBERRIES TO UNDERSERVED REGIONS OF SOUTHEASTERN U.S.* United States Department of Agriculture, National Agricultural Library. https://www.nal.usda.gov/research-tools/food-safety-research-projects/expanding-southern-highbush-blueberries-underserved-regions-southeastern-us

Takeda, F., Li, C., DeVetter, L. W., Williamson, J., Sargent, S., & Yang, W. Q. (2021, May 11). *Transitioning from Hand to Machine Harvesting of Blueberries for Fresh Market | Progressive Crop Consultant*. https://progressivecrop.com/2021/05/11/transitioning-from-hand-to-machine-harvesting-of-blueberries-for-fresh-market/

USDA. (2017). *Microbial quality of blueberries and hygiene conditions of fresh blueberry packing lines—UNIVERSITY OF GEORGIA*. National Institue of Food and Agriculture. https://portal.nifa.usda.gov/web/crisprojectpages/1014538-microbial-quality-of-blueberries-and-hygiene-conditions-of-fresh-blueberry-packing-lines.html

USDA-NASS. (2024). *Census of Agriculture*. United States Department of Agriculture, National Agricultural Statistics Service.

https://www.nass.usda.gov/Publications/AgCensus/2022/Full_Report/Volume_1,_Chapter_2_US_State_Level/

Valloton, A., Bardsley, C., Edwards, A., & Strawn, L. (2021). *Assessing On-Farm Produce Safety Risks: Production Stage*. https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/FST/fst-403/FST-403.pdf

Wan Ikhsan, S. N., Yusof, N., Aziz, F., Ismail, A. F., Jaafar, J., Wan Salleh, W. N., & Misdan, N. (2021). Superwetting materials for hydrophilic-oleophobic membrane in oily wastewater treatment. *Journal of Environmental Management*, *290*, 112565. https://doi.org/10.1016/j.jenvman.2021.112565

Xi, F., Huang, Y., Zhao, Y., Liu, Y., Dai, W., & Tian, Y. (2022). Effects of Substrate Roughness on Microstructure and Fatigue Behavior of Plasma Electrolytic Oxidation-Coated Ti-6Al-4V Alloy. *Materials (Basel, Switzerland)*, *15*(12), 4256. https://doi.org/10.3390/ma15124256

Xu, Q. F., Liu, Y., Lin, F.-J., Mondal, B., & Lyons, A. M. (2013). Superhydrophobic $TiO_2$ – Polymer Nanocomposite Surface with UV-Induced Reversible Wettability and Self-Cleaning Properties. *ACS Applied Materials & Interfaces*, *5*(18), 8915–8924. https://doi.org/10.1021/am401668y

Yemmireddy, V., Adhikari, A., & Moreira, J. (2022). Effect of ultraviolet light treatment on microbiological safety and quality of fresh produce: An overview. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.871243

Zhang, Z.-X., Li, Y., Ye, M., Boonkerd, K., Xin, Z., Vollmer, D., Kim, J. K., & Deng, X. (2014). Fabrication of superhydrophobic surface by a laminating exfoliation method. *J. Mater. Chem. A*, *2*(5), 1268–1271. https://doi.org/10.1039/C3TA14204C

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J., & Hwang, G. (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.643722

# Chapter 5

## 5.0 Efficacy of Postharvest Sanitizers on *Salmonella enterica* Reduction in Living Lettuce Roots and their Impact on Leaf Quality

### 5.1 Abstract

Over the past few years, several outbreaks of foodborne illnesses have been associated with *Salmonella enterica*, particularly in commonly consumed raw foods such as fresh produce. More recently, indoor-grown lettuce has emerged as a food safety concern due to its enclosed growing environment, which may promote pathogen persistence and cross-contamination during postharvest handling. To effectively prevent and mitigate microbial contamination in fresh produce, it is crucial to implement good agricultural practices throughout the entire food production chain. A strategy to prevent cross-contamination involves using chemical sanitizers during postharvest produce washing. This study evaluated the efficacy of three chemical sanitizers, 200 ppm chlorine, 80 ppm peroxyacetic acid (PAA) and 3% hydrogen peroxide ($H_2O_2$), in reducing *S. enterica* populations on living lettuce roots with and without media plugs at 0 h, 24 h, and 7 days of storage. In addition, the impact of these treatments were assessed on leaf color (L*, a*, b*, chroma and hue angle) via a handheld colorimeter and weight loss during storage. Initial *Salmonella* concentrations on non-rinsed control treatment were 5.78 log CFU/g (roots without media plugs) and 7.41 log CFU/g (roots with media plugs). Both chlorine and PAA significantly reduced *S. enterica* counts, achieving final recoveries of 1.35 log CFU/g (roots without media plugs) and 0.79 CFU/g (roots without media plugs) after 7 days, respectively. All color characteristics (L*, a*, b*, chroma and hue) remained stable across treatments and storage time points and weight loss was minimal (2.67% to 4.51%) at day 7. These findings demonstrate that root sanitation

using chlorine or PAA effectively reduce *S. enterica* contamination in living lettuce without compromising visual quality, suggesting its potential as a postharvest intervention strategy.

**Keywords:** food safety, postharvest, living lettuce root, *Salmonella,* sanitizers, color measurement.

## 5.2 Introduction

Fresh produce is an important source of essential nutrients and contributes significantly to a balanced diet (WHO, 2025). While both food safety and food quality are crucial to consumer well-being, they represent distinct aspects of food management (Losasso et al., 2012). Food safety is non-negotiable, as it directly impacts public health. Fresh produce is susceptible to contamination by foodborne pathogens such as *Salmonella,* pathogenic *Escherichia coli,* and *Listeria monocytogenes,* which can be introduced at various points along the supply chain, including during production, processing, packaging, and transportation (CDC, 2024c). Major routes of contamination include soil, animal manure, and irrigation water (Park et al., 2012). Consumption of contaminated produce can result in serious foodborne illness (Malka & Park, 2022).

Due to the increasing number of outbreaks associated with fresh fruits and vegetables, produce safety has become a growing public health concern in the United States (U.S.) and globally (Mensah et al., 2024). In 2024, the U.S. Centers for Disease Control and Prevention (CDC) reported several foodborne outbreaks, including *Salmonella* infections linked to fresh basil (CDC, 2024d), two separate outbreakes involving cucumbers (CDC, 2024b, 2024e), and an *E. coli* O157:H7 outbreak related to organic carrots (CDC, 2024a).

To mitigate contamination risks, several interventions have been adopted along the produce supply chain, including the implementation of good agricultural practices (GAPs) (FDA, 2024), worker hygiene and sanitation of harvest tools (Beuchat, 2006) and sanitary design of food-contact equipment (USDA FSIS, 2015). Postharvest washing is a common practice, and the addition of sanitizers during this step can reduce microbial load and prevent cross-contamination. When properly managed, washing combined with sanitizers can effectively lower the presence of pathogens on produce surfaces (López-Gálvez et al., 2021). Sanitizers are especially important as alternatives to more aggressive preservation methods, such as high temperature or pressure, which may negatively impact product quality (De Siqueira Oliveira et al., 2018).

Common sanitizers used in the produce industry include chlorine, peroxyacetic acid (PAA), hydrogen peroxide ($H_2O_2$), ozone, and organic acids (Gómez-López, 2012). Chlorine is the most widely used due to its broad antimicrobial spectrum (bacteria, molds, yeast, and viruses), although it is ineffective against spores (Chinchkar et al., 2022). PAA has emerged as an environmentally friendly alternative to chlorine (Pironti et al., 2021). While $H_2O_2$ may lose effectiveness in the presence of organic matter, it has recently shown promise for microbial control in hydroponic systems (Olaimat & Holley, 2012; Sela Saldinger et al., 2023). Regardless of the sanitizer used, application must follow the U.S. Food and Drug Administration (FDA) guidelines, as exceeding recommended concentrations may cause damage to the produce (FDA, 2018).

In addition to microbial safety, maintaining visual and nutritional quality is essential for consumer acceptance of fresh produce. Attributes such as color, texture, and flavor

influence purchasing decisions, with color often serving as a visual cue for freshness, ripeness, and nutrient content (Francis et al., 2012; Schifferstein et al., 2019). Given that sanitizers may alter the physical appearance of produce, particularly leaf color, it is important to evaluate potential quality changes alongside microbial reductions (Malka & Park, 2022; Pahariya et al., 2019; Rosli et al., 2022). In the U.S., various trends have emerged to extend the shelf life and quality of fresh produce while maintaining food safety (De Corato, 2020). One such trend is the sale of living lettuce, a product gaining popularity at farmers markets (Riemenschneider, 2017). Living lettuce is hydroponically grown in greenhouses and sold with its roots intact, packaged in plastic clamshells (Waitt et al., 2014). Although hydroponic systems promote rapid growth of leafy greens, the enclosed, high-humidity environments used in indoor farming may inadvertently support the growth of pathogens (Topalcengiz et al., 2024).

Given the limited research on microbial risks associated with living lettuce, the objective of this study was to evaluate the efficacy of chemical sanitizers (chlorine, PAA, and $H_2O_2$) in reducing *Salmonella enterica* on living lettuce roots, with and without media plugs. Additionally, the study aims to assess the impact of these sanitizers on leaf quality and post-treatment weight loss. We hypothesize that PAA and chlorine will be the most effective sanitizers for reducing *S. enterica* on living lettuce roots, both with and without a media plug. Additionally, minimal effects on leaf physiology and weight loss are expected across all treatments.

## 5.3 Material and methods

*5.3.1 Preparation of Bacteria Culture.*

A *Salmonella enterica* cocktail was prepared using three serovars: *S. enterica* serovar Enteritidis (ATCC 13076), *S. enterica* serovar Newport (ATCC 6962), and *S. enterica* serovar Typhimurium (ATCC 14028), all obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Freeze-dried bacteria were rehydrated in Nutrient Broth (BD Difco™ Nutrient Broth, Becton Dickinson and Company, Sparks, MD, USA) and incubated at 37°C for 24 h. After incubation, 500 µL of each culture was mixed with 500 µL of sterile glycerol:d$H_2O$ solution (1:1 v/v; EMD Chemicals, Darmstadt, Germany) in 2 mL screw-cap tubes and stored at -80°C for further use.

Each *S. enterica* serovar was gradually adapted to resistance against 50 ppm nalidixic acid (Sigma-Aldrich, Saint Louis, MO, USA) and 50 ppm rifampicin (Sigma-Aldrich, Saint Louis, MO, USA), according to the method described by Cimowsky et al. (2022). The adaptation was conducted in Triple Sugar Iron agar (TSI; BD Difco, Sparks, MD, USA), starting with 10 ppm antibiotic concentrations and increasing the levels by 10 ppm at each transfer. Cultures were incubated at 37°C for 24 h. The adaptation continued until the cultures grew in TSI containing 50 ppm nalidixic acid and 50 ppm rifampicin.

The bacterial cocktail was poured into sterile glass cuvette and adjusted to a McFarland scale of 0.5 using approximately 300 mL of phosphate-buffered solution (PBS; CSH Protocols; 2006). The final *S. enterica* concentration was approximately 1.5 x $10^8$ CFU/mL.

*5.3.2 Inoculation of Bacteria Culture on Lettuce Root.*

Fresh butterhead lettuces (*Lactuca sativa* var. Rex) with intact roots were obtained from the Vertical Farm in the Department of Horticulture at Auburn University (Auburn, AL, USA). The lettuce heads were classified in two groups based on the root type: (i) roots with a peat moss media plug, and (ii) intact roots without a media plug. After classification, lettuce heads were placed on a sterilized aluminum foil tray and exposed to ultraviolet light at 254 nm for 10 min to reduce background microflora. Following UV treatment, the lettuce heads were weighed and stored in sterile plastic clamshells containers (Plastic Container City, Petersburg, VA, USA).

Lettuce roots were submerged in the inoculum without agitation for 2 min, then air-dried in a biosafety cabinet for 2 h (Pizzo et al., 2023). To assess the initial bacteria load, a no-rinse (NR) control was included, in which inoculated roots received no sanitizer treatment. The NR control samples were prepared in triplicate for each root type.

*5.3.3 Preparation of Washing Treatments.*

Four washing treatments were used in this study: 200 ppm chlorine, 80 ppm PAA, 3% $H_2O_2$, sterile water. The chlorine solution was prepared by diluting commercial bleach (The Clorox® Company, Oakland, CA, USA) in sterile deionized water to achieve a final concentration of 200 ppm. The pH of the solution was adjusted to 7.0 ± 0.02 using a 1 M citric acid solution (Sigma-Aldrich, Saint Louis, MO, USA) and measured with a Cole-Parmer pH meter (Model 05669-00; Vernon Hills, IL, USA). The PAA solution was prepared by diluting Sanidate 5.0 (BioSafe Systems, East Hartford, CT, USA) in sterile deionized

water to a final concentration of 80 ppm. The $H_2O_2$ solution was prepared by diluting 12% Food Grade Hydrogen Peroxide (Bulk Peroxide, Phoenix, AZ, USA) in sterile deionized water to obtain a final concentration of 3%. All sanitizer treatments were freshly prepared prior to use and handled according to the respective manufacturers' guidelines. Sterile water was used to simulate washing treatment in the absence of sanitizer.

The experiment utilized a completely randomized design (CRD) with five different treatments: chlorine (50 ppm), PAA (80 ppm), $H_2O_2$ (3%), deionized (DI) water, and a control. Each treatment was applied to two types of living lettuce roots: with media plugs and without media plugs, resulting in a total of ten treatment combinations. Each combination was replicated four times. The living lettuces were inoculated with *S. enterica* and incubated for 2 h to allow for bacterial attachment. The roots were then washed in their respective sanitizer solution for 2 m. Microbial recovery was assessed at three time points: 0 h, 24 h, and 7 d post-treatment. To evaluate postharvest quality, additional non-inoculated lettuce heads were treated similarly, and leaf physiology and weight loss assessed at the same time points. In total, 120 microbial samples were collected (5 treatments × 2 root types × 4 replicates × 3 time points), along with 40 physiological samples for postharvest evaluation. This experimental design allowed for the assessment of both the efficacy of the sanitizers in reducing *Salmonella* and their potential effects on product quality.

*5.3.4 Simulated Postharvest Washing.*

The simulated postharvest washing procedure was adapted from Cimowsky et al., (2022) and Pizzo et al., (2023). Briefly, inoculated lettuce roots were submerged in 200 mL of the respective washing treatment for 2 min without agitation. Samples were then removed

from the wash solution using sterile tweezers, placed into sterile Whirl-Pak® bags, and diluted 1:5 (w/v) with PBS containing 0.2% Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA) and 0.1% sodium thiosulfate (Fisher Scientific, Fair Lawn, NJ, USA). Samples were placed in a stomacher (Stomacher® 400 CIRCULATOR, Seward Inc, Bohemia, NY) and processed at 300 rpm for 30 sec to detach bacteria from the root surface. The rinsates were serially diluted in buffered peptone water (BPW; Fisher Scientific, Fair Lawn, NJ, USA) and spread-plated on Xylose Lysine Deoxycholate agar (XLD, Oxoid Ltd, Lasingstoke, Hants, UK) supplemented with 50 ppm rifampicin and 50 ppm nalidixic acid. The plates were incubated at 37°C for 24 h. Colony counts were log transformed before statistical analysis and results were presented as log CFU/g. Each washing treatment was performed in quadruplicate.

*5.3.5 Lettuce Shelf-Life Assessment.*

After washing, lettuce heads were stored in sterile clamshells containers at 4°C. At each storage time (24 h and 7 days), samples were diluted, processed using the stomacher, plated onto XLD agar, incubated at 37°C for 24 h, and colonies were enumerated. Bacteria counts were log-transformed prior to statistical analysis and results were presented as log CFU/g. Washing treatment in each storage time was performed in quadruplicate.

*5.3.6 Lettuce Weight Loss and Color Quality.*

For leaf quality and weight loss analysis, samples were not inoculated with bacteria but underwent the same washing and storage procedures described previously. Weight loss

was determined by weighing the entire living lettuce (including roots and leaves) at 24 h and 7 days using equation (1).

$$\%\, Weight\ loss = \frac{Weight\ loss\ 24h - Weight\ loss\ 7\ days}{Weight\ loss\ 24h} x\ 100 \qquad \text{Equation (2)}$$

Color measurements were obtained at 5 different points on each lettuce head using a Konica Minolta CR-400 colorimeter (Konica Minolta Sensing, Wayne, NJ, USA). The average values of L*, a*, b*, hue angle, and chroma (c*) were recorded. L* indicates lightness (0 = black, 100 = white); a* represents the green-red axis (– 60 to +60), and b* the blue-yellow axis (–60 to +60). Chroma, also called saturation, indicates the intensity of the color, while hue angle is calculated as arctangent (b*/a*).

To visualize the final color of living lettuce samples, we converted the recorded CIELAB values (L*, a*, b*) into corresponding HEX color codes using R software version 4.5.1. These HEX codes simulate how each color would appear to the human eye. The conversion process involved transforming the CIELAB coordinates into RGB values before converting them into HEX format. Finally, a simple tile plot was created to display the color associated with each treatment. This visual representation facilitated an intuitive comparison of color differences among treatments, time points, and root types.

Additionally, the L*, a* and b* values were used to calculate the browning index (BI) using equation (3). The total color difference ($\Delta E$) of lettuce samples was also determined during storage by using equation (4), where $L_0^*$, $a_0^*$ and $b_0^*$ are L*, a* and b* values of NR control at the corresponding time point. Whiteness index (WI) was calculated using equation (5). This index shows the tendency of the samples to be light (white), and the closer its value

is to 100, the lighter the samples are (Min et al., 2017; Nourzad et al., 2024; Pahariya et al., 2019).

$$BI = \frac{[100\ (x-0.31)]}{0.17} \qquad \text{Equation (6)}$$

$$where: \quad x = \frac{(a^* + \ 1.75L^*)}{[5.645L^* + \ (a^* - 3.012b^*)]}$$

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad \text{Equation (7)}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \qquad \text{Equation (8)}$$

*5.3.7 Statistical Analysis.*

All statistical analyses were conducted using SAS Studio 5.2. A generalized linear model (GLM) was performed to address non-normal distributed data. In the GLM, the dependent variables of *S. enterica* population values (log CFU/g), colorimetric parameters (L*, a*, c*, hue angle, $\Delta E$, WI, and BI), and weight loss were analyzed using washing treatment, root type, time, and their interactions as fixed effect. A multifactorial analysis of variance (3-Way ANOVA) was also performed and least squares means were compared using Tukey's HSD post-hoc test at a significance level of 0.05.

## 5.4 Results

### *5.4.1 Effects of Treatments on Inoculated S. enterica Lettuce Roots over Time*

The *Salmonella* population on inoculated lettuce roots varied significantly ($p \leq 0.05$) depending on the type of treatment (NR, water, $H_2O_2$, chlorine, and PAA), root type (with or without media plug), and time (0 h, 24 h, and 7 days) (Table 5.1) All washing treatments resulted in significant reductions ($p \leq 0.05$) in bacteria counts compared to the NR control, across all time points and for both root types. There were no statistically significant three-way interactions among root type, treatment, and time. Significant two-way interactions were observed between treatment and root type, treatment and time, and root type and time ($p \leq 0.05$).

The *S. enterica* population in NR control samples ranged from 4.96 to 5.42 log CFU/g for roots without media plug and from 6.88 to 7.36 log CFU/g for roots with media plug. Water washing alone significantly ($p \leq 0.05$) reduced *S. enterica* populations compared to NR, with reductions ranging from 1.69 to 2.11 log CFU/g in roots without plugs, and up to 2.62 log CFU/g in roots with plugs by day 7.

Among the sanitizer treatments, PAA and chlorine were the most effective across all time points. In roots without media plugs, PAA reduced bacteria populations to 2.32 log CFU/g at 0 h, 1.77 log CFU/g at 24 h, and 1.35 log CFU/g at 7 days. Chlorine showed similar efficacy, with counts of 2.96, 2.40, and 0.79 log CFU/g at the same time points. In contrast, $H_2O_2$ had moderate antimicrobial activity, resulting in *S. enterica* levels of 3.62, 2.84, and 2.45 log CFU/g at 0 h, 24 h, and 7 days, respectively.

A similar trend was observed in roots with media plugs, although bacterial loads were significantly higher ($p \leq 0.05$) than in the roots without media plugs. At 0 h, PAA and chlorine reduced *S. enterica* levels to 4.49 and 4.35 log CFU/g, respectively, and continued to be effective over time, achieving final counts of 3.07 and 3.21 log CFU/g after 7 days, respectively. In contrast, $H_2O_2$ treated roots with media plugs maintained significantly high bacteria levels across all time points, remaining above 5.5 log CFU/g.

Across all treatments, *S. enterica* populations were significantly lower ($p \leq 0.05$) in roots without media plugs compared to those with media plugs. The most significant bacterial reduction was observed in chlorine-treated roots without plugs after 7 days of storage, reaching 0.79 log CFU/g, corresponding to a reduction of over 4 log CFU/g compared to the NR control.

**Table 5.1**. Microbial population of *Salmonella enterica* recovered from root-inoculated lettuce (with and without media plug) under different washing treatments.

| Treatments | Roots with media plug (log CFU/g) | Roots without media plug (log CFU/g) |
|---|---|---|
| NR | 7.14 ± 0.12 aA | 5.16 ± 0.18 aB |
| Water | 4.70 ± 0.05 bA | 3.26 ± 0.13 bB |
| Sanitizers | | |
| 3% H2O2 | 5.57 ± 0.05 cA | 3.08 ± 0.19 bB |
| 200 ppm Chlorine | 4.45 ± 0.30 dA | 2.16 ± 0.31 cB |
| 80 pppm PAA | 3.94 ± 0.28 dA | 1.81 ± 0.21 cB |

Values followed by similar lowercase letters indicate no significant differences ($p > 0.05$) among treatments (rows) within media plug type (columns). Values followed by similar uppercase letters indicate no significant differences ($p > 0.05$) among media plug type (columns) within treatments (rows). Abbreviations: NR: No-rinse; $H_2O_2$: hydrogen peroxide; PAA: peroxyacetic acid.

For the treatment and root type interaction (Table 5.1), chlorine and PAA showed the most effective reductions in both root types. In roots without media plug, PAA and chlorine achieved recovered counts of 1.81 and 2.16 log CFU/g, respectively, representing reductions of 3.32 and 3 log CFU/g compared to NR. In roots with media plug, recovered levels for PAA and chlorine were 3.94 and 4.45 log CFU/g, corresponding to reductions of 3.21 and 2.69 log CFU/g, respectively.

**Table 5.2**. Microbial population of *S. enterica* recovered from root-inoculated lettuce immediately after washing treatments (0 h), after 24 h and 7 days of storage.

| Treatments | Storage times (log CFU/g) | | |
|---|---|---|---|
| | **0 h** | **24 h** | **7 d** |
| NR | 5.94 ± 0.40 aA | 6.39 ± 0.40 aA | 6.09 ± 0.66 aA |
| Water | 3.99 ± 0.27 bA | 4.23 ± 0.26 bA | 3.72 ± 0.33 bA |
| Sanitizers | | | |
| 3% H2O2 | 4.29 ± 0.35 bA | 3.77 ± 0.56 bA | 4.01 ± 0.62 bA |
| 200 ppm Chlorine | 3.65 ± 0.31 cA | 3.93 ± 0.61 cA | 2.00 ± 0.55 cB |
| 80 pppm PAA | 3.41 ± 0.46 cA | 3.01 ± 0.54 cAB | 2.21 ± 0.40 cB |

Values followed by similar lowercase letters indicate no significant differences ($p > 0.05$) among treatments (rows) within storage time (columns). Values followed by similar uppercase letters indicate no significant differences ($p > 0.05$) among storage times (columns) within treatment (rows). Abbreviations: NR: No-rinse; $H_2O_2$: hydrogen peroxide; PAA: peroxyacetic acid.

Regarding the treatment and time interaction (Table 5.2), PAA and chlorine again demonstrated superior performance. At 0 h, both sanitizers produced significant reductions ($p \leq 0.05$), with PAA achieving 3.41 log CFU/g (2.53 log CFU/g reduction) and chlorine 3.65 log CFU/g (2.29 log CFU/g reduction) compared to the 5.94 log CFU/g observed in NR.

After 24 h, PAA showed the lowest recovery at 3.01 log CFU/g, corresponding to a reduction of 3.38 log CFU/g compared to NR (6.39 log CFU/g). By day 7, PAA and chlorine remained the most effective, with final counts of 2.21 and 2.00 log CFU/g, representing reductions of 3.88 and 4.09 log CFU/g, respectively, compared to NR (6.09 log CFU/g).

**Table 5.3.** Microbial population of *S. enterica* recovered from root-inoculated lettuce (with and without media plug) over time (0 h, 24 h and 7 days)

| Root type | Storage times (log CFU/g) | | |
|---|---|---|---|
| | **0 h** | **24 h** | **7 d** |
| Root with media plug | 5.18 ± 0.24 aA | 5.48 ± 0.29 aA | 4.53 ± 0.36 aB |
| Root without media plug | 3.46 ± 0.21 bA | 3.22 ± 0.31 bA | 2.28 ± 0.32 bB |

Values followed by similar lowercase letters indicate no significant differences ($p > 0.05$) among root type (rows) within storage time (columns). Values followed by similar uppercase letters indicate no significant differences ($p > 0.05$) among storage times (columns) within root type (rows).

Finally, for the root type and time interaction (Table 5.3), lettuce roots without media plug exhibited the lowest *S. enterica* levels at all time points (0 h, 24 h, and 7 days). Recovery levels in these samples were 3.46 log CFU/g at 0 h, 3.22 log CFU/g at 24 h, and 2.28 log CFU/g at 7 days. In contrast, roots with media plug had significantly higher *Salmonella enterica* counts, with levels of 5.18, 5.48, and 4.53 log CFU/g at 0 h, 24 h, and 7 days, respectively.

*5.4.2 Effects of Treatments on Color and Weight Loss in Lettuce Roots over Time*

There were no significant differences in the representative visualizations of L*, a*, and b* values for living lettuce leaves harvested from roots with and without media plug following washing and storage (Figure 5.1). Across all treatments, root types, and time points, lettuce leaves maintained stable visual appearance based on these colorimetric parameters.

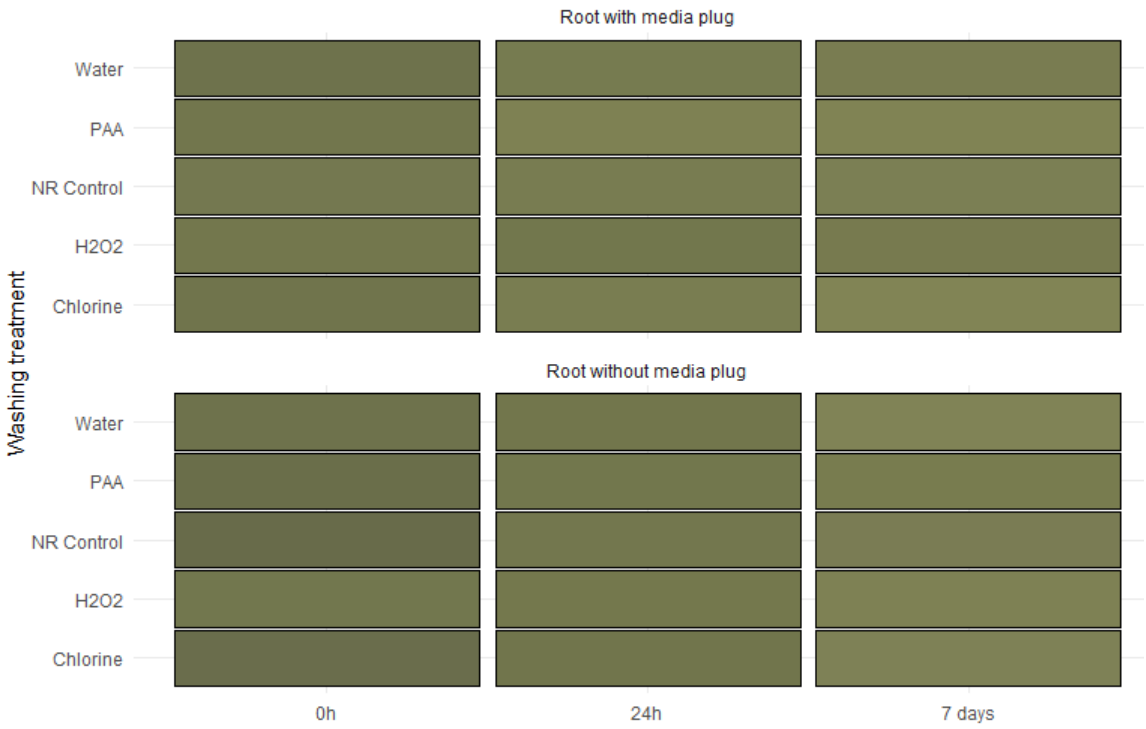


**Figure 5.1**. Representative visualization of L*, a*, and b* colorimetric parameters of living lettuce leaves harvested from roots with and without media plugs after root washing treatments and storage times.

No significant three-way or two-way interactions were observed among treatment, root type, and time for any of the colorimetric parameters or for weight loss ($p > 0.05$) (Table 5.5; Figure 5.2; Table 5.4). Statistically significant main effects were observed: L*, a*, b*

and c* values were influenced by both root type and time ($p \leq 0.05$), while hue angle, BI, WI, and $\Delta E$ were significantly impacted by time alone ($p \leq 0.05$). Additionally, weight loss was significantly affected by root type ($p \leq 0.05$).

Regarding the effect of root type, leaves harvested from roots with media plug had slightly higher L* values (50.28) compared to those without plugs (48.94), indicating consistent mid-level lightness across samples. The a* values remained stable and negative for both groups (–8.75 and –8.38 for leaves harvested from roots with and without plugs, respectively), reflecting a persistent green coloration. Similarly, b* values remained positive and consistent (23.00 and 21.89, for leaves harvested from roots with and without plugs, respectively), indicating a stable yellow tone. The c* values remained 19.19 to 26.98 across root types. Average weight loss was 3.31% for samples with media plug and 4.07 % for those without media plug.

For the main effect of time, there were significant but minor changes across L*, a*, b*, c*, hue angle, BI, WI, and ΔE values. Specifically, L* values were from 46 to 48.15 at 0 h, 48.49 to 50.65 at 24 h, and 52.01 to 52.03 at 7 days. The a* and b* values remained within narrow ranges across time points, indicating sustained green and yellow tones. Chroma and hue angle values also remained stable, suggesting that neither vividness nor tonality of leaf color was negatively affected over time. ΔE values, calculated relative to the NR control, were 3.83 at 0 h, 3.87 at 24 h, and 5.97 at 7 days. Likewise, BI and WI values were 43.31 and 44.09, respectively, suggesting minimal browning and preserved brightness.

In summary, root washing using chlorine, PAA, $H_2O_2$, or water did not significantly affect the color integrity or weight retention of living lettuce leaves throughout time points.

**Table 5.4.** Average of washing treatment, and root type on the colorimetric parameters of living lettuce leaves.

| Color parameters | Mean values ± SE |
|---|---|
| L* | 49.55 ± 0.47 |
| a* | -8.55 ± 0.09 |
| b* | 22.40 ± 0.32 |
| c* | 24.00 ± 0.33 |
| Hue angle (°) | 111.06 ± 0.16 |
| BI | 43.31 ± 0.48 |
| WI | 44.09 ± 0.31 |
| ΔE | 4.56 ± 0.33 |
| Weight loss (%) | 3.69 ± 0.18 |

Values represent means ± standard errors. No statistically significant differences among treatments, root type and storage time for the same color parameter and weight loss ($p > 0.05$). Abbreviations: BI: Browning index; WI: Whiteness index; ΔE: color difference.

**Table 5.5**. Effect of washing treatment, time, and root type on colorimetric parameters and weight loss of living lettuce leaves.

| Treatment | Time | Root type | Color indices | | | % Weight loss at day 7 |
|---|---|---|---|---|---|---|
| | | | ΔE | WI | BI | |
| NR | 0h | Root with Media Plug | * | 43.98 ± 1.12 aA | 42.11 ± 2.22 aA | 3.99 ± 0.48 aA |
| | 24h | | * | 44.65 ± 1.12 aA | 43.98 ± 2.22 aA | |
| | 7 days | | * | 45.82 ± 1.12 aA | 42.11 ± 2.22 aA | |
| | 0h | Root without Media Plug | * | 40.92 ± 1.12 aA | 36.99 ± 2.22 aA | 3.83 ± 0.48 aA |
| | 24h | | * | 44.32 ± 1.12 aA | 45.43 ± 2.22 aA | |
| | 7 days | | * | 45.72 ± 1.12 aA | 40.56 ± 2.22 aA | |
| Water | 0h | Root with Media Plug | 4.39 ± 1.27 aA | 42.42 ± 1.12 aA | 39.93 ± 2.22 aA | 3.05 ± 0.48 aA |
| | 24h | | 4.86 ± 1.27 aA | 44.45 ± 1.12 aA | 42.63 ± 2.22 aA | |
| | 7 days | | 6.24 ± 1.27 aA | 44.80 ± 1.12 aA | 43.85 ± 2.22 aA | |
| | 0h | Root without Media Plug | 4.18 ± 1.27 aA | 42.48 ± 1.12 aA | 41.11 ± 2.22 aA | 3.96 ± 0.48 aA |
| | 24h | | 3.15 ± 1.27 aA | 42.71 ± 1.12 aA | 46.25 ± 2.22 aA | |
| | 7 days | | 4.41 ± 1.27 aA | 46.70 ± 1.12 aA | 44.15 ± 2.22 aA | |
| Sanitizers | | | | | | |
| 3% $H_2O_2$ | 0h | Root with Media Plug | 2.72 ± 1.27 aA | 43.30 ± 1.12 aA | 44.65 ± 2.22 aA | 3.68 ± 0.48 aA |
| | 24h | | 4.09 ± 1.27 aA | 43.08 ± 1.12 aA | 44.94 ± 2.22 aA | |
| | 7 days | | 5.91 ± 1.27 aA | 44.21 ± 1.12 aA | 45.39 ± 2.22 aA | |
| | 0h | Root without Media Plug | 6.48 ± 1.27 aA | 43.41 ± 1.12 aA | 42.37 ± 2.22 aA | 4.21 ± 0.48 aA |
| | 24h | | 3.06 ± 1.27 aA | 43.17 ± 1.12 aA | 46.16 ± 2.22 aA | |
| | 7 days | | 5.04 ± 1.27 aA | 46.29 ± 1.12 aA | 44.76 ± 2.22 aA | |
| 200 ppm Chlorine | 0h | Root with Media Plug | 2.26 ± 1.27 aA | 42.66 ± 1.12 aA | 42.17 ± 2.22 aA | 2.67 ± 0.48 aA |
| | 24h | | 2.33 ± 1.27 aA | 44.91 ± 1.12 aA | 45.19 ± 2.22 aA | |
| | 7 days | | 5.00 ± 1.27 aA | 46.48 ± 1.12 aA | 47.18 ± 2.22 aA | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 80 ppm PAA | 0h | Root without Media Plug | 2.13 ± 1.27 aA | 41.53 ± 1.12 aA | 36.61 ± 2.22 aA | 4.51 ± 0.48 aA |
| | 24h | | 3.80 ± 1.27 aA | 42.67 ± 1.12 aA | 43.58 ± 2.22 aA | |
| | 7 days | | 8.18 ± 1.27 aA | 46.43 ± 1.12 aA | 41.54 ± 2.22 aA | |
| | 0h | Root with Media Plug | 5.29 ± 1.27 aA | 43.10 ± 1.12 aA | 44.50 ± 2.22 aA | 3.16 ± 0.48 aA |
| | 24h | | 4.68 ± 1.27 aA | 46.03 ± 1.12 aA | 46.39 ± 2.22 aA | |
| | 7 days | | 7.43 ± 1.27 aA | 46.20 ± 1.12 aA | 47.32 ± 2.22 aA | |
| | 0h | Root without Media Plug | 3.18 ± 1.27 aA | 41.24 ± 1.12 aA | 40.69 ± 2.22 aA | 3.82 ± 0.48 aA |
| | 24h | | 4.98 ± 1.27 aA | 43.22 ± 1.12 aA | 43.23 ± 2.22 aA | |
| | 7 days | | 5.55 ± 1.27 aA | 44.23 ± 1.12 aA | 46.17 ± 2.22 aA | |

Values represent means ± standard errors. Values followed by similar lowercase letters indicate no significant differences ($p > 0.05$) among treatments within same time point, as determined by Tukey's test. Values followed by similar uppercase letters indicate no significant differences ($p > 0.05$) among each treatment across different time point, as determined by Tukey's test. Abbreviations: $H_2O_2$: hydrogen peroxide; PAA: peroxyacetic acid; NR: no rinse; *: no data as NR was used as the reference value.

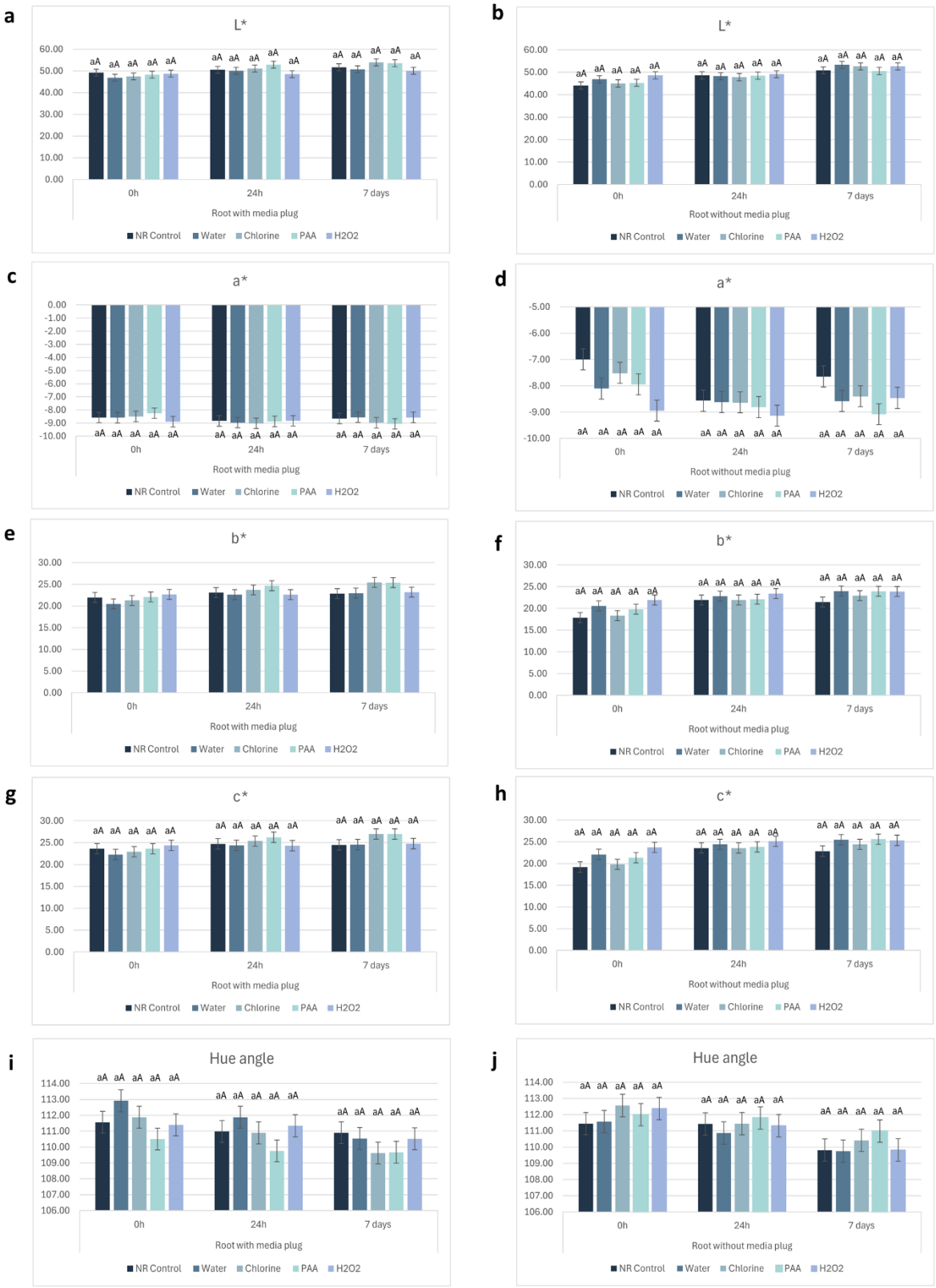
a
L*
60.00
50.00
40.00
30.00
20.00
10.00
0.00
aA
0h
24h
7 days
Root with media plug
NR Control
Water
Chlorine
PAA
H2O2
b
L*
Root without media plug
c
a*
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
-6.00
-7.00
-8.00
-9.00
-10.00
d
a*
-5.00
-6.00
-7.00
-8.00
-9.00
-10.00
e
b*
30.00
25.00
20.00
15.00
10.00
5.00
0.00
f
b*
g
c*
h
c*
i
Hue angle
114.00
113.00
112.00
111.00
110.00
109.00
108.00
107.00
106.00
j
Hue angle

**Figure 5.2.** Effect of root washing treatment, time, and root type on the colorimetric parameters of living lettuce leaves. L* values of living lettuce leaves with (a) and without (b) media plugs; a* values of living lettuce leaves with (c) and without (d) media plugs; b* values of living lettuce leaves with (e) and without (f) media plugs; c* values of living lettuce leaves with (g) and without (h) media plugs; Hue angle (°) values of living lettuce leaves with (i) and without (j) media plugs. Data represents means and error bars represent standard errors. Lowercase letters indicate no significant differences ($p > 0.05$) among treatments within the same time point. Uppercase letters indicate no significant differences ($p > 0.05$) within each treatment across different time points, based on Tukey's test. Abbreviations: c*: chroma (saturation); H2O2: hydrogen peroxide; PAA: peroxyacetic acid;

## 5.5 Discussion

To meet the growing demand for leafy greens, producers have adopted various strategies to enhance shelf life and marketability, including the use of controlled environment agriculture (CEA) systems, including hydroponic setups that enable year-round cultivation (Ferris et al., 2023). One emerging product of this system is living lettuce, typically sold with intact roots in plastic clamshells. Marketed for its extended shelf life and visual freshness, living lettuce is gaining popularity among consumers. More importantly there is currently no scientific evidence confirming that intact roots extend shelf life, and their presence may introduce additional food safety risks. The enclosed environment of hydroponic systems, combined with the moist conditions provided by media plugs and intact roots, can promote the growth of foodborne pathogens such as *S. enterica*. Notably, a 2021 outbreak of *S.* Typhimurium linked to packaged leafy greens underscores the need for evidence-based handling practices in these systems (FDA, 2021).

The results of this study indicate that a root sanitation step using chlorine, PAA, and $H_2O_2$, can effectively reduce *S. enterica* population without adversely affecting lettuce leaf quality, supporting its inclusion as a best practice for leafy green producers. Implementing a postharvest washing step can mitigate and reduce the risk of cross-contamination between the roots and leaves.

Chlorine (200 ppm) is among the most widely used sanitizers in the fresh produce industry due to its low cost and broad-spectrum antimicrobial activity (Dankwa et al., 2021). It inactivates microorganisms by altering the permeability of the cytoplasmic membrane, damaging DNA via chloramines, and inhibiting cell wall synthesis enzymes (Yoon & Lee,

2018). In this study, chlorine significantly reduced *S. enterica* populations achieving up to 4.17 log CFU/g reduction after 7 days in roots without media plugs. These reductions are comparable or superior to previously reported log recovery values ranging from <1 to 2.8 log CFU/g, on other produce such as romaine lettuce and pepper (Cimowsky et al., 2022; Dunn et al., 2019). Yet, chlorine's efficacy is known to be reduced in the presence of organic matter (Dankwa et al., 2021), highlighting the importance of pre-washing and maintaining low organic loads during treatment.

PAA (80 ppm) demonstrated consistent and robust antimicrobial activity across all conditions tested, achieving similar pathogen reductions in roots both with and without media plugs, suggesting its efficacy under various hydroponic packaging conditions. As a strong oxidant agent, PAA acts primarily by generating reactive oxygen species that damage cells membranes, proteins, and nucleic acids (Unruh et al., 2021). PAA also exhibits greater stability than chlorine in the presence of organic matter (Hoorfar, 2014), a critical advantage in real-world processing environments. The results of this study align with previous studies that reported PAA to be equally or more effective than chlorine in reducing pathogens on fresh produce, with reductions of up to 3.95 log CFU/g for *E. coli* O157:H7 population (Pahariya et al., 2019), 6.8 log CFU/g on tomato, and 4.5 log CFU/g in cantaloupe for *S.* Typhimurium DT104 (Singh et al., 2018).

$H_2O_2$ (3%), although less effective than chlorine and PAA, with maximum reductions of 2.58 log CFU/g, still provided significant reductions compared to the NR control. Although, the moderate antimicrobial performance of 3% $H_2O_2$ observed in this study supports previous findings that its efficacy may be limited by organic load or insufficient

contact time (Olaimat & Holley, 2012). Moreover, $H_2O_2$ remained above 5.5 log CFU/g on media plug roots at all time points, indicating it may not be a reliable stand-alone treatment for more heavily contaminated surfaces. These findings are consistent with previous studies showing limited pathogen inactivation on fresh produce, including fresh-cut melon, strawberries, and red bell peppers, even with concentrations up to 5% (Alexandre et al., 2012; Ukuku et al., 2005).

A key finding of this study was the consistent difference in *S. enterica* recovery between lettuce roots with and without media plugs. Roots with media plugs harbored significantly higher pathogen loads, likely due to the porous structure and moisture retention of the peat-based material, which may provide shelter for microbial attachment and growth (Jechalke et al., 2019). This highlights the importance of considering root substrate or packaging material when designing microbial reduction strategies for living lettuce and similar crops.

Beyond microbial safety, maintaining product quality during storage is critical for consumer acceptance. In this study, none of the sanitizer treatments adversely affected the visual quality or weight of the living lettuce leaves. Colorimetric parameters, including L*, a*, b*, c*, hue angle, ΔE, WI, and BI remained stable throughout storage, regardless of treatment or root type. This is consistent with findings from previous studies showing that chemical sanitizers, when used at recommended concentrations, typically do not affect the surface pigments of leafy greens (Francis et al., 2012; Mensah et al., 2024; Poimenidou et al., 2016). Similarly, the observed total color differences ($\Delta E < 6.5$) remained within the

threshold for perceptible but minor changes (Mao et al., 2024), further supporting the conclusion that visual quality was preserved.

Weight loss across treatments ranged between 2.67% and 4.51% after 7 days and was not significantly affected by sanitizers, root type, or storage time. These results suggest that the applied washing solutions did not disrupt the lettuce's moisture retention capacity (Rosli et al., 2022), even for roots in direct contact with chemical agents. Moreover, the differences in weight loss between roots with and without plugs may relate to root morphology and water availability rather than treatment effects alone (Balliu et al., 2021; Rouphael & Colla, 2005).

These findings support the potential application of chlorine and PAA as effective sanitizers for reducing *S. enterica* contamination in living lettuce production systems without compromising product quality. The differential outcomes based on root substrate emphasize the need for tailored interventions depending on the presence of media plugs or other growing media. Future work should further explore sanitizer efficacy in more complex indoor farming or commercial hydroponic systems, especially under varying organic load and environmental conditions. Furthermore, research is needed to evaluate the risk of cross-contamination that could result in the transfer of *S. enterica* from the lettuce roots to the leaves.

## 5.6 Conclusion

This study demonstrates that root sanitation using chemical agents such as PAA, chlorine, and $H_2O_2$ can effectively reduce *S. enterica* populations on living lettuce roots, with minimal impact on the visual quality and weight of the leaves during short-term cold storage. Among the sanitizers tested, 80 ppm PAA and 200 ppm chlorine were the most effective,

achieving up to 4-log CFU/g reductions, particularly in roots without media plugs. $H_2O_2$ showed limited efficacy, resulting in smaller reductions across treatments. Importantly, root sanitation did not significantly alter colorimetric parameters (L*, a*, b*, c*, hue angle, BI, WI, and ΔE), suggesting that these treatments preserve the postharvest quality of living lettuce leaves. Furthermore, the presence of media plugs was associated with higher pathogen survival, highlighting a potential risk factor for food safety. These findings support the inclusion of a targeted root sanitation step in the postharvest handling of living lettuce to enhance microbial safety without compromising product quality. Future studies should explore the potential for root-to-leaf cross-contamination and evaluate longer storage periods to validate the efficacy and shelf-life implications of root disinfection strategies.

## 5.7 References

Alexandre, E. M. C., Brandão, T. R. S., & Silva, C. L. M. (2012). Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. *Food Control*, *27*(2), 362–368. https://doi.org/10.1016/j.foodcont.2012.04.012

Balliu, A., Zheng, Y., Sallaku, G., Fernández, J. A., Gruda, N. S., & Tuzel, Y. (2021). Environmental and Cultivation Factors Affect the Morphology, Architecture and Performance of Root Systems in Soilless Grown Plants. *Horticulturae*, *7*(8), 243. https://doi.org/10.3390/horticulturae7080243

Beuchat, L. R. (2006). Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *British Food Journal*, *108*(1), 38–53. https://doi.org/10.1108/00070700610637625

CDC. (2024a). *E. coli Outbreak Linked to Organic Carrots*. https://www.cdc.gov/ecoli/outbreaks/e-coli-o121.html

CDC. (2024b). *Salmonella Outbreak Linked to Cucumbers*. https://www.cdc.gov/salmonella/outbreaks/cucumbers-11-24/index.html

CDC. (2024c, April 24). *How Food Gets Contaminated: The Food Production Chain*. https://www.cdc.gov/foodborne-outbreaks/foodproductionchain/index.html

CDC. (2024d, June 18). *Salmonella Outbreak Linked to Fresh Basil | CDC*. https://www.cdc.gov/salmonella/basil-04-24/index.html

CDC. (2024e, July 2). *Salmonella Outbreak Linked to Cucumbers | CDC*. https://www.cdc.gov/salmonella/africana-06-24/index.html

Chinchkar, A. V., Singh, A., Singh, S. V., Acharya, A. M., & Kamble, M. G. (2022). Potential sanitizers and disinfectants for fresh fruits and vegetables: A comprehensive review. *Journal of Food Processing and Preservation*, *46*(10). https://doi.org/10.1111/jfpp.16495

Cimowsky, S., Kumar, G. D., Biscaia Ribeiro Da Silva, A. L., White, E., Kerr, W. L., Rodrigues, C., Juneja, V. K., & Dunn, L. L. (2022). Postharvest control of Escherichia coli O157:H7 on romaine lettuce using a novel pelargonic acid sanitizer. *LWT*, *154*, 112168. https://doi.org/10.1016/j.lwt.2021.112168

CSH Protocols. (2006). Phosphate-buffered saline (PBS). *Cold Spring Harbor Protocols*, *2006*(1), pdb.rec8247. https://doi.org/10.1101/pdb.rec8247

Dankwa, A. S., Machado, R. M., & Perry, J. J. (2021). Sanitizer efficacy in reducing microbial load on commercially grown hydroponic lettuce. *Journal of the Science of Food and Agriculture*, *101*(4), 1403–1410. https://doi.org/10.1002/jsfa.10753

De Corato, U. (2020). Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: A comprehensive critical review from the traditional technologies into the most promising advancements. *Critical Reviews in Food Science and Nutrition*, *60*(6), 940–975. https://doi.org/10.1080/10408398.2018.1553025

De Siqueira Oliveira, L., Eça, K. S., De Aquino, A. C., & Vasconcelos, L. B. (2018). Hydrogen Peroxide (H2O2) for Postharvest Fruit and Vegetable Disinfection. In *Postharvest Disinfection of Fruits and Vegetables* (pp. 91–99). Elsevier. https://doi.org/10.1016/B978-0-12-812698-1.00004-2

Dunn, L. L., Harness, M. L., Smith, D. M., Gorman, S. J., Zhong, Q., Davidson, P. M., & Critzer, F. J. (2019). Essential Oil Emulsions as Postharvest Sanitizers To Mitigate Salmonella Cross-Contamination on Peppers. *Journal of Food Protection*, *82*(1), 159–163. https://doi.org/10.4315/0362-028X.JFP-18-190

FDA. (2018). *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. FDA. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-fruits-and-vegetables

FDA. (2021). *Factors Potentially Contributing to the Contamination of Packaged Leafy Greens Implicated in the Outbreak of Salmonella Typhimurium During the Summer of 2021*. https://www.fda.gov/food/outbreaks-foodborne-illness/factors-potentially-contributing-contamination-packaged-leafy-greens-implicated-outbreak-salmonella

FDA. (2024, September). *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption: What You Need to Know About the FDA Regulation: Guidance for Industry Small Entity Compliance Guide*. https://www.fda.gov/media/107298/download

Ferris, W., Tate, S., & Ulaky, A. (2023). *Controlled Environment Agriculture Strategy and Roadmap in GO Virginia Region 3*. https://cece.vt.edu/content/dam/cece_vt_edu/projects/CEA%20Strategy%20and%20Road%20Map%20Final%20Report.pdf

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors Affecting Quality and Safety of Fresh-Cut Produce. *Critical Reviews in Food Science and Nutrition*, *52*(7), 595–610. https://doi.org/10.1080/10408398.2010.503685

Gómez-López, V. M. (Ed.). (2012). *Decontamination of fresh and minimally processed produce*. Blackwell Pub. 10.1002/9781118229187

Hoorfar, J. (Ed.). (2014). *Global safety of fresh produce: A handbook of best practice, innovative commercial solutions and case studies*. Woodhead Publishing.

Jechalke, S., Schierstaedt, J., Becker, M., Flemer, B., Grosch, R., Smalla, K., & Schikora, A. (2019). Salmonella Establishment in Agricultural Soil and Colonization of Crop Plants Depend on Soil Type and Plant Species. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00967

López-Gálvez, F., Allende, A., & Gil, M. I. (2021). Recent progress on the management of the industrial washing of fresh produce with a focus on microbiological risks. *Current Opinion in Food Science*, *38*, 46–51. https://doi.org/10.1016/j.cofs.2020.10.026

Losasso, C., Cibin, V., Cappa, V., Roccato, A., Vanzo, A., Andrighetto, I., & Ricci, A. (2012). Food safety and nutrition: Improving consumer behaviour. *Food Control*, *26*(2), 252–258. https://doi.org/10.1016/j.foodcont.2012.01.038

Malka, S. K., & Park, M.-H. (2022). Fresh Produce Safety and Quality: Chlorine Dioxide's Role. *Frontiers in Plant Science*, *12*, 775629. https://doi.org/10.3389/fpls.2021.775629

Mao, Z., Qiu, H., Shih, C., & Kang, Z. (2024). P-13.12: The Delta E Color Dissimilarity Analysis of LCD Panels. *SID Symposium Digest of Technical Papers*, *55*(S1), 1404–1414. https://doi.org/10.1002/sdtp.17382

Mensah, A. A., Lewis Ivey, M. L., Moodispaw, M. R., & Ilic, S. (2024). Effectiveness of Chemical Sanitizers against Salmonella Typhimurium in Nutrient Film Technique (NFT) Hydroponic Systems: Implications for Food Safety, Crop Quality, and Nutrient Content in Leafy Greens. *Foods*, *13*(12), 1929. https://doi.org/10.3390/foods13121929

Min, S. C., Roh, S. H., Boyd, G., Sites, J. E., Uknalis, J., Fan, X., & Niemira, B. A. (2017). Inactivation of Escherichia coli O157:H7 and Aerobic Microorganisms in Romaine Lettuce Packaged in a Commercial Polyethylene Terephthalate Container Using Atmospheric Cold Plasma. *Journal of Food Protection*, *80*(1), 35–43. https://doi.org/10.4315/0362-028x.jfp-16-148

Nourzad, S., Naghdi Badi, H., Kalateh Jari, S., Mehrafarin, A., & Saeidi-Sar, S. (2024). Investigation of the qualitative and appearance characteristics of *Eryngium caeruleum* L. based on colorimetric and browning indices in storage conditions. *Food Science & Nutrition*, *12*(9), 6690–6698. https://doi.org/10.1002/fsn3.4243

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, *32*(1), 1–19. https://doi.org/10.1016/j.fm.2012.04.016

Pahariya, P., Choudhary, R., & Fisher, D. J. (2019). Antimicrobial Effect of Sanitizing Solutions on Fresh Romaine Lettuce. *2019 Boston, Massachusetts July 7- July 10, 2019*. 2019 Boston, Massachusetts July 7- July 10, 2019. https://doi.org/10.13031/aim.201901623

Park, S., Szonyi, B., Gautam, R., Nightingale, K., Anciso, J., & Ivanek, R. (2012). Risk Factors for Microbial Contamination in Fruits and Vegetables at the Preharvest Level: A Systematic Review. *Journal of Food Protection*, *75*(11), 2055–2081. https://doi.org/10.4315/0362-028x.jfp-12-160

Pironti, C., Dell'Annunziata, F., Giugliano, R., Folliero, V., Galdiero, M., Ricciardi, M., Motta, O., Proto, A., & Franci, G. (2021). Comparative analysis of peracetic acid (PAA) and permaleic acid (PMA) in disinfection processes. *Science of The Total Environment*, *797*, 149206. https://doi.org/10.1016/j.scitotenv.2021.149206

Pizzo, J. S., Pelvine, R. A., Da Silva, A. L. B. R., Mikcha, J. M. G., Visentainer, J. V., & Rodrigues, C. (2023). Use of Essential Oil Emulsions to Control Escherichia coli O157:H7 in the Postharvest Washing of Lettuce. *Foods*, *12*(13), 2571. https://doi.org/10.3390/foods12132571

Poimenidou, S. V., Bikouli, V. C., Gardeli, C., Mitsi, C., Tarantilis, P. A., Nychas, G.-J., & Skandamis, P. N. (2016). Effect of single or combined chemical and natural antimicrobial interventions on Escherichia coli O157:H7, total microbiota and color of packaged spinach and lettuce. *International Journal of Food Microbiology*, *220*, 6–18. https://doi.org/10.1016/j.ijfoodmicro.2015.12.013

Riemenschneider, P. (2017, January 24). Living lettuce takes root. *Produce Market Guide*. https://www.producemarketguide.com/news/living-lettuce-takes-root

Rosli, S. Z., Noranizan, M. A., Radu, S., Karim, R., Mohd Adzahan, N., Aadil, R. M., & Koh, P. C. (2022). Impact of sanitizer solutions on microbial reduction and quality of fresh-cut pennywort (Centella asiatica) leaves. *Journal of Food Science and Technology*, *59*(3), 1211–1220. https://doi.org/10.1007/s13197-021-05131-3

Rouphael, Y., & Colla, G. (2005). Growth, yield, fruit quality and nutrient uptake of hydroponically cultivated zucchini squash as affected by irrigation systems and

growing seasons. *Scientia Horticulturae*, *105*(2), 177–195. https://doi.org/10.1016/j.scienta.2005.01.025

Schifferstein, H. N. J., Wehrle, T., & Carbon, C.-C. (2019). Consumer expectations for vegetables with typical and atypical colors: The case of carrots. *Food Quality and Preference*, *72*, 98–108. https://doi.org/10.1016/j.foodqual.2018.10.002

Sela Saldinger, S., Rodov, V., Kenigsbuch, D., & Bar-Tal, A. (2023). Hydroponic Agriculture and Microbial Safety of Vegetables: Promises, Challenges, and Solutions. *Horticulturae*, *9*(1), 51. https://doi.org/10.3390/horticulturae9010051

Singh, P., Hung, Y., & Qi, H. (2018). Efficacy of Peracetic Acid in Inactivating Foodborne Pathogens on Fresh Produce Surface. *Journal of Food Science*, *83*(2), 432–439. https://doi.org/10.1111/1750-3841.14028

Topalcengiz, Z., Chandran, S., & Gibson, K. E. (2024). A comprehensive examination of microbial hazards and risks during indoor soilless leafy green production. *International Journal of Food Microbiology*, *411*, 110546. https://doi.org/10.1016/j.ijfoodmicro.2023.110546

Ukuku, D. O., Bari, M. L., Kawamoto, S., & Isshiki, K. (2005). Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces. *International Journal of Food Microbiology*, *104*(2), 225–233. https://doi.org/10.1016/j.ijfoodmicro.2005.01.016

Unruh, D. A., Stull, K. J., Pliakoni, E. D., & Gragg, S. E. (2021). A Bisulfate of Soda and Peroxyacetic Acid Solution Reduces Salmonella on Fresh-Cut Spinach. *Food Protection Trends*, *41*(4), 409–415.

USDA FSIS. (2015). *RESPONSE TO QUESTIONS POSED BY THE DEPARTMENT OF DEFENSE REGARDING MICROBIOLOGICAL CRITERIA AS INDICATORS OF PROCESS CONTROL OR INSANITARY CONDITIONS*. https://www.fsis.usda.gov/sites/default/files/media_file/2020-12/Response%20to%20Questions%20Posed%20By%20the%20Department%20of%20Defense%20Regarding%20Microbiological%20Criteria%20As%20Indicators%20of%20Process%20Control%20or%20Insanitary%20Conditions%20.pdf

Waitt, J. A., Kuhn, D. D., Welbaum, G. E., & Ponder, M. A. (2014). Postharvest transfer and survival of *Salmonella enterica* serotype enteritidis on living lettuce. *Letters in Applied Microbiology*, *58*(2), 95–101. https://doi.org/10.1111/lam.12170

WHO. (2025). *Healthy diet*. https://www.who.int/initiatives/behealthy/healthy-diet

Yoon, J.-H., & Lee, S.-Y. (2018). Review: Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. *Critical Reviews in Food Science and Nutrition*, *58*(18), 3189–3208. https://doi.org/10.1080/10408398.2017.1354813

# Chapter 6

## 6.0 Future Considerations

The use of nano-textured coatings in food production has the potential to effectively reduce *E. coli* O157:H7 infections within the food industry. It is important to consider practical factors such as cost-effectiveness, ease of application, and regulatory compliance. Notably, the active ingredients in the coating formulations evaluated are not currently food-grade. Therefore, their application on food-contact surfaces would require additional safety evaluations and regulatory approvals before they can be commercially implemented.

As living lettuce gains popularity, minimizing cross-contamination during post-harvest handling becomes increasingly important. Utilizing chemical sanitizers as a washing step for the roots could effectively mitigate *Salmonella enterica*. It is equally critical to determine whether these treatments can prevent pathogen transfer to the edible leaves. Future studies should not only focus on microbial reductions at the root level but also investigate the potential for internalization or surface contamination of the shoot system.

## 6.1 Conclusion

The findings underscore the potential effectiveness of nano-textured coatings and chemical sanitizers in addressing foodborne pathogens during both harvest and postharvest handling. The application of superhydrophobic coatings on food-contact surfaces has shown an ability to repel pathogens, which may yield operational benefits such as decreased water usage and lower cleaning costs in large-scale operations. However, the comprehensive implementation of these technologies within the food industry necessitates further assessment regarding regulatory approval, durability, and long-term effectiveness.

Chemical sanitizers continue to be widely used in the food industry. In this study, their use as a postharvest washing step for living lettuce demonstrated an effective reduction of *Salmonella enterica* without adversely affecting the appearance of the leaves. These results support the integration of root-level sanitation practices to enhance the microbial safety of leafy greens for small growers, particularly as consumer preferences for minimally processed and hydroponically grown produce grow.

Together, these strategies serve as promising tools to bolster food safety interventions. Although, their large-scale adoption must take into account factors such as practicality, regulatory compliance, economic viability, and environmental sustainability. Continued research is essential to validate their efficacy in commercial settings and ensure alignment with current food safety standards and consumer expectations.