CONTROL OF *LISTERIA MONOCYTOGENES* ON FOOD CONTACT SURFACES USING PHOTON-BASED INTERVENTIONS

by

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(Under the Direction of Govindaraj Dev Kumar)

ABSTRACT

Food contact surfaces (FCS) in produce packing houses can accrue microbial soil during processing operations and serve as reservoirs for foodborne pathogens. A surveillance study to determine the microbial load of FCS and proximate environments was carried out in peach packing facilities. Brushes used to apply wax to produce surfaces in the packing houses were the most contaminated with a bacterial population of 5.49±0.89 log CFU/cm^2. Antimicrobial blue light (aBL; 405 nm) and far UVC (222 nm) were evaluated as interventions against foodborne pathogens on FCS. It was observed that surface color for high-density polyethylene (HDPE) surfaces influenced the efficacy of aBL. A *L. monocytogenes* reduction of 1.84±0.08 log CFU/cm^2 resulted after 8h (1016.6 J/cm^2). Far UV-C exposure for 8h resulted in *L. monocytogenes* and *S. enterica* reductions of 3.26±0.33 log CFU/cm^2 and 1.01±0.32 log CFU/cm^2, respectively. The results indicate that aBL and far UVC are effective antimicrobial hurdles.

INDEX WORDS: aBL, UV-C (222 nm), *L. monocytogenes*, *S. enterica*, HDPE plastic coupons, peach packing houses.

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DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Fresh peaches carry a potential risk of microbiological contamination and food borne illnesses as evident from recent food borne outbreaks reported in the U.S. from past few years (CDC, 2020; FDA 2019; Jackson et al., 2015). Frequently, these incidents were traced back to produce packing facilities, where the specific strains responsible for the outbreaks were identified from fruit contact surfaces or the surrounding environment (Jackson et al., 2015). Several of such fresh produce associated outbreaks and recalls have necessitated the need for routine environmental monitoring and testing procedures in packing facilities.

The Food Safety Modernization Act (FSMA) provided control measures intended to minimize *L. monocytogenes* contamination of ready to eat food when exposed to environment prior to packaging or in case where food will not receive a lethal treatment prior to consumption (FDA, 2017). While pathogen specific testing is often incorporated in environmental monitoring program (EMP), quantification of indicator organisms such as total aerobes, yeast and molds, coliforms, *Pseudomonas* spp. and *Listeria* spp. are standard practices in routine sampling (FDA, 2017; NACMCF, 2015). Besides, the detection of foodborne pathogens within packing environments could be a resource intensive and time-consuming process due to their low prevalence and sporadic occurrences (Van Pelt et al., 2018). Hence, detection and quantification of indicator organisms are preferred to evaluate plant specific hygiene and sanitation regime (Cinar & ONBAŞI, 2020).

Among several foodborne pathogens, *S. enterica* and *L. monocytogenes* are commonly associated with several fresh produce outbreaks (CDC, 2020; CDC 2015; CDC 2012). They are ubiquitous in nature and could thrive adverse environmental conditions such as low temperatures

(*L. monocytogenes; -18 °C*) (Santos et al., 2019) and dry processing conditions (*S. enterica;* aw: 0.2) (Janning et al., 1994). Owing to their ability to attach and survive on food contact surfaces, they were repeatedly isolated from fresh produce packing line, such as waxer brushes, rollers, conveyor belts and sorting machines during outbreak investigations (Simonetti et al., 2021; Tan et al., 2019). Current practices of sanitation procedure involve extensive use of chemical-based sanitation treatments on food contact surfaces as a part of pathogen mitigation strategy. However, certain unit operations in packing line are incompatible for wet sanitation using chemicals and daily manual cleaning of non-uniform surfaces such as automatic sorting system, rollers, washer and waxer brushes would be time consuming and labor intensive (Williamson et al., 2018). Furthermore, there is an emergence of antimicrobial resistant pathogenic bacteria in the food processing environment. These bacteria could withstand the concentrations of sanitizers, persist on FCS and subsequently lead to cross-contamination of fresh produces. Thus, it has become imperative to find alternate strategies to prevent or delay their accumulation on FCS.

Light based interventions for decontamination are novel approach to compliment traditional chemical-based sanitation due to their comparable, if not superior bactericidal properties (von Hertwig et al., 2023). A wide spectrum of emission wavelengths broadly classified as ultraviolet (100-400 nm) and visible light (400-470 nm) have been investigated for their antibacterial efficacy. Antimicrobial blue light (aBL; 405 nm) is a light emitting diode (LED) based technology that has the potential to be used as a dry sanitation method in fresh produce industries. Bacterial exposure to aBL triggers the excitation of endogenous photosensitizers present in their cells leading to the production of reactive oxygen species (ROS) and cell death (Wang et al., 2017). While several studies reported the efficacy of aBL against foodborne pathogens in suspensions or food matrices, further studies are required to optimize their effectiveness against pathogens that are attached to

food-contact surfaces. Another novel photon-based intervention that gained researchers attention is the use of far UV-C (222 nm) for inactivation of pathogenic microorganisms. This light source targets the genetic materials (DNA and RNA), proteins structures and cell wall membrane components of pathogenic microorganisms leading to cell death (Maguluri et al., 2023). Both aBL (405 nm) and far UV-C (222 nm) are reliable technique that are harmless to humans and hence could be deployed in real world systems.

Overall, this research has three major objectives. Initially, we quantified the microbial load of hygiene indicators such as total aerobes and coliforms on food contact and non-contact surfaces of fresh peach packing facilities in Georgia. Secondly, we evaluated the physical parameters such as treatment time and surface color that contributed to potentiality of aBL (405 nm) against *L. monocytogenes* cocktail on high density polyethylene coupons. Lastly, we assessed the effectiveness of far ultraviolet-C (222 nm) light in inactivating dry inoculated gram-positive and gram-negative bacteria on high-density polyethylene (HDPE) coupons surfaces.

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CHAPTER 2

LITERATURE REVIEW

2.1 Fresh Peach Industry

Peaches and nectarines are fresh produces of high economic importance, with a global production value of 21.58 million tons reaching 2021 as reported by Food and Agriculture Organization of the United Nations (FAO, 2021). China, Spain, Italy, and United States are the top four producers of Peaches and Nectarines (FAO, 2021). In 2022, the total utilized production of peaches in the US was 6.11 million tons with a total crop value of $651 million. Although Georgia, US is called as the peach state, they stand third in total utilized production of peaches whereas California, US is the largest peach producing state (NASS, 2023). There are majorly two commercial peach growing regions in Georgia, with central region (Peach County) contributing to a production of 30.00 million pounds worth of $25.20 million annually (UGA Extension, 2021). The ideal months for peach harvest in Georgia are from mid-May through August, and the fresh peach packing industries are at their peak operation during this time (Cox, 2023; Belisle et al., 2018).

A traditional peach packing house typically involves the following unit operations (Figure 2.1) such as fruit receiving, hydrocooling, cold storage, washing, waxing, sorting, boxing, storage, and shipment (Crisosto & Valero, 2008; Williamson et al., 2018). Peaches that come from the field are dumped into peach bins and are carried for hydrocooling to remove post-harvest field heat (Bernat et al., 2017). They undergo a wash step to remove dirt or debris and conveyed to washer brushes to remove the trichomes. Peaches are often wax coated along with fungicide treatment and dried. They are sorted automatically using a sorting system and segregated based on quality

parameters. Finally, they are packed manually in boxes and are stored at cold temperature until shipped. Since peaches are consumed fresh, utmost importance should be given to ensure the safety and hygienic practices in handling peaches in the packing houses.

2.2 Hygienic Status of Peach Packing Facilities

Upon harvest, fresh fruits surfaces could retain up to 3-5 log CFU/cm^2 of microbial load when they arrive at the packing houses (Pao & Brown, 1998). This is because fresh produce is often in contact with soil, insects, water, animals, and humans which are considered as vectors of microbial contamination (Narsaiah et al., 2012). The unit operations in peach packing facilities are intended to reduce the microbial load on fruit surfaces. However, previous studies reported the prevalence of microbial contaminants and foodborne pathogens in produce contact surfaces and recirculated water used for hydrocooling or washing and their ability to cross transfer and internalize the fresh produce (Macarisin et al., 2017). The hygienic status of four fresh peach packing lines of Georgia were investigated previously by Wang et al. (2021). They identified that optical sizer (sorting cups), manual sorting areas, washer brushes and rollers were heavily contaminated. Similarly, higher microbial persistence was observed on peach surfaces and on carriers (sorting cups) during automatic sorting operations in peach packing facilities (Williamson et al., 2018). Wang et al. (2023) found that peaches collected from weighing areas after manual sorting has higher total aerobic and coliform counts compared to peaches collected from dumping area. They identified that that the hand gloves worn by peach handlers had higher incidences of thermo-tolerance coliform (39.39 %) and enterococci (7.58 %). Simonetti et al. (2021) conducted a multiyear longitudinal surveillance in tree packing houses to determine the prevalence of *L. monocytogenes* on non-food contact surfaces. Out of 17.5 % prevalence, persistent contamination was observed on packing line area. This could be attributed to high moisture and fruit debris

accumulation on packing equipment and floors during peak production times. Furthermore, *Escherichia coli* O157:H7 was isolated from peach crates in sorting line and from packers' hands during peach packing operations which indicates the inadequate sanitation and personal hygiene practices within the packing facility (Duvenage & Korsten, 2017). With multiple reports on microbial cross contamination within fruit packing facilities, it is imperative to monitor the hygiene regime of the processing facility and prevent cross contamination of foodborne pathogens with fresh peaches.

2.3 Hygiene Indicator Organisms

The sporadic occurrences and lower prevalence could hamper the detection of foodborne pathogens in fresh produce packing facilities; thus their quantification could be a resource intensive and time-consuming process (Van Pelt et al., 2018). Additionally, the testing process could be cumbersome and takes up a significant amount of product's shelf life. In some cases, the entire batch of products needs to be retained and potentially withdrawn from the market. Moreover, the test outcomes might not necessarily pinpoint the origin of the contamination (Zoellner et al. 2016). Hence, detection and quantification of indicator organisms such as total aerobes, yeast and molds, coliforms, *Pseudomonas* spp. and *Listeria* spp. are used to evaluate plant specific hygiene and sanitation regime (Ruiz-Llacsahuanga et al., 2022; Ruiz-Llacsahuanga et al., 2021b; Tan et al., 2019).

2.3.1 Aerobic Plate Counts (APC)

Aerobic plate counts (APC) or standard plate count is extensively used in the food industry as a part of environmental monitoring of microorganisms. It is an estimate of aerobic microorganisms that can grow at mesophilic temperatures (20-45 °C). A food contact surface with high aerobic plate count indicates insufficient sanitation practices (Knutson, 2020). An aerobic

plate counts less than 1000 CFU (Colony Forming Units) per food contact surface is an indicative of a clean surface (Knutson, 2020). However, till date, no direct correlation of aerobic plate counts to occurrence of pathogens or toxins are established (Gordon, 2020).

2.3.2 Coliform Counts

Coliforms are indicators of fecal contamination and general sanitation effectiveness. They are Gram negative facultative anaerobic rod-shaped bacterium that can grow at mesophilic temperature and ferment lactose to produce acid and gas (Batt & Tortorello 2014). Coliforms include *Citrobacter freundii, Enterobacter cloacae, Enterobacter aerogenes, E. coli*, and *Klebsiella pneumoniae* that belong to the *Enterobacteriaceae* family. Among these, *Citrobacter* and *Enterobacter* could be of plant origins and are not necessarily associated with public health concerns (Feng et al., 2014). However, increased levels of thermotolerant coliforms such as *E. coli* and *Klebsiella* that can grow at 44.5±0.2 °C indicates potential enteric pathogenic contamination (Pan et al., 2015).

2.3.3 Pseudomonas spp.

Pseudomonas spp. are ubiquitous microorganisms isolated from plants, animals, soil, and water (Urgancı et al., 2022). They are Gram-negative, rod-shaped psychrotrophic aerobes commonly associated with spoilage of multiple food products including fresh produces. The majority of *Pseudomonas spp.* are non-pathogenic; however, some species such as *Pseudomonas syringae, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas fluorescens* (Little et al., 1998; Li et al., 2023) are associated with plant or animal pathogenic infections. *Pseudomonas spp*. are known for its ability to form dense networks of mono or multi species biofilms (Meliani & Bensoltane, 2015). The association and interaction of *Pseudomonas* with other foodborne pathogens in a complex biofilm matrix was established in some studies (Xu et al., 2019; Dong et

al., 2022). For instance, *L. monocytogenes* exhibited improved attachment and viability on condensate forming stainless-steel surfaces with pre-existing *P. putida* biofilms (Hassan et al., 2004). The dual species biofilm formation by *L. monocytogenes* and *P. fluorescens* has been reported widely in dairy processing industries (Maggio et al., 2021). *L. monocytogenes* on food contact surfaces evaded cinnamomum zeylanicum treatment due to protection provided by *P. fluorescens* (Maggio et al., 2023). *Pseudomonas spp.* was used as a hygiene indicator in meat slaughtering process in a study conducted by Ghafir et al. (2008). The study revealed that despite a lower *Pseudomonas* count observed, there is a likelihood of isolating *Salmonella* and *Campylobacter* from broiler carcasses. Moreover, such multispecies biofilms demonstrated increased resistance to antimicrobial treatments (Fatemi & Frank, 1999). Hence, it is relevant to use *Pseudomonas* as a hygienic indicator in food industry as it shows association with foodborne pathogens.

2.4 Foodborne Pathogens in Production Surfaces

Foodborne pathogens are inherently present in the environment. They are introduced into the food processing facilities via soil and dust, surface adherence on fresh produce, processing water, cross-contamination from raw food ingredients, and poor personal hygiene. The accumulation of organic matter and debris on produce or food contact surfaces in post-harvest processing facilities could lead to microbial persistence and cross-contamination. Food pathogens such as *L. monocytogenes*, *S. enterica*, *E. coli* O157:H7, *S. aureus*, and *B. cereus* are known to survive in moist organic debris and are even capable of strong biofilm formation (Sibanyoni & Tabit, 2019). The cantaloupe Listeriosis outbreak in 2011 and the caramel apple outbreak in 2014 were linked to *L. monocytogenes* cross-contamination from FCS in packing houses (Angelo et al., 2017; McCollum et al., 2013). The *L. monocytogenes* strains isolated during environmental

samplings of FCS such as conveyor belts, brushes, wooden bins, and felt rollers in these packing houses were matched with the outbreak strains (Angelo et al., 2017). The prevalence of *Listeria* spp. in five commercial apple packing houses was investigated by Ruiz-Llacsahuanga et al. (2021a). Several samples collected from FCS such as polishing brushes, stainless steel dividers, brushes, and drying rollers tested positive for *Listeria* spp. with the highest prevalence of the pathogen found on wax coating equipment. Another example of investigation on the contamination source of *L. monocytogenes* outbreak strains which were isolated from stone fruits was traced back to its packing house environment (Chen et al., 2016). The prevalence rate of *L. monocytogenes* isolated from food and environmental samples of 48 food processing facilities in Ireland was around 4.6% (Leong et al., 2014). They also provided evidence of pathogen transfer from the food processing environment to food (same pulsotype detected in both) in four out of seven food processing facilities tested. Similarly, Muhterem-Uyar et al. (2015) demonstrated the ability of *L. monocytogenes* to transfer from non-food contact surfaces (NFCS) to FCSs in food processing environments. A potential challenge in eradicating *L. monocytogenes* from food processing facilities is due to its ability to grow at refrigeration conditions, survive acid and sanitizer stresses, and produces strong biofilms (Spanu & Jordan, 2020). Thus, this literature indicates the inefficacy of current sanitation treatments and justifies the need to develop novel strategies to eliminate pathogens from food processing environments.

2.4.1 Listeria monocytogenes

L. monocytogenes is a Gram positive, rod shaped, non-sporulating, facultative anaerobic bacterium capable of surviving a wide range of environmental conditions (Yassoralipour et al., 2023). This pathogen is ubiquitous in its nature; they are found in water, air, soil, and fresh produce (Baquero et al., 2020). They are also found to be frequently persistent in food production and

processing facilities (Mazaheri et al., 2021) (Table 2.1). Among 14 serotypes of *L. monocytogenes* described, 1/2a, 1/2b, and 4b serotypes are causative pathogens in vast majority of clinical cases (Borucki & Call, 2003). This intracellular pathogen could cause severe illnesses and mortality in humans and livestock (Borucki & Call, 2003). The consumption of food contaminated with *L. monocytogenes* has contributed to a total of 77 outbreaks with 50 multistate outbreaks, 786 illnesses, 690 hospitalizations, and 128 mortalities over the past decade (2010-2020) in the United States (CDC NORS, 2022). It is estimated that there are around 1600 cases of Listeriosis per year with 1,400 hospitalizations and 250 deaths reported in the United States (Da Silva et al., 2013). There is a zero-tolerance policy implemented for *L. monocytogenes* in ready-to-eat products which are regulated and approved by U.S. Food and Drug Administration (Archer, 2018). The clinical manifestation of listeriosis varies from mild infections such as diarrhea, fever, and headache to severe cases of fatality rate among neonatal, pregnant women, elderly, and immuno-compromised individuals (Allerberger & Wagner, 2010; Kumar et al., 2016; Fan et al., 2019).

In food processing facilities, the survivability of *L. monocytogenes* is attributed to its ability to form biofilms on diverse types of food contact surfaces, quorum sensing and adaptation due to the presence of antibiotic resistance genes (Yassoralipour et al., 2023; Da Silva et al., 2013). The initial step in biofilm formation is the attachment and *L. monocytogenes* is capable of growing biofilms on stainless steel, Teflon®, nylon, and polyester floor sealant, glass, plastic, stainless steel, metal, and wood surfaces (Torlak & Sert, 2013; Blackman & Frank, 1996; Adetunji & Isola, 2011). *L. monocytogenes* forms a monolayer biofilm with population reaching 4 to 7 log CFU/cm^2 as compared to other bacteria that produces three dimensional multilayer biofilms with cell density of 9 log to 12 log CFU/cm^2 (Gram et al., 2007). The biofilm forming ability of *L. monocytogenes* varies among strains and they are classified into weak, moderate, and strong biofilm formers

(Harvey et al., 2007). Studies show that persistent serotypes of *L. monocytogenes* that form biofilms are more resistant to antimicrobials and sanitizers compared to wild type strains (Lundén et al., 2000). Thus, persistence and survival of *L. monocytogenes* and its ability to resist traditional sanitation practices demands for a novel strategy such as photon-based interventions to prevent the bacterial establishment in the food processing environments.

2.5 Sanitizers Used for Surface Decontamination

In fresh produce industries, adequate sanitary practices are required to prevent both and produce debris accumulation and microbial adhesion to food contact surfaces (Harada & Nascimento, 2021). Overnight sanitation is a practice employed in peach processing facilities wherein peach packing lines are cleaned and sanitized to remove waste, adhered debris, dust, and other potential sources of contamination after the completion of daily operations (Cox, 2023). Decontamination of food contact surfaces involves using disinfectants that are indented to reduce the number of viable microorganisms to a desirable level (Skåra & Rosnes, 2016). In the fresh produce industry, a commonly used chemical sanitizer for surface decontamination is chlorine which is typically utilized in the form of sodium hypochlorite (NaOCl). An active concentration of up to 200 ppm was employed for sanitation purposes (Joshi et al., 2013). Another food grade chemical sanitizer approved by the Food and Drug Administration (FDA) for sanitation of food contact surfaces is peracetic acid (Zoellner et al., 2018). The usage of peracetic acid at a concentration of 160 ppm could inactivate mixed culture biofilms generated by *Pseudomonas* and *L. monocytogenes (*Fatemi & Frank, 1999). Moreover, the bactericidal effects of benzalkonium chloride, an industrial sanitizer and quaternary ammonium compounds, against foodborne pathogens such as S*taphylococcus aureus, L. monocytogenes, Bacillus cereus, Salmonella typhimurium, Escherichia coli and Pseudomonas aeruginosa* were investigated (Skåra & Rosnes,

2016). These chemical sanitizers were found to be effective against Gram positive strains compared to Gram negative bacterium.

Dry sanitation techniques are employed in areas in produce processing lines such as sorting cups and roller conveyors. Since these surfaces are difficult to access and clean, the unnecessary introduction of moisture would lead to potential microbial growth niches or biofilms (Burnett & Hagberg, 2014). Dry sanitation involves procedures that do not use a notable amount of moisture. This may include wiping, dusting, spraying alcohol-based suspensions, application of dry heat (or steam), gaseous antimicrobial agents such as ozone, and physical treatment such as UV (Ultraviolet) light or irradiation (Burnett & Hagberg, 2014). Harada & Nascimento (2021) conducted a comparison study on different dry sanitation techniques such as UV-C light, dry heat, gaseous ozone, 70% ethanol, and a commercial sanitizer against *Bacillus cereus* biofilms on stainless steel and polypropylene surfaces. Their studies indicate that dry sanitary techniques were least efficient compared to sodium hypochlorite suspension (wet sanitary method). In another research, Wesolowski, (2019) evaluated the sanitizing efficacy of ethanol mist to reduce *Salmonella* populations on the surfaces of tomatoes and cantaloupes. They used a Biomist system that uses carbon dioxide gas as a carrier to atomize ethanol. The Biomist based sanitation technique would eliminate the necessity for a wiping/ rinsing step that reduces the risks of cross contamination (Biomist Anonymous, 2023). Nevertheless, a major challenge associated with the application of chemical-based sanitizers is the potential development of residues on food contact surfaces. These chemical residues, when transferred to fresh produce, pose a high toxicity risk upon human consumption. Furthermore, there is an emergence of several antimicrobial resistance pathogens isolated from food contact surfaces (Iwu & Okoh, 2019). Hence, novel interventions are required to address the drawbacks of chemical-based sanitation systems.

2.6 Photon Based Interventions

Light based technologies for microbial decontamination are a novel intervention that holds potential to be applied in the food industry for sanitation practices. These light-based methods offer cost-effective and user-friendly alternatives for reducing microbial populations on produce and food contact surfaces. The bactericidal property of light-based technology lies in the spectrum of electromagnetic radiation ranging from 100 nm to 600 nm (Figure 2.2). Ultra-violet irradiation (wavelength of 100-400 nm), a promising germicidal technique are classified into 4 distinct groups; UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm) and vacuum UV (100-200 nm) (Cassar et al., 2020). Antimicrobial blue light (aBL) is a novel light-based technology that is known to exhibit microbicidal properties in the visible spectrum of 400-460 nm (Hadi et al., 2020). In most cases, light-based technologies exhibit bactericidal properties by inducing damage to bacterial genetic material (DNA or RNA) upon light exposure (Chen & Moraru, 2023). Although UV-C (254 nm) produced by mercury lamps with monochromatic output were proved to be most effective in killing the foodborne pathogens, they are prone to mammalian skin damage (Shin et al., 2020). Hence, we assessed the efficacies of two light-based interventions that have less cytotoxic effects on humans against foodborne pathogens.

2.6.1 UV-C Technology (222 nm)

According to FDA regulations (FDA, 2001), UV irradiation is a safe technology to inactivate pathogens on food contact surfaces and liquid food products. The application of UV-C in disinfection treatment was established previously. Far-UV-C light (207 to 222 nm) has exhibited a comparable efficacy to traditional germicidal UV light (254 nm) in inactivating the microorganisms (Buonanno et al., 2020). The killing mechanism of far UV-C (222 nm) involves destruction of bacterial cell membrane and protein molecules (Maguluri et al., 2023). The

penetration power of far UV-C is much larger than the size of bacteria and viruses, however, is limited to penetrate on biological surfaces such as skin and cornea of eye. For this reason, it can efficiently eliminate microorganisms without affecting human cells compared germicidal UV-C (254 nm) (Buonanno et al., 2020). Regarding the light source used for UV-C, many studies reported the utilization of a Krypton Chloride (KrCl) excimer lamps with a primary emission at a wavelength of 222 nm (Buonanno et al., 2020). Excimer lamp offers several design features such as rapid warm-up, possess extended lifetimes, exhibit wavelength-selectivity, provide design flexibility and moreover, is safer compared to mercury-based lamps (Rahmani et al., 2009).

The efficacy of UV-C light sources of three different wavelengths (222, 254, 282 nm) against inactivation of *Escherichia coli* O157:H7 in apple juice matrix was evaluated by Yin et al. (2015). They found that far UV-C (222 nm) provided significantly higher reduction (2.81 log CFU/ml) of pathogen compared to other light sources exposed at same fluences (75 mJ/cm^2). The reactivation potential of *E coli* O157:H7 after far UV-C exposure was also significantly lower. Narita et al. (2020) studied the germicidal effect of 222 nm excilamps (KrCl) on live bactericidal cells, endospores, fungal hyphae and spores, and viruses. They found that far UV-C (222 nm) had the potential to inactivate a wide spectrum of foodborne pathogens. Moreover, exposure to far UV-C (222 nm) had a strong detrimental effect on bacterial endospores possibly due to presence of peptide bonds and amino acids present in the cell walls. In another study, the KrCl excilamps that emit UV-C (22 nm) light were used for inactivation of Gram-positive and Gram-negative pathogens such as *L. monocytogenes*, *S. aureus E. coli* O157:H7, *P. aeruginosa* in liquid films (1.2 mm thickness) and biofilms on stainless steel surfaces (Chen & Moraru, 2023). An exposure dosage of 354 mJ/cm^2 minimized the biofilm formation and increased the susceptibility of surviving cells to sodium hypochlorite by two folds. Shin et al. (2020) studied the combined effect

of 222 nm excilamps and 280 nm Light emitting diode (LED) on inactivation of *Salmonella* Typhimurium and *L. monocytogenes* liquid suspensions. An exposure to combination treatment inactivated foodborne pathogens with reduced cell recovery and increased cell damage. Ha et al. (2017) evaluated the underlying mechanism of 222 nm UV-C excilamps in inactivating foodborne pathogens using a fluorescent staining method. The intracellular enzyme inactivation and cell membrane damage were identified as the primary factors contributing to enhanced bactericidal activity.

2.6.2 Antimicrobial Blue Light Technology

Antimicrobial blue light (aBL) is a novel light-based technology that is known to exhibit microbicidal properties in the visible spectrum of 400-460 nm. In recent years, the application of aBL as a chemical-free, non-antibiotic, disinfectant technology has been investigated against the inactivation of foodborne pathogens and their biofilms. The most accepted explanation of the mechanism in which aBL-mediated cell death occurs is via intracellular reactive oxygen species (ROS) production. The excitation of photosensitizing chromophores such as porphyrins (protoporphyrin, coproporphyrin, and uroporphyrin), found in bacterial and fungal species results in the generation of ROS that leads to damage of cell membrane, genetic material (DNA), and via lipid peroxidation (Hadi et al., 2020; Leanse et al., 2022). The phototoxic effect of blue light technology is relatively less prominent in mammalian cells (host cells) due to its high specificity to higher concentrations of porphyrins present in bacteria (Leanse et al., 2022). The aBL is found to be equally effective against both Gram-negative and Gram-positive bacteria (Dietel et al., 2007). However, their susceptibility varies among different pathogens (Hadi et al., 2020). Apart from that, aBL could regulate multi-cellular behaviors such as intracellular communication via blue light receptors present in bacteria, hence preventing biofilm formation (Dai et al., 2012). Because

of their multiple target characteristics, it is envisioned that the antimicrobial resistance ability to aBL would be rather a slow process compared to antibiotics and other disinfectant chemicals used in the food industry (Dai et al., 2012; Wang et al., 2017). Ultraviolet radiation (wavelength 100 nm- 400 nm), even though proven to have germicidal properties, continuous exposure could lead to damage of host cells (for example; human skin), with potential carcinogenic effects (Trevisan et al., 2006). The antimicrobial blue light on the other hand was found to be relatively innocuous to host cells (human skin) (Enwemeka et al., 2021).

The illumination of aBL can be achieved by using diverse sources of light-based technologies. The most used light source for aBL studies is light emitting diodes (LEDs) comprised of a semiconductor, which are typically made of III-nitride blue super luminescent LED on gallium nitride (GaN, binary direct bandgap semiconductor) with a central wavelength of 420nm and emission bandwidth of ~ 5nm (Feltin et al., 2009). The other available light sources are laser diode consisting of a higher coherence and narrow emission bandwidth and femtosecond lasers, used for pulsed photodynamic therapy that emits pulsed light (10^{-15} s per pulse) both of which are more expensive than LED (Dall Agnol et al., 2009; Tsen et al., 2011). However, Masson-Meyers et al., (2015) found that the antimicrobial efficacy of the blue light is independent of the light source (405 nm; LED and Laser diode) at comparable fluences against methicillin-resistant *Staphylococcus aureus* (MRSA).

The emission wavelength (nm), irradiance (mW/cm^2), dosage (J/cm^2), treatment time (hr), and pulsing light at different % duty cycles, are some of the treatment parameters that could affect the antimicrobial efficacy of blue light in killing a pathogen. Temperature, solid or liquid medium, type of materials exposed to blue light, targeted microorganism, nature of bacterial attachment, availability of nutrients, and surface characteristics also contribute to its microbicidal effects.

Guffey & Wilborn, (2006) compared the bactericidal effects of 405 nm and 470 nm blue light regimes on aerobic (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and anaerobic bacterium (*Propionibacterium acnes*) at different treatment dosages. They found that the bactericidal efficacy of two wavelengths of aBL was dose-dependent. They were effective against aerobic bacteria in comparison to anaerobic bacteria. In another study, blue light treatment of liquid media containing *E. coli, S. aureus,* and *E. faecalis* (405 nm, 5.73 J/ cm^2) was ineffective in killing the pathogens whereas 94.26% of the anaerobic pathogen (*P. nigrescens)* were killed at the same level of exposure (Hope et al., 2016). Hence, these studies indicate the lethal role of oxygen in photodynamic treatments.

Previously, some studies have been conducted on the efficacy of aBL against pathogens attached to food contact surfaces. In a study conducted by Wu et al. (2022), a cocktail of seven Shiga Toxin-producing *E. coli* (STEC) serotypes inoculated on food contact surfaces (304 stainless steel and polyoxymethylene plastic) was subjected to two different types of blue light treatment prototypes (405 nm, 26 mW/$cm^{2,}$ and 405 nm; 50 mW/cm^2) in nutrient-rich and deficient conditions. No STEC cells were recovered from plastic surfaces when prototype 2 was used, and only four serotypes survived on stainless steel, detected using polymerized chain reaction analysis. They reported that the variation in aBL efficacy is because of differences in bacterial affinity or attachment due to variations in microstructural and physical characteristics of surfaces. Also, nutrient-rich conditions could absorb light which diminished the bactericidal efficacy of blue light. Similarly, *Campylobacter jejuni* was inactivated to a higher degree on polyvinylchloride surfaces when subjected to aBL (395 ± 5 nm) than on stainless steel with a difference in 2 log reduction (Haughton et al., 2012). aBL has been used to treat biofilm-forming Gram-negative pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Around 3 $\log_{10}$ colony-forming

units (CFU) inactivation of pathogens (24-hour-old and 72-hour-old biofilms) has been achieved (wavelength of 415 nm and dosage of 432 J/cm^2) indicating its efficacy against biofilm-forming bacteria (Wang et al., 2016). The ability of aBL (405nm) in preventing the biofilm formation of *L. monocytogenes* on stainless steel and acrylic coupons supplemented with salmon exudate was evaluated by Li et al. (2018). An illumination temperature of 25° C for 8 h significantly reduced both the biofilm population and planktonic cells on both coupons. They also found that performed *Listeria* biofilms were highly susceptible to sanitizer treatment (200 ppm quaternary ammonium compound) post LED blue light treatment. This study shows the potential of combining aBL with chemical sanitation treatments in preventing biofilm formation. Hence, it would be interesting to study the efficacy of aBL on multispecies biofilms with the most resistant and susceptible pathogens causing foodborne illnesses.

2.7 References

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Table 2.1: *L. monocytogenes* outbreaks associated with fresh produce and cross-contamination from food contact surfaces reported in United States (2011-2022).

Year	Food associated with outbreak	Source of contamination	Epidemiological information	References
2021	Packaged salads	Isolated from equipment used to harvest iceberg lettuce at Dole processing facility.	Illnesses: 18 Hospitalizations: 16 Deaths: 3 States: 13	CDC (2022a)
2021	Packaged Salads	Isolated from packaged salad produced at the Fresh Express facility	Illnesses: 10 Hospitalizations: 10 Deaths: 1 States: 8	CDC (2022b)
2016	Frozen vegetables	Isolated from Frozen corn, peas produced by CRF Frozen Foods.	Illnesses: 9 Hospitalizations: 9 States: 4 Deaths: 3	CDC (2016a)
2016	Packaged salads	Isolated from packaged salad produced at Dole processing facility	Illnesses: 19 Hospitalizations: 19 States: 9 Deaths: 1	CDC (2016b)
2014	Caramel apple	Isolated from polishing brushes, stainless steel dividers and brushes under fans/blowers, and dryer rollers in packing houses	Illnesses: 35 Hospitalizations: 34 States: 12 Deaths: 7	Ruiz-Llacsahuanga et al. (2021a), CDC (2015)
2011	Cantaloupe	Isolated from brush and felt rollers	Illnesses: 147 Hospitalizations: 143 States: 28 Deaths: 33	CDC (2012), McCollum et al. (2013)

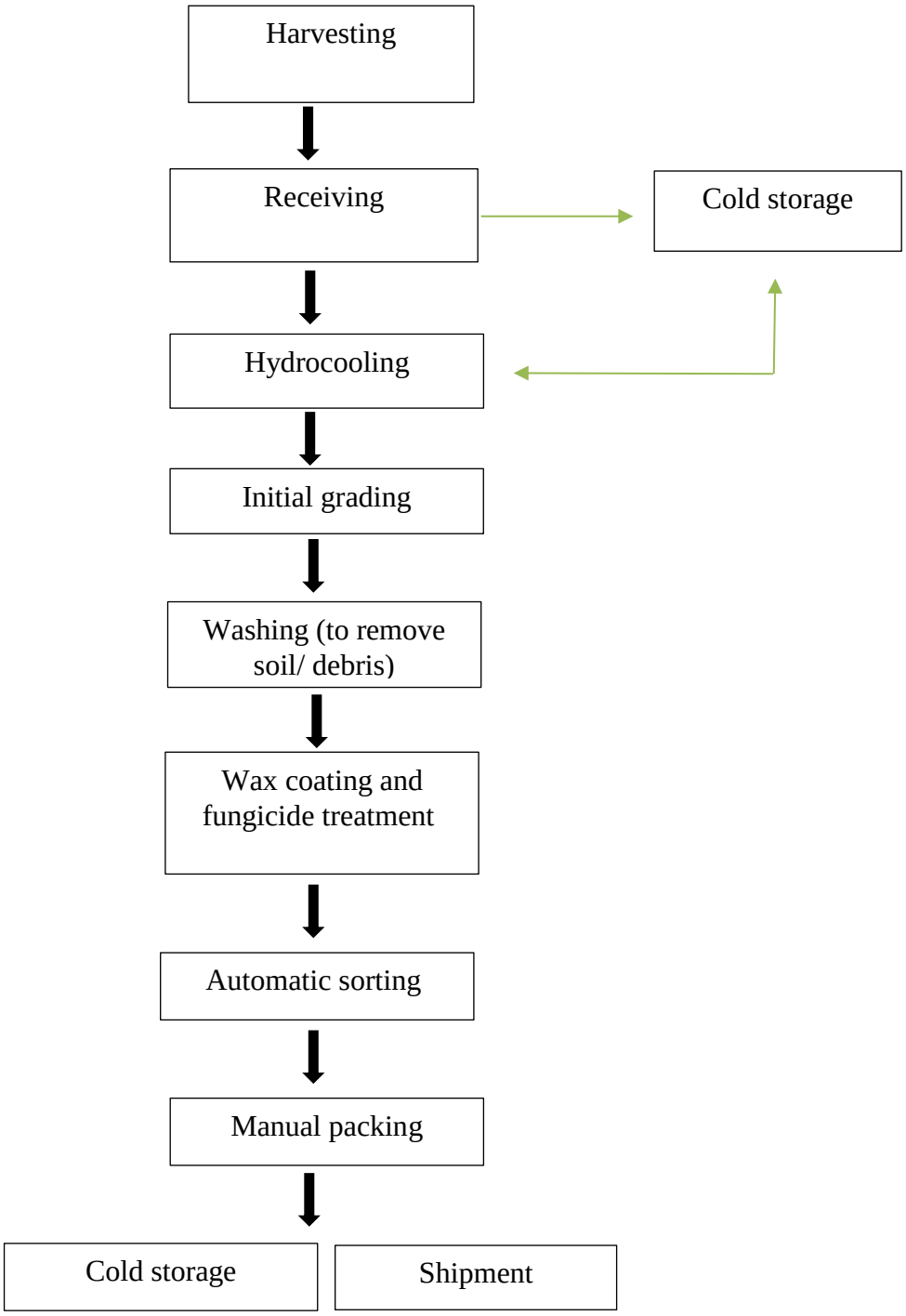

Figure 2.1: Unit operations in a peach packing house.

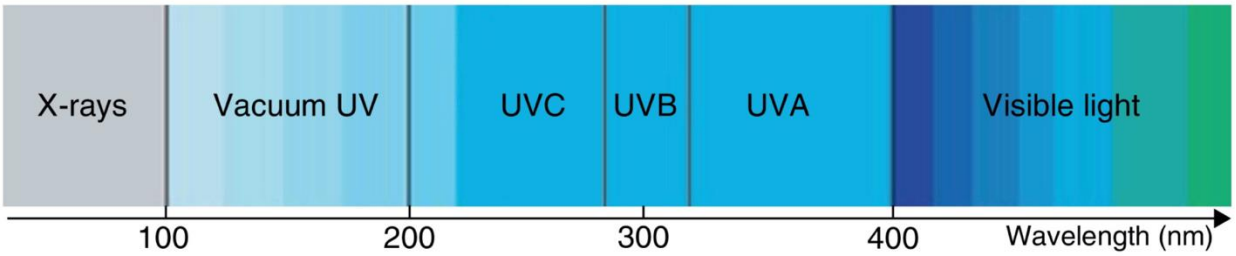

Figure 2.2: The ultraviolet spectrum in electromagnetic radiation (adapted from Yin et al., 2013)

CHAPTER 3

Microbial Indicators and Possible Focal Points of Contamination During Post-Harvest Handling of Peaches

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Abstract

A microbial environmental monitoring of fresh produce packing facilities is crucial in estimating the microbial levels and potential cross contamination risks arising from soiled surfaces within a packing line. In our study, we monitored the microbial population of two distinct peach packing facilities of southern United States (U.S.), throughout different time (early, mid and late) of peach packing season in 2023. Surfaces swabs were collected from eight food contact surfaces (Zone 1) and four non-food contact surfaces (Zone 2 and 3) and enumerated for hygiene indicators such as heterotrophic plate counts (HPC), presence or absence of *E. coli* and *Pseudomonas* spp. using traditional microbiological methods. A significant difference in HPC was observed between two packing facilities ($p \leq 0.05$). Even though overall average HPC (4.22±1.64 log CFU/cm^2) of zone 3 were higher compared to zone 1 and 2, waxer brushes in zone 1 were found to be heavily contaminated (HPC: 5.49±0.89 log CFU/cm^2) ($p \leq 0.05$) among all the surfaces tested. We isolated *P. aeruginosa* from food contact surfaces such as rollers and post wash conveyors during different time intervals within the same packing facility. Enrichment of dust samples collected from zone 2 and 3 of peach packing line has resulted in detection of coliforms, possibly belonging to *Enterobacter cloacae.*

3.1 Introduction

Peaches (*Prunus persica* L.) are sixth largest non-citrus fruit crop produced in U.S., with a total production reaching 612 thousand tons in the year 2022 (NASS, 2023). Fresh peaches are harvested annually from mid-May through August during which peak post-harvest processing takes place (NASS, 2017). The fresh peach packing line generally involves several unit operations such as washing, waxing, conveying, sorting, weighing, and final packing (Wang et al., 2021; Ait-Oubahou et al., 2019).

Fresh peaches can be contaminated by foodborne pathogens and result in outbreaks and product recalls (CDC, 2020a; FDA 2019; Jackson et al., 2015). A total of three foodborne outbreaks have been associated with fresh peaches with 2 multistate outbreaks, 120 illnesses, 30 hospitalizations and one mortality (CDC, NORS, 2022). Majority of outbreaks have been attributed to contamination by foodborne pathogens, notably *Listeria monocytogenes* and *Salmonella* Enteritidis (CDC, 2020a; CDC 2015; CDC 2012). Packing house surfaces and proximate areas can often serve as niches for foodborne pathogens and result in the spread of contamination to fruit (Jackson et al., 2015). Fresh fruit, fresh cut fruit (FDA, 2020; CDC, 2020b) and processed fruit (Angelo et al., 2017) have been implicated in outbreaks and recalls resulting from contamination with packing house surfaces (CDC, 2012; McCollum et al., 2013). The recurrence of such fresh produce associated outbreaks and recalls have necessitated the need for routine environmental monitoring and testing procedures in packing facilities.

The Food Safety Modernization Act (FSMA) introduced comprehensive guidelines aimed at mitigating the environmental contamination of ready-to-eat (RTE) foods with *L monocytogenes* within packing facilities (FDA, 2017). However, current FDA regulations do not advocate the use

of pathogen specific testing on food contact surfaces, instead, use of indicator organisms was suggested (USDA, 2017). Routine surveillance for the detection of foodborne pathogens within packing houses could be a resource intensive and time-consuming process due to their low prevalence and sporadic occurrences (Van Pelt et al., 2018). Hence, detection and quantification of indicator organisms such as total aerobes, yeast and molds, coliforms are used to evaluate plant specific hygiene and sanitation regime (Holland et al., 2021; Wang et al., 2021; Tan et al., 2019). Interestingly, previous studies suggested the use of *Pseudomonas* spp. as an indicator during environmental monitoring due to their ability to coexist and shelter biofilms produced by foodborne pathogens (Tan et al., 2019; Fagerlund et al., 2021; Rolon et al., 2023). For example, *Pseudomonas* spp. was detected in abundance where there was a persistence contamination of *L. monocytogenes* within tree fruit packing facilities (Tan et al., 2019). Hence, it is essential to include *Pseudomonas* spp. in evaluating the sanitation and hygiene practices.

Numerous studies have been conducted to assess the prevalence of foodborne pathogens and the microbial safety of fruit packing operations. The microbial assessment of the hygienic condition of peach packing lines in a Southern United States over two harvest seasons were conducted by Wang et al. (2021). Williamson et al. (2018) studied a specific unit operation (sorting system) in stone fruit packing and their ability to cross contaminate the fruit with foodborne pathogens such as *L. monocytogenes* and *S. enterica*. Furthermore, *E. coli* O157:H7 was isolated from peach crates in a sorting line and from packers' hands during peach packing operations which indicates the inadequate sanitation and personal hygiene practices within the packing facility (Duvenage & Korsten, 2017). The routine evaluation of microbial population from various environmental surfaces provides an assessment of hygiene and aids in targeted sanitation. While visibly soiled

surfaces might be sanitized during post-operational clean up, dusty non-food contact surfaces might often be overlooked as harborage points of bacteria.

Hence, the objective of our study was to (i) quantify the indicator microbial population (heterotrophic plate counts, presence or absence of *E. coli*, and *Pseudomonas* spp.) on environmental samples collected from packing lines across a peach packing season in two packing facilities and (ii) to identify surface types or unit operation that has greater likelihood to harbor foodborne pathogens.

3.2 Materials and Methods

3.2.1 Sample collection

Two commercial peach packing houses in Southern state of U.S. were surveyed during peak harvest season (June-July 2023). The packing houses were selected based on facility design i.e., open air construction (PH1; packing house 1) and enclosed facility (PH2; packing house 2). We classified the sampling sites into different zones based on risk of cross contamination with the fruits. A total of twelve representative sampling sites which includes eight fruit contact surfaces (zone 1), and four non-contact surfaces (zone 2 and zone 3) were selected for the study (Table 1). Zone 2 were areas that are in closer proximity to fruit contact surfaces (zone 1) and zone 3 consisted of non-fruit contact surfaces that are adjacent to zone 2 and poses least chances of cross contamination during normal operational hours.

The samples were collected from both packing houses throughout the fresh peach packing season. Duplicate swab samples were collected from the selected areas after processing fresh peaches in packing lines. The environmental swab samples were collected using a sterile sampling sponge stick (1.27×7.62 cm^2; 3M™, St. Paul, MN, USA) moistened with 10 mL D/E Neutralizing Broth. A sterile stencil (10 × 10 cm^2; square shaped) was used to swab a surface area of 100 cm^2.

Swabbing was performed on both flat and non-flat surfaces from top to bottom, right to left and diagonal strokes ten times. The swab sponges were transferred into sterile sample bags, carried in a portable cooler and processed within 24 h.

3.2.2 Dust sampling

Dust samples were collected from 6 non-food contact surfaces near packing lines (Table 3.1) from one of the packing facilities using sterile wood handled cotton swabs (Fisherbrand™, Thermo Fisher Scientific, MA, USA) and transferred them to a sterile whirl Pak bag (Nasco, Modesto, CA, USA).

3.2.3 Microbiological evaluation of swab samples

To each sample bag containing swab samples, 90 ml of sterile 1x Phosphate Buffered Saline (PBS; VWR chemicals LLC, Solon, OH, USA) was added and stomached for 10 seconds using a stomacher (Corning, Gosselin S-Blender 1, Corning life sciences, NY, USA) at a speed of 550 strokes/min. Hydrocooling water that was collected directly in the sterile swab sampling bags were stomached and enumerated. Serial dilutions of the homogenized samples were performed by transferring 100 µl of solution into 900 µl of 1x PBS.

3.2.3.1 Enumeration for heterotrophic plate counts

Enumeration for Heterotrophic plate counts (HPC) was performed using the droplet plate technique (Kumar et al., 2023; Oguadinma et al., 2022; Herigstad et al., 2001). Total heterotrophic plate counts (HPC) were enumerated on tryptic soy agar (TSA; Neogen, Lansing, MI, USA) by plating 20 µl of samples from each dilution and the colonies within the perimeter of drops were counted after 48 h of incubation at 35 °C. The limit of detection was 1.69 CFU/cm^2.

3.2.3.2 Presence/Absence of *Escherichia coli*

The samples were enumerated on MacConkey agar (BD Difco™ Sparks, MD, USA) using drop plate technique as previously described. Further steps were followed for the identification and confirmation of the presence of *E. coli* (Figure 3.1). The dilutions that have presumptive lactose fermenting strains were identified by pink color colonies surrounded by a zone of acid precipitated bile on MacConkey agar. Selected colonies from MacConkey agar plate were streaked on to 4-Methylumbelliferyl-β-D Glucuronide (MUG) MacConkey agar (HIMedia Lab. Pvt. Mumbai, India) and eosin methylene blue agar (EMB; CRITERION™, Levine, Hardy Diagnostics, CA, USA). The EMB and MUG MacConkey agar plates were incubated at 37° C for 24 h. Lactose fermenting colonies on MUG MacConkey agar were observed for blue-green fluorescence under longwave ultraviolet light (365 nm) and presence of green metallic sheen producing colonies were observed on EMB agar plates. The presumptive positives were further streak plated on CHROMagar (CHROMagar™ STEC base; Alere, Inc., ON, Canada) and incubated for 24 h at 37 °C. The plates were observed for blue or red color colonies to identify the growth of *E. coli.*

A biochemical test was also performed on all suspected colonies that gave positive results on CHROMagar, EMB, and MUG MacConkey agar using Analytical Profile Index API 20E test kit (BioMerieux, NC, USA) according to manufacturer's instruction.

3.2.3.3 *Pseudomonas aeruginosa*

Enumeration of *Pseudomonas* spp. was performed on *Pseudomonas* isolation agar (PIA; Remel™ Thermo Scientific™, KS, USA) using the drop plate technique and observed for the presence of typical colonies after 48 h of incubation at 35 °C. Blue-green pigmented bacterial colonies on PI agar were isolated and streak plated on sterile PI agar plates followed by incubation for 24 h at 37 °C. The bacterial colonies were observed under longwave ultraviolet light (365 nm) for blue

fluorescence. The colonies were used for detection and confirmation of *Pseudomonas aeruginosa*. Further, the API 20E was also used for species level characterization of *Pseudomonas* spp. isolated on PI agar.

3.2.4 Microbiological evaluation of dust samples

To each sampling bag containing dust samples, 10 ml of 1X PBS was added and stomached for 10 sec using a stomacher at a speed of 550 strokes/min. Initially, the samples were serial diluted and enumerated on TSA, MacConkey agar and *Pseudomonas* isolation agar using drop plate technique. Followed by that, all the dust samples were enriched by transferring 1 ml of homogenized dust sample from each sampling bag to 1 ml of 2X tryptic soy broth (TSB; Neogen, Lansing, MI, USA). The samples were incubated at 37 °C for 24 h and streak plated a 10µl loopful of suspension on MacConkey agar and *Pseudomonas* Isolation agar in duplicates. The agar plates were incubated at 37 °C for 24 h and bacterial colonies if detected, were streak plated on MUG MacConkey agar, EMB agar, and CHROMagar.

3.2.5 Whole genome sequencing

A whole genome sequencing was performed on presumptive *E. coli* and *Pseudomonas aeruginosa* strains isolated on respective selective agar medium. The genomic double stranded DNA (1 µg of gDNA) was extracted and purified by using the ZymoBIOMICS™ DNA Miniprep kit (Zymo Research Corp., Irvine, CA, USA). The purity of the gDNA was evaluated using a 260/280 nm spectrophotometric ratio on a BioTek Cytation3 multimode microplate reader (Agilent Technologies, Inc., Santa Clara, CA). Extracted gDNA was shipped to Plasmidsaurus (Plasmidsaurus Eugene, OR) for whole genome sequencing.

3.2.6 Statistical Analysis

All the statistical data analysis was conducted using JMP Pro 17 (SAS Institute Inc., Cary, NC). A significant difference in average HPC was analyzed using Analysis of variance (ANOVA) and pairwise comparisons between means were analyzed using Students' t test (PH1 and PH2) or Tukey's HSD (honestly significant difference) test.

3.3 Results

3.3.1 Temporal variation in microbial load through peach packing season

Statistical analyses were conducted on the comprehensive microbial populations present in the environmental samples collected throughout the peach packing season (Table 3.2). When overall microbial population of both PH1 and PH2 were compared, the mean HPC during end and mid of packing seasons was significantly higher ($p \leq 0.05$) than the early-packing season. When individual packing houses were taken into consideration, a significant increase in the mean HPC was observed for PH1 (5.78±1.15 log CFU/cm^2), at the end of the packing season compared to early and mid-packing season. However, in the case of PH2, the mean HPC during mid-packing season was significantly higher than early packing season ($p \leq 0.05$) (Table 3.3). Based on API 20E test, *Klebsiella pneumoniae* and *Citrobacter freundii* were detected in sampling sites of PH1 during early and end season of sampling (Table 3.5).

3.3.2 Focal points of contamination

The results showed that packing zone and sampling sites had significant effect on mean HPC ($p \leq 0.05$). The mean HPC between PH1 and PH2 showed a statistical significance ($p \geq 0.05$), with a high level of HPC (4.28±1.67 log CFU/cm^2) observed in PH1. Zone 3 had the highest average HPC (4.22±1.64 log CFU/cm^2) and compared to zone 1 and 2 ($p \leq 0.05$).

When microbial load on sampling sites were compared, waxer brushes (zone 1) were found to have a higher mean HPC (5.49±0.89 log CFU/cm^2) ($p \leq 0.05$). The HPC were least for peach bins (2.96±1.09 log CFU/cm^2), packing tables (2.05±0.7 log CFU/cm^2), rollers (pre-wax/wash) (3.03±1.35 log CFU/cm^2), and post wash conveyor (3.28±1.53 log CFU/cm^2). For PH1, the mean HPC did not significantly vary across different sampling sites. In contrast, for PH2, the mean HPC on peach bins (2.42±1.14 log CFU/cm^2) were significantly higher than those on waxer brushes (5.60±0.74 log CFU/cm^2) (Table 3.4). Even though higher mean HPC was observed on zone 3 compared to zone 1 and zone 2 (in both packing houses) ($p \leq 0.05$), comparable HPC (>5 log CFU/cm^2) were observed on a fruit contact surface (waxer brushes; zone 1). Coliforms such as *Klebsiella pneumoniae* and *Citrobacter freundii* were isolated from all three different zones which includes peach bins, waxer brushes (zone 1); waxer washer area (zone 2) and main production drains (zone 3) (Table 3.5).

Pseudomonas aeruginosa were isolated from environmental swabs that were collected from the fruit contact surfaces of PH2. The sampling sites that tested positive for *Pseudomonas aeruginosa* were rollers (early packing season) and post wash conveyors (mid packing season).

3.3.3 Microbial population in dust samples

Initially, no microbial growth was observed on TSA, MacConkey agar and PI agar when dust samples were enumerated. However, an enrichment of dust samples allowed the growth of stressed microorganisms leading to isolation of coliforms from dust samples collected from motor near the post wash conveyor (zone 2). The API 20 E test showed that the isolated strains could possibly be *Citrobacter freundii* (38.7 %). The whole genome sequencing results of microbial colonies isolated from dust samples indicated the presence of *Enterobacter cloacae* strain R11 plasmid pASM4 (99.9%). However, *Pseudomonas* spp were not detected on dust samples.

3.3 Discussion

Environmental monitoring of fresh produce packing facilities is critical to identify microbial hotspots and their potential sources of contamination. Numerous factors such as seasonal, spatiotemporal and geographical location, field practices and interventions steps, hygiene and sanitation protocols followed contribute to dynamic microbial population within a packing facility. In this study, facility construction type, packing season, sampling sites, and zones were considered to evaluate the microbial population after packing operations.

A higher HPC on PH1 which has an open air construction, justifies the possibility of elevated airborne transmission and deposition of microbial population on different surfaces swabbed. When considering seasonal variations, we observed a gradual increase in microbial load towards the end of the season. This increase could be attributed to the deposition of a complex matrix of dust, microbes, moisture, and debris on inaccessible parts that are difficult to clean using current sanitation protocols (Kumar et al., 2018). Our findings indicated that both packing facilities exhibited a higher HPC exceeding 5 log CFU/cm^2 on waxer brushes, followed by washer brushes and sorting cups in zone 1. Likewise, zones 2 and 3 showed an HPC population of more than 3.5 log CFU/cm^2 in PH1. Prior studies also reported a significant accumulation of microorganisms (HPC) on waxing and washer brushes (3.26 log CFU/cm^2) and sorting cups (exceeding 4 log CFU/cm^2) before packing house sanitation (Wang et al., 2021; Duvenage & Korsten, 2017). Comparable results were observed for coliform counts. Even though, foodborne pathogens were not isolated in our study, these sampling sites were identified as potential harborage for foodborne pathogens such as *Escherichia coli* O157:H7, *L. monocytogenes*, and *Staphylococcus aureus* (Gil et al., 2023; Simonetti et al., 2021; Duvenage & Korsten, 2017), as reported in previous studies. A higher prevalence of *L. monocytogenes* was observed in zone 3 (51%), followed by zone 2 (14%)

and zone 1 (13%) in a fresh-cut processing facility handling fruits, vegetables, and salads (Gil et al., 2023). Similarly, high prevalence rates of *L. monocytogenes* (100%) were found in waxing areas, floor blow sorting lines (33%), and main floor drains (91.7%) in tree fruit packing facilities (Simonetti et al., 2021). A higher prevalence of *Listeria* spp. on waxing brushes was attributed to the ability of brush bristles to trap the microorganism and wax residues accompanied by inadequate sanitation procedures (Ruiz-Llacsahuanga et al., 2021a). Furthermore, *E. coli* O157:H7 was isolated from floors, crates, sorting lines, and cold storage rooms in a peach packing facility in South Africa (Duvenage & Korsten, 2017). Hence, it is imperative to implement adequate intervention steps in these areas to mitigate cross-contamination of fresh produce.

Coliforms are gram negative non spore forming facultative anaerobic bacillus that are capable of fermenting lactose to produce acid and gas at 35±2 °C within 24-48 h (Halkman & Halkman, 2014). These include majorly four genera of *Enterobacteriaceae* family such as *Klebsiella, Enterobacter, Citrobacter,* and *Escherichia* (Halkman & Halkman, 2014). Pathogenic non-lactose producing *Enterobacteriaceae* such as *Proteus, Salmonella, Yersinia,* and *Shigella* could also grow on MacConkey agar to produce colorless colonies (AuWerter, 2021). Apart from that bacterium belonging to genus *Erwinia,* a plant pathogen, and *Aeromonas* are capable of fermenting lactose, however, are not classified as coliforms (Elrod, 1942). A positive correlation between aerobic plate count (APC) and coliform counts (0.617) were observed on livestock samples (meat such as chicken, beef, and pork) collected from retail store (Kim & Yim, 2016). In contract a rapid test (ATP adenosine triphosphate swab test) could not establish a strong association between *E. coli* counts and APC while monitoring the microbial load of food contact surfaces of apple packing facilities after sanitation procedures (Ruiz-Llacsahuanga et al., 2021b). Hence, adequate intervention steps are required in these areas to eliminate cross contamination of fresh produce.

Pseudomonas spp. is ubiquitous and predominant microbiota in food processing environments (Gu et al., 2019; Xu et al., 2022). Recent studies revealed their ability to coexist with foodborne pathogens such as *L. monocytogenes* and *S. enterica*, sheltering them by forming biofilms (dos Santos et al., 2023; Chen et al., 2022; Tan et al., 2019; Fagerlund et al., 2021; Rolon et al., 2023). *Pseudomonas* spp are not only causative organism for food spoilage, but also could be employed in food industry as a hygiene indicator, especially to monitor *L. monocytogenes* (Chen et al., 2022). In our study, we isolated *P. aeruginosa* from post wash conveyors and rollers (zone 1) of PH2. Similarly, *Pseudomonas* and *Acinetobacter* were the predominant microflora in zone 1 of fresh cut vegetables processing facility of China (Xu et al., 2022). Also, they were abundant in non-food contact surfaces such as production floors (zone 3) and psychotropic environments in a fresh cut produce processing facility before sanitation procedures (Gu et al., 2019). Previous studies reported the ability of *P. aeruginosa* to form dense network of biofilms with *L. monocytogenes*, in a simulated chicken processing environment (Dong et al., 2022). Moreover, a multispecies biofilm involving *P. aeruginosa* and *S. aureus* with *L. innocua* demonstrated increased survival rates when exposed to QAC (quaternary ammonium compound) disinfection treatments (Kocot & Olszewska, 2020). Therefore, detecting *P. aeruginosa* on food contact surfaces is a serious concern due to the potential of mixed biofilm formation and the risk of cross-contamination.

Dust is a fine particulate matter in air that could carry foodborne pathogens and travel several distances. The implication of dust associated contamination is evident in recent *Salmonella* Enteritidis outbreak associated with peaches (Kumar et al., 2018; FDA, 2021). Fugitive dust, originated from cattle or poultry farm operations, was identified as a potential source of contamination (FDA, 2021). Despite significant importance having been given to improving the sanitation and hygiene practices in fresh produce industry, the dust associated microbial

contamination is mostly overlooked. When post-harvest operations for peaches take place in open-air constructions (PH1), it is difficult to eliminate dust accumulation and the associated contamination of the produce. Enough attention should be given not only to cleaning and sanitizing food-contact surfaces but also to prevent the deposition of dust particles in zones 2 and 3. In our study, we detected the presence of coliform *Enterobacter cloacae* after enriching the dust samples. Previous studies have reported that *L. monocytogenes* can survive on surrogate dust particles for more than 5 months (De Roin et al., 2003). *E coli* O157:H7, a coliform responsible for the bagged spinach outbreak in 2006, was isolated from soils and sediments near a cattle farm (Jay et al., 2007). Similarly, *S.* Newport in surrogate air particles exhibited the ability to survive and cross-contaminate tomato blossoms, and even internalized in the calyx and fruits (Kumar et al., 2017). An overrepresentation of coliforms on romaine lettuce during summer has been linked to the immigration of bacteria associated with dust storm, particularly from the *Enterobacteriaceae* family, and their subsequent deposition on leaf surfaces (Rastogi et al., 2012). Hence, implementing a cleaning protocol to prevent dust accumulation is crucial.

3.4 Conclusion

Our study indicated that there was a gradual increase in both HPC during peach packing season which could be due to the insufficient cleaning and sanitation prior to packing operation, higher initial microbial load on peaches, or inadequate manufacturing practices employed in the packing facilities. The waxer brushes, washer brushes, and sorting cups were found to be highly contaminated food contact surfaces and suitable sanitation protocols are required to reduce the microbial load on these surfaces. Isolation of *P. aeruginosa* during different packing seasons from food contact surfaces possesses the risk of biofilm formation with foodborne pathogens and cross

contamination. Additionally, microbial contamination originating from dust accumulation need to be further explored in fresh produce packing facilities.

3.6 References

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Table 3.1: Sampling sites of peach packing line

Zone	No.	Location
Zone 1	1	Peach bins
	2	Hydrocooling water
	3	Packing tables
	4	Rollers pre-wax/wash
	5	Post wash conveyor
	6	Waxer brushes
	7	Washer brushes
	8	Sorting cups
Zone 2	9	Washer waxer area
	10	Final packing area
Zone 3	11	Cold storage drains
	12	Main production drains
Dust samples (Packing facility 1)	1	Window near sorting area
	2	Near the peach bin loading site
	3	Sorting cup machine
	4	Motor near post-wash conveyor
	5	Switchboard near sorting area
	6	Fan after wax wash area

Table 3.2: Mean HPC population on environmental surface samples collected during the study. Different connecting letters followed by mean±standard deviation within the same comparative variable (Packing house, zones, season, sampling site) and same column indicates statistically significant ($p<0.05$).

	No. of samples	Mean HPC population (log CFU/cm^2)
Packing houses		
Packing house 1	70	4.28±1.67 ^{A}
Packing house 2	72	3.38±1.42 ^{B}
Sampling zones		
Zone 1	94	3.73±1.53 ^{A}
Zone 2	24	3.77±1.84 ^{A}
Zone 3	24	4.22±1.64 ^{A}
Packing season		
Early	48	3.06±1.22 ^{B}
Mid	48	4.12±1.68 ^{A}
End	46	4.24±1.63 ^{A}
Sampling sites		
Peach bins	12	2.96±1.09 ^{B}
Hydrocooling water (log CFU/ml)	10	3.38±1.02 ^{B}
Packing tables	12	2.95±1.45 ^{B}
Rollers pre-wax/wash	12	3.03±1.35 ^{B}
Post wash conveyor	12	3.28±1.53 ^{B}

Waxer brushes	12	5.49±0.89 [A]
Washer brushes	12	4.05±1.82 [AB]
Sorting cups	12	4.15±1.48 [AB]
Washer waxer area	12	3.54±1.73 [AB]
Final packing area	12	3.74±1.81 [AB]
Cold storage drains	12	4.53±1.43 [AB]
Main production drains	12	4.68±1.65 [AB]

Table 3.3: HPC population (log CFU/cm^2) on samples collected from two peach packing facilities across fresh peach packing season. Different connecting letters followed by mean±standard deviation indicates statistically significant ($p<0.05$). No. of samples = 24. * No. of sample = 22

Packing season	Packing facility 1	Packing facility 2
Early season	3.67±1.48 ^{B}	2.94±1.01 ^{B}
Mid-season	3.51±1.35 ^{B}	3.93±1.42 ^{A}
End season	5.78±1.15 ^{A}	3.27±1.63 ^{AB}

Table 3.4: HPC population (log CFU/cm^2) on samples collected from different sampling sites of two peach packing facilities. Different connecting letters followed by mean±standard deviation indicates statistically significant ($p<0.05$). No. of sample = 6. * No. of sample = 4

Sampling Sites	Packing facility 1	Packing facility 2
Peach bins	3.51±0.77 ^{A}	2.42±1.14 ^{B}
Hydrocooling water (log CFU/ml)	3.77±1.02 ^{A*}	3.11±1.01 ^{AB}
Packing tables	2.89±1.71 ^{A}	3±1.29 ^{AB}
Rollers pre-wax/wash	3.3±1.67 ^{A}	2.77±1.02 ^{B}
Post wash conveyor	3.35±2.08 ^{A}	3.22±0.89 ^{AB}
Waxer brushes	5.6±0.74 ^{A}	5.37±1.08 ^{A}
Washer brushes	5.49±1.09 ^{A}	2.61±1.06 ^{B}
Sorting cups	4.84±1.66 ^{A}	3.47±0.98 ^{AB}
Washer waxer area	4.33±1.91 ^{A}	2.75±1.19 ^{B}
Final packing area	3.76±2.11 ^{A}	3.72±1.66 ^{AB}
Cold storage drains	5.18±1.02 ^{A}	3.88±1.57 ^{AB}
Main production drains	5.14±1.29 ^{A}	4.21±1.95 ^{AB}

Table 3.5: Presumptive positive results for coliforms and *Pseudomonas* spp isolated from Packing house 1

Season	Sampling site (Zone)	API 20E test results
Early season	Peach bins (Zone 1)	Possibility of *Erwinia spp (Pantoea spp.2 (57.1 %), Citrobacter freundii (26.1 %))*
Early season	Waxer brushes (Zone 1)	Possibility of *Erwinia spp (Pantoea spp.2 (57.1 %), Citrobacter freundii (26.1 %))*
Early season	Main production drains (Zone 3)	*Klebsiella pneumoniae (81.1 %)*
End season	Washer waxer area (Zone 2)	Possibility of *Erwinia spp (Pantoea spp.2 (52.6 %), Citrobacter freundii (12.6%))*

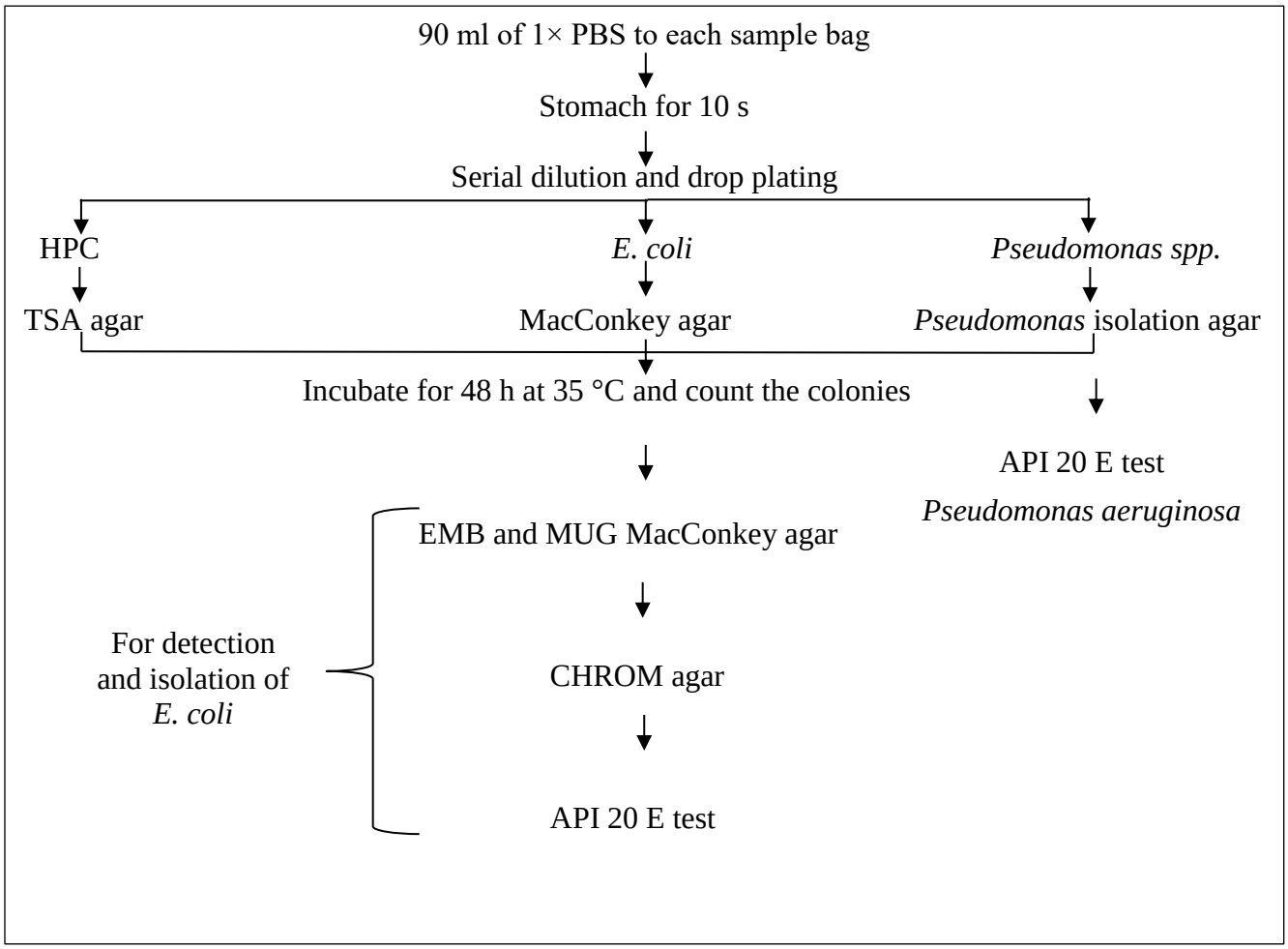

Figure 3.1: Diagrammatic representation of procedures used for enumeration, detection and isolation of microbial population from environmental surface swabs

CHAPTER 4

Impact of Surface Color on the Efficacy of Antimicrobial Blue Light Against *Listeria monocytogenes*

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Abstract

Listeria monocytogenes is a foodborne pathogen of concern that can contaminate food contact surfaces and persist in food processing environments. Blue light at certain wavelengths has been known to have antimicrobial properties against foodborne pathogens. The objective of this study was to evaluate the influence of surface color (red, blue, black, green, and white) and treatment time (1, 4, and 8 h) on the antimicrobial efficacy of blue light (aBL; 405nm, 35.3 mW/cm^2) in inactivating a five-strain cocktail of *L. monocytogenes* on high-density polypropylene plastic (HDPE) coupons. Negative and positive controls consisted of inoculated coupons placed in the dark or exposed to light from a fluorescent bulb light (0.2 mW/cm^2). The population of *L. monocytogenes* was enumerated on non-selective media (TSAYE; tryptic soy agar with 0.6% yeast extract) and selective media (Rapid *L. mono* plates®). A significant difference in bacterial recovery was observed between Rapid *L. mono* plates® and TSAYE ($p \leq 0.05$) after aBL treatment. The surface color of the coupons and treatment time (dosage; J/cm^2) had a significant impact on the efficacy of the aBL against *L. monocytogenes* ($p \leq 0.05$). Among the five colors of HDPE coupons exposed to aBL for 4 h, the highest reduction ($p \leq 0.05$) in *L. monocytogenes* population was observed when blue color coupons were used (1.76±0.61 log CFU/ cm^2). After 1 h of aBL exposure, *L. monocytogenes* reduction on green coupons was similar to red and white coupons ($p \geq 0.05$), whereas higher reductions were observed on black and blue coupons ($p \leq 0.05$). While treating controls under dark (0 J/cm^2) and fluorescent bulb light (5.76 J/cm^2) for 8 h, an average of 0.42±0.28 log CFU/cm^2 (dark) and 0.44±0.34 log CFU/cm^2 (fluorescent bulb light) reductions were observed. The temperature during aBL treatment was found to be negatively correlated to *L. monocytogenes* reductions with a correlation coefficient ($r = -0.21$). The study

indicates that material color and treatment time are important factors contributing to the efficacy of aBL treatment.

4.1 Introduction

L. monocytogenes is a Gram-positive facultative anaerobic bacterium, known for its ability to survive in a wide range of environmental conditions such as low and high temperatures, pH, saline, and oxygen concentration (Roberts et al., 2020). This pathogen is ubiquitous in its nature; they are found in water, air, soil, and fresh produce (Baquero et al., 2020). They are also found to be frequently persistent in food production and processing facilities (Mazaheri et al., 2021). The consumption of food contaminated with *L. monocytogenes* has contributed to a total of 77 outbreaks with 50 multistate outbreaks, 786 illnesses, 690 hospitalizations, and 128 mortalities over the past decade (2010-2020) in the United States (CDC NORS, 2022). In the United States, there is a zero-tolerance policy implemented for *L. monocytogenes* in ready-to-eat products which are regulated and approved by U.S. Food and Drug Administration (Archer, 2018). The clinical manifestation of listeriosis varies from mild infections such as diarrhea, fever, and headache to severe cases of fatality rate among neonatal, pregnant women, elderly and immuno-compromised individuals (Allerberger & Wagner, 2010; Kumar et al., 2016; Fan et al., 2019). Investigations on the *Listeriosis* outbreak associated with cantaloupe in 2011 and caramel apple in 2014 led to the conclusion that they were prevalent on food contact surfaces (FCS) of the equipment such as brushes and rollers detected during environmental assessments (Ruiz-Llacsahuanga et al., 2021). The inadequacy in sanitary hygienic practices could lead to direct or indirect cross-contamination. For example, during Schnitzel production, sorting plates made of plastic material and conveyor belts made of rubber sheets repeatedly tested positive for *L. monocytogenes* (Stessl et al., 2022).

Many studies reported the ability of *L. monocytogenes* to attach to food contact surfaces and form biofilms (Romanova et al., 2007; Djordjevic et al., 2002; Trang et al., 2023; Doijad et al., 2015). Silva et al. (2008) conducted a study on adherence and viability of *L. monocytogenes* on various FCS made of stainless steel (304), marble, polypropylene, granite, glass, silestone, etc. out of which, polypropylene materials exhibited the highest viability of bacteria. Yassoralipour et al. (2023) found a transfer rate of 70.08% (cabbage, chicken fillet) and 81.21% (bread) from a plastic board artificially inoculated with *L. monocytogenes.* Thus, there is an inherent risk of *L. monocytogenes* food contamination by sessile cells and biofilms, it has become imperative to find alternate strategies to prevent or delay their accumulation on FCS.

Among various decontamination technologies, antimicrobial blue light (aBL) is gaining attention due to its bactericidal properties at visible wavelengths (400-470 nm). aBL is a non-thermal, sustainable, cheap, and cost-effective sanitation technology commonly used to treat hospital-acquired infections and for decontamination of hospital environments (Cabral & Ag, 2019; Maclean et al., 2014). The aBL results in the excitation of endogenous photosensitizers present in microbes leading to the production of reactive oxygen species (ROS) and cell death (Wang et al., 2017). Recently, the application of aBL in eliminating foodborne pathogens from food contact surfaces and food matrices has been investigated in several studies. The efficacy of aBL treatment of FCS such as glass, stainless steel, and plastic materials such as acrylic polymer has been studied previously (McKenzie et al., 2013; Baek et al., 2021). Around 5-7 $\log_{10}$ reduction of *Escherichia coli* biofilms were achieved using aBL treatment (405 nm,140 mW cm^{-2} for 5–60 min) on acrylic and glass surfaces. Blue light treatment (466 nm, 150 J/cm^2) of stainless coupons inoculated with *Staphylococcus aureus* induced damage to cell membranes and leakage of intracellular components (Baek et al., 2021). While the parameters such as transmissibility of aBL through

surfaces (McKenzie et al., 2013), surface morphology such as roughness, hydrophobicity, or hydrophilicity (Deák et al., 2022), the temperature of FCS (Gunther et al., 2016), presence of organic matter (Wu et al., 2022) were evaluated for contributing factors to aBL efficacy, no research is available evaluating the role FCS color. In food-based industrial settings, conveyor belts, brushes, produce harvesting baskets, etc. could be made of different colored plastic materials. Interestingly, Lang et al. (2022) reported that the reflectivity properties of different food contact materials could be linked to the inactivation of yeasts. Also, Zhao et al. (2022) studied the role of the color of plastics in influencing the absorption of light with different wavelengths and energy leading to the photoaging of plastics. Hence, our objective is to evaluate whether the food contact surface color would contribute to increased efficacy of aBL in inactivating *L. monocytogenes* on HDPE coupons. Other parameters such as treatment time, and dual plating system (TSAYE and Rapid *L. mono* plates®) to determine stress response, temperature, and relative humidity, were also evaluated.

4.2 Materials and Methods

4.2.1 *L. monocytogenes* culture preparation

Five outbreak strains of *L. monocytogenes* (Table 4.1) were obtained from the University of Georgia-Center for Food Safety, culture collection. The cultures were revived from frozen glycerol stocks by transferring a loopful (10µl) of culture to Brain Heart Infusion Broth (BHIB, Becton Dickinson Microbiology Systems, Sparks, MD, USA) and incubating at 37 °C for 42-48 h. Subculturing was performed by streaking a loopful (10µl) of each strain on tryptic soy agar with 0.6% yeast extract (TSAYE; Neogen, Lansing, MI, USA) and incubating at 37 °C for 42-48 h, followed by confirmation for typical colony on modified oxford agar (MOX; Oxford Medium Base plus Modified Antimicrobic Supplement, Difco, Becton Dickinson Microbiology Systems, Sparks,

MD, USA) and Rapid *L. mono* plates® (Bio-Rad, Marne La Coquette, France). Typical colonies of each *L, monocytogenes* isolates were sub-cultured on TSAYE plates at 37 °C for 48 h and used for experiments.

4.2.2 *L. monocytogenes* cocktail

Stocks of each *L. monocytogenes* strain from the TSAYE plates were prepared by flooding the plate with 5ml of sterile 1x Phosphate Buffered Saline (PBS; VWR chemicals LLC, Solon, OH, USA) and scraping off the colonies using a 10 µl inoculation loop. The PBS *L. monocytogenes* slurry (approximately 3 ml) was transferred to a sterile 15 ml centrifuge tube (Corning; NY, USA) and vortexed (3000 rpm) using an analog vortex mixer (V0000, Argos Vortamix, Houston, TX, USA) until uniformly mixed. Each stock solution was serially diluted once by adding 1 ml to 9 ml of PBS. A cocktail of the 5 strains was prepared by mixing the 10 ml diluted suspensions to achieve a total volume of 50 ml with a final inoculation concentration of 8.79±0.29 log CFU/ml.

4.2.3 HDPE coupon preparation

Five different colors (red, black, blue, green, and white) of high-density polyethylene plastic cutting boards (HDPE; Thirteen chefs, Redmond, WA, USA) were cut into 3.4 x 2.1 Inch blocks in the Griffin campus machine workshop. Prior to use, the coupons were washed using a detergent (Ajax, Colgate-Palmolive, NY, USA) and air dried. The coupons were then treated with 70% ethanol and air dried at room temperature before dry sterilization in an autoclave at 121 °C for 15 min.

4.2.4 Inoculation of coupons

The surface of the HDPE coupons was inoculated with ten- 100 µl aliquots (1ml) of the *L. monocytogenes* strain cocktail. The coupons were dried overnight (12-14 h) inside a biosafety cabinet.

4.2.5 aBL treatment set-up

Exposure of coupons to aBL was performed inside a hydroponic mylar grow tent with dimensions of 123x61x153 cm (Vivosun, Ontario, Canada). aBL was emitted from 20-panel light-emitting diode (LED) lamp (Fasttobuy, Guangdong, China) with 200W output, and peak emission at 405 nm. Irradiance from the lamp was measured using a radio photometer (Honle UV Technology, UVAHAND 250GS). Objects to be treated with aBL were placed on a metal tray at a distance of 23 cm from the lamp to obtain an irradiance of *35.3* mW/cm^2 (Figure 4.1). The treatment intensity (Table 4.2) was calculated using the formula; *Irradiance* (*W/cm^2)* × *Exposure Time (in seconds) = J/cm^2* (Maclean et al., 2009). Ice packs were kept under the metal tray to negate heating by the lamps and maintain an average temperature (20±3 °C) during the experiment. The temperature and relative humidity were monitored using a WIFI thermocouple (SensorPush, Brooklyn, NY, USA). Thermal images of HDPE coupons subjected to aBL treatment were captured at 1, 4, and 8 h using a FLIR One Pro LT iOS Pro-Grade Thermal Camera for Smartphones (Teledyne FLIR, Wilsonville, OR, USA).

4.2.6 Treatment of coupons with aBL

For each treatment, 3 coupons were placed under the aBL-LED lamp for durations of 1, 4, and 8 h. Coupons were also exposed to a control treatment consisting of light from a fluorescent bulb with an irradiance of 0.2 mW/cm^2 or placed in the dark (irradiance- 0 mW/cm^2) for durations of 1, 4, and 8 h. The comparative control consisted of coupons inoculated with *L. monocytogenes* cocktail enumerated at 0 h. Coupons were evaluated for the decrease in *L. monocytogenes* population after treatments.

4.2.7 Enumeration of surviving *L. monocytogenes* population coupons

Immediately after treatment, the coupons were transferred into 24-ounce Whirl-Pak® filter bags (Nasco, Modesto, CA, USA) containing 100 ml of 1X PBS. The bags containing the coupons were placed in a sonicating water bath (22-23 °C) and were sonicated for 5 min at 42 kHz (FS30H sonicator, Fisher Scientific, Pittsburgh, PA, USA) to aid in the release of attached cells. Serial dilutions of the PBS buffer containing the released cells were performed by transferring 100 µl of solution into 900 µl of 1x PBS and the cells were enumerated on TSAYE and Rapid *L. mono*® plates using the droplet plate technique (Kumar et al., 2023; Oguadinma et al., 2022; Herigstad et al., 2001). The limit of detection was 1.69 log CFU/ml. Colonies were counted after 48 h of incubation at 37 °C.

4.2.8 Statistical analysis

All the experiments were conducted in three technical and biological replicates. A randomized block design was used to design the experiment by blocking replication. The differences in the *L. monocytogenes* population were compared using the analysis of variance (ANOVA; JMP Pro 15, SAS Institute Inc., Cary, NC, USA). The significant differences between the mean counts were established using Tukey's honestly significant difference (HSD) test at a 95 % significance level. Significant differences are denoted by $p \leq 0.05$ whereas the highest significant difference is defined at $p \leq 0.0001$. The effect of temperature and humidtiy during treatment on *L. monocytogenes* reduction were estimated by calculating the Pearson's correlation coefficient on $\log_{10}$ transformed microbial counts.

4.3 Results

4.3.1 aBL efficacy on *L. monocytogenes* inactivation

The treatment dosages for aBL at 1, 4, and 8 h were 127.08, 508.32, and 1016.64 J/ cm^2 respectively (Table 4.2). The treatment time and surface color of HDPE coupons were found to be significant ($p \leq 0.05$) contributing factors towards efficacy of aBL in inactivating *L. monocytogenes*. The interaction effect of treatment time and surface color were also found to be significant ($p \leq 0.05$) with blue coupons exposed to aBL for 8 h having the highest reduction. The bacterial counts on selective media (Rapid *L. mono* plates®) and non-selective media (TSAYE) showed significant differences ($p \leq 0.05$) post aBL treatment. The average reduction of *L. monocytogenes* on HDPE coupons enumerated on selective media were 1.30±0.38, 1.38±0.43, and 1.63±0.37 log CFU/ cm^2, whereas non-selective media showed a reduction of 1.05±0.48, 1.26±0.40, and 1.53±0.30 log CFU/cm^2 for 1, 4 and 8 h of aBL treatment (Table 4.3). The mean log reduction of *L. monocytogenes* on HDPE coupons treated with fluorescent bulb light and plated on selective and non-selective media were 0.50±0.47, -0.08±0.25, 0.45±0.42 log CFU/cm^2 and 0.35±0.30, 0.03±0.30, 0.44±0.34 log CFU/cm^2 respectively. Similarly, the mean log reduction of *L. monocytogenes* on HDPE coupons placed in dark and plated on selective and non-selective media were 0.22±0.39, -0.16±0.23, 0.39±0.33 and 0.11±0.31, -0.13±0.26, 0.42±0.28 log CFU/cm^2 respectively for three treatment times.

4.3.2 Effect of Surface color on aBL efficacy

Among the five different colors of HDPE coupons subjected to aBL treatment, higher reduction in *L. monocytogenes* population was observed on blue color coupons in comparison to green and white coupons ($p \leq 0.05$) when plated on TSAYE. However, no significant difference in bacterial

reduction was observed on blue color coupons in comparison to other coupons colors ($p \geq 0.05$) expect for green color coupons when plated on Rapid *L. mono* plates®.

The highest *L. monocytogenes* reduction of 1.98±0.16 log CFU/cm^2 was observed on blue coupons exposed to aBL for a duration of 8 h on selective media. The least reduction of bacterial population after aBL treatment was observed on green coupons (0.63±0.28 log CFU/cm^2) after 1 h of exposure ($p \leq 0.05$) plated on non-selective agar. aBL treatment for 1 and 4 h on blue coupons resulted in *L. monocytogenes* population reduction by 1.47±0.42 and 1.70±0.67 log CFU/cm^2 and 1.34±0.55 and 1.76±0.61 log CFU/cm^2 on Rapid *L. mono* plates® and TSAYE respectively. After 1 h of aBL exposure, *L. monocytogenes* reduction on green coupons was similar to red and white coupons; whereas higher reductions were observed on black and blue coupons ($P \leq 0.05$) (Table 4.3).

The color of the coupons did not significantly contribute ($p \geq 0.05$) to the reduction of bacteria kept in the dark for 4 and 8 h of treatments. Similarly, no significant difference ($p \geq 0.05$) was observed on bacterial reduction among coupons subjected to fluorescent bulb light, for 1,4 and 8 h of treatment.

4.3.3 Treatment time

It was found that the duration of aBL exposure had a significant impact on *L. monocytogenes* reduction ($p \leq 0.0001$) on the HDPE coupon surfaces. There was a significant difference in bacterial inactivation among coupons exposed for 1, 4, and 8 h with a mean reduction of 1.05±0.48, 1.26±0.40, and 1.53±0.30 log CFU/cm^2, among all colored coupons (TSAYE). Treatment time in combination with surface color had a significant impact on the efficacy of aBL against *L. monocytogenes*. Based on the bacterial counts obtained from TSAYE agar and Rapid *L. mono* plates®, blue-colored coupons exposed to aBL for 1 h had similar bacterial reduction as black and

white coupons exposed to aBL for 8 h (Table 4.3). Furthermore, no significant difference in reduction was observed on blue coupons exposed to aBL for 1, 4, and 8 h ($p \geq 0.05$).

Reduction of *L. monocytogenes* population on coupons exposed to fluorescent bulb light and dark was influenced by treatment time ($p \leq 0.05$). However, the interaction effect of color and treatment time were insignificant for both controls ($p \geq 0.05$). Coupons exposed to fluorescent bulb light (control) had reductions of 0.36±0.30, -0.03±0.22 and 1.44±0.34 log CFU/cm^2 after 1, 4 and 8 h respectively ($p \leq 0.05$). Coupons exposed to dark (control) had reductions of 0.11±0.31, -0.13±0.26 and 0.42±0.28 log CFU/cm^2 after 1, 4 and 8 h respectively ($p \leq 0.05$) (Table 4.4).

4.3.4 Treatment temperature and humidity

The mean temperature of the coupons exposed to aBL for 1, 4, and 8 h was found to be 21.12±1.68 °C, 17.6±1.09 °C, and 17.15±1.15 °C (Figure 4.2). The mean relative humidity for three different treatment times was found to be 48.45±8.24 %, 48.24±9.63 %, and 48.15±8.29 %. The temperature during aBL treatment was found to be negatively correlated to *L. monocytogenes* reductions ($r = -0.21$). However, the correlation coefficient of relative humidity and aBL reductions were not correlated ($r = 0.01$). Similarly, the temperature and humidity did not play a role in the log reduction of *L. monocytogenes* on coupons kept at dark light and light controls (correlation coefficient < 0.1). The thermal images of HDPE coupon surfaces were taken during aBL treatment (Figure 4.3). Based on the FLIR thermal images during aBL exposure, we found that both treatment time and coupon color significantly contributed to the mean temperature measured on HDPE coupon surfaces ($p \leq 0.05$). There was an overall increase in mean temperature from 19.6±1.64 °C (0 h) to 28.86±1.12 °C after 8 h of aBL exposure ($p \leq 0.05$). The highest mean temperature was recorded on black HDPE coupon surface (26.75±3.13 °C) followed by blue (26.33±3.81 °C) and green (27.57±4.69 °C) HDPE coupons, while lowest mean temperature was

recorded on white (24.43±4.25 °C) HDPE coupon surface ($p \leq 0.05$) (Table 4.5). However, the surface temperature did not contribute to log reduction of *L. monocytogenes* on coupons subjected to aBL (correlation coefficient < 0.1).

4.4 Discussion

Listeria monocytogenes is a foodborne pathogen of concern that can contaminate and persist on food contact surfaces. Antimicrobial blue light (aBL) is a photo-mitigation strategy that has been explored at wavelengths ranging between 400 and 450 nm. aBL induces cellular damage by reacting with endogenous porphyrins to generate reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, singlet oxygen (Ghate et al., 2019; Nitzan et al., 2004).

Commonly used strategies to improve the efficacy of aBL include the use of exogenous photosensitizers such as methylene blue, d-aminolevulinic acid, Na-chlorophyllin and curcumin, (Pinto et al., 2017; Nitzan et al., 2004; Luksiene & Paskeviciute, 2011; Vengatesan, 2023). The characteristic colors of these chromophores contribute towards absorption of certain wavelengths (Pinto et al., 2017). The reflection of red light and far-red light off white and black plastic mulches had differential influences on crop growth, seedling establishment, quality as well as quantity of the yield (Decoteau et al., 1988). Similarly, we hypothesized that color of food contact surfaces could influence the efficacy of aBL. In this study, we evaluated the factors that could increase the antimicrobial efficacy of 405-nm low-power aBL in inactivating *L. monocytogenes* on HDPE plastic coupons. We observed that material color and treatment time had a significant impact on the efficacy of the aBL treatment against *L. monocytogenes* populations ($p \leq 0.05$).

Blue-colored plastic coupons had significantly higher inactivation of *L. monocytogenes* when subjected to aBL than green and white colored coupons when exposed for 1 h ($p \leq 0.05$). The

highest reduction (1.98±0.16 log CFU/cm^2) of *L. monocytogenes* was observed on blue coupons that were exposed to aBL for 8 h. Coupons were placed in the dark or exposed to light from a fluorescent bulb light as comparative controls for the aBL treatment. Compared to the controls, aBL treated coupons had significantly higher reductions in population over all three treatment times tested ($p \leq 0.05$). However, *L. monocytogenes* population on coupons exposed to light from a fluorescent bulb did not have significant population reduction after 8 h ($p \geq 0.05$).

L. monocytogenes can sense the blue light via Lmo0799, a flavin mononucleotide-containing sensory protein that activates general stress response sigB (σ^B) (O'Donoghue et al., 2016). Genes that involve σ^B regulon also regulate multiple stress responses such as antibiotic resistance (Palmer et al., 2009), quorum sensing (Guerreiro et al., 2022), biosynthesis of flagella (Palmer et al., 2011), response to temperature variations (Dorey et al., 2019) etc. Previous research has indicated that exposure of bacteria to light can result in sub-lethal stress and result in phenotypic changes such as the transition to the viable but non-culturable (VBNC) state (Oguadinma et al., 2022). Differences in recovery of stressed cells on non-selective and differential media has been reported after antimicrobial treatment or exposure to environmental stress (Kumar et al., 2016). No significant differences in recovery were observed among *L. monocytogenes* cells after exposure to aBL.

Previous studies reported temperature variations during blue light treatment. Few studies reported contrasting results on increase in temperature of stainless-steel coupons subjected to aBL treatment. Sommers et al. (2017) reported a rise in the temperature of stainless-steel coupons to 50-56° C (405 nm, 150-300 mW/cm^2/s, 180 J/cm^2), whereas a temperature increment of only 2.5° C was observed when stainless steel coupons were illuminated with aBL (26 mW/cm^2, 749 J/cm^2) for 8 h (Li et al., 2018). Hence, it can be inferred that the temperature of the FCS subjected to aBL

varies based on irradiance (mW/cm^2) of blue light illumination. In our study, the irradiance during treatment was set at 35.3 mW/cm^2 which did not significantly increase the temperature of coupons and the temperature during treatment was regulated (20±3 °C) using ice packs below metal trays on top of which the coupons were placed. However, the negative correlation between aBL reduction and temperature could be attributed due to ROS production in *L. monocytogenes* with a decrease in temperature (Azizoglu & Kathariou, 2010). Similarly, higher inactivation rates are found on different bacterial strains when exposed to sunlight at a temperature below 26 °C (Oguadinma et al., 2022).

4.5 Conclusion

The results from this study indicate that surface color of HDPE coupons could influence the antimicrobial efficacy of aBL against *L. monocytogenes*. Selecting surface colors of FCS that accentuate the antimicrobial action of aBL could result in an effective photo-mitigation strategy for the control of *L. monocytogenes*.

2.6 References

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Table 4.1: Five *L. monocytogenes* strains used in this study (Olszewska & Diez-Gonzalez, 2021)

Strain id	Serotype (if known)	Source	Reference
19115	4b	Human isolate	ATCC
19117	4d	Animal isolate (sheep)	ATCC
Coleslaw	4b	Food (coleslaw)	UGA
G1091	4b	Coleslaw	UGA
2011L-2626		Cantaloupe outbreak	CDC

UGA, University of Georgia; ATCC, American Type Culture Collection; CDC, Centers for Disease Control and Prevention.

Table 4.2: aBL and fluorescent bulb light treatment dosages (J/cm^2) used for the study

Treatment time (h)	Light Intensity (J/cm^2)	
	aBL	Fluorescent bulb light
1	127.08	0.72
4	508.32	2.88
8	1016.6	5.76

Table 4.3: *L. monocytogenes* reduction during aBL treatment plated on selective and non-selective agar. Different letters (A, B, C, D, E) within a column following the mean ± standard deviation values indicate statistically significant ($p \leq 0.05$).

Treatment time (h)	HDPE coupon color	Log reduction in L. monocytogenes population (Log CFU/cm^2)	
		TSAYE	Rapid *L. mono* plates®
1 h	Black	1.28±0.62^{BCD}	1.48±0.26^{ABC}
	Blue	1.34±0.55^{ABCD}	1.47±0.42^{ABC}
	Green	0.63±0.28^{E}	0.99±0.51^{C}
	Red	1.15±0.32^{CDE}	1.4±0.23^{ABC}
	White	0.87±0.16^{DE}	1.15±0.17^{BC}
4 h	Black	1.11±0.12^{CDE}	1.36±0.22^{BC}
	Blue	1.76±0.61^{AB}	1.7±0.67^{AB}
	Green	1.1±0.21^{CDE}	1.19±0.26^{BC}
	Red	1.2±0.15^{CD}	1.26±0.28^{BC}
	White	1.13±0.29^{CDE}	1.41±0.47^{ABC}
8 h	Black	1.32±0.18^{ABCD}	1.51±0.33^{ABC}
	Blue	1.84±0.08^{A}	1.98±0.16^{A}
	Green	1.52±0.27^{ABC}	1.64±0.34^{AB}
	Red	1.63±0.33^{ABC}	1.46±0.22^{ABC}
	White	1.34±0.25^{ABCD}	1.56±0.5^{ABC}

Table 4.4: *L. monocytogenes* reduction during control treatments (FBL and dark) plated on non-selective agar. Different letters (A, B, C, D, E) within a column following the mean ± standard deviation values indicate statistically significant ($p \leq 0.05$).

Treatment time (h)	HDPE coupon color	Log reduction in L. monocytogenes population (Log CFU/cm^2) FBL	Dark
1 h	Black	0.32 ±0.30 ^{ABCD}	0.12 ±0.19 ^{ABCDE}
	Blue	0.46 ±0.32 ^{AB}	-0.01 ±0.34 ^{BCDE}
	Green	0.33 ±0.30 ^{ABCD}	-0.04 ±0.33 ^{CDE}
	Red	0.47 ±0.33 ^{AB}	0.35 ±0.32 ^{ABC}
	White	0.14 ±0.17 ^{ABCD}	0.15 ±0.23 ^{ABCDE}
4 h	Black	-0.08 ±0.14 ^{CD}	-0.22 ±0.18 ^{E}
	Blue	0.02 ±0.35 ^{BCD}	-0.08 ±0.43 ^{CDE}
	Green	-0.13 ±0.13 ^{D}	-0.12 ±0.21 ^{DE}
	Red	0.06 ±0.16 ^{BCD}	-0.08 ±0.26 ^{CDE}
	White	-0.04 ±0.23 ^{CD}	-0.18 ±0.17 ^{E}
8 h	Black	0.31 ±0.41 ^{ABCD}	0.32 ±0.28 ^{ABCD}
	Blue	0.37 ±0.18 ^{ABC}	0.52 ±0.30 ^{A}
	Green	0.46 ±0.20 ^{AB}	0.44 ±0.18 ^{AB}
	Red	0.53 ±0.45 ^{A}	0.50 ±0.36 ^{A}
	White	0.55 ±0.38 ^{A}	0.31 ±0.25 ^{ABCD}

Table 4.5: Mean temperature (°C) recorded on surfaces of HDPE coupons during aBL exposure. Different letters (A, B, C, D, E) within a column following the mean ± standard deviation values indicate statistically significant ($p \leq 0.05$).

	Mean Temperature (°C)			
HDPE coupon color	0 h	1 h	4 h	8 h
Red	19.77 ± 1.05^{EF}	28.53 ± 0.75^{ABCD}	27.1 ± 0.66^{BCD}	29.1 ± 1.04^{ABC}
Blue	20.4 ± 0.79^{E}	26.9 ± 0.26^{CD}	29.87 ± 0.4^{AB}	28.17 ± 1.46^{BCD}
Black	22.07 ± 0.25^{E}	26.7 ± 0.79^{CD}	29.17 ± 1.5^{ABC}	29.1 ± 0.96^{ABC}
White	17.57 ± 1.18^{F}	25.77 ± 1.03^{D}	26.87 ± 0.47^{CD}	27.53 ± 0.65^{BCD}
Green	20 ± 0.36^{EF}	29.7 ± 0.56^{AB}	31.3 ± 1.32^{A}	29.3 ± 0.89^{ABC}

Figure 4.1: Pictorial representation of aBL set up used for the study. (a) aBL set up (b) HPDE coupons before treatment (c) HDPE coupons during Blue light treatment

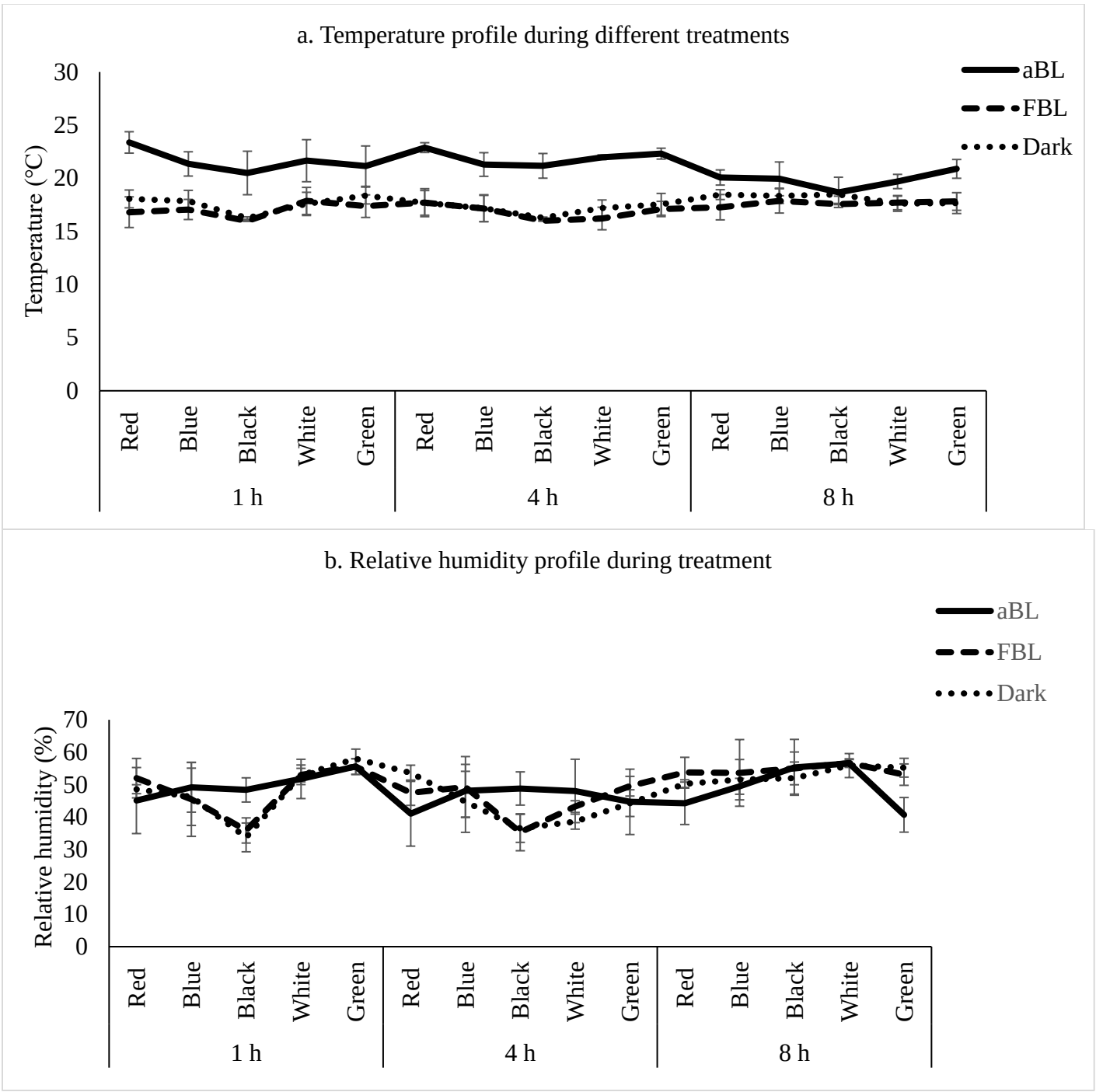


Figure 4.2: Temperature, °C (a) and relative humidity, % (b) profile during aBL (405 nm) and control (dark and FBL) treatments.

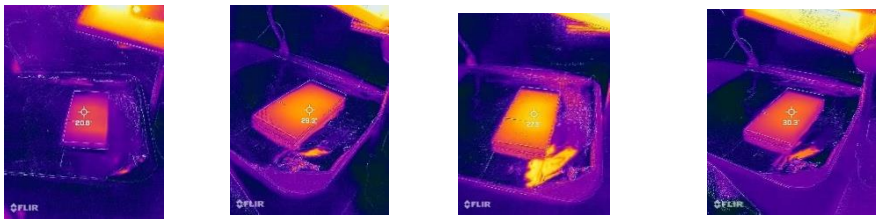
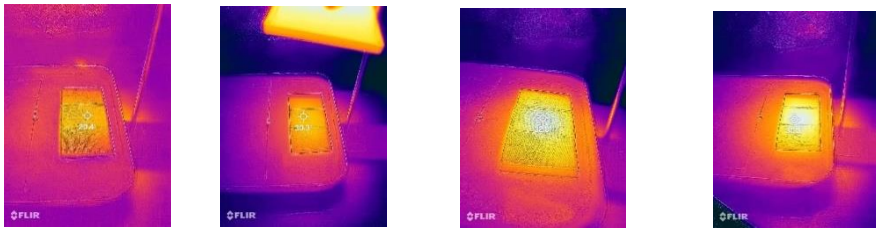
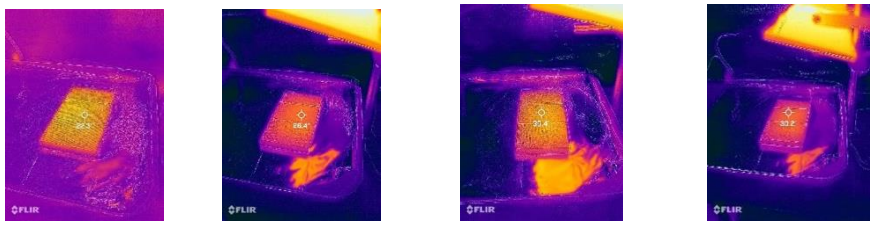
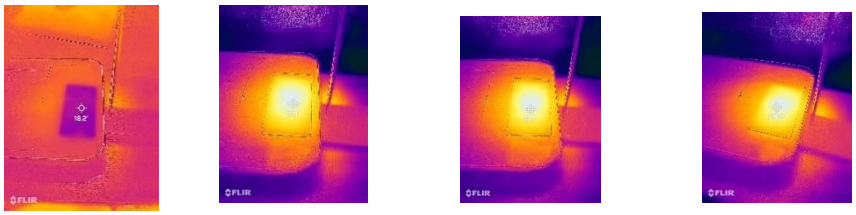
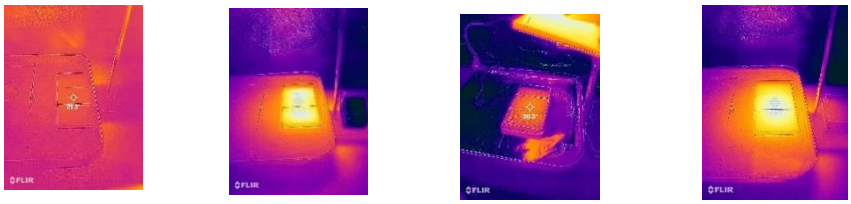
HDPE coupon color	Treatment time (h) 0 h	1 h	4 h	8 h
Red				
Green				
Black				
White				
Blue				

Figure 4.3: Thermal images of HDPE coupons recorded at different aBL exposure time

CHAPTER 5

Antimicrobial efficacy of Far UV-C (222 nm) against *L. monocytogenes* and *S. enterica* contaminated HDPE surfaces

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Abstract

L. monocytogenes and S. enterica can attach to food contact surfaces and result in the cross-contamination of fresh produce and ready-to-eat foods. Far UV-C (200-230 nm) is recognized for its antimicrobial efficacy against foodborne pathogens while being benign to mammalian cells due to limited depth of penetration. The objective of this study was to investigate the efficiency of Far UV-C (222 nm; irradiance: 106 µW/ cm^2) treatment on eliminating a five strains cocktail of *L. monocytogenes* and *S. enterica* dry inoculated on high-density polyethylene (HDPE) coupons surfaces after an exposure duration of 1, 4 and 8 h. Negative and positive controls consisted of inoculated coupons placed in the dark or exposed to light from a fluorescent bulb light (FBL; 0.2 mW/cm^2). The population of *L. monocytogenes* was enumerated on non-selective media (TSAYE; tryptic soy agar with 0.6% yeast extract) and selective media (MOX agar). There were no significant differences in bacterial recovery observed between MOX agar and TSAYE ($p \geq 0.05$) after Far UV-C (222 nm) treatment. There was a significant reduction in bacterial population on HDPE coupons exposed to Far UV-C (222 nm) ($p \leq 0.05$) compared to both FBL and dark treatment conditions (< 0.2 log CFU/cm^2), indicating the effectiveness of Far UV-C (222 nm). The time of exposure (h) had a significant impact on the efficacy of the Far UV-C (222 nm) against *L. monocytogenes* and *S. enterica* ($p \leq 0.05$). An exposure period of 8 h led to a significant reduction of *L. monocytogenes* population by 3.26±0.33 log CFU/cm^2, while *S. enterica* on HDPE coupons exhibited a reduction of only 1.01±0.32 log CFU/cm^2 ($p \leq 0.05$). The study indicated sthat treatment time is an important factor contributing to the efficacy of Far UV-C (222 nm) treatment and the susceptibility to Far UV-C (222 nm) is bacterial-specific.

5.1 Introduction

The fresh produce industry in the United States (U.S.) is a dynamic sector, constantly evolving to meet consumer demands for healthier and safe food (Kumar et al., 2016). According to USDA NASS (2019), the total fruit and tree nut production in the U.S. has increased significantly over years (2000-2018) reaching a value worth $30 billions and vegetable production achieving $ 20 billions (USDA, 2019). However, the rise in fresh produce consumption has been associated with an increase in occurrences of foodborne outbreaks (Aiyedun et al., 2021). Among several causative foodborne pathogens, *L. monocytogenes* and *S. enterica* have been implicated in recent foodborne outbreaks (CDC, 2020; CDC 2015; CDC 2012). In multiple cases, these outbreaks have been traced back to the isolation of these pathogens within the produce processing facilities or directly from food contact surfaces (FCS) (Ruiz-Llacsahuanga et al., 2021). For instance, outbreak-associated isolates of *L. monocytogenes* were identified from produce contact surfaces during the investigation of two multi-state Listeriosis outbreaks linked to cantaloupe and caramel apples (Angelo et al., 2017; CDC, 2012; CDC, 2015). The prevalence of *Listeria* spp. on several FCS of apple packing facilities post-sanitation exhibits the inadequacy of current sanitation practices (Dev Kumar et al., 2017; Ruiz-Llacsahuanga et al., 2021). Furthermore, *L. monocytogenes* was responsible for transient and persistent cross-contamination events in commercial tree fruit packing houses (Chen et al., 2022). Similarly, *Salmonella* serovars were able to persist and survive on FCS such as sponge rollers, conveyor belts, wooden surfaces, and cross-contaminate tomatoes in a tomato packing line (Allen et al., 2005). Another study demonstrated a higher risk of cross-contamination of fresh cauliflowers with *S. enterica* from reusable plastic crates made of polypropylene plastic surfaces (López-Gálvez et al., 2021). Moreover, *S.* Enteritidis present on

poultry skin demonstrated the capability to cross-contaminate tomatoes even after the disinfection and cleaning of cutting boards (Soares et al., 2012). Hence, it is crucial to explore mitigation strategies to reduce microbial persistence in produce processing environments and FCS.

The use of light-based technologies for dry sanitation is an innovative concept and has found its application in the disinfection of several food products, water, air, and food contact surfaces (Koutchma et al., 2008; Calle et al., 2021). Within the ultraviolet irradiation spectrum (100-400 nm), UV-C light (200-280 nm) has shown excellent germicidal properties due to their ability to disrupt the molecular bonds, inducing dimerization of pyrimidine bases, in the genetic material (DNA and RNA) of microorganisms (Pedrós-Garrido et al., 2018**).** Previous studies have reported that Far UV-C (222 nm) does not cause mammalian skin or eye damage upon exposure due to their limited penetrability to biological tissues but has comparable antimicrobial efficacy to germicidal UV-C (254 nm) light (Buonanno et al., 2017; Tavares et al., 2023; Kaidzu et al., 2019). The cell membrane damage induced by the inactivation of enzyme (respiratory chain dehydrogenase), lipid peroxidation, and DNA damage induced by ROS production were identified as key bactericidal mechanisms of Far UV-C (222 nm) (Kang et al., 2018). The exposure of Far UV-C (222 nm) krypton chloride excilamp against biofilms produced by Gram-positive and Gram-negative strains on stainless steel surfaces at a cumulative dosage of 354 mJ/cm^2 resulted in reduced biomass accumulation (Chen & Moraru, 2023). Further studies are required to investigate the application of Far UV-C (222 nm) against foodborne pathogens on FCS. In this study, we assessed the efficiency of Far UV-C (222 nm) treatment against dry inoculated Gram-positive (*L. monocytogenes*) and Gram-negative (*Salmonella enterica)* on high-density polyethylene (HDPE) coupons surfaces for an exposure

duration of 1, 4 and 8 h. We also compared the differences in susceptibility of both pathogens during treatment.

5.2 Materials and Methods

5.2.1 Bacterial culture preparation

Five outbreak strains of *L. monocytogenes* previously used in another study (Olszewska & Diez-Gonzalez, 2021) such as *L. monocytogenes* 19115 (4b; human isolate), *L. monocytogenes* 19117 (4d; animal isolate), *L. monocytogenes* Coleslaw (4b; coleslaw), *L. monocytogenes* G1091 (4b; coleslaw), *L. monocytogenes* 2011L-2626 (cantaloupe) and *S. enterica strains* such as *S. enterica* Enteriditis 2020AM-1539 (peach outbreak), *S. enterica* Newport 2020AM-0919 (onion outbreak), *S.* Agona (Alfalfa outbreak) *S.* Montevideo (Tomato outbreak), *S.* St. Paul (Pepper outbreak) were obtained from the University of Georgia, culture collection. *L. monocytogenes* cultures were revived from frozen glycerol stocks by transferring a loopful (10µl) of culture to Brain Heart Infusion Broth (BHIB, Becton Dickinson Microbiology Systems, Sparks, MD, USA) and incubating at 37 °C for 42-48 h. Subculturing was performed by streaking a loopful (10µl) of each strain on tryptic soy agar with 0.6% yeast extract (TSAYE; Neogen, Lansing, MI, USA) and incubating at 37 °C for 42-48 h, followed by confirmation for typical colony on modified oxford agar (MOX; Oxford Medium Base plus Modified Antimicrobic Supplement, Difco, Becton Dickinson Microbiology Systems, Sparks, MD, USA) and Rapid *L. mono* plates® (Bio-Rad, Marne La Coquette, France). Typical colonies of each *L, monocytogenes* isolates were sub-cultured on TSAYE plates at 37 °C for 48 h and used for experiments. All the *S. enterica* strains used in the study were adapted to rifampicin resistance at a concentration of 80 µg/ml. They were revived from frozen glycerol stocks by transferring a loopful (10µl) of culture to tryptic soy broth containing 80 ppm rifampicin (TSBR, Neogen, Lansing, MI, USA) and incubating at 37 °C for

42-48 h. Subculturing was performed by streaking a loopful (10µl) of each strain on tryptic soy agar with rifampicin (TSAR; Neogen, Lansing, MI, USA) and incubating at 37 °C for 16-24 h. Typical colonies of each *S. enterica* isolate were sub-cultured on TSAR plates at 37 °C for 24 h and were used for experiments.

5.2.2 Bacterial cocktail preparation

Stocks of each *L. monocytogenes* strain from the TSAYE plates were prepared by scraping off all the colonies using a 10 µl inoculation loop and suspending them in 10 ml of sterile 1x Phosphate Buffered Saline (PBS; VWR chemicals LLC, Solon, OH, USA). The PBS *L. monocytogenes* slurry was vortexed (3000 rpm) using an analog vortex mixer (V0000, Argos Vortamix, Houston, TX, USA) until uniformly mixed. A cocktail of the 5 strains was prepared by mixing the 10 ml diluted suspensions to achieve a total volume of 50 ml with a final inoculation concentration of 9.47±0.15 log CFU/ml. A Similar procedure was employed for the preparation of *S. enterica* cocktail, by scraping the microbial colonies grown in the TSAR plates. Subsequently, these colonies were suspended in 10 ml of 1X PBS and diluted together to obtain a total volume of 50 ml with a final inoculation concentration of 9.26±0.16 log CFU/ml.

5.2.3 HDPE coupon preparation

Blue-colored high-density polyethylene plastic cutting boards (HDPE; Thirteen Chefs, Redmond, WA, USA) were cut into 3.4 x 2.1-inch blocks in the Griffin campus machine workshop. Prior to use, the coupons were washed using a detergent (Ajax, Colgate-Palmolive, NY, USA) and air dried. The coupons were then treated with 70% ethanol and air dried at room temperature before dry sterilization in an autoclave at 121 °C for 15 min.

5.2.4 Inoculation of coupons

The surface of the HDPE coupons was inoculated with ten- 100 µl aliquots (1ml) of the bacterial cocktail (*L. monocytogenes or S. enterica)*. The coupons were dried overnight (16 h) inside a biosafety cabinet.

5.2.5 Far UV-C (222 nm) treatment set-up

Exposure of coupons (HDPE) containing *L. monocytogenes and S. enterica* to Far UV-C (222 nm) lamp was performed inside a hydroponic mylar grow tent with dimensions of 123x61x153 cm (Vivosun, Ontario, Canada). A Far UV-C low-pressure discharge lamp, and peak emission at 222 nm (Foton defender^{TM}, Safe disinfecting Inc; California, USA) was procured for the study (Figure 5.1). Irradiance from the lamp was measured using a handheld light meter for 222 nm and intensity simulations provided by Ushio (UIT2400 Handheld Light meter; Cypress, CA, USA). Coupons to be treated with Far UV-C (222 nm) were placed on a metal tray at 1 ft (30.48 cm) distance from the lamp to obtain an average irradiance of 66.4-124.5 µW/cm^2 with an average irradiance of 106 µW/ cm^2 based on simulation measurements (Figure 5.2). The treatment intensity was calculated using the formula; Irradiance (µW/cm^2) / 1000 × Exposure Time (in seconds) = mJ/cm^2 (Chen & Moraru, 2023). The temperature and relative humidity were monitored using a WIFI thermocouple (SensorPush, Brooklyn, NY, USA). Thermal images of HDPE coupons subjected to Far UV-C (222 nm) were captured at 1, 4, and 8 h using a FLIR One Pro LT iOS Pro-Grade Thermal Camera for Smartphones (Teledyne FLIR, Wilsonville, OR, USA). Control treatments included placing coupons in the dark as well as exposure to fluorescent bulb light (FBL). After treatments, coupons were transferred to 1X PBS and sonicated for 5 min at 42 kHz (FS30H sonicator, Fisher Scientific, Pittsburgh, PA, USA) to aid in the release of attached cells. Serial dilutions of the PBS buffer containing the released cells was performed and the cells were enumerated on TSAYE and MOX

agar plates (*L. monocytogenes*) and TSAR (*S. enterica*) using the droplet plate technique (Herigstad et al. 2001; Kumar et al., 2023; Oguadinma et al., 2022). Colonies were counted after 48 h of incubation at 37 °C.

5.2.6 Treatment of coupons with Far UV-C (222 nm)

For each treatment, a total of nine coupons were placed under the Far UV-C (222 nm) lamp that was configured to pulse for a duration of 16.65 min with a 1 s interval off for a duration of 1, 4, and 8 h. Coupons were also exposed to a control treatment consisting of light from a fluorescent bulb with an irradiance of 0.2 mW/cm^2 or placed in the dark (irradiance - 0 mW/cm^2) for durations of 1, 4, and 8 h. The comparative control consisted of coupons inoculated with *L. monocytogenes* or *S. enterica* cocktail enumerated at 0 h. Coupons were evaluated for the decrease in *L. monocytogenes* and *S. enterica* population after treatments.

5.2.7 Enumeration of surviving *L. monocytogenes* population coupons

Immediately after treatment, the coupons were transferred into 24-ounce Whirl-Pak® filter bags (Nasco, Modesto, CA, USA) containing 100 ml of 1X PBS. The bags containing the coupons were placed in a sonicating water bath (22-23 °C) and were sonicated for 5 min at 42 kHz (FS30H sonicator, Fisher Scientific, Pittsburgh, PA, USA) to aid in the release of attached cells. Serial dilutions of the PBS buffer containing the released cells were performed by transferring 100 µl of solution into 900 µl of 1x PBS and the cells were enumerated on TSAYE and Rapid *L. mono*® plates using the droplet plate technique (Kumar et al., 2023; Oguadinma et al., 2022; Herigstad et al., 2001). The limit of detection was 1.69 log CFU/ml. Colonies were counted after 48 h of incubation at 37 °C.

5.2.8 Statistical analysis

All the experiments were conducted in three technical and biological replicates. A randomized block design was used to design the experiment by blocking replication. The differences in the *L. monocytogenes* or *S. enterica* population were compared using the analysis of variance (ANOVA; JMP Pro 15, SAS Institute Inc., Cary, NC, USA). The significant differences between the mean counts were established using Tukey's honestly significant difference (HSD) test at a 95 % significance level. Significant differences are denoted by $p \leq 0.05$ whereas the highest significant difference is defined at $p \leq 0.0001$.

5.3 Results

5.3.1 Parameters affecting Far UV-C (222 nm) against bacterial inactivation

The treatment dosages for Far UV-C (222 nm) at 1, 4, and 8 h were 381.6 mJ/cm^2, 1526.4 mJ/cm^2, and 3052.8 mJ/cm^2 respectively. The treatment time was a significant ($p \leq 0.0001$) contributing factor towards the efficacy of Far UV-C (222 nm) against both bacterial strains studied. The overall temperature (19.30±1.30 °C) and relative humidity (50.90±3.91%) remained stable in both the Far UV-C (222 nm) and control treatments, showing no significant variations and negligible influence on bacterial inactivation ($p \geq 0.05$) (Figure 5.3). Likewise, thermal images of HDPE coupon surfaces confirmed temperature uniformity during Far UV-C (222 nm) treatment (Figure 5.4). Both *S. enterica* and *L. monocytogenes* inoculated on HDPE coupons were significantly susceptible to Far UV-C (222 nm) treatment in comparison to controls (FBL and dark exposure) ($p \leq 0.05$). The cocktail of *L. monocytogenes* isolates on HDPE coupons was found to be highly susceptible to Far UV-C (222 nm) treatment compared to the cocktail of *S. enterica* isolates ($p \leq 0.0001$).

5.3.2 Efficacy of Far UV-C (222 nm) on *L. monocytogenes* inactivation

The *L. monocytogenes* counts on selective media (MOX) and non-selective media (TSAYE) did not show significant differences ($p \geq 0.05$) post-UV-C treatment and for dark and fluorescent bulb light controls. Since the MOX agar plates could not differentiate the extent of *L. monocytogenes* cell injury, further results were based on bacterial counts from TSAYE plates. It was found that the duration of Far UV-C (222 nm) exposure had a significant impact on *L. monocytogenes* reduction ($p \leq 0.0001$) on the HDPE coupon surfaces. The average reduction of *L. monocytogenes* on HDPE coupons was 2.50±0.33, 2.44±0.09, and 3.26±0.32 log CFU/cm^2 for 1, 4 and 8 h of Far UV-C (222 nm) treatment respectively. When *L. monocytogenes* inoculated coupons were used for the study, there was a significant reduction in bacterial population when coupons were exposed to Far UV-C (222 nm) for 8 h in comparison to coupons exposed for 1 and 4 h ($p \leq 0.05$) (Table 5.1).

The mean log reduction of *L. monocytogenes* on HDPE coupons placed in the dark were 0.81±0.20, 0.11±0.20, and 0.18±0.24 log CFU/cm^2 respectively for three treatment times. Similarly, the mean log reduction of *L. monocytogenes* on HDPE coupons exposed to fluorescent bulb light were 0.52±0.14, 0.51±0.32, and 0.19±0.11 log CFU/cm^2 respectively.

5.3.3 Efficacy of Far UV-C (222 nm) on *S. enterica* inactivation

Significant reductions in *S. enterica* population of 1.18±0.17 and 1.00±0.32 log CFU/cm^2 occurred after 1 and 8 h of treatment, however, a recovery of 0.47±0.15 log CFU/cm^2 in cell population was observed after 4 h ($p \leq 0.05$). Recovery of *S. enterica* population at 4h was also observed in *S. enterica* cell population exposed to FBL and dark control treatments. The mean reduction in *S. enterica* population on HDPE coupons exposed to FBL for 1, 4 and 8 h were 0.36±0.20, 0.08±0.10, 0.16±0.14 log CFU/cm^2, after 1, 4 and 8h respectively. For HDPE couponskept at the dark, a

mean reduction of 0.49±0.08 and 0.01±0.15 log CFU/cm^2 were observed at 1 and 8 (Table 5.1) h. A recovery of 0.06±0.14 log CFU/cm^2 was observed in the *S. enterica* population after 4 h of dark exposure.

5.3.4 Comparison in reduction between bacterial strains

HDPE coupons inoculated with *S. enterica* were significantly susceptible to Far UV-C (222 nm) treatment ($p \leq 0.05$) in comparison to controls (FBL and dark exposure). Similar results were observed when *L. monocytogenes* was exposed to three treatment conditions. A higher reduction of *L. monocytogenes* (2.49±0.33 log CFU/cm^2) was observed in comparison to *S. enterica* (1.18±0.17 log CFU/cm^2) within 1 h of Far UV-C (222 nm) treatment ($p \leq 0.05$). However, the mean reduction of *S. enterica* and *L. monocytogenes* population on HDPE coupons exposed to FBL and dark for 1 and 8 h were comparable ($p \geq 0.05$), whereas a reduction in population was observed for *L. monocytogenes* but not for *S. enterica* when exposed to FBL for 4 h ($p \leq 0.05$).

The reduction of the bacterial population on HDPE coupons exposed to Far UV-C (222 nm) light and FBL was influenced by an interaction between treatment time and the pathogen under study ($p \leq 0.0001$). Exposing *L. monocytogenes* inoculated coupons to Far UV-C (222 nm) for a duration of 8 h resulted in the highest reduction (3.26±0.32 log CFU/cm^2) compared to other treatments studied. Reduction in *L. monocytogenes* population after 1 h of Far UV-C (222 nm) exposure was significantly higher than reduction in *S. enterica* after 8 h of Far UV-C (222 nm) exposure ($p \leq 0.0001$). This result showed the increased susceptibility of the *L. monocytogenes* in comparison to the *S. enterica*.

The population of *S. enterica* and *L. monocytogenes* exposed to FBL changed significantly with exposure times ($p \leq 0.05$) while the populations of pathogen on HDPE coupons kept in the dark did not have significant changes over time.

5.4 Discussion

L. monocytogenes and *S. enterica* are two foodborne pathogens that are frequently linked to contamination in ready-to-eat products and fresh produce. *L. monocytogenes* is known to survive or even grow at low temperature conditions (Roberts et al., 2020), while *S. enterica* is known for their ability to thrive in dry environments (Finn et al., 2013). Both of these pathogens are capable of attaching to food contact surfaces and cross contaminate food products (Corcoran et al., 2014; Ruiz-Llacsahuanga et al., 2021). Since current chemical sanitation procedures performed in food processing and fresh produce industries cannot completely eradicate these pathogens additional interventions such as photonFar UV-C (222 nm) was tested for its efficacy against these pathogens.FCS

Filtered Far UV-C (222 nm) krypton chloride excimer lamps are promising technology used for disinfection purposes. The application of excimer lamps was investigated against airborne pathogens mainly, viruses, wide spectrum of human pathogens found on environmental and health care facilities, foodborne pathogens and their biofilms. (Eadie et al., 2022; Navarathna et al., 2023; Ha et al., 2016). The antimicrobial mechanism of UV-C irradiation assisted disinfection involves, primarily, damage to genetic material (DNA and RNA), by dimerization of pyrimidine molecules, specifically thymine to produce cyclobutene dimers. This prevents nucleic acid replication and eventually loss the cell viability (Buonanno et al., 2020). In addition to genetic material damage, Far UV-C (222 nm) excilamps specifically target the cell membrane integrity by inactivation of key enzymes responsible for maintaining membrane potential such as respiratory chain dehydrogenase and esterase (Kang et al., 2028; Ha et al., 2017). Apart from that, higher lipid peroxidation and intracellular reactive oxygen species (ROS) generation were found to be secondary causes of impaired cell membrane integrity. (Kang et al., 2018). However, considering

the limited penetration depth (< 3-6 μm) of Far UV-C (222 nm) in biological materials such as human skin and eyes, it is safe to be used for disinfection purposes (Welch et al., 2023).

Our study results showed that the efficacy of Far UV-C (222 nm) is dependent on the dosage applied. We found a significant reduction in *L. monocytogenes* populations (3.26±0.33 log CFU/cm^2) when exposed to a dosage of 3052.8 mJ/cm^2 (8 h), compared to 381.6 mJ/cm^2 (1 h). Our findings are consistent with prior research involving the irradiation of *S.* Typhimurium, *L. monocytogenes*, and *Escherichia coli* 0157:H7 in water (initial population of 7 log CFU/ml) using a UV-C excimer lamp (222 nm). A treatment dose of 2.03 mJ/cm^2 for 20 s (irradiance of 101.61 μW/cm^2) led to a mean reduction of 2-3 log CFU/ml in comparison to a 5 s treatment with an average reduction of only 0.5 log CFU/ml (Ha & Kang, 2018). Similarly, exposing stainless steel surfaces inoculated with foodborne pathogens to Far UV-C (222 nm) irradiation for less than 7.08 mJ/cm^2 over 30 s yielded comparable reductions whereas a higher irradiance of 236 mJ/cm^2 (1000 s) resulted in a significant reduction of *L. monocytogenes* (4.5 log CFU/cm^2) compared to *Pseudomonas aeruginosa* and *E. coli* (1.5 log CFU/cm^2) (Chen & Moraru, 2023). Interestingly, our study results indicated no significant differences in the reduction of *S. enterica* populations on HDPE coupons (~ 1 log CFU/cm^2) between the two exposure dosages (381.6 mJ/cm^2 and 3052.8 mJ/cm^2). This demonstrates that the impact of Far UV-C (222 nm) in pathogen reduction is species dependent. It is worth noting that in our study, despite subjecting HDPE coupons to a substantial treatment dosage of 3052.8 mJ/cm^2 during an 8 h exposure to Far UV-C, we observed a maximum reduction of only 3 log CFU/cm^2 in cocktail *L. monocytogenes* population. Therefore, further research is warranted to explore and compare surface properties that contribute to pathogen inactivation.

Previous studies have demonstrated differences in susceptibility between Gram positive and Gram negative bacteria to Far UV-C (222 nm) (Kang et al., 2018; Shin et al., 2016; Shin et al., 2020). This could be attributed to differences in cell wall structure and composition of Gram-negative and Gram-positive bacteria. Shin et al. (2016) reported a higher resistance of *L. monocytogenes* suspension compared to *S.* Typhimurium when subjected to an irradiance of 422.15 $\mu W/cm^2$ during Far UV-C (222 nm) treatment. This could be due to a thick outer peptidoglycan layer present in Gram positive bacteria (Kang et al., 2018; Shin et al., 2016; McKinney & Pruden, 2012). In contrast, our observations on HDPE coupons revealed that *L. monocytogenes* were highly susceptible to Far UV-C (222 nm) treatment compared to *S. enterica* when exposed to all three treatment durations. *L. monocytogenes* and *S. aureus* (Gram-positive) exhibited greater sensitivity to Far UV-C (222 nm) treatment than *E. coli* and *P. aeruginosa* (Gram-negative) when exposed to a treatment dose of 236 mJ/cm^2 on stainless steel surfaces (Chen & Moraru, 2023), consistent with our findings. The varying susceptibilities among Gram-negative pathogens can also be noted, with *P. aeruginosa* in stainless steel sufaces exhibiting highly resistance to Far UV-C (222 nm) treatment compared to *E. coli*, which signifies the strain-dependent nature of Far UV-C (222 nm) responses (Chen & Moraru, 2023). Far UV-C (222 nm) primarily targets lipoproteins and cellular enzymes due to the peak absorption of photons by peptide bonds present in proteins and phospholipids (Ha et al., 2017). Furthermore, it induces damage to Gram-positive cell structures through the generation of reactive oxygen species (ROS) (Tavares et al., 2023). Importantly, Far UV-C (222 nm) exhibits limited penetration depth, which restricts its ability to reach inner cellular components, especially in Gram-negative organisms with multiple layers of cell membranes, making it less effective (Chen & Moraru, 2023). Additionally, It is also worth noting that all the cocktail *S. enterica* strains used in our study were resistant to an antibiotic (rifampicin; 80 ppm).

Previous studies reported the requirement of higher Far UV-C (222 nm) doses (200–400 mJ/cm^2) to inactivate bacterial strains containing antibiotic resistant genes by 3-4 log CFU/ml, indicating the limited efficacy of Far UV-C (222 nm) against antibiotic-resistant bacteria for disinfection purposes (McKinney & Pruden, 2012).

5.5 Conclusion

Far UV-C (222 nm) showed promising results against persistence of *L. monocytogenes on food contact surfaces.*

5.6 References

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Table 5.1. *L. monocytogenes* and *S. enterica* reduction during different treatment conditions (UV-C (222 nm), fluorescent bulb light, dark). Different letters (A, B, C, D, E, F, G, H, I) within a column following the mean ± standard deviation values indicate statistically significant ($p \leq 0.05$).

		Mean bacterial reduction (log CFU/cm^2)		
Bacterial Strains	**Time (h)**	**UV-C**	**Dark**	**FBL**
L. monocytogenes	1	2.49 ± 0.33^{B}	0.81 ± 0.20^{DE}	0.52 ± 0.1^{EF}
	4	2.44 ± 0.09^{B}	0.11 ± 0.20^{HI}	0.51 ± 0.33^{EF}
	8	3.26 ± 0.33^{A}	0.18 ± 0.25^{FGHI}	0.19 ± 0.12^{FGHI}
S. enterica	1	1.18 ± 0.17^{C}	0.49 ± 0.08^{EFG}	0.36 ± 0.19^{FGH}
	4	0.71 ± 0.12^{DE}	$-0.06 \pm 0.14^{I*}$	0.08 ± 0.10^{HI}
	8	1.01 ± 0.32^{CD}	0.01 ± 0.15^{HI}	0.16 ± 0.14^{GHI}

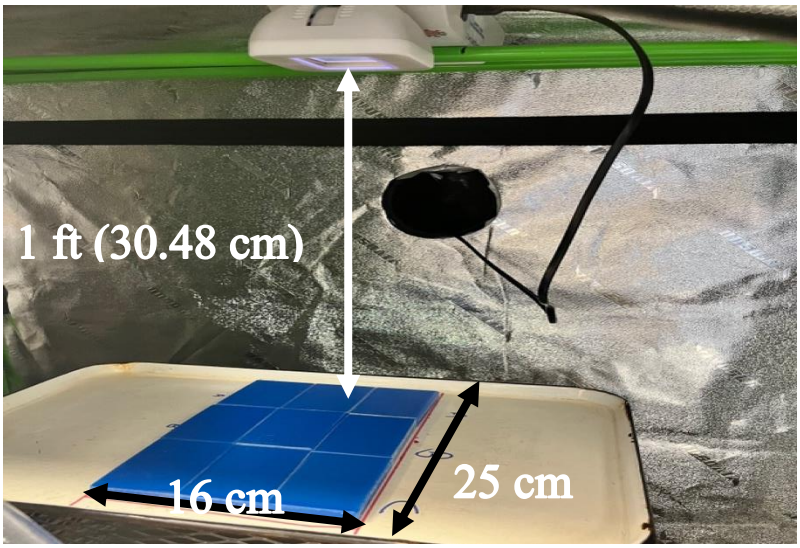


Figure 5.1: UV-C (222 nm) experimental setup. HDPE coupons consists of three technical and biological replicates of bacterial strains used for the study.

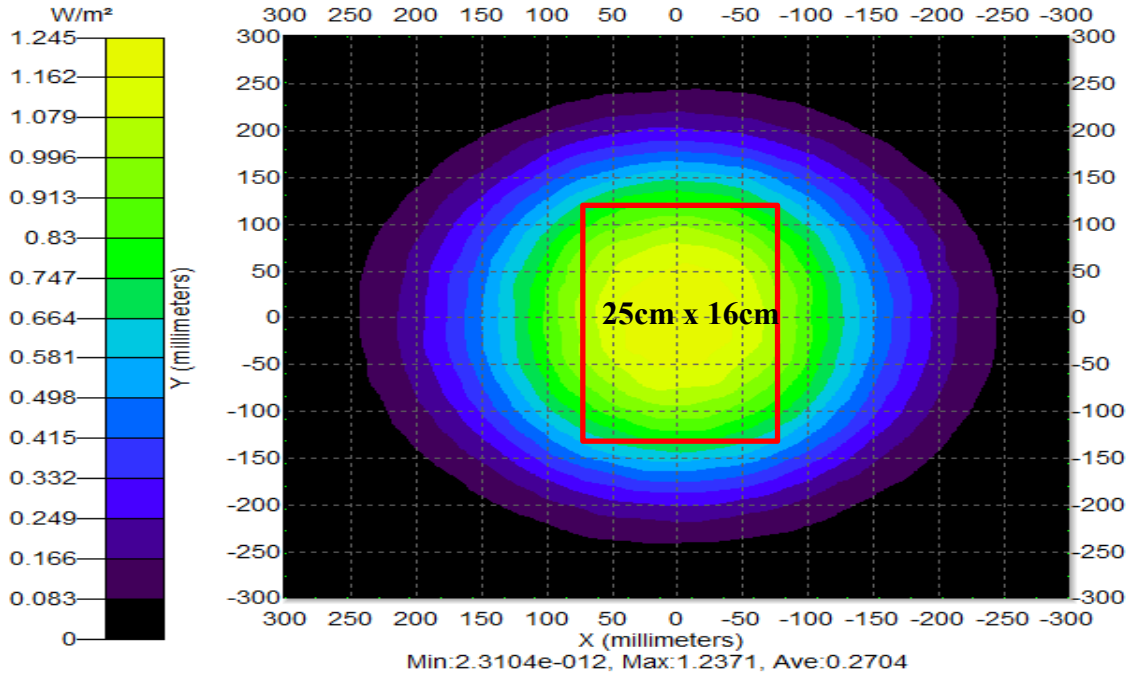


Figure 5.2: Simulation results UV-C (222 nm) irradiance measurement using UV-light meter.

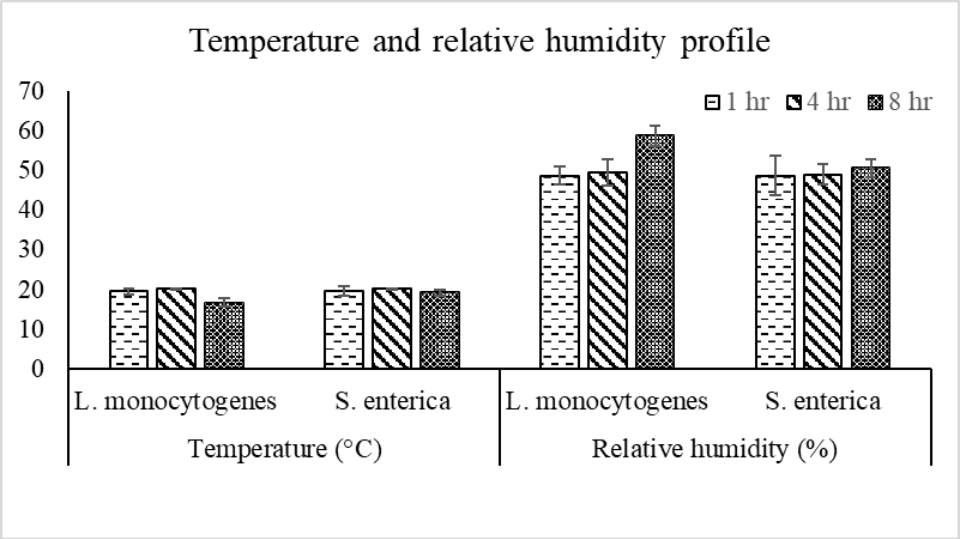


Figure 5.3: Temperature (°C) and relative humidity (%) during UV-C and control treatment

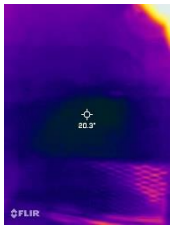
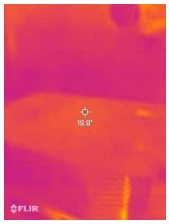
Treatment Time	0 h	1 h	4 h	8 h
Thermal images of HDPE coupons during UV-C (222 nm) treatment				

Figure 5.4: Thermal images of HDPE coupons captured during UV-C (222 nm) treatment for 1, 4, and 8 h.

CHAPTER 6

CONCLUSION AND SUMMARY

Peaches are an economically and culturally important fresh produce commodity of Georgia, USA, with peak harvesting and packing from June to August annually. Microbial assessment of fresh peach packing lines is necessary to identify areas or surfaces with the highest microbial growth and cross-contamination probability. Due to the lack of inefficiencies in current sanitation practices in the fresh produce industry, cost-effective, sustainable, and effective decontamination strategies are in need to reduce the pathogenic load on food contact surfaces. In this thesis, we explored two photon-based interventions: antimicrobial blue light technology (aBL) with a peak emission of 405 nm and far UV-C with a wavelength of 222 nm against foodborne pathogens on plastic surfaces.

In the first study, we assessed the microbial load of twelve environmental surfaces (Zone 1-3) and dust collected within distinct packing facilities in Georgia at different time intervals during peach packing season. Based on enumeration for hygiene indicators such as heterotrophic plate counts, coliform counts, and *Pseudomonas* spp., we found that a fruit contact surface (zone 1), waxer brushes were heavily contaminated with an HPC of 5.49±0.89 log CFU/cm^2 throughout the packing season in both packing facilities. This could be attributed to the complex structure of brush bristles that traps the debris or wax, which eventually supports the accumulation of microorganisms, including pathogens. We isolated *P. aeruginosa* from the fruit contact surfaces, such as rollers and post wash conveyor, in one of the peach packing facilities. Identifying *P. aeruginosa* on fruit contact surfaces poses a concern as they could form dense mixed species biofilms with foodborne pathogens. A higher microbial load on fruit contact surfaces indicated the

need for a novel dry sanitation technique that has superior or comparable efficacy of a chemical sanitation system and, at the same time, is reliable.

Antimicrobial blue light (aBL) is gaining popularity due to its bactericidal properties at visible wavelengths (400-460 nm). The second study optimized the critical physical parameters such as HDPE surface color and exposure duration (h) in improving the efficacy of aBL on sessile *L. monocytogenes* cells. Among five different colors of HDPE plastic coupons (red, blue, green, white, and black) and three different time points (1, 4, and 8 h) tested, we found that an exposure duration of 8 h on blue-colored coupons was able to inactivate five strain cocktail of *L. monocytogenes* cells on HDPE coupons by 1.8 log CFU/cm^2. In food-based industrial settings, conveyor belts, brushes, and produce harvesting baskets could be made of different colored plastic materials. In order to achieve a higher reduction of pathogens, future studies should be explored to evaluate the role of exogenous food-grade photosensitizers on food contact surfaces.

The antimicrobial efficacy of UV-C irradiation is well established and was implemented in various food processing operations. In our third objective, we assessed the effectiveness of far ultraviolet-C (UV-C) light at a wavelength of 222 nm in inactivating dry inoculated Gram-positive (*L. monocytogenes*) and Gram-negative (S. *enterica*) bacteria on high-density polyethylene (HDPE) coupons surfaces. A UV-C (222 nm) exposure duration of 8 h on coupons inoculated with *L. monocytogenes* was reduced by 3.26 log CFU/cm^2 compared to *S. enterica*, which was only reduced by 1.01 log CFU/cm^2. This indicated a selective susceptibility among different strains used for the study. The inactivation of pathogens was also influenced by exposure time.

To conclude, both aBL and far UV-C (222 nm) technology showed the ability to inactivate foodborne pathogens on food contact surfaces. They could be deployed as a dry sanitation technique in the dynamic environment of the fresh produce industry, where the conveyor belts,

brushes, sorting cups, and produce handling baskets are in continuous movement. However, further research is required to amplify the efficacy of these technologies by using a hurdle approach by combining multiple intervention steps for thoroughly irradicating the pathogens.

DECLARATION

During the preparation of this work, I used CHATGPT to correct the grammatical mistakes, and to improve the overall brevity and readability of the thesis. After using this tool/service, we reviewed and edited the content as needed and take full responsibility for the content of the publication.