EVALUATION OF INDICATORS OF AIR QUALITY AND HYGIENE DURING PEACH PRODUCTION AND PACKING

by

Brandon Cox

(Under the Direction of Govindaraj Dev Kumar)

ABSTRACT

Peaches are susceptible to foodborne pathogen contamination during pre-harvest cultivation and post-harvest handling. Hence, the microbiological quality of air in peach orchards was evaluated. Further, the hygiene and sanitation efficiency of peach packing houses was also determined. In air samples at the peach orchards (n=9), there was a moderate correlation between coliform populations and bile (r=0.64). For the two tested packinghouses, sanitation programs at both packinghouses were most effective against coliform populations with an average reduction in population of 0.89±0.73 log MPN/cm^2 and 0.80±0.94 log MPN/cm^2 for packinghouse 1 and 2, respectively. The most contaminated surfaces were sorting cups and washer/waxer brushes. Peptones from different sources were evaluated for capture buffer (CB) preparation during air sampling. Bacto™ Malt Extract showed the highest retention (3.44 ± 0.23 log CFU/cm^3) and stability of airborne *Escherichia coli* K12. The results highlight the importance of monitoring hygiene during peach cultivation and packing.

INDEX WORDS: Packinghouses, Orchards, Bioaerosols, Bile

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by

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EVALUATION OF INDICATORS OF AIR QUALITY AND HYGIENE DURING PEACH PRODUCTION AND PACKING OPERATIONS

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DEDICATION

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CHAPTER 1

INTRODUCTION

The detection and quantification of microbial and chemical indicators of hygiene are some of the most commonly used methods to evaluate food safety, environmental contamination and sanitation quality (Jay et al., 2005). These indicators are crucial to many different food operations and their maintenance of safe, uncontaminated products and facilities. Some of the typical indicators for food safety are coliforms or *Escherichia coli* (Jones et al., 2020). Bile is another potential indicator tied to fecal contamination from either humans or animals (Elhmmali et al., 2000; Tyagi et al., 2009). Bile may serve as a novel indicator of microbial presence in both surface and airborne environments. The fresh produce industry is one area of concern for food safety as their products have the potential to become contaminated by a large number of different types and severities of hazards (Hussain & Gooneratne, 2017). These products could be contaminated at various points in the production process from pre-harvest on a farm to post-harvest in packinghouse environments (Machado-Moreira et al., 2019). Environmental monitoring and product testing in the fresh produce industry are integral, especially in recent years, to have the ability to pinpoint contamination areas and vectors as the number of outbreaks associated with these products continues to persist or grow (Carstens et al., 2019).

A less examined component of food safety is the presence of bioaerosols. These bioaerosols can be broadly defined as airborne particulate matter carrying or composed of viable microorganisms or other biological matter (Fabian et al., 2005; Lindsley et al., 2017). The dispersal of these bioaerosols is a potentially substantial vector for spreading various foodborne

pathogens in both pre- and post-harvest environments. The bioaerosol level assessment largely depends on the methodology used to collect and analyze air samples. Some common techniques include passive methods, such as plate settling, or active methods, such as impaction or impingement (Reponen, 2017). When implementing the impingement method, the choice of what collection media to use has the potential to affect the overall recovery of microbes in indoor air samples (Chang & Wang, 2015). However, there are increasing variables when performing air sampling in an outdoor, pre-harvest environment. The collection of outdoor bioaerosols provides potential issues in maintaining the natural characteristics (physiological state, viability, etc.) of the given sample through sampling length and the storage/transportation of the sample back to the laboratory for analysis (Šantl-Temkiv et al., 2020). For the impingement method, the choice of collection media requires consideration. This is because the collection media must have enough nutrient value for microorganisms to retain their viability, but they need to not have too much nutrient value to where the microorganisms can grow in the media during sample collection or transportation, especially when the sample is to be enumerated.

This research aims to evaluate the presence of indicators in both a pre- and post-harvest environment for air quality and hygiene in the peach industry. It is also aimed at gaining more understanding of the potential differences between collection media in an air impingement system for the improvement of the collection/recovery of bioaerosols.

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CHAPTER 2

LITERATURE REVIEW

Peaches & Peach Industry

Peaches are a stone fruit that originated in eastern Asia and are now commonly grown in various areas around the globe (Chin et al., 2014). Stone fruits, typically genus *Prunus*, are defined as "a fruit with a lignified endocarp surrounding the stone (seed), a fleshy mesocarp, and a thin exocarp" (Brady, 1993; Fadón, 2020). Common examples of stone fruits include peaches, apricots, plums, cherries, and nectarines (Fadón, 2020; Riva et al., 2020). Peaches, along with the typical internal structure found in stone fruits, are covered with fuzz on their surfaces known as trichomes (Yan et al., 2020). Trichomes are often defined as appendages (multi- or unicellular) that grow outward on the surface of plants from epidermal cells (Werker, 2000; Fernández et al., 2011). The surface of peaches is covered by a 0.4-1.0 mm thick layer of trichomes with lengths of 0.1 to 1.0 mm (Fernández et al., 2011). It has been shown that microbial cells are able to be seen, through scanning electron microscopy, on the surface of the peach trichomes (Kaur et al., 2017). Sugar content and constitution can greatly affect the taste and perception of peaches (Cirilli et al., 2016). The sugar content of peaches is comprised primarily of fructose, sucrose, and glucose in varying amounts depending on peach type and variety (Byrne et al., 1991) It has been shown that higher quality peaches will have higher levels of fructose, but lower-quality peaches will have higher levels of glucose (Robertson et al., 1988). Peach fruits also typically contain various phenolic compounds, such as flavonols, flavan-3-ols, anthocyanins, and hydroxycinnamic acid (Tomás-Barberán et al., 2001). The phenolic compound content of red-

fleshed peaches has been shown to be higher than both yellow and white fleshed peaches (Vizzotto et al., 2007). Phenolic compounds are important antioxidants as they possess the ability to both act as a metal chelator and neutralize reactive oxygen species (ROS) (Zhang & Tsao, 2016). The peaches' metabolic composition undergoes several changes or shifts throughout their development (Drincovich, 2021). The highest levels of polyphenols have been observed in peaches during their early stages of development (Belhadj et al., 2016). Metabolomic changes also can differ in the various sections and tissues of the peach. For example, a high synthesis of lignin in the endocarp layer of the peach can be observed at the early stages of peach development (Rodriguez et al., 2019). Peaches have been found to have a relatively short shelf life due to their high perishability (Mahajan et al., 2014). It has been shown that the shelf life of peaches stored at refrigeration temperatures can be reduced by the onset of chilling injury (Lurie & Crisosto, 2005). Chilling injury typically includes the onset of flesh browning, loss of juiciness, and flavor loss (Crisosto et al., 1999). While it has been shown that chilling injury can begin at between 1-2 weeks of cold storage, off-flavors can be detected ~5 days before chilling injury symptoms become visible (Lurie & Crisosto, 2005; Crisosto et al., 1999).

Peaches are produced in over eight states across the United States with California being the highest producing state ahead of South Carolina and Georgia at second and third, in 2021 (USDA-NASS, 2022). While peaches can be further processed, they are often treated and packaged to be eaten as a raw commodity, much like other fruits and vegetables (Duvenage & Korsten, 2017). In the southeast region of the United States, the peach harvesting season will generally run from May to August with some variation based on environmental conditions and harvest yields (Belisle et al., 2018). Peaches are typically harvested by hand via field workers and placed into bins for transport to a processing or packing facility. Upon arrival to a packing

facility, a primary priority is to remove field heat from the peaches via a hydrocooling process to assist in quality preservation and halt potential bacterial growth (Han et al., 2017). The water used in the hydrocooling process is often recirculated throughout a day of operations and is supplemented with chlorine (Suslow, 2000). After hydrocooling, peaches are typically moved to the packing line or moved to cold storage until they are moved to the packing line. Typically, the first step in the packing process is for the peaches to be inspected and graded to eliminate any damaged peaches or potential debris from the line (Guarnieri et al., 2014). The primary decontamination step in packing fresh peaches is a wash step followed by applying a surface wax. Often, this step is accomplished via an overhead spraying system to apply the sanitizing water and wax (Wang et al., 2021a). This style of overhead application system is often used in conjunction with rotating brushes to assist in the removal of potential dirt or debris from the surface of the peach (Pao et al., 2012). Once the peaches are washed and waxed, they must be graded and sorted for packaging and labeling purposes. Some operations, typically smaller ones, can undergo this process via manual grading/sorting via hands-on workers (Londhe et al., 2013). For larger commercial operations, an automatic sorting system that analyzes the peaches via optical sensors and scanners is often used (Crisosto & Valero, 2008). Versions of this style of the system will move the peaches through the automatic sorter on individual carrier cups (Londhe et al., 2013). The surfaces of these cups are difficult to clean as they must be cleaned in place; each system is comprised of a large numbers of cups, so disassembly for daily cleaning is unrealistic (Williamson et al., 2018). The sorted peaches are then distributed by the automatic sorting system to their respective packaging area to be packaged and prepared for shipping. Often, the brushes found in the washer/waxer are among the most contaminated sites across packing lines of fresh produce capable of harboring various foodborne pathogens (Wang et al., 2021b;

Portman et al., 2002). The general flow of a peach packinghouse operation has been visualized to show the typical flow routes of peaches through the process (Figure 2.1). One practice found in some peach packinghouse operations is using overnight sanitation. Overnight sanitation is where a crew comes in to clean and sanitize the facility and packing lines, after daily operations are complete, to remove waste, debris, or any other potential contaminant before the following day's operations (Møretrø & Langsrud 2017).

Peach-Related Outbreaks & Recalls

Throughout the years, there have been a number of outbreaks and/or recalls associated with peaches in the United States. In 2020, there was an outbreak of *Salmonella enterica* subsp. *enterica* serovar Enteritidis across the United States tracked to peaches from California (FDA, 2020). While this outbreak did not cause any deaths, it did cause 101 illnesses and 28 hospitalizations across 17 states (FDA, 2020). Upon investigation, there was no *Salmonella* found in the packinghouses, but they did find *Salmonella* in the orchards and the poultry and dairy operations that were in close proximity (FDA, 2020). This sort of proximity of fresh produce farms to animal operations has the potential to lead to more incidents similar to this one. According to the FDA (2020) investigation, it was surmised that the likely culprit for the contamination was dust originating from the poultry and dairy operations nearby, based upon the isolation of *Salmonella enterica* subsp. *enterica* serovar Alachua (chicken isolate) and *Salmonella enterica* subsp. *enterica* serovar Montevideo (cattle isolate) from peach tree leaves near the animal operations. In 2014, a packing company in California recalled various stone fruits, including peaches, for potential *Listeria monocytogenes* contamination (FDA, 2014). The stone fruits from this recall were found to be potentially associated with illnesses in multiple states (Jackson et al., 2015). In 2019, a Jac. Vandenberg, Inc. issued a recall of fresh peaches,

nectarines, and plums due to potential contamination with *L. monocytogenes* (FDA, 2019). According to the FDA (2019), the fruits were distributed to 6 retailers across over 15 states, but no recorded illnesses were reported or associated with this recall. In 2022, Brookshire Grocery Company issued a recall of peaches due to potential *L. monocytogenes* contamination (FDA, 2022).

Produce Contamination

Contamination throughout the production process for various fresh produce products has been a food safety concern for many years. In a pre-harvest environment, various vectors (soil, water, dust, etc.) can contaminate fresh produce and allow bacteria to survive on either the surface or within the tissues of the commodity (Beuchat, 2006). Various strains of *S. enterica* are isolatable from water in areas of high fresh produce production (Gorski et al., 2011). Agricultural water may be contaminated via fecal contamination from either animals or humans via runoff (Wei & Kniel, 2010). The use of soil amendments, such as poultry litter, can potentially increase the chance of produce contamination with foodborne pathogens, such as *S. enterica*, due to these amendments being natural reservoirs for foodborne pathogens (Gu et al., 2018). Soil contamination can also perpetrate *L. monocytogenes* contamination in fresh produce given its ability to persist and survive in soil (Miceli & Settanni, 2019).

Dust is one vector of potential contamination for fresh produce, at both the pre- and post-harvest stage, that is largely unexplored, in comparison to other vectors (Dev Kumar et al., 2018). There have been a number of occasions where dust has been the culprit of food contamination. In 1999, a *L. monocytogenes* outbreak from ready-to-eat (RTE) meats was attributed to dust contamination originating from construction occurring outside the processing plant (De Roin et al., 2003). The 2020 *S.* Enteritidis outbreak associated with peaches was

attributed to dust contamination from animal operations (FDA, 2020). This spread of pathogens from dust originating at animal operations has been explored in multiple instances. In one instance on a pig farm, it was found that methicillin-resistant *Staphylococcus aureus* was detected in air and dust samples at various ranges within 300 meters of the barns housing the pigs (Schulz et al., 2012). *E. coli* O157:H7 and *Salmonella* were detectable in dust samples at a cattle feed yard, but they were only detectible in the dust samples taken after the dust was agitated/generated in the yard (Miller et al., 2008). *E. coli* was also found to be detectable on growing spinach samples in proximity to a cattle feed yard after dust agitation, but it was not detectible before exposure to the area (Yanamala et al., 2011). Studies such as these expand on the concern for operating fresh produce farming within a certain proximity to potential animal operations at the risk of food safety.

A number of routes of contamination can affect fresh produce as it moves to a post-harvest environment. Some of the potential post-harvest contamination vectors are dust, food handlers, or contaminated food contact surfaces (Beuchat, 2002). The washing of and wax application to fresh produce commodities is common practice to clean the product's surface and improve its appearance and shelf life (Dhall, 2013). This washing step is typically supplemented with a sanitizer, such as chlorine or peroxyacetic acid, as it is the primary decontamination step for whole, fresh produce (Barrera et al., 2012; Beuchat et al., 2004). However, it has been shown that this wax application process has the potential to spread contaminants further down the packing line process after the wax has been applied (Ruiz-Llacsahuanga et al., 2022).

Foodborne Pathogens of Concern in Produce

In the United States, the most prevalent foodborne pathogens of interest, for fresh produce, are *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* (Beuchat, 1996). *E. coli*

O157:H7 is a shiga-toxin-producing strain (STEC) that causes gastroenteritis or possible hemolytic uremic syndrome (HUS) upon infection (Machado-Moreira et al., 2019). Between 2003-2012, there were 255 outbreaks of *E. coli* O157:H7 from food sources (meat, poultry, fruit, vegetables, etc.; Heiman et al., 2015). After field applied spray inoculation of *E. coli* O157:H7 onto whole heads of red and green cabbage, the pathogen was found to still be detectable via enrichment after 22 days in field conditions (Erickson et al., 2019). *E. coli* O157:H7 has also been shown to have resistance to various antimicrobial treatments that are commonly used in the food industry. *E. coli* O157:H7 has strongly attached itself to the surface of Valencia oranges has shown the ability to exhibit higher tolerance/resistance to lactic acid, chlorine, and hot water treatments, compared to loosely attached cells (Martínez-Chávez et al., 2019). *E. coli* O157:H7 was also shown to survive on the surface of kale, in a refrigeration environment at 4 °C, for up to 19 days (Bywater et al., 2023). In another instance, *E. coli* O157:H7 was shown to survive on the surface of fresh strawberries at both room and refrigeration temp, and the bacteria showed improved survival on strawberries with a cut surface (Knudsen et al., 2001). This improved survivability of *E. coli* O157:H7 on compromised or damaged fresh produce surfaces is not an isolated instance. Regarding fresh lettuce, *E. coli* O157:H7 was shown to have increased survivability on damaged leaves compared to non-damaged leaves (Aruscavage et al., 2008). Instances such as these emphasize the importance of proper handling of fresh produce to prevent unnecessary damage to the product.

Non-typhoidal *Salmonella* is one of the leading causes of foodborne bacterial-related illnesses, hospitalizations, and deaths in the United States (Scallan et al., 2011). *Salmonella* has been implicated in a number of produce-related outbreaks, including tomatoes, peaches, & leafy greens (Krug et al., 2020; FDA, 2020; Carstens et al., 2019). Salmonellosis typically leads to

gastroenteritis, diarrhea, nausea, and human bowel inflammation (Coburn et al., 2007). *Salmonella* is adept at surviving on a number of fresh produce commodities. *S. enterica* was shown to attach and survive on mangos through ambient conditions, ripening storage, and cold storage without substantial decreases in populations (Mathew et al., 2018). There has also been evidence that *Salmonella* can infiltrate and internalize into mangoes through both the stem and blossom end of the mango (Penteado et al., 2004). Like *E. coli* O157:H7, *S. enterica* was shown to survive on field-inoculated heads of cabbage for up to 22 days post-inoculation (Erickson et al., 2019). On cucumbers, *S. enterica* has been shown to exhibit a 1 log improvement in survival, after 24 h, on waxed cucumbers in comparison to non-waxed cucumbers (Callahan & Micallef, 2019). Persistence, such as this, is of concern as many fresh produce commodities are waxed before shipment to consumers.

L. monocytogenes is a widespread environmental organism among the most significant causative agents of foodborne illness in the United States (Buchanan et al., 2017). Listeriosis can be associated with gastroenteritis, fever, flu-like symptoms, and seizures (Swaminathan & Gerner-Smidt, 2007; CDC, 2022). *L. monocytogenes* is of primary concern for immunocompromised individuals and pregnant women. Pregnant women who become infected with *L. monocytogenes* are at risk of miscarriage, infection, or death of the baby (Swaminathan & Gerner-Smidt, 2007). The primary strains of *L. monocytogenes* associated with foodborne illnesses are 1/2a, 1/2b, and 4b (Borucki & Call, 2003). Due in large part to the high mortality rate (15-30%) of *L. monocytogenes*, the United States Food and Drug Administration (FDA) implemented a "zero tolerance" policy in regard to *L. monocytogenes* in foods (FDA, 2012; Farber et al., 2021). In 2015, there was an outbreak of Listeriosis associated with caramel apples which led to 35 illnesses, 34 hospitalizations, and 7 deaths across 12 states (CDC, 2015).

Another outbreak of Listeriosis was associated with cantaloupes, from a farm in Colorado, which caused 147 illnesses, 143 hospitalizations, and 33 deaths across 28 states, in 2011 (CDC, 2012). Due to the nature of the illness, most of the cases of Listeriosis will often lead to hospitalizations. *L. monocytogenes* has been said to show increased persistence in food processing environments due to its ability to physically adapt to its environment through formation of biofilms and surface attachment. However, this increased persistence has shown to not be related to any increase in resistance to antimicrobial sanitizers that are often used on these surfaces (Holah et al., 2002; Pan et al., 2006). Another reason that special care must be taken for the prevention of *L. monocytogenes* is that is has a high cold tolerance that allows it to be resistant to and grow at typical refrigeration temperatures used in the storage and handling of many food products (Tasara and Stephan, 2006). It has been shown that *L. monocytogenes* exhibits greater survival on fresh cut produce, in comparison to whole fresh produce, at refrigeration temperatures (Kroft et al., 2022). This increased survival on cut produce is telling as to why produce needs to be handled properly to prevent surface damage to the commodity, and this would assist in the prevention of internalization of *L. monocytogenes* into the product, where it can grow more effectively in comparison to on the surface.

Contamination of produce is not limited to those who grow in or on the ground. Given outbreaks like the *Salmonella* outbreak with peaches in 2020 where contamination occurred at pre-harvest/harvest, the contamination of tree fruits growing off the ground is a real threat and possibility (FDA, 2020). There have also been *Salmonella* outbreaks with almonds, in 2000-2001 specifically, where the bacteria were isolated from the soil, so the contamination might have occurred through dust production rising and infecting the nuts (Uesugi et al., 2007).

Microbial Indicators of Hygiene

The use of indicators for investigating food safety and hygiene levels is a common practice throughout the food industry. In microbiology, there are both indicator organisms and index organisms. An indicator organism is generally considered to be an organism or group of organisms whose presence indicates the overall microbial presence of the surface or area, and an index organism is an organism or group of organisms whose presence indicates the presence of specific, similar pathogens (Ashbolt et al., 2001).Coliforms are one of these potential indicator organisms that are often used in assessing potential contamination in water or food products (Martin et al., 2016). Coliforms are typically defined as a group of gram-negative, rod-shaped bacteria that ferment lactose and don't form spores (Paruch & Mæhlum, 2012; Molina et al., 2015). Coliforms, as a group, are often associated with fecal contamination (Rompré et al., 2002). The group of coliform bacteria includes various bacteria of interest, such as *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter* (Halkman & Halkman 2014). The food industry sometimes tests for these coliform bacteria, in place of pathogenic bacteria, as part of regular operations in order to ensure hygiene standards are being maintained in the given facility (Kaydan et al., 2020). Research studies taking place in a variety of food processing facilities or packinghouses have used coliforms as a metric of measuring surface sanitation and fecal contamination (van Dyk et al., 2016; Williamson et al., 2018; Ruiz-Llacsahuanga et al., 2021).

Another potential indicator of hygiene is *Pseudomonas*. *Pseudomonads* are Gram-negative rod-shaped bacteria that are found in many environments and conditions. *Pseudomonas* spp. are generally soil based organisms that are readily found in the environment and have been shown to both be able to survive and be detected in dust and air samples (Täubel et al., 2009). Exposure to dust has been shown to have little to no effect on the growth of *Pseudomonas* (Bado

et al., 2018). Actually, there have been instances where, upon exposure to dust in a nutrient-poor environment, *Pseudomonas* has exhibited both improved growth and enhanced biofilm production capabilities (Suraju et al., 2015). There have also been ties from one strain of *Pseudomonas*, *P. syringae*, to bacterial canker on stone fruit trees, which is a bacterial disease that causes cankers, oozing, or tree death in some cases (Bophela et al., 2020; Hattingh et al., 1989). One cause for concern regarding *Pseudomonas* in the food industry is its ability to form biofilms in packinghouse or processing facility environments. In regard to this, *Pseudomonas* has been shown to be able to form multispecies biofilms (Chmielewski & Frank, 2003; Sasahara & Zottola, 1993). There have also been studies conducted on the relationship between *Pseudomonas* and *L. monocytogenes*. For instance, there has been evidence of *L. monocytogenes* having a greater potential for surface attachment when there was a preexisting *Pseudomonas* biofilm on the given surface (Hassan et al., 2004). It has been shown that *L. monocytogenes* also tend to attempt to colonize inside of the bottom layers of *P. fluorescens* biofilms in order to potentially effectively protect themselves from potential damage (Puga et al., 2018). It is believed that in the formation of a multispecies biofilm of *Pseudomonas* and *L. monocytogenes*, *Pseudomonas* tends to protect *L. monocytogenes* from potential threats via washing or sanitation of the surface (Ripolles-Avila et al., 2022). In comparison to a pure *L. monocytogenes* biofilm, a mixed culture biofilm of *L. monocytogenes* and *P. aeruginosa*, at 12 °C, showed less susceptibility to sanitizers (Lourenço et al., 2011). *Pseudomonas* is also known to be a potential spoilage organism for various fresh produce products (Raposo et al., 2016). While most isolates of *Pseudomonas* are spoilage-type organisms, there are some types that are pathogenic in nature. *P. aeruginosa* is an opportunistic human and plant pathogen (Rhame et al., 2000). It is generally of concern for individuals that are immunocompromised, such as individuals with cystic fibrosis,

or are currently in an intensive care unit (de Bentzmann & Plésiat, 2011). According to the CDC, multidrug-resistant *P. aeruginosa* was responsible for roughly 32,600 hospitalizations and 2,700 deaths in 2017 (CDC, 2019).

Chemical Indicators of Hygiene

Aside from microbial based indicators, a number of chemical indicators exist to be used in the prediction of hygiene and possible microbial contamination. One commonly used method is the utilization of ATP swabs. These swabs are typically used to get a fast, relatively inexpensive result for the "sanitation level" of a sampled surface, typically in a processing facility or packinghouse, but the presence of sanitizers has the potential to interfere/invalidate the swab reading (Vasavada, 2001). The swab often works by using luciferin and luciferase to react with any ATP present on the collected swab and oxygen to produce light, which is in turn measured using some form of luminometer (Vasavada, 2001). While this method is fairly common in the food industry, its narrow view of contamination and various pitfalls in consistency and interference make it not the ideal solution.

One possible concern when evaluating hygiene in the food industry is the possibility of bacteria transitioning to the viable but non-culturable (VBNC) state. The VBNC state is when a bacterium is alive but will not grow on conventional culturing media, and the bacteria has the potential to be resuscitated given proper growth conditions (Oliver, 2000). Bile and endotoxins are two examples of potential biomarkers that circumvent these concerns for VBNC presence (Tyagi et al., 2009; Tager et al., 2010; Heidelberg et al., 1997). Bile acids are steroid acids found and produced in animal digestive systems (Bull et al., 2002). Bile acid is an indicator of fecal contamination as it is a commonly excreted product from both humans and animals in feces (Elhmmali et al., 2000; Tyagi et al., 2009). Bile has large potential as a biomarker because some

bile acids possess the ability to persist and resist degradation over extended periods, and this would allow for the bile presence to give a broader view of contamination over time when individual bacteria may die or lose culturability (Obuseng et al., 2013). A study evaluating fecal sterols and bile acids to determine fecal pollution and its sources revealed that, for runoff and fresh manure, various sterols and bile acids showed consistent results as biomarkers for various animals, suggesting that sterols and bile acids can be used to identify sources and occurrence of fecal matter (Tyagi et al., 2009).

Endotoxins are lipopolysaccharides (LPS) found as a component present in the membrane of gram-negative bacteria, such as *E. coli* or *Salmonella* (Gehring et al., 2020; Thorne & Duchaine, 2007). It has been shown that endotoxin concentrations can generally be higher when closer to concentrated animal feeding operations (CAFO), compared to further distances (Tager et al., 2010). It is common for workers at CAFOs to be routinely exposed to airborne endotoxins during operations, such as animal waste management (Ko et al., 2010). These bioaerosols, originating from CAFOs, can negatively impact human health and contaminate crops that are present in close proximity to these facilities (Thu, 2002; Von Essen & Auvermann, 2005). Finding and enumerating endotoxins in collected air/samples containing particulate matter is possible (van Leenen et al., 2021). Measuring endotoxin concentrations in air samples, at CAFOs, using the Limulus amebocyte lysate (LAL) assay has allowed for the assessment of potential differences and improvements between various methods and implementations of waste treatments and waste management technologies to reduce the risk of contamination from the operations (Ko et al., 2010).

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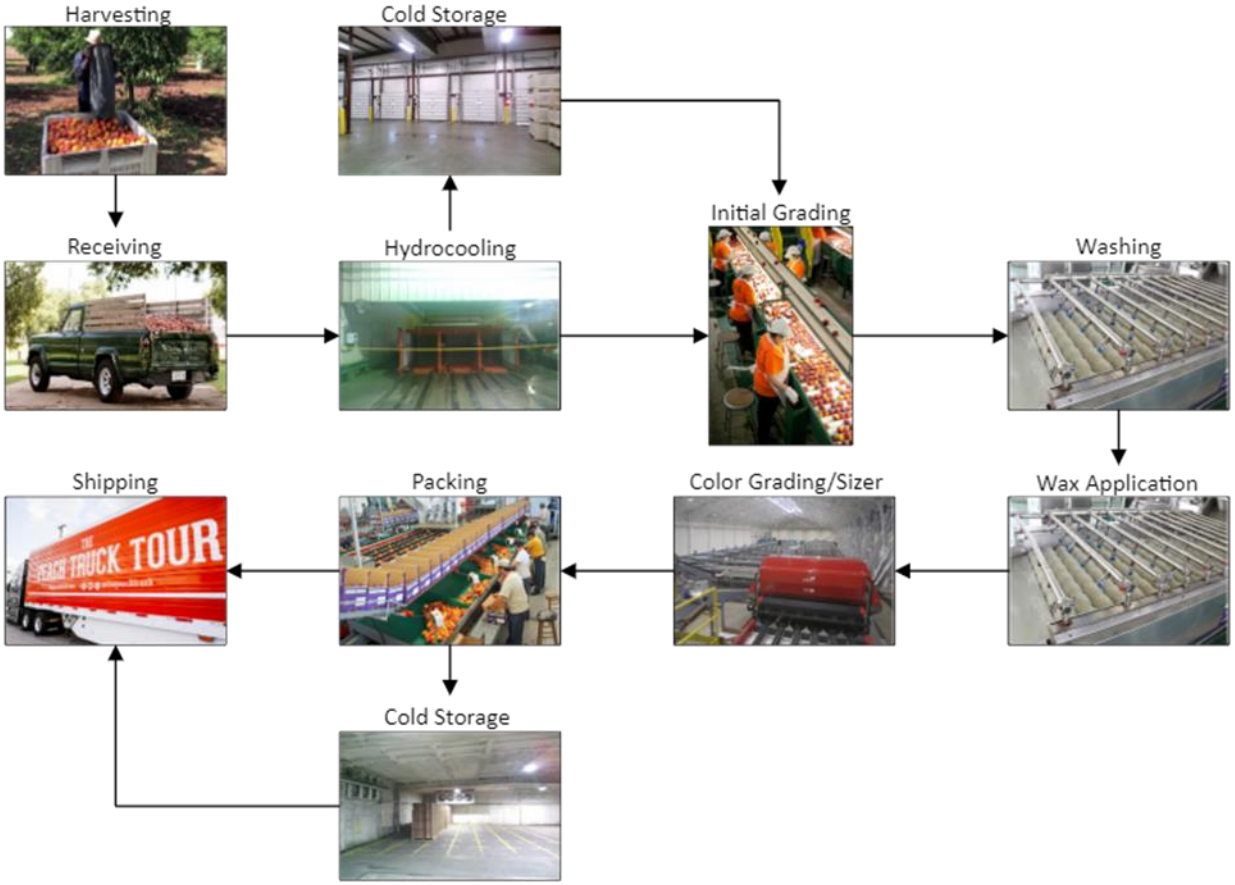
Figure 2.1
General packinghouse processing procedure of fresh, whole peaches



CHAPTER 3

INDICATORS OF AIR QUALITY, HYGIENE, AND SANITATION EFFICACY IN PEACH PACKINGHOUSES [1]

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Abstract

Sanitation program efficacy and various air quality parameters were examined in two peach packinghouses. To evaluate the effectiveness of the sanitation programs, samples were collected prior to sanitation and post-sanitation. The samples were evaluated for heterotrophic plate count (HPC), coliforms, and *Pseudomonas* populations to evaluate the effectiveness difference against various microbes. The sanitation programs at both packinghouses were most effective against coliform populations with an average reduction in population of 0.89±0.73 log MPN/cm^2 and 0.80±0.94 log MPN/cm^2 for packinghouse 1 and 2, respectively. Particulate matter (0.3 µm, 2.5 µm, 10 µm) showed low to slightly negative correlations with the tested microbial populations in the air samples. Sanitation was effective on pre-wash/wax rollers and conveyors and some packing tables. Sanitation was less effective on the sorting cups for the automatic sorting systems and brushes for wax application. This study indicates a need for more focus on improved sanitation practices for automatic sorting system cups.

Introduction

Effective sanitation practices are a critical food safety tool to reduce the potential for post-harvest contamination. Food handlers, dust, or contaminated food contact surfaces could be potential contamination sources to fresh produce (Beuchat, 2002). In some fresh produce processing facilities or packinghouses, cleaning and sanitizing occurs during the third shift (i.e., night shift). The crew responsible will clean and sanitize the facility and packing lines after daily operations are complete, and will also remove waste, debris, and any other contaminants before the following day's operations (Møretrø & Langsrud 2017). In a fresh produce packing environment, the lack of a full kill step, such as heat treatment, can be substituted with washing steps where sanitizers, such as chlorine or peroxyacetic acid, are added to primarily prevent cross-contamination (Barrera et al., 2012; Beuchat et al., 2004). It has been shown that *L. monocytogenes* can be detectable and persistent in tree fruit packinghouses on a large variety of surfaces (Simonetti et al., 2021). The common practice of applying wax to fresh produce for product appearance and quality can spread contaminants down the packing line after the wax application (Ruiz-Llacsahuanga et al., 2022). The potential for contamination of peaches from surfaces in a packinghouse environment serves to increase the importance of quality sanitation procedures for said packinghouse.

Air quality is another area where food and worker safety can be potentially jeopardized. There have been instances where contamination of food products in a processing environment has been attributed to airborne/dust contamination from construction occurring outside the facility (De Roin et al., 2003). Bacterial survival in airborne dust leaves the possibility of them settling and growing on food contact surfaces in a peach packinghouse to contaminate either the surface or peaches further. The presence of high levels of particulate matter, of various sizes, can

have a number of potential adverse health effects on nearby people and workers (McCray et al., 2010). Measuring these particulate matter levels may potentially serve as a sort of indicator of microbial populations in the air.

A number of methods are used to monitor the sanitation and safety of food production facilities, including packinghouses. For non-culture-based methods, ATP swabs are sometimes used to get a fast, relatively inexpensive result for the sanitation level of the sampled surface. Still, the presence of sanitizers has the potential to interfere/invalidate the swab reading (Vasavada, 2001). For culture-based methods, indicator organisms are often used in place of specific pathogens (Jones et al., 2020). For example, some use Aerobic Plate Count as the indicator of total microbial population and coliforms or *E. coli* as an indicator of potential fecal contamination (Jones et al., 2020).

The objective of this study was to evaluate the efficacy of sanitation practices at two peach packinghouses. Pre- and post-sanitation surface and air samples were taken and enumerated for microbial populations (Heterotrophic Plate Count, coliforms, *Pseudomonas*). Correlations between microbial populations and other environmental measurements were evaluated. The results of this study are intended to clarify the efficacy of the sanitation practices and identify potentially problematic surfaces for food safety and surface hygiene.

Materials and Methods

Sample Collection. All samples were collected from two packinghouses on three dates during the 2022 peach season. Surface samples were gathered throughout the packing line and ancillary storage areas at each facility. All samples were collected before and after third shift sanitation. Surface samples were collected using a 3M Sponge-Stick with 10 mL D/E Neutralizing Broth (SSL10DE, 3M, Saint Paul, MN, USA) from a ~15 cm x 15 cm square area

using ten swipes each in horizontal, vertical, and diagonal directions. Each packinghouse consisted of 14 grouped samples areas, with three sponge swabs per group, and three individual samples based on each packinghouse's surfaces of concern (total samples n=204). For the grouped samples, the individual sponge samples were combined into one bag on site prior to storage and transport to the lab. ATP readings were taken using Hygiena UltraSnap ATP Swabs (Hygiena, Camarillo, CA, USA) per manufacturers recommendations. Air samples were collected from four locations at each packinghouse for microbial enumeration. Air samples were collected using AGI-30 glass impingers (7540, ACE GLASS Inc., St. Petersburg, FL, USA) containing 90 ml of 0.1% peptone (Fisher BioReagents™, Pittsburgh, PA, USA). The impingers were fitted to Gilian BDX-II vacuum pumps (Sensidyne, St. Petersburg, FL, USA) for 1 h of total collection at a flow rate of 2.5 ml/min. Environmental measurements (Particle count, Air temperature, Relative humidity, Dew point, & Wet bulb) using an air particle meter (PCE-MPC-20, PCE Instruments, Jupiter, FL, USA) were also taken at each air sampling location using an air particle meter. Description of surface samples/groups and air sampling locations are noted (Tables 3.1 & 3.2). The general layouts for each packinghouse are described (Figures 3.1 & 3.2).

Microbial Enumeration. For heterotrophic plate count (HPC) and coliform enumeration, IDEXX's HPC for Quanti-Tray and Colilert (IDEXX, Westbrook, ME, USA) were used, respectively. Surface sample bags were diluted using 90 ml of sterile deionized water (SDW) and stomached in a Gosselin S-Blender 1 stomacher (Corning, Corning, NY, USA) at 550 rpm for 30 seconds. For HPC and coliform analysis of the grouped samples, 100 mL of the sample was transferred to an IDEXX 120 ml sample vessel with sodium thiosulfate (IDEXX, Westbrook, ME, USA). Then, a reagent packet for the respective test was added and shaken to homogenization. For the non-grouped samples, 10 mL of the sample was transferred to an

IDEXX 120 ml sample vessel with sodium thiosulfate (IDEXX, Westbrook, ME, USA) along with 90 mL of SDW. The 100 mL of homogenized liquid was then transferred to a Quanti-Tray 200 tray and sealed in an IDEXX Quanti-Tray Sealer PLUS. Sealed HPC trays were incubated at 35 °C for 44-72 hours and read for results via the manufacturer's instructions. Sealed Colilert trays were incubated at 35 °C for 24-28 hours and read for results via the manufacturer's instructions. All well counts were converted to MPN data using IDEXX's MPN Generator.

Pseudomonas spp. enumeration was done via a 96-well plate, resazurin-based MPN. The assay is a miniaturized version of a traditional 15-tube MPN (Tillett, 1987). Modified Cephaloridine–Fucidin–Cetrimide (CFC) broth base supplemented with 25 mg/L of triclosan (Sigma-Aldrich, Inc., St. Louis, MO, USA) was used as the media. All wells were supplemented with 0.1 ml of resazurin (Thermo Fisher Scientific, Waltham, MA, USA). To the first set of dilution wells, 0.5 mL of sample was combined with 0.5 ml of 2X supplemented CFC broth. These wells were then serially diluted to the remaining two sets of wells, each containing 0.9 ml of 1X supplemented CFC broth. After dilution was complete, the 96 well plate was incubated at 35 °C for 48 hours prior to reading for results. A well showing a color change from dark blue/purple to pink was a presumptive positive well. All presumptive positive wells were plated for confirmation via drop plating on *Pseudomonas* Isolation Agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The plates were incubated at 35 °C for 48 hours prior to reading for final results. Confirmed positive counts were converted to MPN data using FDA's BAM MPN Calculator.

Statistical Analysis. All statistical data analysis was performed using JMP Pro 16 (SAS Institute Inc., Cary, NC). Significant differences in HPC, coliform, and *Pseudomonas* populations before and after sanitation were analyzed through ANOVA. Pairwise comparisons

between individual group means were analyzed using Tukey's HSD (honestly significant difference) test and least square mean estimation for mixed effect models with a significance interval of 95%. Pearson's correlation coefficient was calculated between $\log_{10}$ transformed microbial counts, ATP, and environmental measurements.

Results

Surface Microbial Analysis. Microbial enumeration was performed on all surface samples from Packinghouse 1 and Packinghouse 2 for HPC, coliform, and *Pseudomonas* populations (Tables 3.3 & 3.4). ATP swab results are noted for Packinghouse 1 and 2 (Tables 3.5 & 3.6). The change in microbial populations from the sanitation practices are noted for each packinghouse and surface. (Figures 3.3 & 3.4).

Among all Packinghouse 1 surfaces before sanitation, the sorting cups had the highest level of HPC (>6.03±0 log MPN/cm^2) and coliform (>6.03±0 log MPN/cm^2) populations. The sorting cups also had the highest level of HPC counts (5.87±0.27 log MPN/cm^2) among the Packinghouse 1 surfaces after sanitation. While there was no significant difference among any of the samples, the final packing area before sanitation exhibited the highest *Pseudomonas* population (4.31±0.44 log MPN/cm^2) for packinghouse 1. The highest reduction in HPC (1.57±1.06 log MPN/cm^2) and coliform (2.38±0.94 log MPN/cm^2) from the overnight sanitation was found on the Pre-Wash/Wax Rollers, for Packinghouse 1. There was an increase in *Pseudomonas* population (0.82±2.05 log MPN/cm^2) on the Waxer Exit Brushes between the pre- and post-sanitation samples.

For packinghouse 2, the Washer Entry Brushes before sanitation had the highest HPC population overall (>6.03±0 log MPN/cm^2). The pre-sanitation Waxer Exit Brushes showed the highest populations of both coliforms (5.80±0.39 log MPN/cm^2) and *Pseudomonas* (4.05±0.52

log MPN/cm^2). There was no significant difference in *Pseudomonas* populations among the surfaces of packinghouse 2. The Pre-Sort/Grade Conveyors & Rollers showed the largest reduction in coliform population (2.19±0.70 log MPN/cm^2). The highest reduction in HPC population (1.06±0.19 log MPN/cm^2) was from the Final/Secondary Packing. On the Initial Sorting Cup Area samples, there was an increase in coliform population (1.42±0.63 log MPN/cm^2) overnight.

Air Quality Analysis. In the air samples, microbial populations were enumerated for 4 locations at Packinghouse 1 and Packinghouse 2 (Tables 3.7 & 3.8). Particulate matter counts and environmental measurements were taken at all sampling sites for both Packinghouse 1 (Tables 3.9 & 3.10) and Packinghouse 2 (Tables 3.11 & 3.12).

For Packinghouse 1, the Packing Table Area before sanitation showed the highest HPC population (2.69±0.32 log MPN/100 cm^3). The pre-sanitation Receiving Area had the highest coliform population (1.45±0.64 log MPN/100 cm^3). The Mid-Packing Line Area, prior to sanitation, exhibited the highest *Pseudomonas* population (1.69±0.39 log MPN/100 cm^3). There was no significant difference in coliform or *Pseudomonas* populations among the samples. The post-sanitation Receiving Area showed the highest particulate matter counts for both 0.3 µm (56229.00±32214.34) and 2.5 µm (250.33±113.07). The Mid-Packing Line Area, prior to sanitation, showed the highest particulate matter count for 10 µm (20.33±0.58).

For Packinghouse 2, the highest HPC population was found at the Employee Area (2.17±0.93 log MPN/100 cm^3) and Packing Area (2.17±1.19 log MPN/100 cm^3). The Receiving Area, before sanitation, showed the highest coliform population (1.93±1.61 log MPN/100 cm^3). The pre-sanitation Packing Area also showed the highest population of *Pseudomonas* (1.93±0.64 log MPN/100 cm^3). There was no significant difference in HPC, coliform, or *Pseudomonas*

populations between any of the samples. The post-sanitation Employee Area showed the highest particulate matter counts for 0.3 µm (35663.37±18551.61), 2.5 µm (220.33±88.87), and 10 µm (22.33±15.01).

Correlation Analysis. Pearson Correlation Coefficients were determined between all microbial counts on surface samples and ATP swab results (Table 3.13) and microbial counts and environmental measurements for air samples (Table 3.14). For surface samples, there was a moderate correlation between HPC and *Pseudomonas* populations (r = 0.66), and a strong correlation between HPC and coliform populations (r = 0.88). There was a moderate-to-low correlation between ATP readings and HPC (r = 0.40), coliform (r = 0.38), and *Pseudomonas* (r = 0.32) populations.

For air samples, there was a moderate correlation between both HPC & coliforms (r = 0.53) and HPC & *Pseudomonas* (r = 0.51) populations. There was a moderate correlation between air temperature (r = 0.49) and wet bulb (r = 0.47) with HPC populations. There was a low or slightly negative correlation between the three sizes of particulate matter counts with the three microbial populations.

Discussion

Environmental monitoring is an essential step in the maintenance of proper sanitation practices in fresh produce packinghouses to ensure food safety (Marriott et al., 2018). The use of *Pseudomonas* as an indicator in a packinghouse environment serves a potentially strong measure of sanitation program efficacy as it has been shown to have generally high tolerance to factors such as low temperatures, low pH, low nutrients, disinfectants, and high shear forces (Møretrø & Langsrud 2017). The association of *Pseudomonas* with *L. monocytogenes* in fresh produce environments serves to further its strength as a potential indicator (Hassan et al., 2004).

The moderate correlation between HPC & *Pseudomonas* (r = 0.66) populations and strong correlation between HPC and coliforms (r = 0.88) exhibit the potential to be used as an indicator of microbial presence on packinghouse surfaces. The low correlation between coliform population and ATP (r = 0.38) is in agreement with other studies that have found ATP is not a suitable indicator of microbial contamination in the packinghouse environment (Townsend et al., 2023). The lack of correlation between the microbial populations and the ATP readings can be affected by the variable ATP levels on surfaces from both microbial, food, and soil residue, as well as the potential interference of the assay due to residual sanitizer on the surface after sanitation (Vasavada, 2001).

Packinghouse 1 employed a degreaser onto their washing and waxing brushes and a quaternary ammonium sanitizer (QUAT) as their primary sanitizer across all surfaces. Packinghouse 2 chose not to disclose their chosen sanitizers. The sanitation practices at Packinghouse 1 were more effective at reducing the coliform populations (0.89±0.73 log MPN/cm^2) than HPC (0.38±0.41 log MPN/cm^2) or *Pseudomonas* (0.62±0.72 log MPN/cm^2) populations. This was also the case for Packinghouse 2 where the coliform population reduction (0.80±0.94 log MPN/cm^2) was greater than the HPC (0.30±0.40 log MPN/cm^2) and *Pseudomonas* (0.22±0.54 log MPN/cm^2) population reductions. The lower reduction in *Pseudomonas* populations could potentially be attributed to its earlier stated high tolerance to the typical forces and properties associated with packinghouse sanitation practices (Møretrø & Langsrud 2017). The lower HPC reduction could be due to the lack of selection to purely bacteria in the test as it broadly includes heterotrophic organisms.

The use of sorting cups in conjunction with automatic sorting scanners is often used to expedite the process of sorting and color grading various fruits, including stone fruits. The

surfaces of these cups are difficult to clean as they must be cleaned in place as they are often complex systems with large numbers of cups in each system, so disassembly for daily cleaning is unrealistic in terms of cost and time (Williamson et al., 2018). In conjunction with the potential complexity of the systems, Packinghouse 1 may have shown the highest HPC populations before and after sanitation, in comparison to the other Packinghouse 1 surfaces before and after sanitation, because of the difficulty with reaching the cups. At Packinghouse 1, the sorting cup area is elevated above the production floor and only has a walkway on one side of the apparatus. These factors all can contribute to difficulty in properly cleaning these surfaces. There must also be extra care in cleaning the cups around the automatic sorters to not alter or damage the various electronics and sensors required to ensure the system functions properly (Williamson et al., 2018).

Brushes used in various types of fresh produce packinghouses for washing and wax application can be implicated in being high-risk areas for contamination (Ruiz-Llacsahuanga et al., 2021). The various brushes in Packinghouse 2 showing the highest microbial populations before sanitation speak to the ability for these areas to harbor bacteria throughout a day of operations. Studies in peach packinghouses show that the brushes found in washer and waxer areas can be among the most contaminated sites throughout the packing line (Wang et al., 2021). *L. monocytogenes* has been found to be detectable in this type of area of brushes in apple packing lines as well (Portman et al., 2002).

Conclusion

In summary, a need exists for an expanded and reworked sanitation program for the packinghouses in the study. The lack of many significant reductions on the surfaces at both packinghouses is likely cause for further research into the cause of these results. The

ineffectiveness of the sanitation could be due to a number of factors, such as the choice of sanitizers or improper application of the program by the overnight workers. Some areas that may need to be further focused on would be brushes in the washer & waxer areas, sorting cups at the automatic sorter, and certain packing table areas, which are primarily Zone 1 surfaces. Proper sanitation for the sorting cups warrants further research to improve the program's efficacy from a food safety standpoint.

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Table 3.1
List of Surface Sampling Areas for Packinghouse 1 & 2

Packinghouse 1	Packinghouse 2
Cold Storage Floor	Hydrocooler/Cold Storage Drains
Cold Storage Floor Drains	Peach Bins
Hydrocooler Entry	Hydrocooler
Peach Bins	Packing Line Entrance
Packing Entry Conveyors	Pre-Sort/Grade Conveyors & Rollers
Rollers Pre-Wash/Wax	Grade/Sort Area
Washer/Waxer Area	Washer/Waxer Area
Post-Wash Conveyors	Drying Area
B-Grade Packing	Post-Drying Area
Packing Tables	Initial Sorting Cup Area
Packing Area	Post-Initial Sorter
Final Packing Line Entrance	Second Sizing/Sorting Area
Final Packing Area	Final/Secondary Packing
Main Production Drains	Final Cold Storage
Washer Brushes *	Washer Entry Brushes*
Waxer Brushes *	Intermediate Washer/Waxer Brushes*
Sorting Cups *	Waxer Exit Brushes*

* Samples followed by a (*) are non-grouped samples
* n=204, six per surface

Table 3.2
Air Sampling Locations per Packinghouse

Packinghouse 1	Packinghouse 2
Cold Storage	Employee Area
Receiving Area	Packing Area
Initial Grading	Sorting Area
Packing Area	Receiving Area

Table 3.3
Microbial Populations Present in Packinghouse 1 Surface Samples Before and After Sanitation

	HPC (log MPN/cm^2)		Coliforms (log MPN/cm^2)		*Pseudomonas* (log MPN/cm^2)	
Sample	PRE	POST	PRE	POST	PRE	POST
B-Grade Packing	4.34±0.36 ^{CDa}	4.30±0.22 ^{ABCa}	4.00±0.65 ^{ABCDa}	3.58±0.36 ^{Aba}	3.67±0.72 ^{Aa}	2.81±1.00 ^{Aba}
Cold Storage Floor	3.52±0.79 ^{Da}	3.41±0.69 ^{Ca}	2.73±0.84 ^{DEa}	1.97±1.00 ^{Ba}	2.31±0.55 ^{Aa}	2.01±0.49 ^{ABa}
Cold Storage Floor Drains	3.84±0.28 ^{Da}	3.49±0.66 ^{Ca}	2.85±1.25 ^{CDEa}	2.40±0.27 ^{Ba}	2.52±0.91 ^{Aa}	2.11±0.62 ^{ABa}
Final Packing Area	>4.55±0 ^{BCDa}	4.52±0.05 ^{ABCa}	4.29±0.34 ^{ABCDa}	3.79±0.58 ^{ABa}	4.31±0.44 ^{Aa}	3.90±0.68 ^{ABa}
Final Packing Line Entrance	4.01±0.24 ^{Da}	3.56±0.09 ^{Cab}	3.21±0.43 ^{CDEab}	1.67±0.45 ^{Bc}	3.01±0.34 ^{Ab}	1.74±0.02 ^{Bc}
Hydrocooler Entry	3.55±0.35 ^{Da}	3.36±0.70 ^{Ca}	2.46±1.16 ^{DEa}	2.31±1.20 ^{Ba}	2.08±0.60 ^{Aa}	2.21±0.83 ^{ABa}
Main Production Drains	4.15±0.69 ^{Da}	3.94±0.62 ^{Bca}	3.61±1.04 ^{BCDEa}	3.00±1.35 ^{ABa}	3.10±0.95 ^{Aa}	2.80±1.06 ^{ABa}
Packing Area	4.01±0.16 ^{Da}	3.40±0.17 ^{Ca}	3.44±0.04 ^{CDEa}	2.48±0.46 ^{Bbc}	2.57±0.26 ^{Ab}	1.87±0.21 ^{c}
Packing Entry Conveyors	4.37±0.31 ^{BCDa}	3.91±1.11 ^{Ca}	4.21±0.58 ^{ABCDa}	3.02±1.71 ^{ABa}	4.12±0.98 ^{Aa}	2.22±0.81 ^{ABa}
Packing Tables	4.42±0.22 ^{BCDa}	3.92±0.30 ^{BCa}	3.86±0.19 ^{BCDa}	1.65±0.42 ^{Bb}	3.91±0.46 ^{Aa}	2.31±0.55 ^{ABb}
Peach Bins	3.32±0.36 ^{Da}	2.85±0.08 ^{Cab}	1.66±0.44 ^{Eb}	1.74±0.75 ^{Bb}	1.99±0.44 ^{Ab}	1.86±0.23 ^{Bb}
Post-Wash Conveyors	4.30±0.22 ^{CDa}	4.08±0.82 ^{ABCa}	3.81±0.17 ^{BCDa}	2.35±1.29 ^{Ba}	3.50±0.69 ^{Aa}	3.08±1.49 ^{ABa}
Rollers Pre-Wash/Wax	4.42±0.22 ^{BCDa}	2.86±1.14 ^{Cab}	4.11±0.09 ^{ABCDa}	1.73±0.98 ^{Bb}	3.63±0.62 ^{Aa}	<1.73±0 ^{Bb}
Sorting Cups	>6.03±0 ^{Aa}	5.87±0.27 ^{Aa}	>6.03±0 ^{Aa}	5.33±0.24 ^{Aa}	3.92±1.07 ^{Ab}	3.19±0.24 ^{ABb}
Washer Brushes	5.48±0.94 ^{ABCa}	4.40±1.09 ^{ABCa}	4.87±1.03 ^{ABCa}	3.33±1.19 ^{ABa}	3.19±1.70 ^{Aa}	2.68±0.83 ^{ABa}
Washer/Waxer Area	4.42±0.22 ^{BCDa}	4.12±0.45 ^{ABCa}	3.73±0.47 ^{BCDa}	3.66±0.60 ^{ABa}	3.20±1.27 ^{Aa}	3.19±1.24 ^{ABa}
Waxer Brushes	5.68±0.60 ^{ABa}	5.84±0.33 ^{ABa}	5.64±0.68 ^{ABa}	5.37±0.48 ^{Aa}	3.56±1.20 ^{Aa}	4.38±1.34 ^{Aa}

* Values followed by different uppercase letters in each column are significantly different ($p<0.05$)
* Values followed by different lowercase letters in each row are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
*mean ± standard deviation

Table 3.4
Microbial Populations Present in Packinghouse 2 Surface Samples Before and After Sanitation

	HPC (log MPN/cm^2)		Coliforms (log MPN/cm^2)		*Pseudomonas* (log MPN/cm^2)	
Sample	PRE	POST	PRE	POST	PRE	POST
Drying Area	3.98±0.57 ^{Ba}	4.11±0.11 $^{BC\ a}$	3.04±1.10 ^{CDEa}	2.97±0.71 ^{ABCa}	2.65±1.00 ^{ABCDa}	2.94±1.11 ^{Aba}
Final Cold Storage	3.58±0.99 ^{BCa}	3.72±0.76 ^{Ca}	2.17±0.50 ^{EFa}	2.30±1.95 ^{BCa}	1.86±0.23 ^{BCDa}	2.71±1.70 ^{ABa}
Final/Secondary Packing	4.05±0.34 ^{Ba}	3.00±0.34 ^{CDab}	3.19±0.86 ^{CDEab}	<1.17±0 ^{Cc}	2.81±1.00 ^{ABCDab}	1.73±0 ^{Bbc}
Grade/Sort Area	4.35±0.35 ^{Ba}	4.30±0.43 ^{ABCa}	4.14±0.38 ^{ABCDa}	3.65±0.45 ^{ABCab}	2.80±0.74 ^{ABCDb}	2.59±0.40 ^{ABb}
Hydrocooler	2.56±0.68 ^{Ca}	1.86±0.44 ^{Dab}	<1.17±0 ^{Fb}	<1.17±0 ^{Cb}	<1.73±0 ^{Dab}	<1.73±0 ^{Bab}
Hydrocooler/Cold Storage Drains	3.50±0.70 ^{BCa}	3.22±0.24 ^{CDab}	3.05±0.36 ^{CDEabc}	1.72±0.96 ^{c}	1.94±0.36 ^{BCDbc}	<1.73±0 ^{Bc}
Initial Sorting Cup Area	4.17±0.33 ^{Bab}	4.42±0.22 ^{ABCa}	2.74±0.23 ^{CDEFbc}	4.16±0.44 ^{ABCab}	2.20±0.81 ^{ABCDc}	2.72±1.07 ^{ABbc}
Intermediate Washer/Waxer Brushes	5.90±0.22 ^{Aa}	5.48±0.15 ^{ABab}	5.19±0.67 ^{ABab}	3.64±1.32 ^{ABCbc}	3.76±1.00 ^{ABbc}	2.78±0.55 ^{ABc}
Packing Line Entrance	4.42±0.22 ^{Ba}	3.74±0.44 ^{Cab}	4.34±0.25 ^{ABCa}	2.66±0.57 ^{ABCb}	3.01±0.89 ^{ABCDab}	2.38±0.58 ^{ABb}
Peach Bins	3.31±0.51 ^{BCa}	3.47±0.94 ^{Cda}	2.58±0.62 ^{DEFa}	1.91±1.28 ^{Ca}	1.74±0.02 ^{CDa}	1.74±0.02 ^{Ba}
Post-Drying Area	3.99±0.67 ^{Ba}	2.95±1.04 ^{CDab}	2.87±0.88 ^{CDEab}	1.71±0.94 ^{Cb}	2.13±0.35 ^{ABCDab}	<1.73±0 ^{Bb}
Post-Initial Sorter	4.28±0.23 ^{Ba}	3.99±0.96 ^{BCa}	3.63±0.40 ^{BCDEa}	3.06±1.64 ^{ABCa}	2.38±0.56 ^{ABCDa}	2.38±0.58 ^{ABa}
Pre-Sort/Grade Conveyors & Rollers	4.42±0.22 ^{Ba}	4.05±0.46 ^{BCa}	4.00±0.42 ^{BCDa}	1.81±1.10 ^{Cb}	2.89±0.65 ^{ABCDab}	1.86±0.23 ^{Abb}
Second Sizing/Sorting Area	4.40±0.20 ^{Ba}	3.93±0.65 ^{BCa}	3.48±0.44 ^{CDEab}	1.46±0.51 Cc	2.10±0.61 ^{ABCDbc}	2.10±0.64 ^{ABbc}
Washer Entry Brushes	>6.03±0 ^{Aa}	5.90±0.22 ^{Aa}	5.73±0.27 ^{Aa}	5.29±0.80 ^{Aba}	3.69±0.19 ^{ABCb}	3.88±0.72 ^{Ab}
Washer/Waxer Area	4.42±0.22 ^{Ba}	4.09±0.46 ^{BCa}	4.33±0.32 ^{ABCa}	3.76±0.66 ^{ABCab}	2.70±0.53 ^{ABCDbc}	2.01±0.49 ^{ABc}
Waxer Exit Brushes	5.90±0.22 ^{Aa}	5.81±0.38 ^{Aa}	5.80±0.39 ^{Aab}	5.37±1.14 ^{Aabc}	4.05±0.52 ^{Abc}	3.68±0.74 ^{ABc}

* Values followed by different uppercase letters in each column are significantly different ($p<0.05$)
* Values followed by different lowercase letters in each row are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
*mean ± standard deviation

Table 3.5
ATP Levels in Packinghouse 1 Surface Samples Before and After Sanitation

	ATP (log RLU)	
Sample	PRE	POST
B-Grade Packing	3.78±0.21 [A]	2.19±0.11 [A]
Cold Storage Drains	3.11±0.10 [A]	2.90±0.20 [A]
Cold Storage Floor	3.10±0.09 [A]	3.06±0.13 [A]
Final Packing Area	3.79±0.44 [A]	2.30±0.75 [A]
Final Packing Line Entrance	3.65±0.41 [A]	2.84±0.38 [A]
Hydrocooler	2.43±0.12 [A]	2.17±0.37 [A]
Main Production Drains	3.24±0.46 [A]	3.00±0.42 [A]
Packing Area	2.87±0.01 [A]	2.64±0.24 [A]
Packing Entry Conveyors	3.56±0.12 [A]	2.85±0.56 [A]
Packing Tables	4.10±0.14 [A]	2.36±0.20 [A]
Peach Bins	3.48±0.29 [A]	2.13±0.65 [A]
Post-Wash Conveyors	3.74±0.05 [A]	2.01±0.63 [A]
Pre-Wash/Wax Rollers	3.20±0.28 [A]	1.76±0.22 [A]
Sorting Cups	3.28±0.25 [A]	3.60±0.43 [A]
Washer Brushes	3.27±0.56 [A]	1.84±0.15 [A]
Washer/Waxer Area	3.72±0.11 [A]	2.29±0.35 [A]
Waxer Brushes	3.28±0.33 [A]	2.57±0.58 [A]

* Values followed by different letters are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.6
ATP Levels in Packinghouse 2 Surface Samples Before and After Sanitation

	ATP (log RLU)	
Sample	PRE	POST
Drying Area	3.22±0.40 [A]	2.76±0.29 [A]
Final Cold Storage	2.60±0.40 [A]	2.78±0.60 [A]
Final/Secondary Packing	3.70±0.24 [A]	2.75±0.57 [A]
Grade/Sort Area	3.28±0.41 [A]	2.88±0.17 [A]
Hydrocooler	1.57±0.13 [A]	1.30±0.41 [A]
Hydrocooler/Cold Storage Drains	2.87±0.36 [A]	2.90±0.06 [A]
Initial Sorting Cup Area	3.72±0.26 [A]	2.85±0.19 [A]
Intermediate Washer/Waxer Brushes	3.48±0.11 [A]	3.46±0.22 [A]
Packing Line Entrance	3.34±0.33 [A]	2.73±0.89 [A]
Peach Bins	2.50±0.37 [A]	2.77±0.43 [A]
Post-Drying Area	3.56±0.18 [A]	2.37±0.38 [A]
Post-Initial Sorter	3.48±0.46 [A]	2.60±0.04 [A]
Pre-Sort/Grade Conveyors & Rollers	3.49±0.32 [A]	3.31±0.34 [A]
Second Sizing/Sorting Area	3.76±0.31 [A]	2.82±0.06 [A]
Washer Entry Brushes	3.56±0.45 [A]	2.54±0.21 [A]
Washer/Waxer Area	2.92±0.43 [A]	2.96±0.60 [A]
Waxer Exit Brushes	3.50±0.11 [A]	2.52±0.69 [A]

* Values followed by different letters are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.7
Microbial Populations Present in Packinghouse 1 Air Samples Before and After Sanitation

	HPC (log MPN/100 cm^3)		Coliforms (log MPN/100 cm^3)		*Pseudomonas* (log MPN/100 cm^3)	
Sample	PRE	POST	PRE	POST	PRE	POST
Cold Storage Area	1.59±1.12 ^{Aa}	1.14±0.41 ^{Aa}	<1.00±0 ^{Aa}	<1.00±0 ^{Aa}	<1.56±0 ^{Aa}	<1.56±0 ^{Aa}
Mid Packing Line Area	2.40±0.17 ^{Aa}	1.53±0.25 ^{Abcd}	1.03±0.23 ^{Acd}	<1.00±0 ^{Ad}	1.69±0.39 ^{Ab}	<1.56±0 ^{Abc}
Packing Table Area	2.69±0.32 ^{Aa}	1.17±0.23 ^{Ab}	1.17±0.47 ^{Ab}	<1.00±0 ^{Ab}	<1.56±0 ^{Ab}	<1.56±0 ^{Ab}
Receiving Area	2.24±0.64 ^{Aa}	<1.00±0 ^{Ab}	1.45±0.64 ^{Aab}	<1.00±0 ^{Ab}	<1.56±0 ^{Aab}	<1.56±0 ^{Aab}

* Values followed by different uppercase letters in each column are significantly different ($p<0.05$)
* Values followed by different lowercase letters in each row are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.8
Microbial Populations Present in Packinghouse 2 Air Samples Before and After Sanitation

	HPC (log MPN/100 cm^3)		Coliforms (log MPN/100 cm^3)		*Pseudomonas* (log MPN/100 cm^3)	
Sample	PRE	POST	PRE	POST	PRE	POST
Employee Area	2.17±0.93 ^{Aa}	1.51±0.36 ^{Aab}	<1.00±0 ^{Ab}	<1.00±0 ^{Ab}	1.59±0.06 ^{Aab}	<1.56±0 ^{Aab}
Packing Area	2.17±1.19 ^{Aa}	1.31±0.23 ^{Aa}	<1.00±0 ^{Aa}	<1.00±0 ^{Aa}	1.93±0.64 ^{Aa}	<1.56±0 ^{Aa}
Sorting Cup Area	2.09±1.63 ^{Aa}	1.73±0.64 ^{Aa}	1.93±1.61 ^{Aa}	<1.00±0 ^{Aa}	1.77±0.36 ^{Aa}	<1.56±0 ^{Aa}
Receiving Area	1.91±0.79 ^{Aa}	1.54±0.22 ^{Aa}	<1.00±0 ^{Aa}	<1.00±0 ^{Aa}	<1.56±0 ^{Aa}	<1.56±0 ^{Aa}

* Values followed by different uppercase letters in each column are significantly different ($p<0.05$)
* Values followed by different lowercase letters in each row are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.9
Airborne Particulate Matter Counts for Packinghouse 1 Air Sampling Sites

Sample	PM (0.3 µm)		PM (2.5 µm)		PM (10 µm)	
	PRE	POST	PRE	POST	PRE	POST
Cold Storage Area	11705.67±4228.90 ^{AB}	9808.00±4782.65 ^{B}	43.33±13.65 ^{B}	65.33±45.21 ^{B}	2.00±1.73 ^{B}	3.67±2.89 ^{AB}
Mid Packing Line Area	28699.00±2429.22 ^{AB}	42973.00±22818.25 ^{AB}	175.67±57.14 ^{AB}	185.00±52.68 ^{AB}	20.33±0.58 ^{A}	8.67±1.53 ^{AB}
Packing Table Area	24796.00±10275.28 ^{AB}	30125.33±14233.67 ^{AB}	183.00±85.08 ^{AB}	128.33±42.02 ^{AB}	20.00±16.52 ^{A}	7.33±1.53 ^{AB}
Receiving Area	18651.33±9614.83 ^{AB}	56229.00±32214.34 ^{A}	91.67±28.87 ^{AB}	250.33±113.07 ^{A}	6.33±2.31 ^{AB}	11.33±3.21 ^{AB}

* Values followed by different letters in each size set (0.3, 2.5, 10 µm) are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples; PM = Particulate Matter
* mean ± standard deviation

Table 3.10
Environmental Measurements at Packinghouse 1 Air Sampling Sites

Sample	Air Temperature (°C)		Relative Humidity (%)		Dew Point (°C)		Wet Bulb (°C)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Cold Storage Area	25.57±2.96 ^{A}	21.77±1.04 ^{A}	40.33±3.42 ^{B}	42.80±2.01 ^{B}	10.97±1.32 ^{B}	8.50±0.30 ^{B}	17.27±1.78 ^{AB}	14.73±0.57 ^{B}
Mid Packing Line Area	26.80±4.78 ^{A}	22.10±2.60 ^{A}	76.13±9.83 ^{A}	89.67±8.13 ^{A}	22.07±2.49 ^{A}	20.20±1.08 ^{A}	23.37±2.97 ^{A}	20.80±1.49 ^{AB}
Packing Table Area	27.50±4.36 ^{A}	22.97±0.91 ^{A}	75.20±10.02 ^{A}	89.37±7.81 ^{A}	22.53±1.95 ^{A}	21.03±0.71 ^{A}	23.93±2.52 ^{A}	21.60±0.36 ^{AB}
Receiving Area	26.70±5.05 ^{A}	19.93±5.00 ^{A}	78.00±10.65 ^{A}	87.87±5.12 ^{A}	22.33±2.55 ^{A}	17.80±4.00 ^{A}	23.53±3.16 ^{A}	18.47±4.20 ^{AB}

* Values followed by different letters in each parameter set (Air Temperature, Relative Humidity, Dew Point, Wet Bulb) are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.11
Airborne Particulate Matter Counts for Packinghouse 2 Air Sampling Sites

	PM (0.3 µm)		PM (2.5 µm)		PM (10 µm)	
Sample	PRE	POST	PRE	POST	PRE	POST
Employee Area	10645.00±918.53 [A]	35663.67±18551.61 [A]	88.67±14.84 [A]	220.33±88.87 [A]	9.33±4.04 [A]	22.33±15.01 [A]
Packing Area	8917.00±465.75 [A]	27493.33±14036.32 [A]	74.00±21.00 [A]	150.33±39.88 [A]	9.33±3.06 [A]	12.33±3.79 [A]
Sorting Cup Area	10482.67±5283.79 [A]	22271.67±13234.50 [A]	120.33±66.52 [A]	129.00±60.23 [A]	17.33±14.57 [A]	13.67±7.64 [A]
Receiving Area	8630.00±2635.08 [A]	29960.67±16706.68 [A]	61.00±19.52 [A]	181.00±67.54 [A]	7.00±2.65 [A]	12.33±3.51 [A]

* Values followed by different letters in each size set (0.3, 2.5, 10 µm) are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples; PM = Particulate Matter
* mean ± standard deviation

Table 3.12
Environmental Measurements at Packinghouse 2 Air Sampling Sites

Sample	Air Temperature (°C)		Relative Humidity (%)		Dew Point (°C)		Wet Bulb (°C)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Employee Area	31.80±0.70 [A]	24.93±1.46 [A]	59.67±9.44 [A]	73.40±5.05 [A]	22.80±2.97 [A]	19.77±2.12 [A]	25.40±1.87 [A]	21.40±1.70 [A]
Packing Area	31.93±0.58 [A]	25.20±1.14 [A]	57.53±11.75 [A]	76.23±5.71 [A]	22.23±3.85 [A]	20.63±1.80 [A]	25.17±2.33 [A]	22.03±1.42 [A]
Sorting Cup Area	32.30±0.61 [A]	25.57±0.47 [A]	56.67±10.83 [A]	75.23±5.59 [A]	22.33±3.67 [A]	20.77±1.37 [A]	25.33±2.21 [A]	22.23±0.93 [A]
Receiving Area	32.10±0.61 [A]	25.60±0.69 [A]	57.03±11.06 [A]	73.90±6.19 [A]	22.27±3.70 [A]	20.53±1.59 [A]	25.20±2.27 [A]	22.07±1.14 [A]

* Values followed by different letters in each parameter set (Air Temperature, Relative Humidity, Dew Point, Wet Bulb) are significantly different ($p<0.05$)
* PRE = Pre-Sanitation Samples; POST = Post-Sanitation Samples
* mean ± standard deviation

Table 3.13
Pearson's Correlation Coefficients (r) for Packinghouse Surface Microbial Populations and ATP Readings

	HPC (log MPN/cm^2)	Coliform (log MPN/cm^2)	*Pseudomonas* (log MPN/cm^2)	ATP (log RLU)
HPC (log MPN/cm^2)	1.00			
Coliform (log MPN/cm^2)	0.88	1.00		
Pseudomonas (log MPN/cm^2)	0.66	0.76	1.00	
ATP (log RLU)	0.40	0.38	0.32	1.00

Table 3.14
Pearson's Correlation Coefficients (r) for Packinghouse Air Samples' Microbial Populations & Environmental Measurements

	HPC (log MPN/100 cm^3)	Coliforms (log MPN/100 cm^3)	*Pseudomonas* (log MPN/100 cm^3)	PM (0.3 µm)	PM (2.5 µm)	PM (10 µm)	AT (°C)	RH (%)	DP (°C)	WB (°C)
HPC (log MPN/100 cm^3)	1.00									
Coliforms (log MPN/100 cm^3)	0.53	1.00								
Pseudomonas (log MPN/100 cm^3)	0.51	0.40	1.00							
PM (0.3 µm)	-0.24	-0.15	-0.15	1.00						
PM (2.5 µm)	-0.11	-0.11	-0.08	0.86	1.00					
PM (10 µm)	0.11	-0.07	-0.04	0.29	0.66	1.00				
AT (°C)	0.49	0.32	0.36	-0.67	-0.46	-0.04	1.00			
RH (%)	-0.13	-0.06	-0.09	0.69	0.56	0.20	-0.47	1.00		
DP (°C)	0.36	0.25	0.28	-0.04	0.07	0.16	0.55	0.48	1.00	
WB (°C)	0.47	0.32	0.35	-0.34	-0.18	0.08	0.82	0.12	0.93	1.00

* PM = Particulate Matter; AT = Air Temperature; RH = Relative Humidity; DP = Dew Point; WB = Wet Bulb

Figure 3.1
Layout of Packinghouse 1 with Surface and Air Sampling Sites

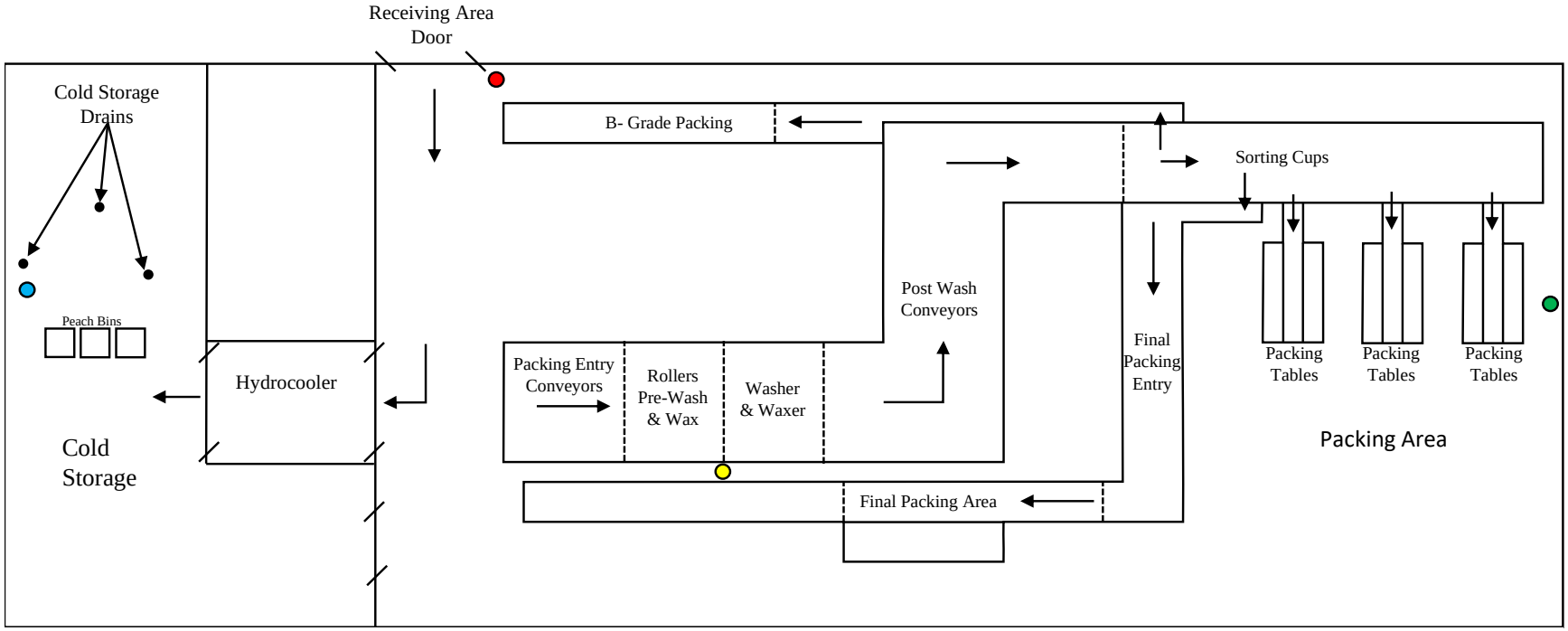


Figure 3.2
Layout of Packinghouse 2 with Surface and Air Sampling Sites

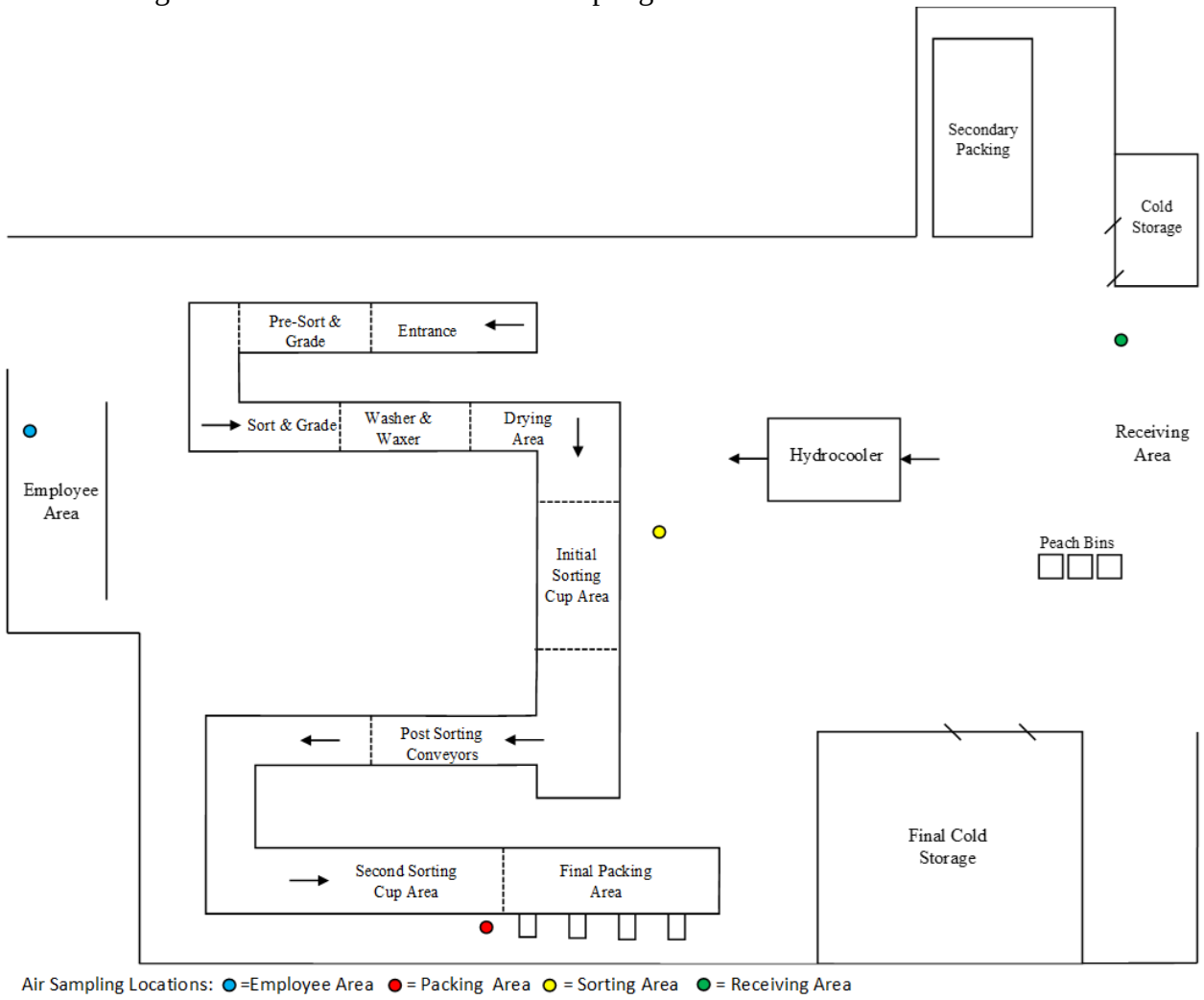


Figure 3.3
Changes in Microbial Populations on Surface Samples from Overnight Sanitation at Packinghouse 1

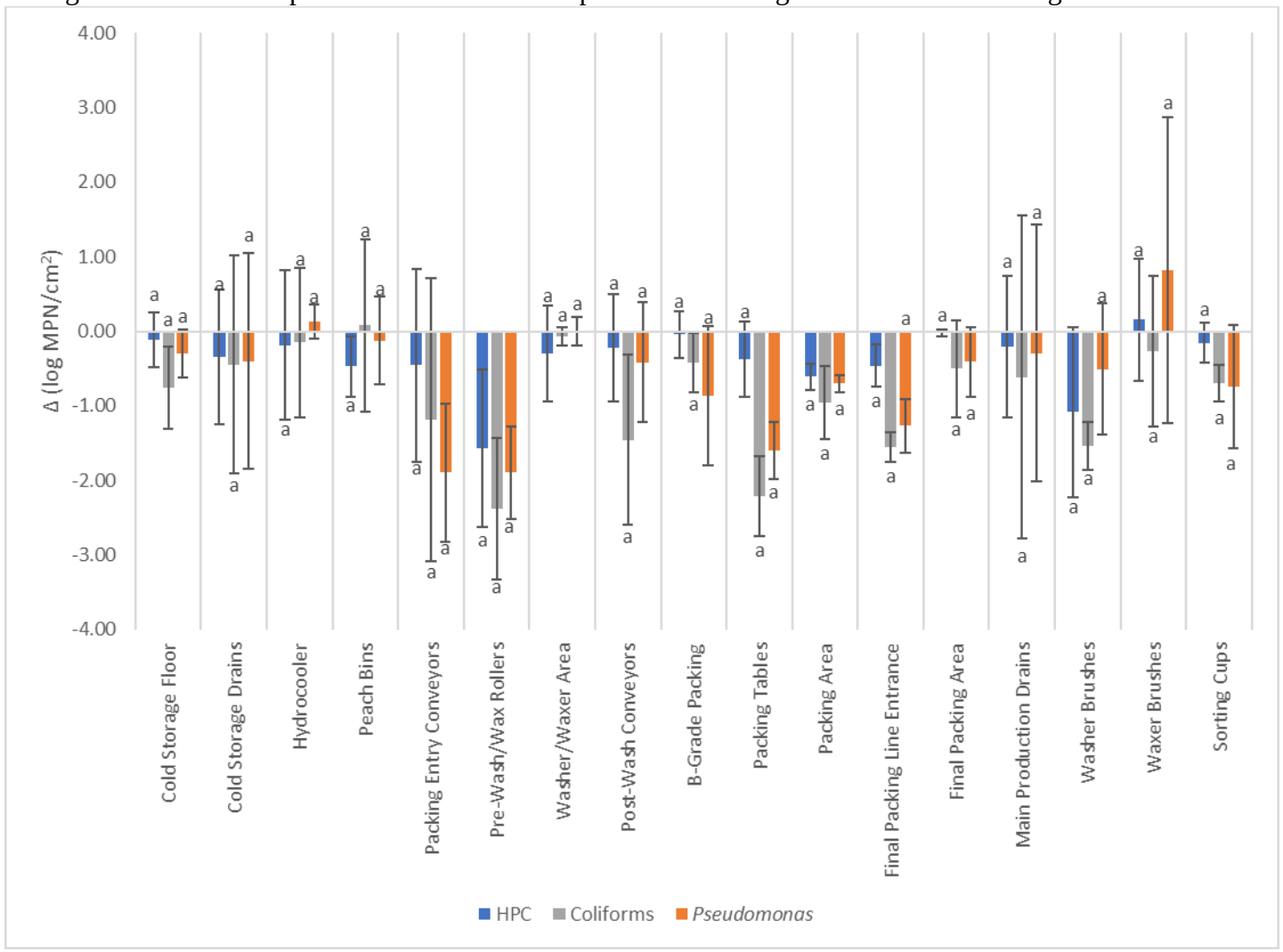
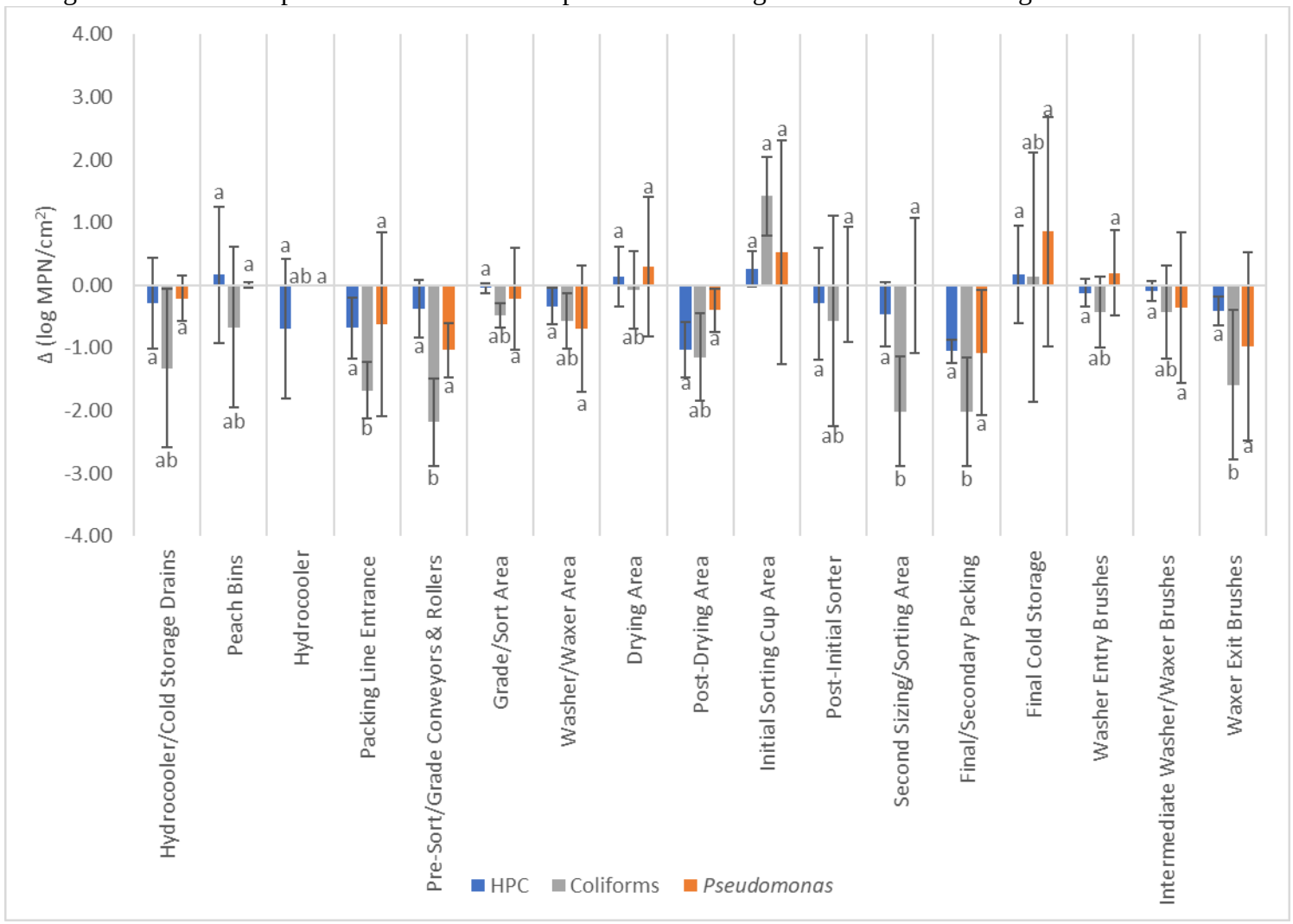


Figure 3.4
Changes in Microbial Populations on Surface Samples from Overnight Sanitation at Packinghouse 2



CHAPTER 4

MICROBIOLOGICAL EVALUATION OF BIOAEROSOLS COLLECTED FROM PEACH ORCHARDS [1]

[1] Brandon Cox, Cameron Bardsley, Kelly Bright, Abhinav Mishra, Laurel Dunn, and Govindaraj Dev Kumar. To be submitted to *Journal of Environmental Sciences*

Abstract

Bioaerosols from peach orchards were evaluated for microbial presence and viability, and other potential biomarkers. Correlations among microbial populations (bioaerosols, soil, leaves) and environmental measurements were evaluated to assess the factors for indicator potential. Nine orchards were sampled based on proximity to potentially relevant infrastructure and animal operations. Bile in the air samples showed a potential for use as a biomarker due to its moderate correlation with coliform counts ($r=0.64$). Particulate matter (0.3 µm, 2.5 µm, 10 µm) showed negative correlations with the air samples' heterotrophic plate count (HPC) populations. Endotoxin showed low or slightly negative correlations with the airborne bacterial populations. There was a moderate negative correlation ($r=-0.63$) between dew point and HPC population on leaf samples, and there was a strongly negative correlation ($r=-0.70$) between dew point and coliform population on leaf samples. There was no significant difference in the microbial populations in each of the air, soil, and leaf samples across the different orchards ($p>0.05$). The study results indicate that bile could be a potential indicator of airborne coliforms in a pre-harvest environment.

Introduction

Foodborne pathogens can potentially spread via bioaerosols from animal operations containing high numbers of animals such as Concentrated Animal Feeding Operations (CAFOs) (Dev Kumar et al., 2018; Gibbs et al., 2006; Predicala et al., 2002). CAFOs must manage large quantities of animal waste and can affect the air quality of the surrounding environment. Bioaerosols from CAFOs can contain dust, bacteria, fungal spores, and antigenic viruses (Rodríguez de Evgrafov et al., 2013). Bioaerosols from CAFOs can negatively impact human health and contaminate crops that are present in close proximity to these facilities (Thu, 2002; Von Essen & Auvermann, 2005). For example, methicillin-resistant *S. aureus* (MRSA) was isolated from air and dust samples surrounding pig farm operations (Schulz et al., 2012). Similarly, *E. coli* O157:H7 and *Salmonella* have been isolated from dust samples of cattle feed yards only after the agitation of dust in the area that resulted from animal activity (Miller et al., 2008). The ability of these pathogens to survive on dust leaves an innate possibility of them contaminating fresh produce in a pre-harvest environment within certain levels of proximity (Yanamala et al., 2011). In 2020, there was an outbreak of *S.* Enteritidis, in the United States, from peaches found to have been contaminated by fugitive dust from cattle and poultry neighboring from the production orchards (FDA, 2020).

Collection and analysis of bioaerosols could be challenging because of uncertainty in sample volume collected or microbial stress that could affect bacterial culturability. Many environmental factors could play a part in the potential spread of bioaerosols. These environmental factors include moisture levels, air temperature, and particle matter counts (Berry et al., 2015). Hence, the efficacy of bioaerosol analysis could be improved by using various biomarkers such as bile and endotoxins. Some examples of biomarkers/bioindicators that have

shown a potential to serve as indicators of bioaerosols are endotoxins or bile (Tyagi et al., 2009; Tager et al., 2010). Endotoxins can be used as indicators of bacterial presence as they are lipopolysaccharides (LPS) found on the outer cell membrane of Gram-negative bacteria, including *E. coli* and *Salmonella* (Thorne & Duchaine, 2007). An advantage of using these biomarkers as indicators for bioaerosols is that they do not rely on the culturability state of the microbe for testing or enumeration. Certain indicators, such as endotoxins or bile, alleviate the concern for the potentially present bacteria to be in the viable but non-culturable (VBNC) state among the airborne bacteria (Heidelberg et al., 1997).

Peaches are among the primary fruit crops grown in the state of Georgia, which produced 35,300 tons of peaches in 2021 (USDA-NASS, 2022). Peaches are most often produced with the intention of them being consumed raw (Duvenage & Korsten, 2017). Among the number of potential contamination vectors, dust and contaminated food contact surfaces have the potential to contaminate the fruit at both the pre- and post-harvest stage (Beuchat, 2006; Beuchat, 2002).

This study aimed to evaluate the efficacy of various biomarkers (bile, endotoxin, coliforms, *Pseudomonas*) and environmental conditions as indicators of bioaerosol presence in peach orchards. Various bacterial populations were evaluated alongside these biomarkers for comparison amongst the biomarkers and microbial populations. The results of this study are intended to expand our knowledge and understanding of the utility of biomarkers as indicators of bioaerosol contamination.

Materials and Methods

Sample Collection. Air samples were collected from nine peach orchards using separate glass impingers containing 90 mL of 0.1% peptone (Fisher BioReagents™, Pittsburgh, PA, USA) for microbial enumeration and 90 mL of sterile deionized water (SDW) for bile and

endotoxin analysis. AGI-30 impingers (7540, ACE GLASS Inc., Vineland, NJ, USA) were fitted to Gilian BDX-II vacuum pumps (Sensidyne, St. Petersburg, FL, USA) for 1 h of total collection at a flow rate of 2.5 ml/min. Leaf and soil samples were collected along with environmental measurements (particle count, air temperature, relative humidity, dew point, & wet bulb) using an air particle meter (PCE-MPC-20, PCE Instruments, Jupiter, FL, USA). Samples were placed on ice for transport to the lab after collection. Samples were collected on three dates throughout the 2022 peach season. Proximity to relevant infrastructure or animal operations is given in table 4.1 (Table 4.1).

Microbial Analysis of Air. For heterotrophic plate count (HPC) and coliform enumeration, IDEXX's HPC for Quanti-Tray and Colilert were used, respectively (IDEXX, Westbrook, ME, USA). For HPC analysis, 10 mL of collected air sample was transferred to an IDEXX 120 ml sample vessel with sodium thiosulfate (IDEXX, Westbrook, ME, USA) along with 90 mL of SDW). To this, one powder packet of IDEXX HPC reagent was added to the vessel and shaken to homogenization. The 100 mL of reagent was then transferred to a Quanti-Tray 2000 tray and sealed in an IDEXX Quanti-Tray Sealer PLUS (IDEXX, Westbrook, ME, USA). Sealed HPC trays were incubated at 35 °C for 44-72 h and read for results using the manufacturer's instructions. The Coliform analysis procedure is the same as HPC with Colilert substituting for the HPC reagent. For Colilert trays, the trays are incubated at 35 °C for 24-28 h and read for results via the manufacturer's instructions. All well counts were converted to MPN data using IDEXX's MPN Generator.

A resazurin-based MPN (most probable number) method was used to enumerate *Pseudomonas* spp. in 96-well plates. The assay is a miniaturized version of a traditional 15-tube MPN (Tillett, 1987). Modified Cephaloridine–Fucidin–Cetrimide (CFC) broth base

supplemented with 25 mg/L of triclosan (Sigma-Aldrich, Inc., St. Louis, MO, USA) was used as the media. All wells were supplemented with 0.1 ml of resazurin (Thermo Fisher Scientific, Waltham, MA, USA). To the first set of dilution wells, 0.5 mL of sample was added to 0.5 ml of 2X supplemented CFC broth. These wells were then serially diluted in 0.9 mL of 1X supplemented CFC broth in the remaining wells. After dilution was complete, the 96 well plate was incubated at 35 °C for 48 h prior to reading for results. A well showing a color change from dark blue/purple to pink is a presumptive positive well. All presumptive positive wells were plated for confirmation via drop plating on *Pseudomonas* Isolation Agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The plates were incubated at 35 °C for 48 h prior to reading for final results. Confirmed positive counts were converted to MPN data using FDA's BAM MPN Calculator.

Microbial Analysis of Soil & Leaves. For both soil and leaf samples, identical procedures were used. Ten grams of sample were homogenized using 90 ml of SDW using a Gosselin S-Blender 1 stomacher (Corning, Corning, NY, USA) at 550 rpm for 1 min. The homogenized samples were then used for the enumeration of HPC, coliforms, and *Pseudomonas* spp. HPC counts were determined via drop plating on Plate Count Agar (Neogen, Lansing, MI, USA) and incubation at 37 °C for 24 h. Coliform counts were determined via drop plating on Violet Red Bile Lactose Agar (VRBA) (MilliporeSigma, Burlington, MA, USA) and incubated at 35 °C for 24 h. *Pseudomonas* spp. counts were determined via plating on *Pseudomonas* Isolation Agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated at 35 °C for 48 h.

Endotoxin and Bile Analysis. The air samples impinged through SDW were used for both the endotoxin and bile assays. Endotoxin presence was quantified using the Pierce

Chromogenic Endotoxin Quant Kit (Thermo Scientific, Waltham, MA, USA). The test uses a chromogenic Lyophilized Amebocyte Lysate (LAL) assay to quantify endotoxins with a standard of *E. coli* O111:B4. The assay was performed according to the manufacturer's instructions.

Bile was quantified using the Sigma-Aldrich Bile Acid Assay Kit (Sigma-Aldrich Inc., St. Louis, MO, USA). This assay is a 96-well plate fluorometric assay using 3-hydroxysteroid dehydrogenase to react with bile acids to convert NAD to NADH, resulting in fluorescence. The assay was performed according to the manufacturer's instructions.

Statistical Analysis. Three replicate air samples were collected at each orchard throughout the peach season. All statistical data analysis was performed using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA). Significant differences between HPC, coliform, *Pseudomonas*, bile, and endotoxin levels were analyzed through ANOVA. Pairwise comparisons between individual group means were analyzed using Tukey's HSD (honestly significant difference) test with a significance interval of 95%. To compare the efficacy of different biomarkers at estimating microbial population counts, a Pearson's correlation coefficient was calculated between $\log_{10}$ transformed microbial counts, bile and endotoxin levels, and environmental measurements.

Results

Microbial Analysis of Air. Microbial enumeration was performed on collected air samples to analyze HPC, coliform, and *Pseudomonas* populations (Table 4.2). There was no significant difference among the HPC, coliform, or *Pseudomonas* populations in the air samples among the nine orchards ($p>0.05$). Orchard 3 had the highest average HPC population of over 4.38±0 log MPN/100 cm^3. Orchard 2 had the highest *Pseudomonas* population of 2.27±1.22 log

MPN/100 cm^3. Orchard 4 showed the highest coliform population of 2.00±1.73 log MPN/100 cm^3.

Microbial Analysis of Soil & Leaves. Microbial enumeration was performed on collected leaf and soil samples to analyze HPC, coliform, and *Pseudomonas* populations (Tables 4.3 & 4.4). There was no significant difference in HPC, coliform, or *Pseudomonas* populations in the soil or leaf samples among the nine orchards ($p>0.05$). Orchard 2 had the highest HPC and *Pseudomonas* population, among leaf samples, of 5.01±1.47 log CFU/g and 3.60±0.32 log CFU/g. Orchard 1 had the highest coliform population among leaf samples of 3.86±1.28 log CFU/g. Orchards 4, 8, and 5 had the highest HPC, coliform, and *Pseudomonas* populations, for soil samples, of 6.48±0.55 log CFU/g, 5.10±0.64 log CFU/g, and 5.41±0.35 log CFU/g, respectively.

Bile and Endotoxin Analysis. Bile and endotoxin presence was quantified for all air samples (Table 4.5). Across the nine orchards, the average bile presence in the air samples was 2.52±3.17 µM, and the average endotoxin presence in the air samples was 0.05±0.01 EU/ml. Orchard 4 had the highest bile presence of 10.89±16.54 µM. Orchard 9 had the highest endotoxin presence of 0.08±0.02 EU/ml.

Environmental Measurement. Various environmental measurements were collected from the orchards during the air sample collection (Table 4.6). There was no significant difference between the orchards for any environmental measurements ($p>0.05$). Orchard 8 had the highest particle count for all 3 sizes of 31507±20943.01 (0.3 µm), 211.67±143 (2.5 µm), and 15.67±8.08 (10 µm).

Correlation Analysis. Pearson Correlation Coefficients were determined among all microbial population counts, bile & endotoxin presence, and environmental measurements

(Table 4.7). There was a moderate correlation between HPC and *Pseudomonas* populations in the air (r=0.40) and leaf samples (r=0.63). In air samples, there was a moderate correlation between HPC and coliform populations (r=0.43). The coliform population on leaf samples showed a moderate correlation with HPC (r=0.57) and coliform (r=0.60) populations in air samples. There was a moderate correlation between the air sample coliform population and bile levels (r=0.64). There was a moderate negative correlation (r=-0.63) between dew point and leaf HPC population. There was a strongly negative correlation (r=-0.70) between dew point and leaf coliform population.

Discussion

Bioaerosols have the potential to harbor various antigens, such as bacteria and fungi (Tyagi et al., 2008). However, many issues, including the consistency of the capture and enumeration of bioaerosols, make airborne bacteria challenging to analyze (Toivola et al., 2002). Using various potential biomarkers or indicators, in place of specific microbes, serves as a potential solution for the issues with bioaerosol analysis.

For this study, the positive correlations found among the different microbial populations in the air samples with both bile presence and microbial populations on leaves show the potential for utilizing these parameters as indicators for bioaerosols. The moderate correlation between the coliform population in air samples and bile levels (r=0.64) shows the highest potential, as an indicator, for use in place of coliforms as an indicator in certain air sampling situations. Bile is most commonly used as a fecal contamination indicator as it is present in both animal and human feces (Elhmmali et al, 2000). The low to negligible correlation between most microbial populations and the environmental measurements may be largely due to the variability of farm conditions among sampling days and times. The moderate correlation between HPC and

coliform populations in the air samples aligns with the general usage of coliforms as a microbial indicator. Orchard 3 had the highest average HPC population (>4.38 log MPN/100 cm^3) which may possibly be due to its close proximity to a field restroom (roughly 5 meters). This is potentially problematic as the grower may need to consider moving the field restroom further from the peach trees, if possible. There was a slightly negative correlation between the HPC and coliform populations in air samples and the particulate matter counts at 0.3 µm (r=-0.29, -0.29), 2.5 µm (r=-0.37, -0.22), and 10 µm (r=-0.07, -0.23), respectively. Other studies have found varying results on the correlation between particulate matter and microbial populations. In one instance, there was a positive correlation between the counts for the three particulate matter sizes and airborne bacteria (Kumari et al., 2016). However, other studies have indicated a negative correlation between particulate matter and airborne microbes (Hong et al., 2021). The lack of consistency in the correlation between particulate matter and microbial populations in this and previous studies leads to a conclusion that particulate matter may not be a useful indicator for bioaerosols. Airborne *Salmonella* inoculated onto tomato blossom has been shown to lead to retention and contamination of both the blossom and the further developed fruit (Dev Kumar et al., 2017). Further studies would need to be conducted to determine peach blossoms' airborne bacterial contamination could lead to fruit contamination after development.

Conclusion

This study's results indicate the potential for using bile as an indicator of airborne microbial populations. The low and negative correlations between airborne microbe populations and endotoxin/particulate matter indicate their potentially low efficacy as an indicator in this environment. However, the inconsistencies in results for particulate matter correlations likely

warrant further investigation in this instance. Further optimizations to capturing bioaerosols improve the consistency of the resulting microbial analysis.

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Table 4.1
List of Sampled Peach Orchards, Nearby Relevant Infrastructure, & Sampling Dates

Sample	Area in Proximity	Distance to Animals (m)	Dates of Samplings
Orchard 1	Dirt roads & woods	N/A	04/28/2022 07/05/2022 07/25/2022
Orchard 2	Dirt roads & woods	N/A	04/28/2022 07/05/2022 07/25/2022
Orchard 3	Orchard restroom	N/A	04/28/2022 07/05/2022 07/25/2022
Orchard 4	Dirt roads & Neighboring Orchards	N/A	04/28/2022 07/05/2022 07/25/2022
Orchard 5	Grazing Cows	~50	04/29/2022 06/15/2022 07/11/2022
Orchard 6	Interstate	N/A	04/29/2022 06/15/2022 07/11/2022
Orchard 7	Grazing Horses	~150	04/29/2022 06/15/2022 07/11/2022
Orchard 8	Woods	N/A	05/06/2022 06/15/2022 07/11/2022
Orchard 9	Roads & Chicken Houses	~700	05/06/2022 06/15/2022 07/11/2022

Table 4.2
Microbial Populations in Air Samples from Sampled Peach Orchards

	log MPN/100 cm^3		
Sample	HPC	Coliform	*Pseudomonas*
Orchard 1	3.31±0.87 ^{Aa}	<1.00±0 ^{Ab}	1.66±0.17 ^{Ab}
Orchard 2	3.11±1.21 ^{Aa}	1.38±0.65 ^{Aa}	2.27±1.22 ^{Aa}
Orchard 3	>4.38±0 ^{Aa}	<1.00±0 ^{Ac}	1.93±0.64 ^{Ab}
Orchard 4	3.23±1.68 ^{Aa}	2.00±1.73 ^{Aa}	1.93±0.64 ^{Aa}
Orchard 5	2.29±1.28 ^{Aa}	1.61±1.05 ^{Aa}	<1.56±0 ^{Aa}
Orchard 6	1.59±0.58 ^{Aa}	<1.00±0 ^{Aa}	<1.56±0 ^{Aa}
Orchard 7	2.31±1.43 ^{Aa}	1.78±1.35 ^{Aa}	<1.56±0 ^{Aa}
Orchard 8	2.17±1.06 ^{Aa}	<1.00±0 ^{Aa}	<1.56±0 ^{Aa}
Orchard 9	2.61±0.78 ^{Aa}	<1.00±0 ^{Aa}	2.04±0.83 ^{Aa}

* Values with different uppercase letters in each column are significantly different ($p<0.05$)
* Values with different lowercase letters in each row are significantly different ($p<0.05$)
* mean ± standard deviation

Table 4.3
Microbial Populations in Leaf Samples from Sampled Peach Orchards

Sample	Leaf Samples (log CFU/g)		
	HPC	Coliform	*Pseudomonas*
Orchard 1	4.41±0.83 Aa	3.86±1.28 Aa	3.03±1.81 Aa
Orchard 2	5.01±1.47 Aa	3.21±1.92 Aa	3.60±0.32 Aa
Orchard 3	2.51±1.36 Aa	1.73±1.26 Aa	<1.00±0 Aa
Orchard 4	4.99±2.01 Aa	3.64±2.44 Aa	3.33±2.21 Aa
Orchard 5	4.69±1.55 Aa	3.24±2.55 Aa	2.29±1.12 Aa
Orchard 6	3.78±1.87 Aa	2.51±2.62 Aa	<1.00±0 Aa
Orchard 7	4.14±1.92 Aa	2.63±2.82 Aa	2.28±2.21 Aa
Orchard 8	2.19±2.06 Aa	<1.00±0 Aa	1.84±1.45 Aa
Orchard 9	4.43±2.22 Aa	2.50±2.60 Aa	1.57±0.98 Aa

* Values with different uppercase letters in each column are significantly different ($p<0.05$)
* Values with different lowercase letters in each row are significantly different ($p<0.05$)
* mean ± standard deviation

Table 4.4
Microbial Populations in Soil Samples from Sampled Peach Orchards

	Soil Samples (log CFU/g)		
Sample	HPC	Coliform	*Pseudomonas*
Orchard 1	6.17±0.49 ^{Aa}	3.39±2.35 ^{Aa}	5.01±0.51 ^{Aa}
Orchard 2	5.95±0.07 ^{Aa}	3.21±0.47 ^{Ac}	5.03±0.32 ^{Ab}
Orchard 3	6.27±0.23 ^{Aa}	4.29±0.41 ^{Ab}	5.07±0.49 ^{Ab}
Orchard 4	6.48±0.55 ^{Aa}	3.84±0.71 ^{Ab}	5.25±0.15 ^{Aa}
Orchard 5	6.10±0.19 ^{Aa}	3.35±2.04 ^{Aa}	5.41±0.35 ^{Aa}
Orchard 6	6.03±0.20 ^{Aa}	2.15±1.99 ^{Ab}	4.37±0.35 ^{Aab}
Orchard 7	6.47±0.18 ^{Aa}	2.55±1.48 ^{Ab}	5.26±0.23 ^{Aa}
Orchard 8	6.38±0.42 ^{Aa}	5.10±0.64 ^{Aa}	5.23±0.58 ^{Aa}
Orchard 9	6.36±0.42 ^{Aa}	2.02±1.77 ^{Ab}	4.88±0.22 ^{Aa}

* Values with different uppercase letters in each column are significantly different ($p<0.05$)
* Values with different lowercase letters in each row are significantly different ($p<0.05$)
* mean ± standard deviation

Table 4.5
Bile and Endotoxin Levels in Air Samples from Sampled Peach Orchards

Sample	Bile (μM)	Endotoxin (EU/ml)
Orchard 1	1.04±0.07 ^{A}	0.07±0.06 ^{A}
Orchard 2	2.33±2.30 ^{A}	0.04±0.02 ^{A}
Orchard 3	<1.00±0 ^{A}	0.06±0.04 ^{A}
Orchard 4	10.89±16.54 ^{A}	0.04±0.06 ^{A}
Orchard 5	2.04±1.81 ^{A}	0.04±0.02 ^{A}
Orchard 6	1.87±1.50 ^{A}	0.04±0.03 ^{A}
Orchard 7	<1.00±0 ^{A}	0.06±0.08 ^{A}
Orchard 8	<1.00±0 ^{A}	0.05±0.03 ^{A}
Orchard 9	1.72±1.24 ^{A}	0.08±0.02 ^{A}

* Values with different letters in each column are significantly different ($p<0.05$)
* mean ± standard deviation

Table 4.6
Environmental Measurements from Sampled Peach Orchards

Sample	PM (0.3 µm)	PM (2.5 µm)	PM (10 µm)	Air Temperature (°C)	Relative Humidity (%)	Dew Point (°C)	Wet Bulb (°C)
Orchard 1	16076.33±3977.07[a]	116.00±85.84[a]	13.33±15.37[a]	28.37±3.67[a]	64.77±22.62[a]	20.70±6.26[a]	23.37±4.05[a]
Orchard 2	11342.33±4373.51[a]	68.00±32.70[a]	8.00±8.66[a]	32.07±3.69[a]	57.77±26.49[a]	21.17±5.90[a]	24.93±2.00[a]
Orchard 3	10772.67±2843.70[a]	75.00±20.42[a]	10.67±5.77[a]	35.20±6.59[a]	53.10±26.83[a]	22.17±4.57[a]	26.37±0.72[a]
Orchard 4	10390.33±2583.38[a]	47.67±13.20[a]	12.33±16.20[a]	31.83±3.66[a]	57.17±24.85[a]	20.97±5.26[a]	24.63±1.65[a]
Orchard 5	15924.33±9650.37[a]	117.67±91.66[a]	8.33±8.74[a]	29.23±3.59[a]	63.87±23.02[a]	20.90±4.31[a]	23.63±2.32[a]
Orchard 6	19400.67±11405.77[a]	148.67±114.92[a]	9.67±7.37[a]	29.27±0.65[a]	60.97±17.03[a]	20.47±4.49[a]	23.37±2.33[a]
Orchard 7	19800.67±12602.05[a]	137.00±116.67[a]	9.67±6.03[a]	29.60±2.44[a]	59.37±19.19[a]	20.13±3.59[a]	23.27±1.19[a]
Orchard 8	31507.00±20943.01[a]	211.67±143.00[a]	15.67±8.08[a]	27.27±4.98[a]	75.30±10.76[a]	22.33±2.47[a]	23.67±3.10[a]
Orchard 9	24828.00±14045.35[a]	139.33±102.63[a]	7.33±4.51[a]	25.97±4.88[a]	79.03±10.53[a]	21.87±2.91[a]	23.00±3.29[a]

* Values with different letters in each column are significantly different ($p<0.05$)
* mean ± standard deviation

Table 4.7
Pearson Correlation Coefficients (r) Between Microbial Population Data and Environmental Measurements

	Air HPC (log MPN/100 cm^3)	Air Coliforms (log MPN/100 cm^3)	Air Pseudo (log MPN/100 cm^3)	Bile (µM)	Endotoxin (EU/ml)	Soil HPC (log CFU/g)	Soil Coliforms (log CFU/g)	Soil Pseudo (log CFU/g)	Leaf HPC (log CFU/g)	Leaf Coliforms (log CFU/g)	Leaf Pseudo (log CFU/g)	PM (0.3 µm)	PM (2.5 µm)	PM (10 µm)	Air Temp (°C)	Relative Humidity (%)	Dew Point (°C)	Wet Bulb (°C)
Air HPC (log MPN/100 cm^3)	1.00																	
Air Coliforms (log MPN/100 cm^3)	0.43	1.00																
Air Pseudo (log MPN/100 cm^3)	0.40	0.05	1.00															
Bile (µM)	0.20	0.64	-0.08	1.00														
Endotoxin (EU/ml)	0.14	-0.26	0.25	-0.23	1.00													
Soil HPC (log CFU/g)	-0.17	-0.18	0.13	-0.23	-0.14	1.00												
Soil Coliforms (log CFU/g)	-0.15	-0.30	-0.06	-0.03	-0.07	0.16	1.00											
Soil Pseudo (log CFU/g)	-0.03	0.23	0.12	0.16	-0.33	0.40	0.41	1.00										
Leaf HPC (log CFU/g)	0.50	0.54	0.31	0.27	-0.10	-0.20	-0.52	-0.03	1.00									
Leaf Coliforms (log CFU/g)	0.57	0.60	0.29	0.29	-0.16	-0.18	-0.50	0.03	0.88	1.00								
Leaf Pseudo (log CFU/g)	0.37	0.32	0.33	0.20	-0.14	0.05	-0.04	0.24	0.63	0.53	1.00							
PM (0.3 µm)	-0.29	-0.29	-0.27	-0.17	0.10	-0.19	0.21	-0.21	-0.22	-0.47	-0.18	1.00						
PM (2.5 µm)	-0.37	-0.22	-0.22	-0.16	-0.15	-0.12	0.30	-0.12	-0.28	-0.39	-0.14	0.79	1.00					
PM (10 µm)	-0.07	-0.23	0.03	-0.17	-0.06	0.25	0.48	0.01	-0.15	-0.20	0.25	0.25	0.61	1.00				
Air Temp (°C)	0.28	0.37	0.05	0.22	-0.44	-0.12	0.23	0.34	0.09	0.21	-0.01	-0.39	-0.17	-0.05	1.00			
Relative Humidity (%)	-0.39	-0.62	-0.14	-0.31	0.40	0.26	0.23	-0.19	-0.45	-0.59	-0.18	0.41	0.22	0.21	-0.78	1.00		
Dew Point (°C)	-0.41	-0.60	-0.22	-0.30	0.14	0.32	0.56	0.03	-0.63	-0.70	-0.28	0.21	0.23	0.37	-0.20	0.74	1.00	
Wet Bulb (°C)	-0.18	-0.30	-0.14	-0.11	-0.13	0.19	0.66	0.24	-0.51	-0.50	-0.25	-0.07	0.08	0.29	0.46	0.18	0.78	1.00

*** PM = Particulate Matter**

CHAPTER 5

EFFECTS OF PEPTONE SOLUTIONS, AS CAPTURE BUFFERS IN AN IMPINGER SYSTEM, ON THE CAPTURE AND RECOVERY OF AEROSOLIZED *ESCHERICHIA COLI* [1]

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Abstract

The microbial sampling of air can be performed using a liquid impinger method, where the air is pumped into a capture buffer (CB) to retain suspended microorganisms. The capture and enumeration of enteric pathogens during air sampling can be challenging due to the low retention of microorganisms in CB or the decline in microbial populations due to stress. Further, sampling buffers should not promote bacterial growth during transfer to the laboratory. Hence, potential CB (n=13), were compared for their ability to capture and recover aerosolized bacteria. *Escherichia coli* K12 was aerosolized in a controlled grow tent and sampled with glass impingers filled with the various CB for enumeration. The CB were also evaluated for their ability to promote growth of *E. coli* and *Salmonella* by measuring optical density (OD_{600}) over a duration of 24 h at both 37 °C and 25 °C. Aside from sterile deionized water (SDW), there was no significant difference in recovery from the remaining 12 CB ($p>0.05$). Five of the CB's (Bacto™ Malt Extract, Bacto™ TC Yeastolate, Bacto™ Yeast Extract, Fisher BioReagents™ Granulated Peptone, Difco™ Yeast Extract) showed significantly higher recovery than SDW ($p<0.05$). Bacto™ Malt Extract showed the highest recovery among the tested media (3.44 ± 0.23 log CFU/cm^3) and did not result in the growth of *E. coli* or *Salmonella* at either temperature. Buffered peptone water showed lower recovery (2.92±0.32 log CFU/cm^3) and more growth than most other media with each bacterium and temperatures. This study provides useful data for the selection of CB to ensure efficacious enumeration of airborne bacteria.

Introduction

Airborne dust particles have the potential to carry and deposit foodborne pathogens onto food and food contact surfaces (Dev Kumar et al., 2018). Foodborne pathogen outbreaks could be associated with airborne transfer to produce. For example, an outbreak of *Salmonella* Enteritidis attributed to peaches resulted in 101 illnesses and 28 hospitalizations across 17 states in 2020 (FDA, 2020). Upon investigation, the FDA discerned that the source of the contamination was dust originating from adjacent dairy and poultry operations (FDA, 2020). In other instances, methicillin-resistant *S. aureus* (MRSA) has been isolated in air and dust samples up to 300 meters from pig farm operations (Schulz et al., 2012). *E. coli* O157:H7 and *Salmonella* have been isolated from dust samples up to fifty meters from surrounding cattle feed yard operations after agitation of the dust in the area as the animals move throughout (Miller et al., 2008; Yanamala et al., 2011). Proper bioaerosol sampling is essential in monitoring and mitigation of pathogen transfer.

There are multiple methods that can be used to capture these airborne bacteria for enumeration or detection. Three popular methods are impingement, impaction, and passive sampling (Haig et al., 2016; Mainelis, 2020). The impaction method is a form of active sampling and uses inertia from an air pump to pull air particulate downward onto the surface of an agar plate (Haig et al., 2016; Xu et al., 2013; Mainelis, 2020). The passive sampling method is driven by gravity naturally pulling airborne particles onto the surface of an agar plate without any artificial force applied to the sampled air (Haig et al., 2016; Mainelis, 2020). The impingement method uses glass impingers to pull bioaerosols into a CB and is among the more effective methodologies for sampling and enumerating bioaerosols (Haig et al., 2016; Mainelis, 2020; Šantl-Temkiv et al., 2017). With the impingement method, the air sample is immediately

suspended in a CB, so the sample can be tested in a number of different ways including immediate enumeration or enrichment for a pathogen of interest. Also, using the impinger method relieves some concerns about desiccation on the surface of an agar plate that may occur when utilizing either the passive sampling or impaction methods (Haig et al., 2016). Other advantages to using an impinger method are that one can easily plate the collected sample on numerous agar media simultaneously, and the sample can be easily serially diluted to account for high variations in population density in the sample (May & Harper, 1957).

One important factor in using the impingement methodology is the selection of CB to be used. The CB to be used needs to be able to keep bacteria alive without promoting their growth in the media for at least the length of time the sample may be transported back to the lab from field sampling sessions. It is essential that the CB assists in bacterial culturability because the bacteria can easily be highly stressed in this sort of condition, and this stress can push the bacteria into a viable but nonculturable (VBNC) state (Cho et al., 2020). The chosen media should ideally be able to bring stressed cells out of this nonculturable condition. Peptones are one group of potential candidates for impinger CB. This is largely due to the fact that peptones are often sources of nitrogen used as supplements for growth media (Rezaee et al., 2022). This nutrient availability present in many peptones, in combination with the use of low-concentration solutions (0.1%) of the peptones, gives them the acute possibility of maintaining or regaining the culturability of bacteria suspended inside of the solutions. Among them, yeast extracts are commonly used as microbial growth medium supplements for various bacteria, such as *L. monocytogenes* or *P. acidilactici* (Nolan et al., 1992; Champagne et al., 2003).

The objective of this study was to evaluate the potential for differences in capture and recovery of aerosolized bacteria, through an impinger system, in various CB. To accomplish

this, *E. coli* K12 was aerosolized inside of a controlled environment and collected in glass impingers filled with various different CB. These collected samples were enumerated to evaluate any differences in recovery. Each media was also used in growth curves with *E. coli* K12 and *Salmonella* Newport, at various temperatures, to evaluate population stability in each media. The results of this study are intended to give guidance on what media may be an ideal candidate for use as a CB.

Materials and Methods

Bacterial Strains. The bacterial strains used in this study were *E. coli* K12 and *Salmonella enterica* subsp. *enterica* serotype Newport 11590 K (beef isolate). Both strains were transformed using the pGFPuv plasmid to insert ampicillin resistance and green fluorescent protein genes. These transformations were performed for prior experiments using a method adapted from Dev Kumar et al. (2017). The transformed strains obtained resistance to 100 µg/ml of ampicillin.

Aerosol Collection, Recovery, and Analysis. For air sample collection, *E. coli* K12 was used as the inoculum. Aerosolization and sampling were done inside a VIVOSUN (VIVOSUN, Ontario, CA) grow tent (48x24x60 inches). The 8 log CFU/ml suspension of the *E. coli* was aerosolized using an AFROG mini diffuser. Air samples were collected using glass impingers connected to Gilian BDX-II vacuum air pumps (Sensidyne, St. Petersburg, FL, USA). 13 different CB of various source types were used in this experiment, with concentrations noted if applicable (Table 5.1).

Each impinger was filled with 100 ml of CB before being placed into the growth tent in baskets 30 cm from the right wall of the growth tent. The diffuser was filled with 100 ml of the 8-log suspension and placed 30 cm from the left wall of the growth tent at 30 cm elevation. Once

all impingers and diffuser were placed in the tent, the diffuser was turned on for 15 minutes (0.5 ml/min). After 15 minutes, the diffuser was turned off, and the impinger's air pumps (2.5 ml/min) were turned on for 1 hour of collection. After the collection time, the samples were removed from the tent for enumeration. Samples were enumerated via drop plating on Tryptic Soy Agar (Neogen, Lansing, MI, USA) supplemented with 100 µg/ml ampicillin. The setup of the growth tent setup is noted (Figure 5.1). Each biological rep for each CB was rotated among the three growth tent positions (A, B, C).

Evaluation of Growth Rates. Both the *E. coli* K12 and *S.* Newport were used for growth rate evaluation. Growth rates were evaluated using 96-well plates inoculated with 20 µl of a 6-log suspension (6.32±0.08 log CFU/ml) of the respective bacteria and 180 µl of CB. For each bacterium, the growth rates were evaluated at 37 °C and 25 °C for 24 hours by measuring optical density (OD_{600}) with a BioTek Cytation 3 Imaging Reader (BioTek Instruments Inc., Winooski, VT, USA).

Statistical Analysis. All statistical data analysis was performed using JMP Pro 16 (SAS Institute Inc., Cary, NC). Significant differences between the recovery of bacteria between the different CB were analyzed through ANOVA and Tukey's HSD (honestly significant difference) tests at a 0.05 significance level. All experiments (air sampling and growth rates) were performed with three biological and three technical replicates.

Results

Analysis Recovery of Aerosolized Bacteria. Microbial enumeration was performed on collected air samples with the various CB to analyze the efficacy of the media at capturing aerosolized bacteria (Table 5.2). Bacto™ Malt Extract showed the highest recovery of aerosolized bacteria among the tested medias (3.44 ± 0.23 log CFU/cm^3). Sterile deionized water

(SDW) showed the lowest recovery rate (1.59±0.34 log CFU/cm^3). Excluding SDW, there was no significant difference in the recovered population of bacteria between the remaining 12 CB ($p > 0.05$). There was no significant difference in the recovery based on the three set sampling positions inside the grow tent (Table 5.3).

Growth Rate Analysis. Growth rate analysis was conducted using the 13 CB and two bacterial strains (*E. coli* K12 and *S.* Newport) at various temperatures to simulate potential conditions when collecting air samples in a field/pre-harvest environment (Figures 5.2 – 5.14). Buffered peptone water (BPW) showed visible growth across both temperature and bacteria, and this media was the only one to facilitate growth for *E. coli* K12 at either incubation temperature (Figure 5.2). Bacto™ TC Yeastolate facilitated *S.* Newport growth at both 37 and 25 °C, but not *E. coli* K12 growth (Figure 5.3). Bacto™ Yeast Extract and Difco™ Soytone also promoted the growth of *S.* Newport at 37 °C (Figures 5.4 & 5.5). Bacto™ Malt Extract showed no discernible growth at either incubation temperature for either *E. coli* K12 or *S.* Newport (Figure 5.6).

Discussion

Proper selection of CB, when utilizing an impinger system, is essential to ensuring the proper and accurate capture/enumeration of airborne bacteria. The use of certain different buffers or peptone solutions allows for a potential increase in the recovery rate of aerosolized bacteria in comparison to using standard SDW (Table 5.2). Using alternative CB, such as tween mixture solutions, have been shown to improve the recovery rate of *S. aureus*, compared to water and PBS, in an impinger system (Chang & Wang, 2015). When utilizing glass impingers, there is a potential for harming the bacterial cells, thus harming cell viability, by the bubbles being pulled into the CB bursting in the media (Terzieva et al., 1996). There were slight variations between growth curve results for Bacto™ TC Yeastolate with *S.* Newport at 37 and 25 °C. At 25 °C, the

bacteria seemed to initiate growth faster in *S.* Newport than at 37 °C. There have been instances, with *S.* Typhimurium, where certain peptone media have been shown to facilitate improved growth for injured bacteria in comparison to that with healthy bacteria (Gray et al., 2008). While BPW might seem to be a decent CB in this circumstance, the increased growth rate for both bacterial cultures and relatively lower recovery of airborne bacteria hurts its potential as a suitable CB for air sampling. This makes sense as BPW is more commonly used as a pre-enrichment media, particularly for *Salmonella*, than as a buffer (Baylis et al., 2000). Based on the tests, Bacto™ Malt Extract seems to be the best CB, for these circumstances, among those tested. It exhibited the highest recovery of airborne bacteria (3.44 ± 0.23 log CFU/cm^3) and showed no growth for either *S.* Newport or *E. coli* K12 at either 37 or 25 °C. While PBS is a more traditional buffer media, its recovery data (2.75±0.56 log CFU/cm^3) was among the lowest of the tested media. It is worth noting that low levels of growth in the CB, in the curves, is negligible in the sense that collected air samples will never reach these conditions, with proper practices and sample handling. The curves primarily exhibit the differences in sample population stability in the different media.

Conclusion

In summary, this study's results indicate that proper selection of CB in an impinger based air sampling setting is essential to accurately capture and recover potential aerosolized bacteria. While there was no significant difference among 12 of the tested medias, 5 of these 12 (Bacto™ Malt Extract, Bacto™ TC Yeastolate, Bacto™ Yeast Extract, Fisher BioReagents™ Granulated Peptone, Difco™ Yeast Extract) showed significantly greater recovery than SDW. This difference indicates that there is an importance to the process of selecting a proper media for this collection. Due to the inconsistent nature of air sampling, testing on a per-situation basis is likely

needed to select media. Further research is likely needed to determine differences between CB in a field-based environment, as opposed to a controlled lab-based environment.

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Table 5.1
List of Collection Buffers Used in Aerosol Recovery and Growth Curves

Collection Buffer	Concentration	Source
Fisher BioReagents™ Granulated Peptone	0.1%	Bovine - Porcine
Phytone™ Peptone	0.1%	Soybean meal/flour
Difco™ Soytone	0.1%	Animal origin-free, Soy
Bacto™ Yeast Extract	0.1%	Saccharomyces cerevisiae
Difco™ Yeast Extract	0.1%	Saccharomyces cerevisiae
Bacto™ Proteose Peptone No. 2	0.1%	Porcine
Bacto™ Proteose Peptone No. 3	0.1%	Porcine
Bacto™ Casamino Acids	0.1%	Bovine
Bacto™ TC Yeastolate	0.1%	Saccharomyces cerevisiae
Bacto™ Malt Extract	0.1%	Malted Barley
BPW	1X	Bovine
PBS	1X	
Sterile Deionized Water		

Table 5.2
Capture and Recovery of Aerosolized *E. coli* K12 in Different Collection Buffers

Collection Buffer	Recovery (log CFU/cm^3)
Bacto™ Casamino Acids	2.99±0.21 ^{AB}
Bacto™ Malt Extract	3.44±0.23 ^{A}
Bacto™ Proteose Peptone No. 2	2.65±0.19 ^{AB}
Bacto™ Proteose Peptone No. 3	2.58±0.33 ^{AB}
Bacto™ TC Yeastolate	3.38±0.53 ^{A}
Bacto™ Yeast Extract	3.24±0.37 ^{A}
BPW	2.92±0.32 ^{AB}
Difco™ Soytone	3.02±0.05 ^{AB}
Fisher BioReagents™ Granulated Peptone	3.22±0.68 ^{A}
1X PBS	2.75±0.56 ^{AB}
Phytone™ Peptone	2.93±1.14 ^{AB}
Sterile Deionized Water	1.59±0.34 ^{B}
Difco™ Yeast Extract	3.17±0.52 ^{A}

* Values followed by different letters are significantly different ($p<0.05$)
* Inoculum = 8.34 ± 0.56 log CFU/ml
* mean ± standard deviation

Table 5.3
Capture and Recovery of Aerosolized *E. coli* K12 at Each Sampling Position in Growth Tent Setup

Tent Position	Recovery (log CFU/cm^3)
A	2.96±0.66 ^{A}
B	2.95±0.57 ^{A}
C	2.83±0.67 ^{A}

* Values followed by different letters are significantly different ($p<0.05$)
* Inoculum = 8.34 ± 0.56 log CFU/ml
* mean ± standard deviation

Figure 5.1
Layout for Aerosolization Experiment in Growth Tent

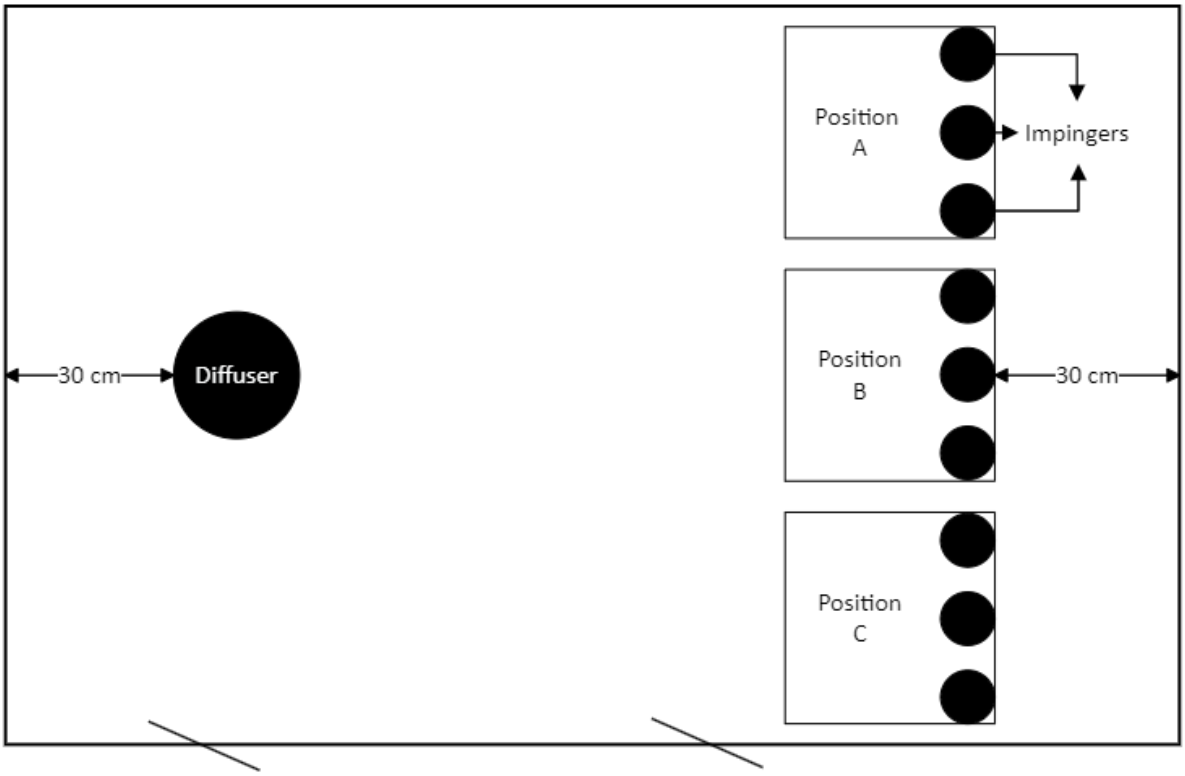


Figure 5.2
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in buffered peptone water (BPW) at 37 ℃ and 25 ℃ for 24 hours

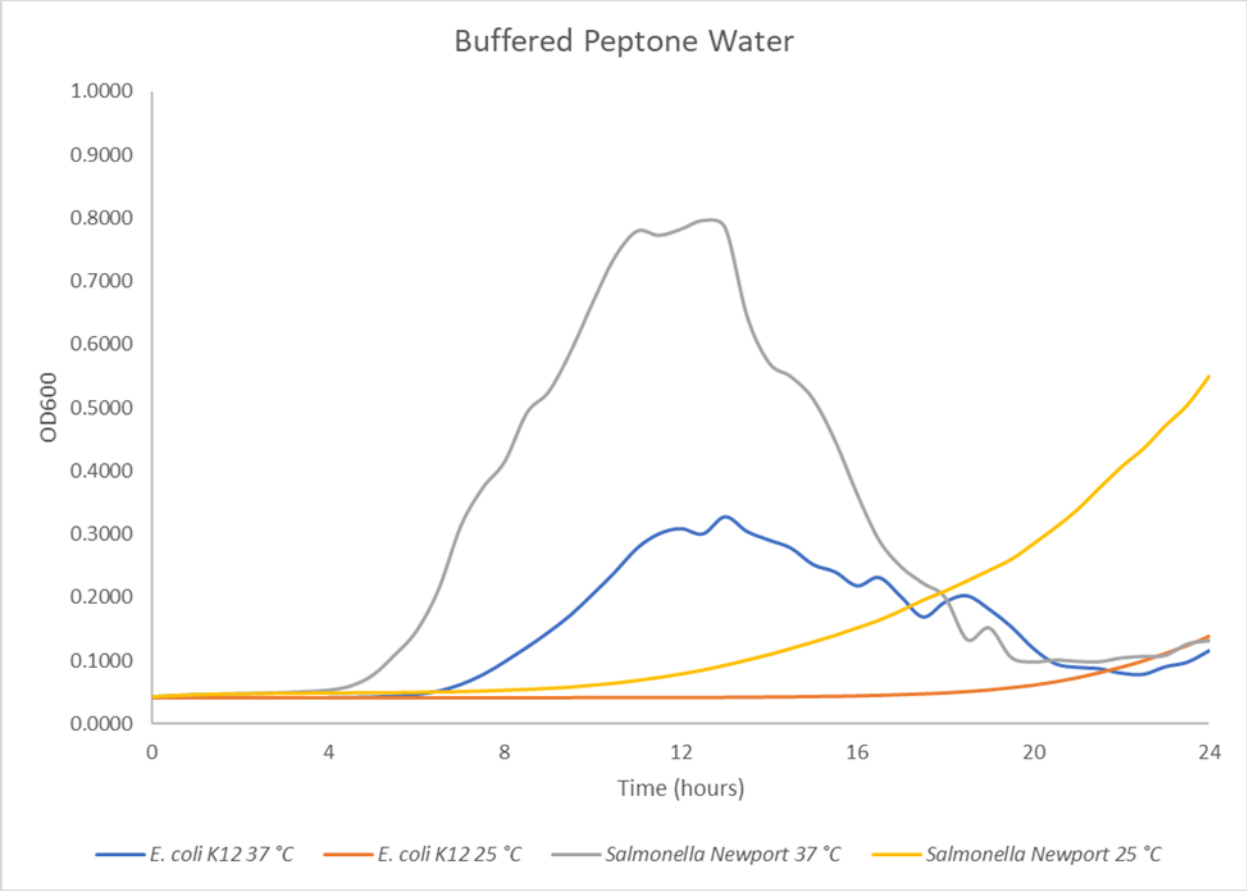


Figure 5.3
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ TC Yeastolate at 37 °C and 25 °C for 24 hours

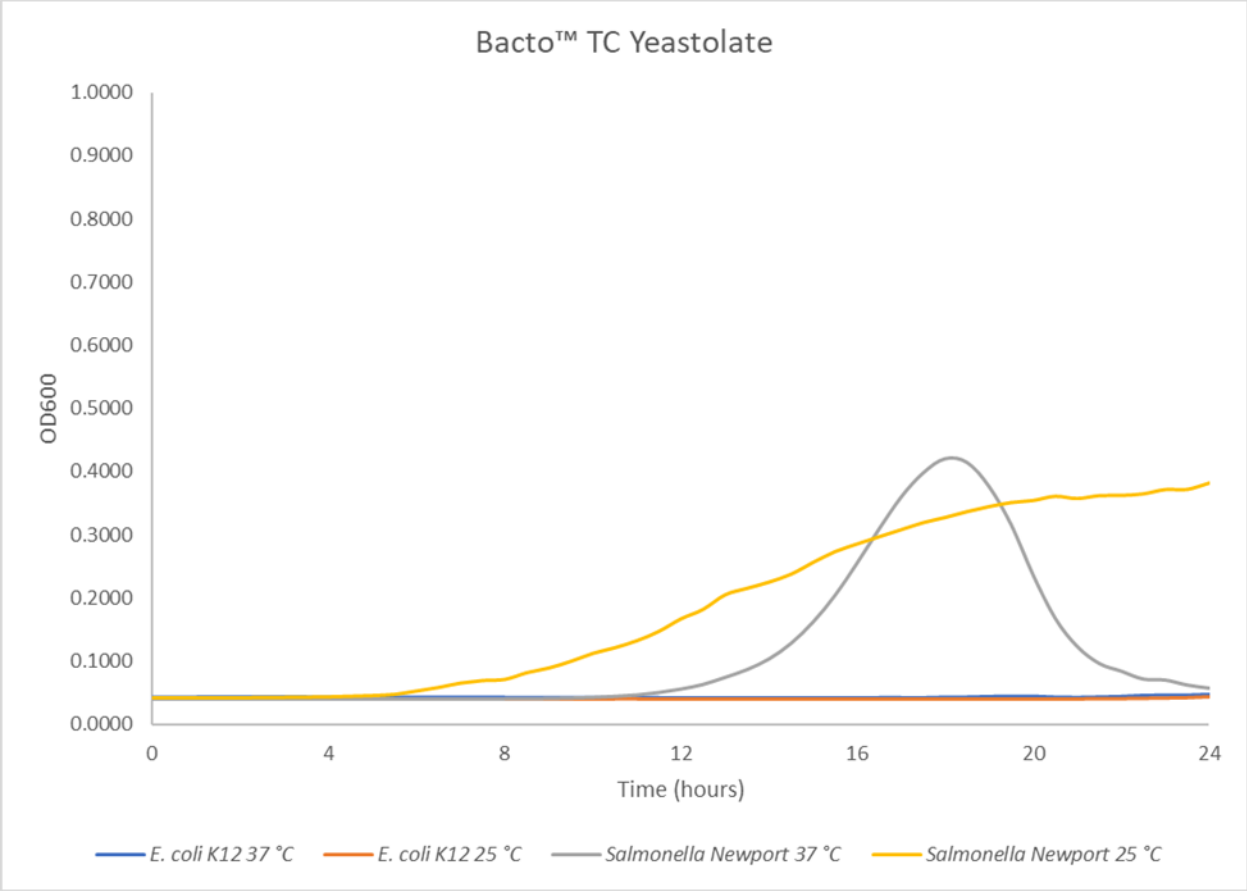


Figure 5.4
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ Yeast Extract at 37 °C and 25 °C for 24 hours

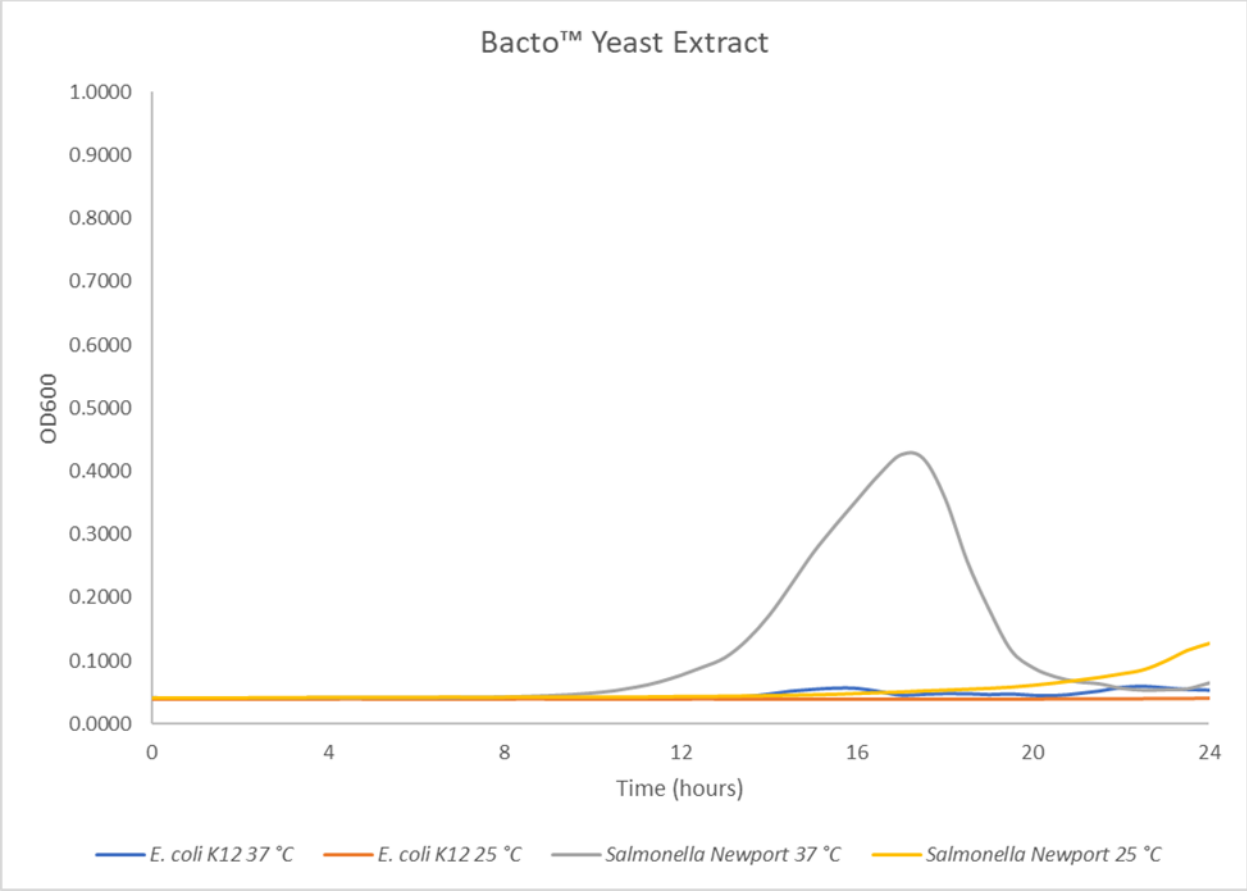


Figure 5.5
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Difco™ Soytone at 37 °C and 25 °C for 24 hours

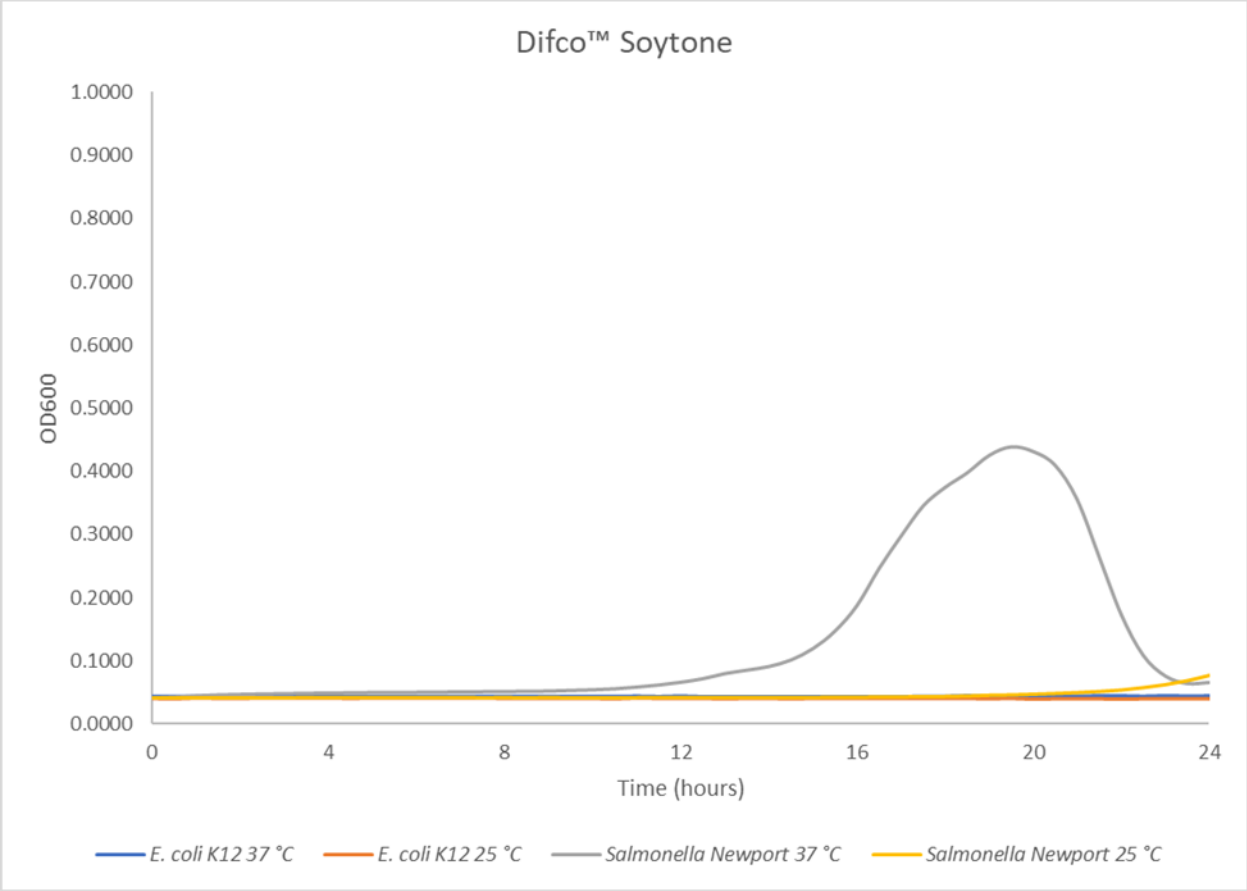


Figure 5.6
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ Malt Extract at 37 °C and 25 °C for 24 hours

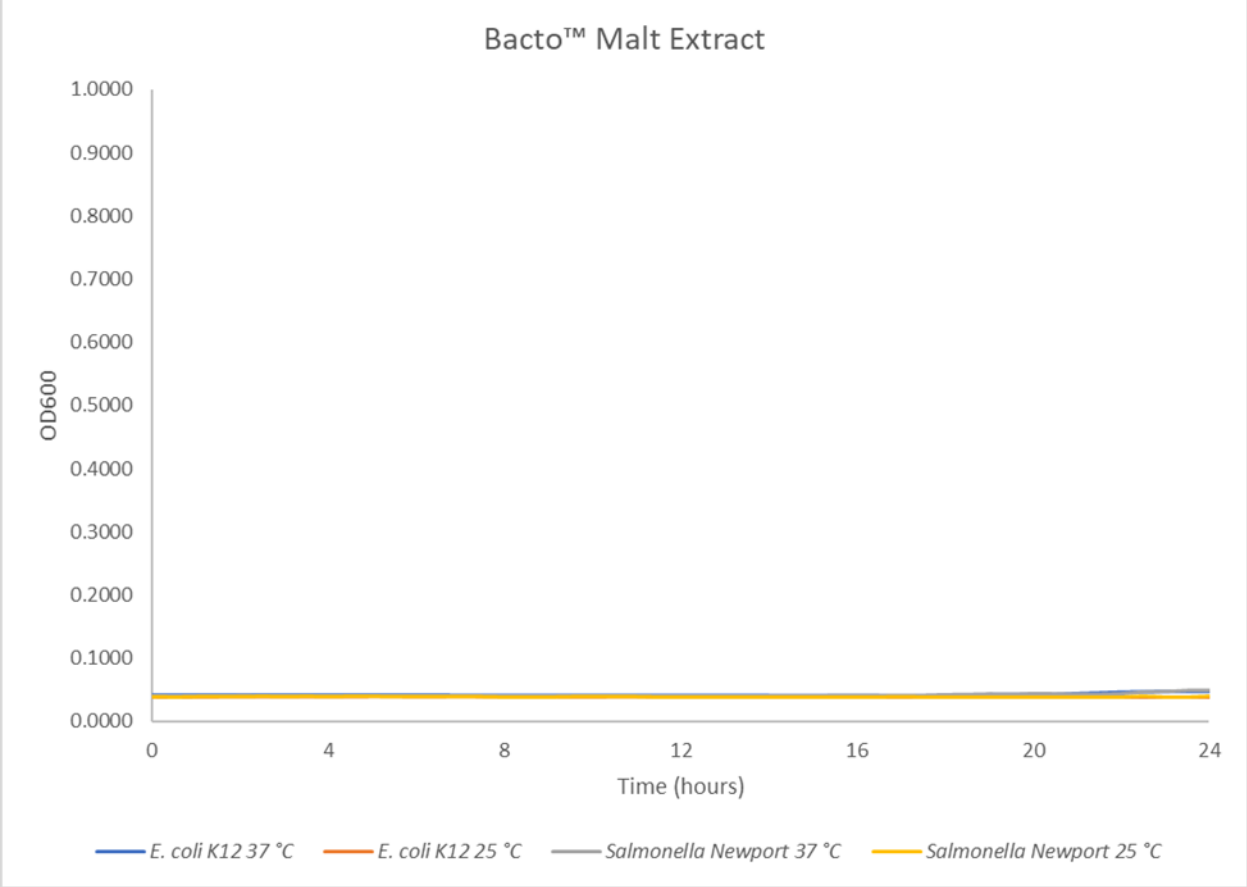


Figure 5.7
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in sterile deionized water (SDW) at 37 ℃ and 25 ℃ for 24 hours

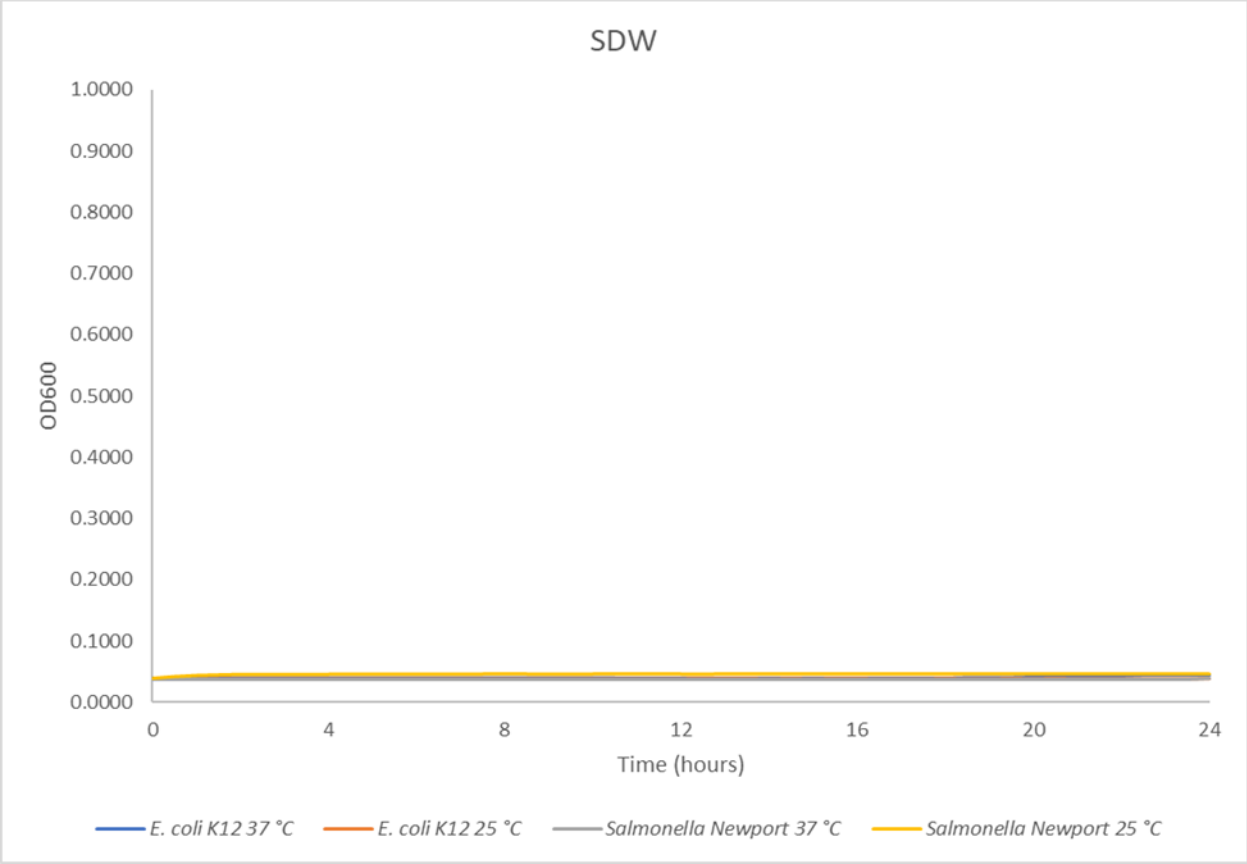


Figure 5.8
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ Proteose Peptone No. 2 at 37 ℃ and 25 ℃ for 24 hours

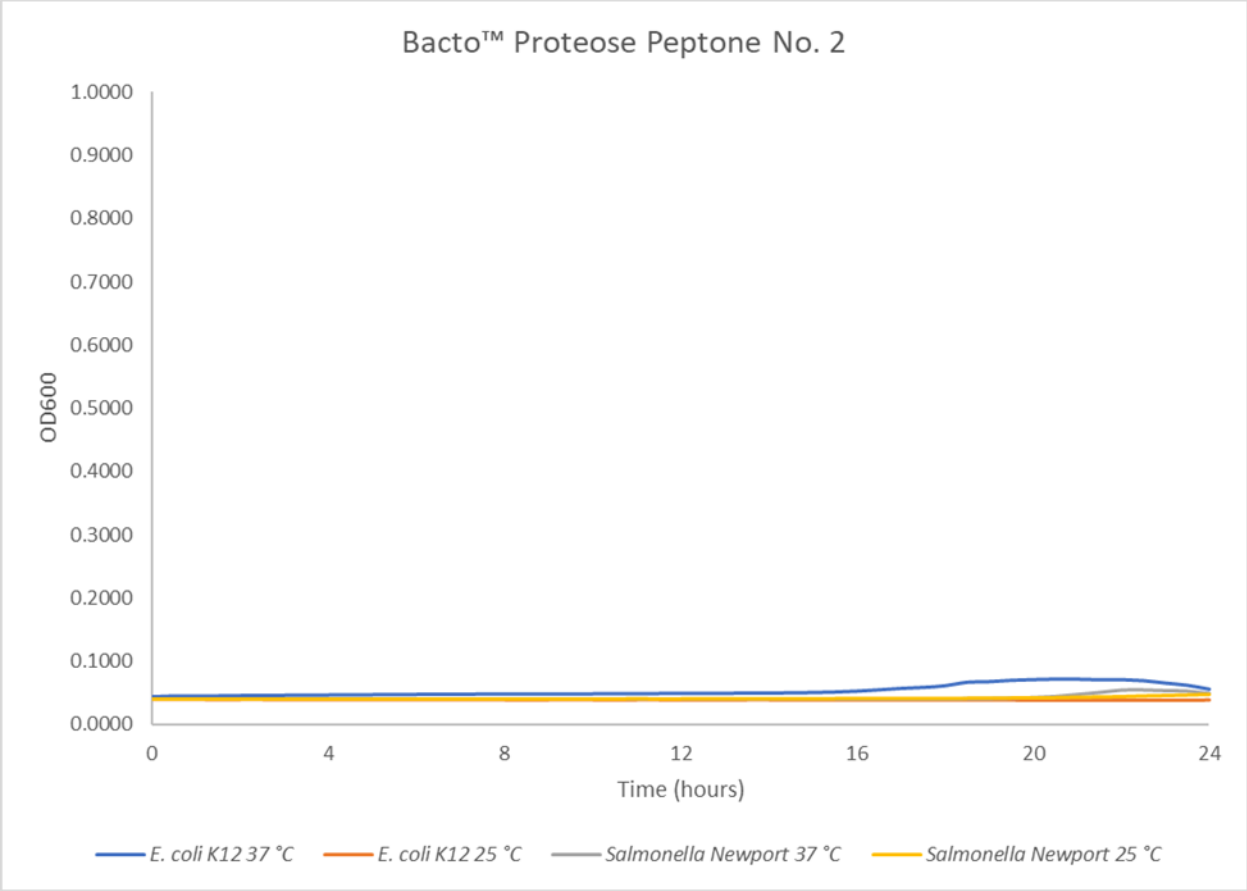


Figure 5.9
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Fisher BioReagents™ Granulated Peptone at 37 ℃ and 25 ℃ for 24 hours

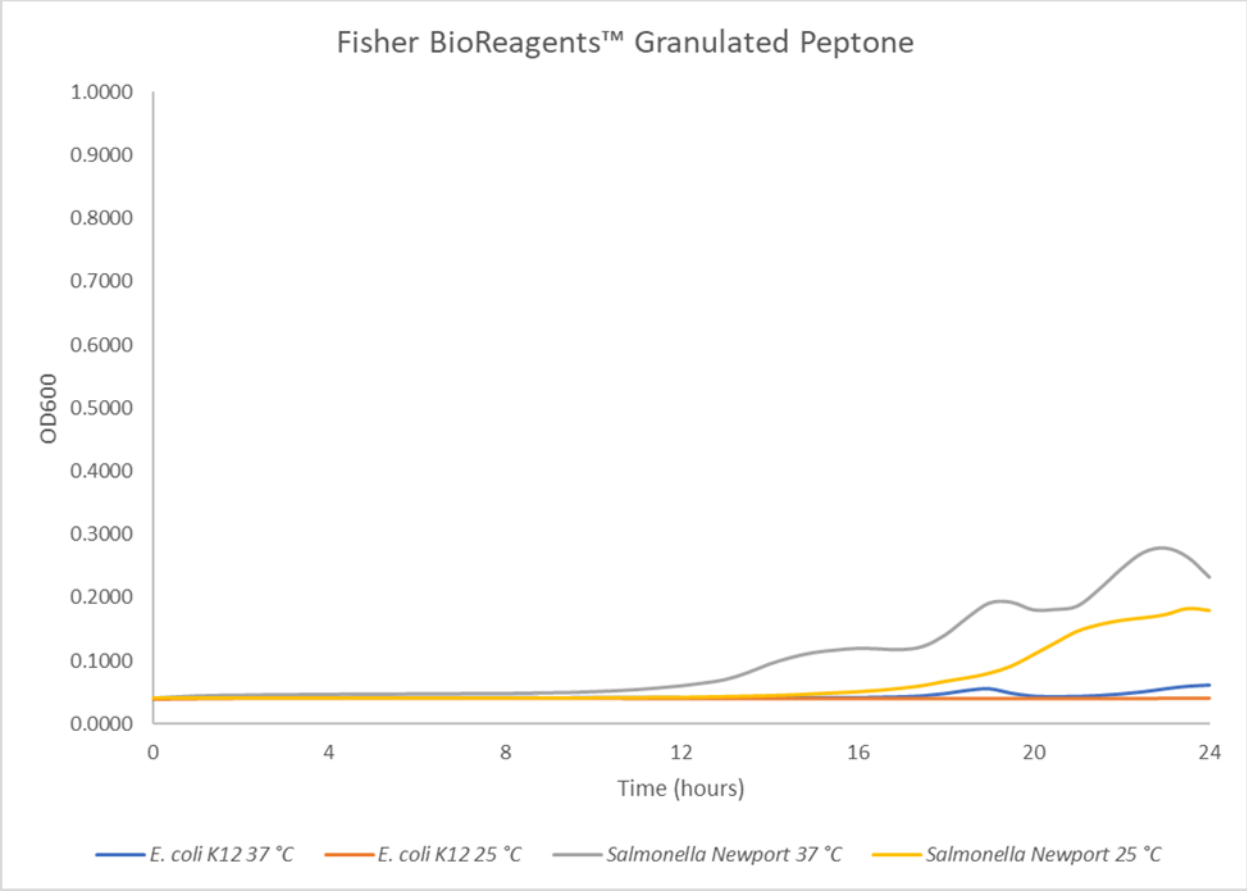


Figure 5.10
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ Proteose Peptone No. 3 at 37 ℃ and 25 ℃ for 24 hours

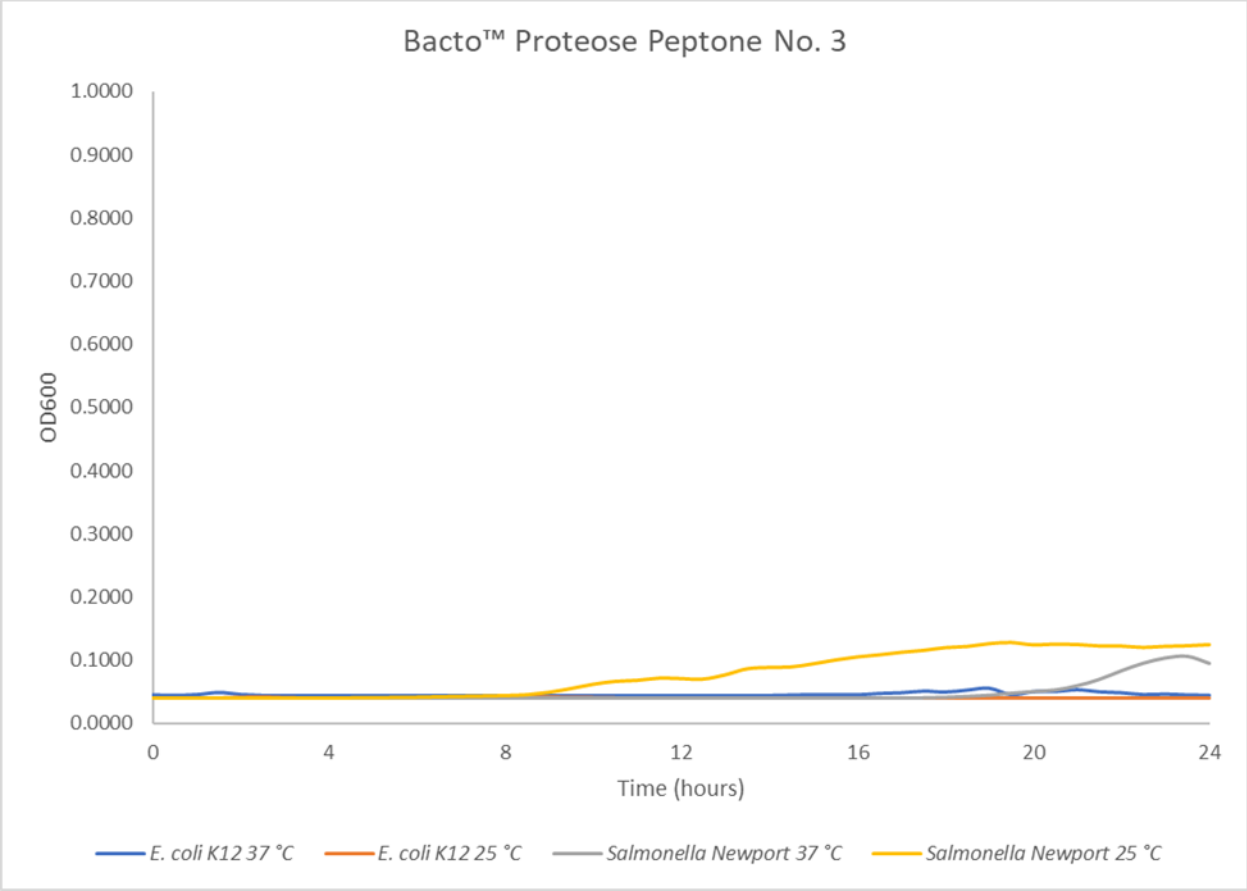


Figure 5.11
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Phytone™ Peptone at 37 °C and 25 °C for 24 hours

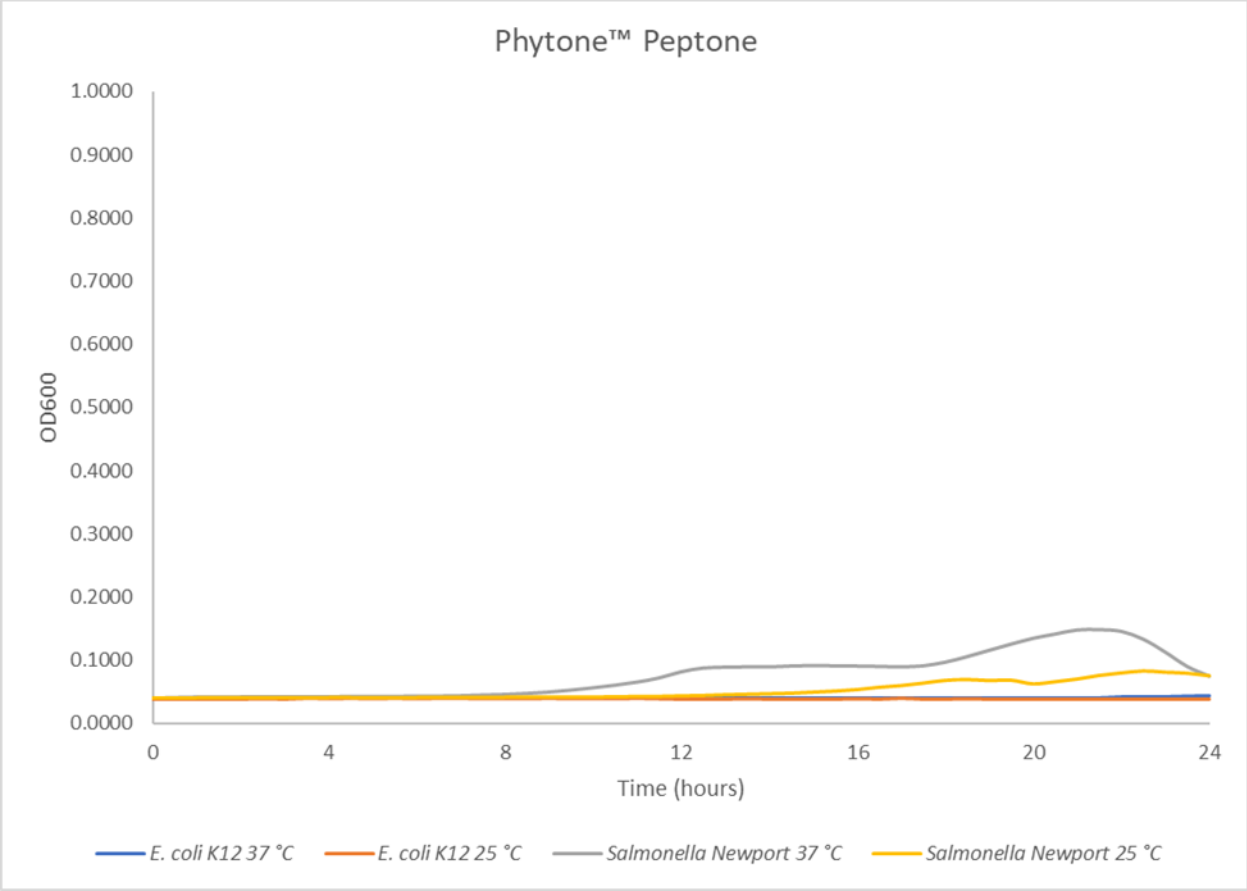


Figure 5.12
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Bacto™ Casamino Acids at 37 ℃ and 25 ℃ for 24 hours

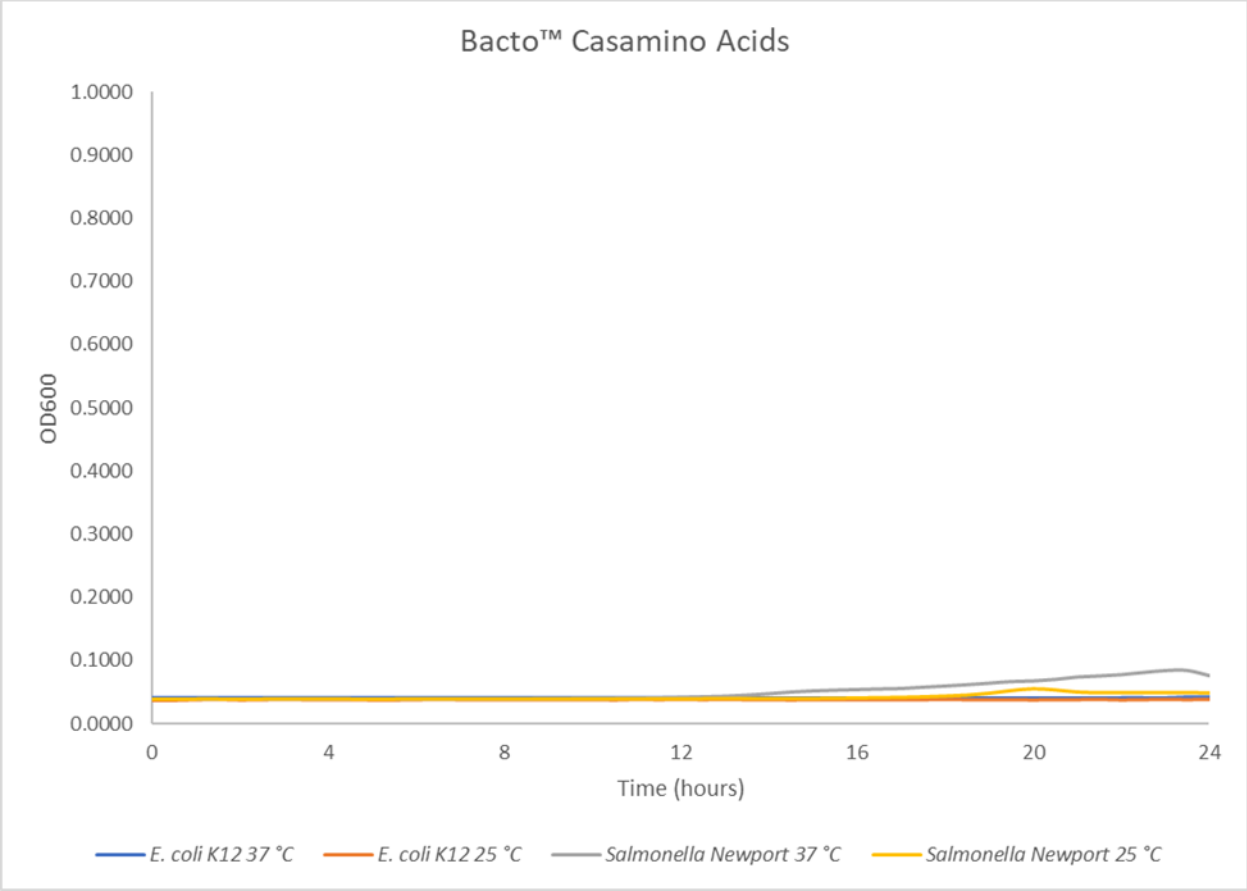


Figure 5.13
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in Difco™ Yeast Extract at 37 ℃ and 25 ℃ for 24 hours

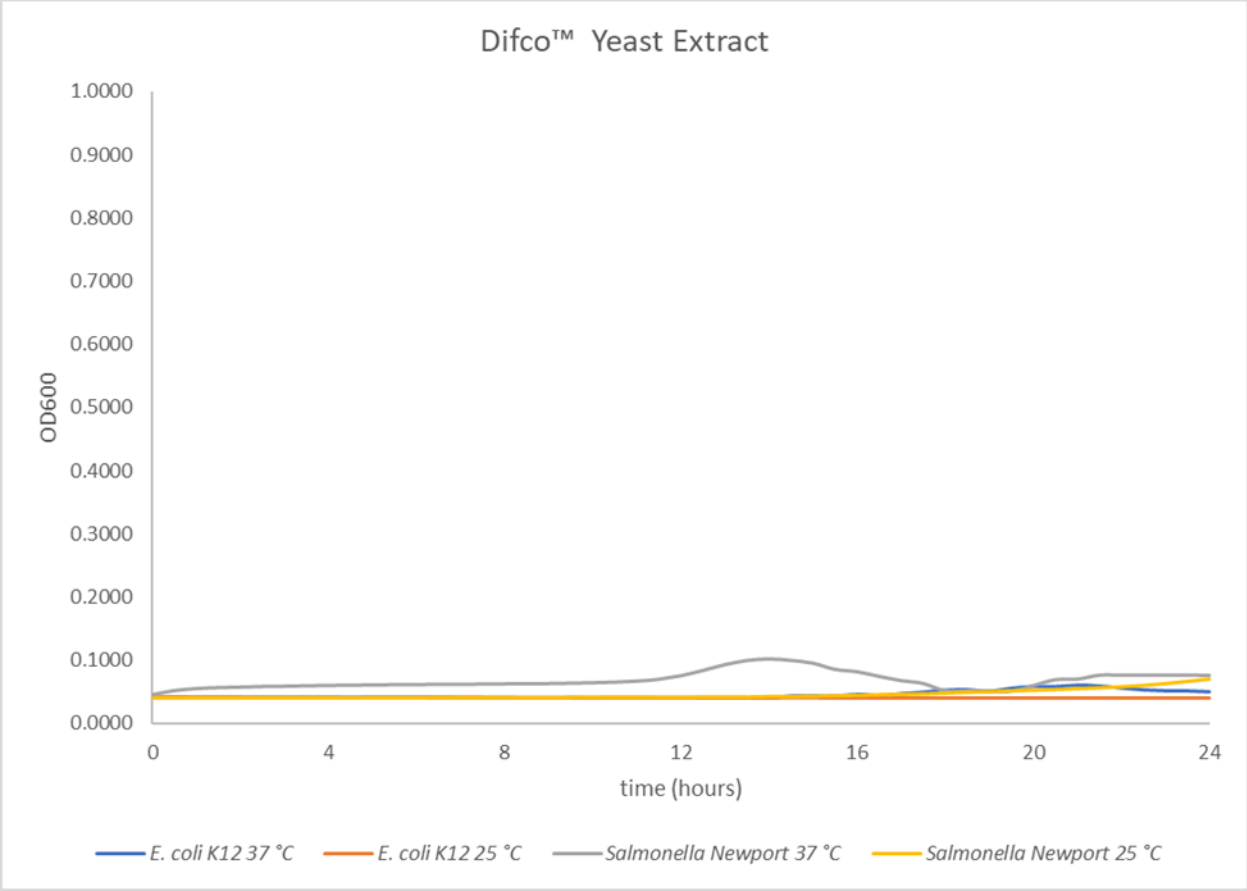
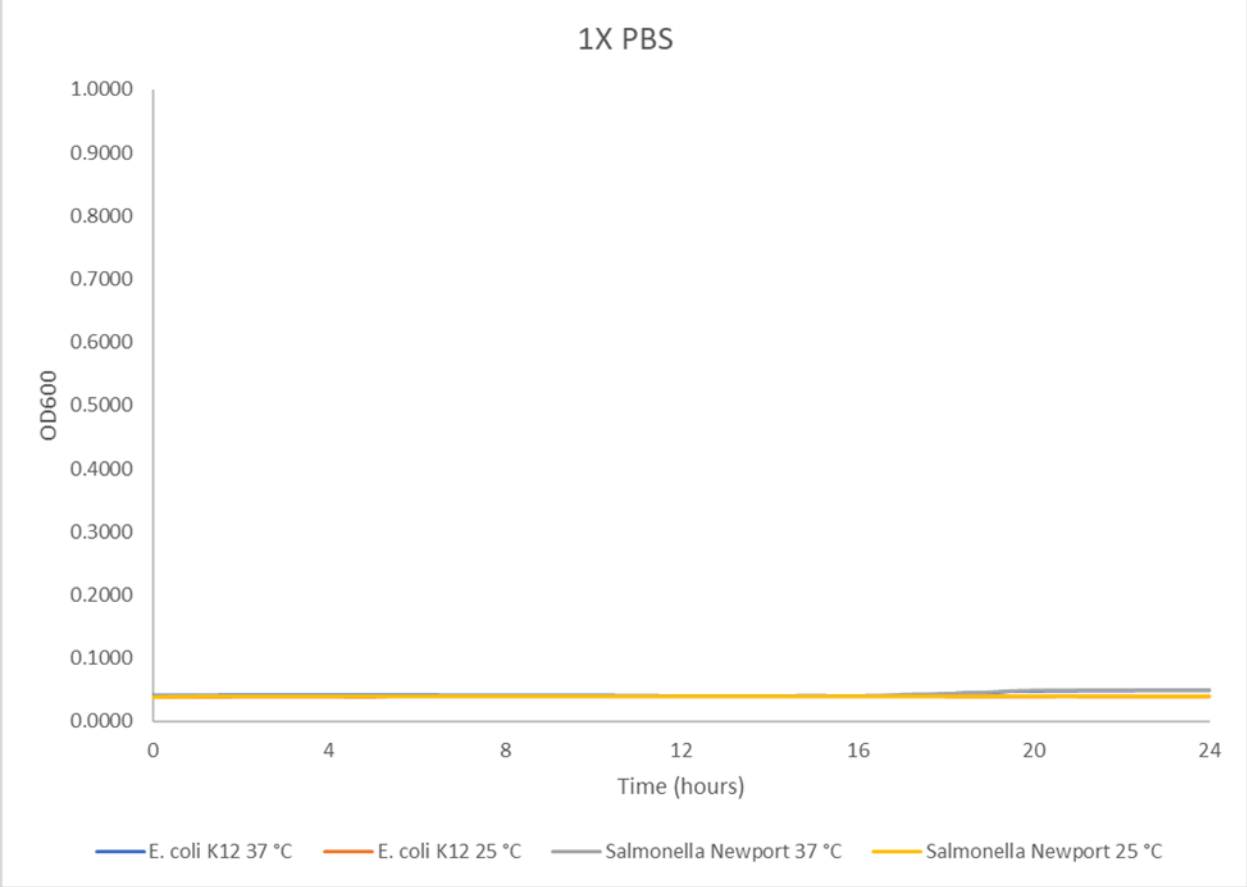


Figure 5.14
Growth rate analysis of *E. coli* K12 and *Salmonella* Newport in 1X PBS at 37 ℃ and 25 ℃ for 24 hours



CHAPTER 6

CONCLUSIONS/SUMMARY

Peach contamination can occur at several points on their journey from pre-harvest to post-harvest environments. Air samples taken from peach orchards were shown to harbor varying levels of microbial contamination through various organisms. Orchard 3 showed the highest airborne HPC population of >4.38±0 log MPN/cm^3 among them. This was likely due to its proximity to an orchard restroom. The air sampling also showed a moderate correlation (r=0.62) between the coliform population and bile levels in the air samples from the orchards. This correlation shows the potential for using bile as an indicator for airborne microbial populations.

Various surfaces in peach packinghouses were shown to harbor high contamination levels. Some of these surfaces were also shown to be not largely affected by the sanitation practices in the packinghouses. Surfaces like the sorting cups for the automatic sorting system, brushes for the washer/waxer, and specific packing tables fall into this category. In certain instances, some surfaces, specifically the sorting cups, showed increased bacterial populations after the sanitation procedures. Hard to-clean surfaces, like the brushes and sorting cups, require a reevaluation or more research to find a more effective method for safely sanitizing these surfaces.

The impinger air sampling procedure relies heavily on the chosen capture buffer. While there was no significant difference in the average recovery of bacteria between the tested buffers (excluding SDW), Bacto™ Malt Extract showed the highest recovery, on average. This buffer

seems to be the most promising option, among those tested, due to its high recovery and the fact that it did not promote bacterial growth upon incubation at 37 ℃ or 25 ℃. BPW was the worst option for a capture buffer as it was on the lower end of recovery and promoted bacterial growth at both incubation temperatures. Results from these studies should give some insight into the importance of proper monitoring practices in peach orchards and packinghouses. Further research is likely needed to assess alternative options for the sanitation of the surfaces in the peach packinghouses. Also, further research is needed to assess if the efficacy of the different capture buffers will be affected by environmental conditions one may find in a peach orchard environment.