HYGIENE CONDITIONS OF FRESH PEACH PACKING LINES AND MICROBIAL QUALITY OF FRESH PEACHES

by

PEIEN WANG

(Under the Direction of Jinru Chen)

ABSTRACT

This project surveyed the hygiene status of fresh peach packing lines and determined the microbial load on peach packers' gloves, on fresh peaches collected at the dumping *vs*. the weighing area, as well as before and after they pass the washing and waxing system (WWS), and in overhead spray water and spent peach wash water. The collected samples were analyzed for the levels of three hygiene indicators (total aerobes, total yeasts and molds, and total coliforms) and the incidence of two pathogen indicators (thermotolerant coliforms and enterococci). The project also assessed the distribution of 7 virulence, and 14 putative adhesins, genes among selected generic *E. coli* isolated from fresh peach packing environments using PCR. The results showed that counts of the hygiene indicators and incidence of the pathogen indicators on fresh peach packing lines increased over time over the course of a packing day. Sites with higher microbial load included optical sizers, brushes/rollers inside the washer, harvest bins, and manual sorting area. The hygiene indicator populations in peach samples from the weighing area were higher than those from the dumping area. The counts of hygiene indicator microorganisms on gloves from employees working at the sorting, and the packing, area were similar. The incidences of thermotolerant coliforms and enterococci in the two areas were 39.39% and 7.58%.

Levels of total aerobes and total coliforms in peach samples collected after the WWS were higher than those collected before the WWS, but an opposite trend was noticed with the total yeast and mold counts. No pathogen indicators or total coliforms were detected in the overhead spray water. The selected virulence genes were not detected, but putative adhesin genes, *sfmA*, *ycbQ*, *csgA*, and *fimA*, were found in all *E. coli* isolates included in the study. The number of adhesin genes varied among tested isolates. This study revealed the ineffectiveness of the packing process and WWS in improving the microbial safety of fresh peaches; suggesting that effective daily post-operation sanitation, good employee hygiene, and handling practice, and functional water safety monitoring program are important to produce safe and wholesome peaches for the fresh market.

INDEX WORDS: Fresh produce, Peach, Packing lines, Gloves, Overhead washing and waxing system, Postharvest water, Indicator organisms, *Escherichia coli*, Virulence genes, Adhesin genes

HYGIENE CONDITIONS OF FRESH PEACH PACKING LINES AND MICROBIAL QUALITY OF FRESH PEACHES

by

PEIEN WANG

B.S., Qilu University of Technology, China, 2014

M.S., Illinois Institute of Technology, 2017

A Dissertation Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2021

HYGIENE CONDITIONS OF FRESH PEACH PACKING LINES AND MICROBIAL QUALITY OF FRESH PEACHES

by

PEIEN WANG

Major Professor:	Jinru Chen
Committee:	Koushik Adhikari Mark A. Harrison Abhinav Mishra

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2021

DEDICATION

This dissertation is dedicated to my parents and families who have always supported me unconditionally in pursuit of my Ph.D. degree. This work is also dedicated to all the food safety professionals that have been working diligently to keep our food safe under the Food Safety Modernization Act in the past 10 years.

ACKNOWLEDGMENTS

Firstly, my deepest gratitude goes to my major professor Dr. Chen, who has not only mentored me for my academic work but also has been loving and caring for me as a person every step along the way. I also would like to give thanks to my committee members, Drs. Koushik Adhikari, Mark A. Harrison, and Abhinav Mishra for their advice, encouragements, and patience during every committee meeting and through our communications.

Secondly, I would like to acknowledge the funding agency of this project, the Georgia Department of Agriculture for financial support through the Special Crop Block Grant Program. I am grateful to our project collaborators, Mrs. Katie B Pitts, Mr. Duke Lanes III, Dr. Dario Chavez, as well as the four peach growers involved in this project and their facility managers for their full support during the project sampling.

Thirdly, my gratitude is towards my peers in the laboratory: Dr. Joycelyn K Quansah, Ms. Himabindu Gazula, Weifan Wu, Seulgi Lee, Minji Hur, Kimberlee Boyd, Miranda J Barr, McKenzie Dallas for their assistant during project sampling. Thanks to our department staff: Mrs. Donna Brown, Karen Simons, Sandra Walker, Sue Ellen McCullough, Mr. Bobby Goss, and Glen Farrell for their supports. In addition, I would acknowledge my friends Dr. Juyoung Kim, Dr. Shaoting Li, Dr. Yingshu He, Ms. Nan Jiang, Ms. Wenxue Zhang, Mr. Fengquan Song, and all my friends in Griffin who have been extremely supportive and helpful during the COVID-19 pandemic.

Last but not the least, I am thankful for being a DAWG.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS v

LIST OF TABLES vii

LIST OF FIGURES x

CHAPTER

1. INTRODUCTION 1
2. LITERATURE REVIEW 8
3. HYGIENE STATUS OF FRESH PEACH PACKING LINES IN GEORGIA 45
4. MICROBIAL LOADS ON FRESH PEACH AND GLOVES WORN BY PEACH PACKERS IN SELECTED PACKING FACILITIES IN GEORGIA 74
5. EFFICACY OF COMMERCIAL OVERHEAD WASHING AND WAXING SYSTEMS ON THE MICROBIOLOGICAL QUALITY OF FRESH PEACHES 100
6. CHARACTERIZATION OF GENERIC *ESCHERICHIA COLI* ISOLATED FROM FRESH PEACH PACKING ENVIRONMENTS 127
7. CONCLUSIONS 146

LIST OF TABLES

Page

Figure 2-1 Fresh peach packing line workflow 15

Table 3-1 Selected sample sites on fresh peach packing lines........ 68

Table 3-2 Overall mean TA, YM, and TC counts in samples collected at different sampling times and sites, and from different packing lines 69

Table 3-3 Mean TA, YM, and TC counts in samples collected from different sites of individual fresh peach packing lines 70

Table 3-4 Mean TA, YM, and TC counts in samples collected from individual PLs at different sampling times 71

Table 3-5 Mean TA, YM, and TC counts in samples collected from various sites at different sampling times 72

Table 3-6 Incidence of TTC and EC in samples collected from various sites of 4 fresh peach packing lines. 73

Table 4-1 The results of the significance test for the effects of packing operation, sampling time, and sampling location on the levels of hygiene indicators on peach samples 93

Table 4-2 Total aerobe, total yeast and mold, and total coliform counts on peach samples collected from different packing operations, at different sampling times and locations 94

Table 4-3 Total aerobe, total yeast and mold, and total coliform counts on peach samples collected at different sample locations and packing operations........ 95

Table 4-4 Incidences of thermotolerant coliforms and enterococci on peach samples collected from different packing operations at different sampling times and locations 96

Table 4-5 Results of significance test for the effects of packing operation, sampling time, and peach handler on the levels of hygiene indicators on glove samples 97

Table 4-6 Levels of total aerobes, total yeasts and molds, and total coliforms on glove samples collected from peach handlers at different packing operations and sampling times 98

Table 4-7 Incidences of TTC and EC on glove samples collected from peach handlers at different packing operations and sampling times 99

Table 5-1 Type III tests for fixed effects by the statistical model of peach sampling 119

Table 5-2 Average counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected from the washing and waxing system at different sampling points and sites in different packing facilities 120

Table 5-3 Mean counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected before and after the washing and waxing system at different sampling points 121

Table 5-4 Mean counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected before and after the washing and waxing system in different packing facilities 122

Table 5-5 Incidences of TTC and EC on peach samples collected from the washing and waxing system at different sampling points and sample sites in different packing facilities................. 123

Table 5-6 Type III tests for fixed effects by the statistical model of water sampling 124

Table 5-7 Average counts of total aerobes, total yeasts and molds, and total coliforms in overhead spray water and spent peach wash water at different sampling points and sample sites in different packing facilities 125

Table 5-8 Incidences of TTC and EC in water samples collected at various sampling points and sample sites in different packing facilities 126

Table 6-1 Oligonucleotide primers used for the screening of virulence genes in the generic *E. coli* isolated from fresh peach packing facilities.. 143

Table 6-2 Putative adhesin gene subunits and primers derived from *E. coli* O157:H7 Sakai strain 144

Table 6-3 Distribution and incidence of putative adhesin genes among the generic *E. coli* isolates included in the study 145

LIST OF FIGURES

Page

Figure 2-1 Fresh peach packing line workflow 15

CHAPTER 1

INTRODUCTION

Driven by the affection for a healthier and more nutritious diet, the consumer demand for fresh produce has enhanced remarkably over the past few decades (Renner, Baker, Fedder, & Upadhyaya, 2020), however, a growing trend of foodborne illnesses and outbreaks associated with the consumption of fresh produce has been observed concurrently (Carstens, Salazar, & Darkoh, 2019). Fresh peaches, as one of the most popular stone fruits consumed in summer, has rarely been linked to foodborne outbreaks before 2014 (Campbell, Merwin, & Padilla-Zakour, 2011; CDC, 2018). In summer 2014, fresh stone fruits, including fresh peaches, were identified as novel vehicles for *Listeria monocytogenes* in a multistate listeriosis outbreak, which resulted in 2 illnesses, 2 hospitalizations, and 1 death in the U.S. (CDC, 2018; Jackson et al., 2014). More recently, fresh peaches sourced from a packing company in California were found to be responsible for a multistate outbreak of *Salmonella* Enteritidis infections resulting in a total of 158 confirmed illnesses and 38 hospitalizations in both U.S. and Canada in summer 2020 (CDC, 2020; PHAC, 2020).

While these outbreak histories connected the potential food safety concerns to commercial fresh peach packing houses, no specific sources of contamination were determined in the outbreak investigations (Chen et al., 2016; U.S. FDA, 2020). Given the fact that even with a low level of inoculation (2.7 log CFU/fruit), foodborne pathogens such as *Listeria monocytogenes* can survive on fresh peaches at 4 °C until the end of the shelf-life (26 days) and have the potential to cause illnesses in susceptible group (De Jesus et al., 2020), it is critical for the fresh peach industry to understand the potential risk factors associated with peach production especially during postharvest handling and have effective microbial intervention measures to improve microbial quality of fresh peaches during packing.

The commercial fresh produce packing process usually involves sorting, grading, and weighing and some operations may also require washing/rinsing (U.S. FDA, 2015). During produce packing, microbial contamination could be introduced on fresh produce via contaminated equipment surfaces, postharvest water, and workers (Chen, Evans, Hammack, Brown, & Macarisin, 2016; Prazak, Murano, Mercado, & Acuff, 2002; Todd, Greig, Bartleson, & Michaels, 2008). Different produce and packing houses may adopt different packing procedures and washing systems, consequently, affecting the microbial quality of fresh produce differently. By far, the detailed studies on hygiene conditions of commercial fresh peach packing facilities are still limited (Duvenage & Korsten, 2017; Williamson et al., 2018).

Escherichia coli are commonly found in the gastrointestinal tract of warm-blooded animals; while most strains are non-pathogenic, a few pathotypes with specific virulence attributes may cause serve gastrointestinal diseases in humans (Kaper, Nataro, & Mobley, 2004). The presence of certain pathogenic *E. coli* in produce and environment can be identified by determining a certain combination of virulence genes conserved by them (Toma et al. 2003). Bacterial adhesins are one of the critical bacterial cell surface structures that could affect the interaction between bacterial cells and their contact surfaces (Tuson & Weibel, 2013). Genes encoding adhesin expression are critically important for the initial bacterial attachment to biotic and abiotic surfaces, and the formation of the secondary structure of biofilms (Berne, Ducret, Hardy, & Brun, 2015).

The objectives of this study were to evaluate the hygiene status of fresh peach packing lines (chapter 3), to determine microbial loads on fresh peach and gloves worn by peach packers (chapter 4), to evaluate the efficacy of the overhead washing and waxing system in improving the microbiological quality of fresh peaches (chapter 5), to explore the distribution of selected virulence, as well as putative adhesin genes among selected generic *E. coli* isolated from the fresh peach packing environments (chapter 6).

This study will provide a better understanding of several key elements linked to the microbial safety of peaches detained for the fresh market during fresh peach packing process, help improve the microbial quality of fresh peaches, and ultimately, increase the sustainability of fresh peach industry.

References

Berne, C., Ducret, A., Hardy, G. G., & Brun, Y. V. (2015). Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiology spectrum*, *3*(4), 10.1128/microbiolspec.MB-0018-2015. https://doi.org/10.1128/microbiolspec.MB-0018-2015

Campbell, O. E., Merwin, I. A., & Padilla-Zakour, O. I. (2011). Nutritional quality of New York peaches and apricots. *New York fruit quarterly, 19*(4), 12-16.

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in Microbiology, 10*(2667). https://doi.org/10.3389/fmicb.2019.02667

CDC. (2018). National Outbreak Reporting System (NORS). NORS dashboard. Retrieved from https://wwwn.cdc.gov/norsdashboard/. Accessed 19 April 2021.

CDC. (2020). Outbreak of *Salmonella* Enteritidis infections linked to peaches. Retrieved from https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 19 April 2021.

Chen, Y., Burall, L. S., Luo, Y., Timme, R., Melka, D., Muruvanda, T., Payne, J., Wang, C., Kastanis, G., Maounounen-Laasri, A., De Jesus, A. J., Curry, P. E., Stones, R., K'Aluoch, O., Liu, E., Salter, M., Hammack, T. S., Evans, P. S., Parish, M., Allard, M. W., Datta, A., Strain, E. A., & Brown, E. W., (2016). *Listeria monocytogenes* in stone fruits linked to a multistate outbreak: enumeration of cells and whole-genome sequencing. *Applied and Environmental Microbiology, 82*(24), 7030-7040. https://doi.org/10.1128/aem.01486-16.

Chen, Y., Evans, P., Hammack, T. S., Brown, E. W., & Macarisin, D. (2016). Internalization of *Listeria monocytogenes* in whole avocado. *Journal of Food Protection, 79*(8), 1440-1445. https://doi.org/10.4315/0362-028X.JFP-16-075

De Jesus, A. J., Sheth, I., Kwon, H. J., Gao, Z., Palmer, J., Hur, M., Hammack, T. S., Macarisin, D., & Chen, Y. (2020). Survival of a serotype 4b strain and a serotype 1/2a strain of *Listeria monocytogenes*, isolated from a stone fruit outbreak investigation, on whole stone fruit at 4 °C. *International Journal of Food Microbiology, 334*, 108801. https://doi.org/10.1016/j.ijfoodmicro.2020.108801

Duvenage, S., & Korsten, L. (2017). Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer. *Food Control, 78*, 374-382. https://doi.org/10.1016/j.foodcont.2017.03.003

Jackson, B.R., Salter, M., Tarr, C., Conrad, A., Harvey, E., Steinbock, L., Saupe, A., Sorenson, A., Katz, L., Stroika, S., Jackson, K. A., Carleton, H., Kucerova, Z., Melka, D., Strain, E., Parish, M., & Mody, R. K. (2015). Listeriosis associated with stone fruit-United States, 2014. (2015). *Morbidity and mortality weekly report(MMWR),* 64 (10).

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

PHAC. (2020) Public Health Notice: Outbreak of *Salmonella* infections linked to peaches imported from the United States. October 15, 2020 - Final Update. Retrieved from https://www.canada.ca/en/public-health/services/public-health-notices/2020/outbreak-salmonella-infections-peaches-imported-united-states.html. Accessed 19 April 2021.

Prazak, A. M., Murano, E. A., Mercado, I., & Acuff, G. R. (2002). Prevalence of *Listeria monocytogenes* during production and postharvest processing of cabbage. *Journal of Food Protection, 65*(11), 1728-1734. https://doi.org/10.4315/0362-028X-65.11.1728

Renner, B., Baker, B., Fedder, C., & Upadhyaya, J. (2020). In an uncertain economy, does fresh produce spoil revenue growth? *Deloitte Insights*. Retrieved from https://www2.deloitte.com/us/en/insights/industry/retail-distribution/future-of-fresh-food-sales/fresh-produce-consumer-trends.html. Accessed 19 April 2021.

Todd, E. C. D., Greig, J. D., Bartleson, C. A., & Michaels, B. S. (2008). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 5. Sources of contamination and pathogen excretion from infected persons. *Journal of Food Protection, 71*(12), 2582-2595.

Toma, C., Lu, Y., Higa, N., Nakasone, N., Chinen, I., Baschkier, A., Rivas, M., & Iwanaga, M. (2003). Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. *Journal of Clinical Microbiology*, *41*(6), 2669–2671. https://doi.org/10.1128/jcm.41.6.2669-2671.2003

Tuson, H. H., & Weibel, D. B. (2013). Bacteria-surface interactions. *Soft Matter, 9*(18), 4368-4380. https://doi.org/10.1039/C3SM27705D

U.S. FDA. (2015) Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. 80 FR 74353 (Docket No. FDA-2011-N-0921). Retrieved from https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption. Accessed 19 April 2021.

U.S. FDA. (2020). Outbreak investigation of *Salmonella* Enteritidis: peaches (August 2020). Retrieved from https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-salmonella-enteritidis-peaches-august-2020. Accessed 19 April 2021.

Williamson, K., Pao, S., Dormedy, E., Phillips, T., Nikolich, G., & Li, L. (2018). Microbial evaluation of automated sorting systems in stone fruit packinghouses during peach packing. *International Journal of Food Microbiology, 285*, 98-102. https://doi.org/10.1016/j.ijfoodmicro.2018.07.024

CHAPTER 2

LITERATURE REVIEW

2.1 Peach

2.1.1 Botanical description and classification

The peach (*Prunus percisa* [L.] Batsch) is a round or flat-shaped drupe (*i.e.,* stone fruit) with a pericarp flesh and an internal pit (Stern, 2006). The flesh is typically in white, yellow, or red and has a unique combined flavor of tanginess and sweetness. The peach with a soft and relatively viscous flesh is called melting fleshed peach, whereas the one with a more condensed flesh is defined as non-melting fleshed peach (Hans, Shah, & Bansal, 2020). In terms of how clingy the flesh is towards the pit, peaches can be grouped into “freestone”, “semi-stone”, and “clingstone”. Clingstones are considered more suitable for processing, whilst the other two types are more popular for fresh consumption (Hans et al., 2020).

According to the taxonomic classification report from the U.S. Department of Agriculture (USDA) National Resources Conservation Service, there are two botanical varieties under the species level of the peach, including the peach with fuzzy trichomes (*Prunus percisa* [L.] Batsch var. *percisa*) and the nectarine with smooth and glossy skin (*Prunus percisa* [L.] Batsch var. *nucipersica* (Suckow) C.K. Schneid) (Cronquist, 1981). Meanwhile, peach breeders more often refer to different types of peaches as different “cultivars”, the cultivated varieties with distinctive and stable characteristics (Brickell et al., 2009; Reig, Iglesias, Gatius & Alegre, 2013). Currently, there are more than 3, 000 peach cultivars worldwide and over 300 in the U.S. (Aubert & Chalot, 2020; Faust & Timon, 1995; Andrews, 2018).

2.1.2 Nutritional value and health benefits

The reference nutrition label provided by the U.S. Food and Drug Administration (U.S. FDA) shows that a medium-sized peach weighted 147 g contains 13 g sugar, 0.5 g total fat, 1 g protein, as well as 2 g dietary fiber which is equivalent to 8% of the daily value (DV); the micronutrients in peaches mainly include potassium (7% DV), iron (2% DV), vitamin A (6% DV), and vitamin C (15%) (U.S. FDA, 2017).

The health benefits of consuming peaches are mainly manifested in the relatively high level of dietary fiber and plant antioxidants. Numerous studies have linked the increased dietary fiber intake to the reduced risk of progressing certain chronic diseases such as certain cancers, cardiovascular diseases, hypertension, *etc.* (Cui et al., 2019; Dahl et al., 2017; Whelton et al., 2005). Antioxidants are well-known for their ability to neutralize free radicals in the human body, consequently, reducing the risk of diseases associated with aging, contrary heart disease, cancer, *etc*. (Bors & Saran, 1987; Cai, Sun, & Corke, 2003; Jamshidi-Kia et al., 2020; Kritchevsky, 1999). The phytochemicals in peaches that have shown obvious antioxidant activities include vitamins, carotenoids, and phenolic compounds such as flavonoids, flavan-3-oils, phenolic acids, anthocyanins, *etc.* (Bento et al., 2020; Hans et al., 2020). The total phenolics in multiple peach cultivars obtained from California was significantly correlated to the antioxidant activity reflected by the free radical scavenging capacity (Gil et al., 2002). Despite the cultivar differences, most studies found that the peel of peaches had more phenolic contents and expressed more antioxidant activities compared to the flesh (Dabbou et al., 2017; Zhao et al., 2015). Given the fact that both carotenoids and phenolic compounds play decisive roles in forming the color of the flesh (Campbell, Merwin, & Padilla-Zakour, 2011), peach cultivars with the red flesh have attracted more research attention due to the significantly high antioxidant activity, as well as the high levels of total phenolics and anthocyanins in them (Aubert & Chalot, 2020; Marcia et al., 2007).

2.1.3 Production

The Food and Agriculture Organization of the United Nations (FAO) database reported that the world's total fresh peach production reached 25.7 million tons in 2019. The world's major peach producers include China, European Union, Turkey, and the U.S. (FAO, 2020). According to the USDA National Agricultural Statistic Service (NASS), the peach industry's value to the American agricultural income in 2019 was near 519 million dollars, and the total production was close to 682 thousand tons consisting of nearly 40% raw and 60% processed peaches (NASS, 2020). Georgia grown peaches are mainly for fresh consumption, with annual production of 9.6, 23.4, and 33.78 thousand tons in 2017, 2018, and 2019, respectively. This increased production in the past few years has made Georgia hold its position as the nation's third-largest peach-producing state, after California and South Carolina (NASS, 2020).

2.1.4 Historical significance of Georgia grown peaches

Peaches were originated in ancient China. Around 2000 years ago, these were brought to Persia through the Silk Road, arrived in Greece, and continuously spread in Europe (Byrne et al., 2012; Hans et al., 2020). Peaches were introduced to the North American continent by Spanish and Portuguese pioneers for the first time in the 1500s and dispersed within the Native American tribes (Byrne et al., 2012). In 1850, peaches were directly imported to the U.S. from China; twenty-five years later, Georgia peach breeder Samuel H Rumph, known as "the Father of Southern Peach Industry", successfully produced a beautiful yellow-fleshed freestone cultivar from the imported "Chinese Cling" seedlings and named it "Elberta" (Faust & Timon, 1995;

Lane, n.d.). The successful cultivation and commercialization of the "Elberta" peach, along with other cultivars developed during the same age (*e.g.*, "J.H. Hale" and "Belle of Georgia"), promoted the exponential growth of peach production in the South (Byrne et al., 2012; Okie, 2012). More importantly, the rise of Georgia peach industry symbolized the expansion of industrial agriculture in the South after the American Civil War (Okie, 2012). The peach became the official state fruit of Georgia and was included into the Georgia Code (GA Code) on April 7, 1995 (GA Code § 50-3-70 [2019]). Thus, a thriving peach industry producing high-quality peaches has been a high priority for Georgia agriculture.

2.2 Foodborne outbreaks associated with fresh peaches

Fresh peaches have rarely been linked to foodborne outbreaks compared to other fruit vehicles, such as melon, mango, papaya, *etc*.; however, the wide distribution of fresh peaches and consumers' patronage towards this climatic commodity has drawn lots of media and public attention when incidences have occurred (Carstens, Salazar, & Darkoh, 2019; Allen, 2014; McCarthy, 2020). According to the records obtained from the National Outbreak Reporting System (NORS) Dashboard developed by the Centers for Disease Control and Prevention (CDC) (2018), as well as the official outbreak investigation reports presented by U.S. FDA (2020), fresh peaches have been involved in a total of two foodborne illness outbreaks with confirmed etiology in the U.S.

The first multistate foodborne outbreak attributed to stone fruit consumption including fresh peaches was reported in 2014, resulting in 1 death and 2 illnesses (CDC, 2018; Jackson et al., 2015). Whole Genome Sequencing data indicated that the clinical *Listeria monocytogenes* isolates from the two patients were highly related to the ones from the stone fruits recalled by a California packing company (Jackson et al., 2015). Further investigations confirmed that fruits and environmental samples collected from this packing facility were genetically matched to the outbreak strain, however, the specific sites of contamination in the facility were unknown (Chen et al., 2016).

The other fresh peach-related multistate outbreak was reported in August 2020 (CDC, 2020). It was believed that *Salmonella*-contaminated peaches shipped from a California peach packing company had caused 101 illnesses in 17 states in the U.S. (CDC, 2020), and 57 cases of salmonellosis in Canada (Public Health Agency of Canada (PHAC), 2020). Surprisingly, no environmental samples collected from the packing facilities tested positive for the outbreak strains. Further root cause investigation was still in process at the time this dissertation was submitted (U.S. FDA, 2020).

2.3 Potential sources of microbial contamination during fresh peach production

At the preharvest level, foodborne pathogens can be transmitted on fresh produce primarily from irrigation water, soil, contaminated manure, livestock, and wild animals (Alegbeleye, Singleton, & Sant'Ana, 2018). Peaches grown on trees usually have limited direct contact to soil and manure; the exposure of peaches to agricultural water may vary depending on different irrigation and spraying system in use. Drip irrigation and micro-sprinkler irrigation systems both have emitters that are close to the ground, hence, water is applied under the tree canopy and rarely reaches to fruits on the tree. However, the sprinklers of the conventional overhead irrigation system are usually erected next to the stem of the peach tree, which can lead to more frequent direct application of irrigation water on peach fruits (Casamali, van Iersel, & Chavez, 2021; Zambrano-Vaca et al., 2018). As a result, it is recommended that if the source of irrigation water raises the risk of microbial contamination, such as the surface water, overhead irrigation should be conducted no later than one week before the harvest activity (Rangarajan et al., 2000). Additionally, domestic, and wild animals are notorious reservoirs for foodborne pathogens. For example, wild birds were found to carry diarrheagenic *Escherichia coli* (*E. coli*) strains such as enteropathogenic *E. coli* and Shiga toxin-producing *E. coli* with multidrug-resistant properties (Borges et al., 2017). As fruit trees can be a natural habitat for birds (Lindell et al., 2016), foodborne pathogens carried by wild birds could be transmitted onto fruits through shedding and excreting.

During harvest, possible sources of microbial contamination for fresh produce include harvesting tools, containers, harvesters, and gloves (Carstens, et al., 2019). Most harvesting containers used in the field are recycled, without proper and frequent cleaning and sanitizing procedures, foodborne pathogens such as *Salmonella* spp. were found to be persistent on the internal surface of the containers and cause subsequential cross-contamination of the following batches of produce (Zhu, Wu, Trmcic, Wang, & Warriner, 2020). Fresh peaches are usually picked manually (Crisosto & Valero, 2008). Pickers' hands may carry human pathogens or facilitate cross-contamination of foodborne pathogens between batches. The only peach supply chain study published so far has reported that multiple swab samples from pickers' hands were found positive for *Listeria* spp. and *Staphylococcus aureus* (Duvenage & Korsten, 2017). Bartz et al. (2017) have demonstrated that a higher prevalence of *E. coli* on fresh produce was observed when farm workers' hands tested positive for *E. coli*. Therefore, it is critical to assure that pickers are well-trained with hygienic practices and following appropriate hand washing steps in the field.

At the postharvest level, foodborne pathogens may be introduced to fresh peaches through direct contact with contaminated postharvest water, such as water used for hydrocooling, washing, waxing, *etc.* It has been well-noted that when circulated water systems such as dumping tanks or fluming are adopted during produce hydrocooling or washing, microbial cross-contamination between different batches of the product may occur if the proper concentration of the antimicrobial chemicals added are not continuously maintained (Gereffi, Sreedharan, & Schneider, 2015; Holvoet, Jacxsens, Sampers, & Uyttendaele, 2012). Furthermore, foodborne pathogens like *L. monocytogenes* can internalize into fresh cantaloupes through stems and stem scars when immersed into contaminated water. Temperature differences between the water and the fruit may facilitate pathogen infiltration (Chen, Evans, Hammack, Brown, & Macarisin, 2016). Under laboratory conditions, the mesocarp of avocado can be contaminated by *L. monocytogenes* penetrated through stem scars from the hydrocooling water (Macarisin et al., 2017). Gomba, Chidamba, and Korsten (2017) found both *E. coli* O157:H7 and *Salmonella* Typhimurium can survive in different commercial fungicide solutions and may have the possibility to infect fresh produce during waxing.

Figure 2-1 illustrates a fresh peach packing line workflow. After being dumped on conveyor belts, fresh peaches are delivered to the washer/waxer and sprayed by sanitizer-added wash water and fungicide-added wax solution consecutively. Manual sorters standing by the following roller conveyors are responsible for rejecting the peaches with surface defects and separating the overly matured peaches from the bulk. Then peaches are single lined up at the optical sizer and conveyed to different weighing areas based on their size and maturity. Thereafter, packers manually fill peaches in packages (*e.g.*, card boxes) at the weighing area.

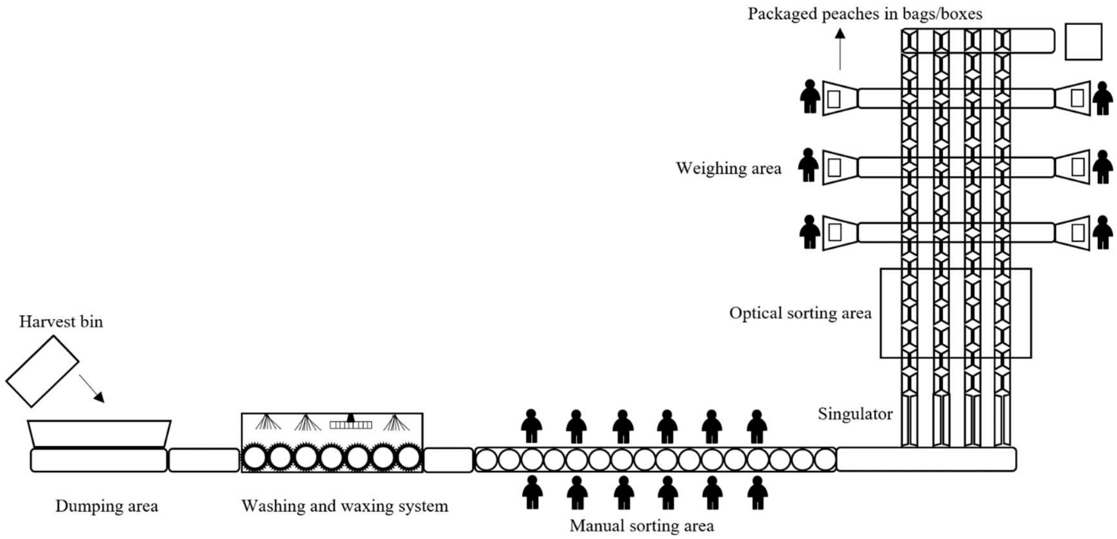

Figure 2-1 Fresh peach packing line workflow

Foodborne pathogens can be transmitted to fresh peaches from contaminated equipment surfaces and/or be dispersed to different batches of fruits due to cross-contamination through series of packing procedures. A recent study found that *Salmonella enterica* carried by fresh cucumbers can subsequently be transferred onto uninoculated ones through brush waxing (Jung & Schaffner, 2020). A series study conducted in a pilot fresh-cut produce packing facility involving *E. coli* O157:H7 inoculated leafy-greens, demonstrated the highest levels of contamination on surfaces were achieved on the conveyor belt and shredder, and the pathogen was continuously detected in the subsequent uninoculated batches (Buchholz, Davidson, Marks, Todd, & Ryser, 2012a; 2012b). In a fresh cabbage packing shed, *Salmonella* found on the conveyor belt was linked to the contamination of processed cabbage samples (Prazak, Murano, Mercado, & Acuff, 2002). The presence of *E. coli* O157:H7 was determined on a sorting contact surface in a commercial fresh peach packing house in South Africa, indicating that the potential microbial contamination on equipment surfaces should be of great concern (Duvenage & Korsten, 2017).

Pathogens can also be introduced to fresh peaches by sorters and packers including the gloves wore by them. Workers with poor hygiene or working while ill can transfer human or foodborne pathogens to food via fecal-oral transmission, especially when hand-washing procedures are not strictly followed (Todd, Greig, Bartleson, & Michaels, 2008). Based on the result from the fresh peach packing house study mentioned previously, the authors pointed out that the presence of both *E. coli* O157:H7 and *Staphylococcus aureus* on packers' hands reflected the failure of workers adhering to the Global Good Agricultural Practices during packing (Duvenage & Korsten, 2017). When glove barriers are used during packing, they are also susceptible to foodborne pathogens through touching contaminated surfaces, resulting in microbial cross-contamination of the produce. A previous laboratory study reported that when preparing fresh-cut produce, the transfer rate of *E. coli* O157:H7 from gloves to carrots can reach as high as 30% (Jensen, Danyluk, Harris, & Schaffner, 2017).

2.4 Current regulatory requirements for fresh produce during packing

The Produce Safety Rule (PSR) contains the "standards for the growing, harvesting, packing, and holding of produce for human consumption" and is the current U.S. federal regulation for mitigating foodborne illnesses caused by fresh produce consumption in the U.S. under the Food Safety Modernization Act (FSMA) of 2011 (U.S. FDA, 2015). Postharvest water, employee training, health and hygiene, equipment, tools, building, and sanitation are the key aspects emphasized for produce packing, generic *E. coli* should be non-detectable in every 100 ml of postharvest water, and periodic water testing should be conducted if the water is not sourced from a certified public water system; supervisors and workers who directly handle the produce or produce-contact surfaces should be trained on hygienic practices and avoid direct contact if they are ill; equipment and containers used during packing should be regularly maintained and properly cleaned (U.S. FDA, 2015). Excluding certain agricultural water requirements that have been postponed until January 26, 2024, covered business entities in different sizes should have complied with the PSR on their respective compliance date no later than January 27, 2020 (U.S. FDA, 2019).

Besides meeting the minimum standards based on the PSR, when certain requests are made by produce buyers such as retail or wholesale markets, growers should be certified by Good Agricultural Practices (GAP) including Good Handling Practices (GHP) programs, or other voluntary audit programs offered by private accredited third-party auditing companies, state departments of agriculture, and USDA (Olsen & Allen, 2017). For example, the "USDA GAP&GHP audit verification program" requires the certified farm to have a documented food safety program, an effective mock recall plan, a documented traceability plan, *etc.* whereas the PSR only requires conducting a hazard analysis (USDA, 2019; Olsen & Allen, 2017).

2.5 Detection of indicator microorganisms for fresh produce and environmental hygiene

The prevalence of foodborne pathogens on fresh produce and in the produce production environments is sporadic, and normally at a low level. The detection of foodborne pathogens could be time-consuming and expensive (Van Pelt et al., 2018). Instead, testing for indicator microorganisms has been widely adopted by the food industry, regulatory agencies, and researchers to evaluate the overall microbial quality of food and water, the hygienic status of the food production environment, and the effectiveness of a sanitation procedure. (Tortorello, 2003).

Total aerobes are one of the most used indicators. A higher level of total aerobes on an equipment surface after cleaning and sanitizing may indicate insufficient sanitation practices (Buchanan & Oni, 2012). Yeasts and molds are two types of fungi that can be indigenous on fresh fruits and vegetables; however, a higher level of yeasts and molds may be linked to a high possibility of food spoilage and the end of the shelf-life (Moss, 2008). Coliforms, including *E. coli*, could indicate potential fecal contamination as *E. coli* are generally present in the gastrointestinal tract of warm-blooded animals. Other genera of the coliform groups such as *Enterobacter*, *Klebsiella,* and *Citrobacter* may be of plant origin or ubiquitous in the environment (Tortorello, 2003). Thermo-tolerant coliforms are defined as a subgroup of coliforms that can ferment lactose at 44.5 ±0.2 °C and produce acid and gas. *E. coli* and some enteric bacteria such as *Klebsiella* spp. are under this category (Feng, Weagant, Grant, & Burkhardt, 2002). The increased levels of thermo-tolerant coliforms may suggest the high probability of enteric pathogen contamination (Pan et al., 2015; Parish, 1998). Both *Enterococcus* and *E. coli* have been used as recommended indicators for human fecal pollution in water, while studies reported enterococci have even better performance than fecal coliforms to reflect specifically the hygiene on hands. (Boehm & Sassoubre, 2014). Considering different indicators can reflect different microbiological properties, a diverse group of indicators should be chosen when evaluating the sanitation practices in a food processing environment and microbial quality of the food.

2.6 Interaction between bacteria and fresh peaches or peach contact surfaces

2.6.1 Peach surface morphology and bacterial attachment on fresh peaches

The surface of a fresh peach is covered by a layer of fuzzy trichomes (*i.e.,* indumentum) composed of polysaccharides, waxes, cutin, and a trace amount of phenolics such as flavonoids (Fernández et al., 2011). Along with the cuticle underneath, the peach surface structure may act as a plant defense measure to prevent the surface from water loss, solar radiation damage, as well as the potential insect and plant pathogen intrusion (Fernández et al., 2011; Karabourniotis, Liakopoulos, Nikolopoulos, & Bresta, 2020; Lu et al., 2015; Stavrianakou et al., 2010). Hence, the mechanical removal of peach trichomes through commercial brushing may create micro-wounds on the peach surface and make it more susceptible to bacterial attachment (Crisosto & Valero, 2008). In addition, a recent electron microscopy study found that *E. coli* O157:H7 can hide within the wax matrix of the cuticle layer and attach to cracks, scars, and the stomata of both intact and hair-removed peaches (Yan, Gurtler, Mattheis & Fan, 2020). It was observed that both *L. monocytogenes* and *Salmonella* Typhimurium can attach on intact peach surfaces immediately after inoculation, while the minimum time needed for the attachment of *E. coli* O157:H7 and *Staphylococcus aureus* was 30 s and 1 h, respectively. (Collignon & Korsten, 2010).

2.6.2 Growth and survival of foodborne pathogens on fresh peaches

Fresh peaches normally have a pH of around 3.30 to 4.05 (Anon, 1962), but it may vary for different varieties. For example, the pH of certain white-fleshed cultivars was observed to be 4.87 (Xi et al., 2017). Although this pH condition is not usually favorable for foodborne pathogens to grow, it is reported that the growth of typical foodborne pathogens such as *Salmonella* spp. and *L. monocytogenes* can be initiated at the pH as low as 4.05 and 4.70, respectively (Chung & Goepfert, 1970; Petran & Zottola, 1989). Furthermore, instead of increasing in population than their initials, foodborne pathogens can survive in low pH food matrixes for a prolonged period. It was noticed that *Escherichia coli* O157:H7 could survive from the acidic stress in apple cider with a pH of 3.6 to 4.0 for 10 to 31 days at 8 °C (Zhao, Doyle, & Besser, 1993). Collignon and Korsten (2010) conducted a case study on the growth and survival of foodborne pathogens on stone fruits under proper time-temperature control which mimics the fresh stone fruit supply chain condition. They concluded that when *L. monocytogenes* and *E. coli* O157:H7 were introduced at the harvesting point with an artificially high inoculation level of 5 log CFU/fruit, they both grew on fresh peaches at 21 °C for the first day of holding, then survived at 0.5 °C for 13 days followed by at 4 °C for 6 days. A very recent study confirmed the long-term survival of *L. monocytogenes* outbreak strains on fresh peaches when 1.7 log CFU/ml inoculated *L. monocytogenes* ST328 remained persistent on fresh yellow peaches for at least 19 days at 4 °C with only 0.8 log CFU/ml reduction (De Jesus et al., 2020). These results indicated the importance of preventing microbial contamination in peach production especially during postharvest handling and having effective microbial intervention measures to improve microbial quality of fresh peaches during packing.

2.6.3 Bacterial adhesion in fresh peach packing environment

In a fresh peach packing environment, bacteria may survive, multiply, and become residents on peach direct and indirect contact surfaces through bacterial adhesion and biofilm formation. Bacterial adhesion is the first and fundamental step of biofilm formation, and it highly depends on the specific interaction between bacterial cells and their contact surfaces (Tuson & Weibel, 2013). Most reviews defined it as a two-phase process (Berne, Ducret, Hardy, & Brun, 2015; Goulter, Gentle, & Dykes, 2009; Van Houdt & Michiels, 2010).

The first phase is a reversible and non-specific attachment mainly affected by different physicochemical forces, such as hydrodynamic and electrostatic forces, acid-based interaction, as well as some weak forces such as van der Waals force and hydrogen bond (Berne et al., 2015; Khelissa, Abdallah, Jama, Faille, & Chihib, 2017). For instance, most bacterial cells are negatively charged (Rivas, Fegan, & Dykes, 2005) and tend to attach to hydrophobic surfaces, with little surface energy, or surfaces that are positively charged (Goulter et al., 2009; Pringle & Fletcher, 1983). At this phase, bacterial cells can experience both repulsive and attractive forces, and they can be easily removed by rinsing without mechanical scrubbing on the surface (Bayoudh, 2004).

The irreversible bacterial attachment is primarily mediated by bacterial surface structures such as fimbria, pili, and flagella (Berne, Ellison, Ducret, & Brun, 2018), that can facilitate bacterial cells to overcome the repulsive forces and eventually lock them onto the abiotic surface (Dunne, 2002). This process is also significantly affected by surface hydrophobicity, surface charges, surface topography, as well as environmental conditions such as pH and salinity (Berne et al., 2015). Once the bacterial cells are fixed irreversibly, they can further multiply and form mature biofilm by producing extracellular components such as exopolysaccharides. Bacterial cells within this insoluble matrix can be more resistant to antimicrobial compounds and require more mechanical forces before sanitizers can penetrate, ultimately, become a challenge for plant sanitation (Dunne, 2002; Khelissa et al., 2017).

2.7 Current commercial fresh produce washing systems

Commercial fresh produce washing systems have been thoroughly reviewed by Pao, Long III, Kim, and Kelsey (2012). The goal of postharvest washing is to remove soil, debris, plant tissue, and microbial contaminants from the surface of produce by mechanical rubbing/brushing, along with water rinsing, cleaning, and sanitizing. The commercial produce washing systems can be classified into two models: immersion and non-immersion systems.

The dumping tank/fluming system is a typical immersion washing system. Ideally, by submerging fresh produce into the sanitizer-added wash water, bacterial cells released into the aquatic system can be more easily inactivated by the dispersed disinfectant (Pao et al., 2012; Pietrysiak, Smith, & Ganjyal, 2019). However, given that water used in the system is usually circulated, organic matters removed from the incoming produce may accumulate in the wash tank and interfere with the efficacy of sanitizer treatment. Consequently, foodborne pathogens that surviving in the water system can cross-contaminate different batches of product, or even cause microbial infiltration (Banach, Sampers, Van Haute, & van der Fels-Klerx, 2015). Numerous studies have mentioned in commercial washing tank systems, due to microbial cross-contamination in wash water, the actual microbial reduction on produce is only 1-2 log (Barrera, Blenkinsop, & Warriner, 2012). Hence, studies on the washing tank system for fresh produce washing usually focused on treating the wash water with sanitizers and maintaining a valid sanitizer concentration to prevent the risk of microbial cross-contamination (Gil, Selma, Lopez-Galvez, & Allende, 2009; Murray, Wu, Shi, Jun Xue, & Warriner, 2017).

The overhead washing system is a representative non-immersion washing system, consisting of overhead sprayers and brush roller beds (Pao et al., 2012). The overhead sprayers can directly apply wash water onto the produce, while brush roller beds can reduce the surface tension of the produce by mechanical scrubbing and stimulate the detachment of the contaminants. One of the advantages of this system is that the concentration of sanitizer added in spray water can be easily controlled, and it uses less water comparing to the dumping tank system. Nevertheless, instead of water, the microbial cross-contamination may be mediated by brush rollers, consequently, hindering the microbial reduction during washing.

2.8 Sanitizers used in fresh produce postharvest washing

Chlorine is the most extensively used and cost-effective sanitizer in the fresh produce industry. The optimal pH for chlorination is 6.0 to 7.5. The recommended concentration is 40-200 ppm (Suslow, 2000). The commercial products include the liquid form of concentrated sodium hypochlorite solution, as well as the solid form of calcium hypochlorite tablets or granules (Suslow, 2000). Both products should be pre-mixed in water to release the hypochlorite ion and combine with the hydrogen ion to form the effective compound, hypochlorous acid. It is a powerful oxidant that can cause a series of bacterial cell damages, such as altering cell membrane permeability, reacting with cellular DNA, inactivating enzymes, etc. (Dukan & Touati, 1996). A previous laboratory study reported that submerging fresh peaches in 100 ppm sodium hypochlorite solution for 5 min reduced total mesophilic aerobic counts on peach surfaces by 2 log CFU/cm^2, and yeasts or molds were non-detectable on peach surfaces after the washing, which resulted in more than 2 log reduction (Calvo, Redondo, Remón, Venturini, & Arias, 2019). With a commercial overhead brush roller washing system, spray washing fresh tomatoes with 100 ppm sodium hypochlorite for 15 s, can reduce at least 3 log CFU/ml *Pectobacterium* from the surface of fresh tomatoes (Balaguero, Sreedharan, & Schneider, 2015). Tokarskyy and Korda (2019) found that the native microflora on fresh tomatoes, total plate count, gram-negative bacteria counts, and yeast and mold counts were reduced by 1.12, 1.19, 1.66 log CFU/fruit, respectively, after treating them with 100 ppm chlorine treatemnt for 1 min by the overhead brush roller washing system.

One significant disadvantage of a chlorine solution is that once the hypochlorous acid and hypochlorite ion join the reactions with organic matters in the wash water, they immediately lose the bactericidal effects and become combined chlorine (Richardson, Thruston, Caughran, Collette, & Patterson, 1998). As a result, when in the washing tank systems, more studies have been exploring the minimum free chlorine needed for preventing cross-contamination. For example, Gómez-López, Lannoo, Gil, and Allende (2014) reported that 5 to 7 ppm free chlorine can inactivate *E. coli* O157:H7 in a washing tank during fresh-cut spinach washing process in a pilot plant. In addition, chlorine combining organic matters can generate disinfectant by-products such as trihalomethanes, haloacetic acids, haloketones, and chloropicrin, which are toxic and associated with potential health risks (Gil et al., 2009; Suslow, 2000).

Chlorine dioxide is a strong oxidant, and mainly reacts with the cell membrane and inactivates different proteins, resulting in disinfection (Gómez-López, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009). The use of chlorine dioxide is permitted by the U.S. FDA to treat wash water for fresh fruits and vegetables. The residual concentration of chlorine dioxide should not be higher than 3 ppm, followed by rinsing with portable water (21 CFR 173). Since it does not generate chlorinated by-products, it has been promoted as a safer alternative sanitizer for chlorine (Gómez-López et al., 2009; Richardson et al., 1998; Wu & Kim, 2007). The oxidation power of chlorine dioxide is 2.5 times higher than chlorine (Benarde, Snow, Olivieri, & Davidson, 1967), which can make it more effective than chlorine in microbial inactivation. For example, in a laboratory condition, 5 ppm chlorine dioxide was 34 times faster than 200 ppm sodium hypochlorite to achieve 1 log reduction of *Salmonella* on fresh mangoes in a water bath with simulated turbidity as found in the packing process (Contreras-Soto, Medrano-Felix, Valdez-Torres, Chaidez, & Castro-Del Campo, 2019). When using overhead washing and roller brush washing system, Pao, Kelsey, and Long Iii (2009) found that spraying fresh tomato surfaces with 5 ppm chlorine dioxide for 10 to 60 s significantly reduced the level of air-dried *Salmonella* by 4.4±0.6 to5.2±0.1 log cycles. One of the limitations of chlorine dioxide is due to the explosive property of the chlorine dioxide gas. It should be prepared onsite by a chlorine dioxide generator and dissolved in the wash water. In a commercially setting, the equipment investment can be higher than the normal chlorine washing system (Wu & Kim, 2007).

Peroxyacetic acid (PAA) is another sanitizer that has the potential to be an alternative to chlorine, as it has less toxic by-product residues and is less combined with organic matter accumulated in a washing tank (Luukkonen & Pehkonen, 2017). It has a wider pH activity ranged from 3.0 to 7.5 (Banach et al., 2015). The PAA solution can be generated by mixing acetic acid and hydrogen peroxide in the wash water. U.S. FDA's standard for PAA in fresh produce wash water is a maximum of 80 ppm (21CFR173.315). Once PAA is dissolved in water, it forms several groups of free radicals, known as reactive oxygen species, resulting in DNA damage, protein denaturation, changed cell membrane permeability, etc. (Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009). The efficacy of PAA in reducing *Salmonella* contamination on fresh mangoes has been compared with other sanitizers in a simulated mango washing tank in a packinghouse. The study found that treating *Salmonella* contaminated fresh mangoes or wash water with 200 ppm chlorine or 80 ppm PAA for 2 min can reduce the initial level of *Salmonella* from 7 log CFU/fruit or 7 log CFU/ml to less than the detection limit. Nevertheless, the mango and water samples treated by 5 ppm chlorine dioxide constantly tested positive for *Salmonella* (Mathew, Muyyarikkandy, Bedell, & Amalaradjou, 2018). Another study illustrated that washing fresh tomatoes with 100 ppm of sodium hypochlorite, 5 ppm of chlorine dioxide or 80 ppm of peracetic acid for 15 s with an overhead spray and roller brush system can achieve a similar reduction of *Pectobacterium* (3 logs) on fresh tomatoes (Balaguero et al., 2015). Therefore, PAA could be a potential alternative sanitizer to chlorine during commercial fresh produce packing.

Ozone, as an antimicrobial agent, is generally recognized as safe to treat bottled water in the U.S. (21CFR184.1563), and it is an approved pesticide chemical to safely use on raw agricultural commodities and water that has direct contact with them during production (21CFR173.368). The oxidating property of ozone is much stronger than chlorine, chlorine dioxide, and PAA (Banach et al., 2015). Through oxidation, ozone can disrupt the integrity of the microbial cell surface causing cellular content leakage, as well as destroy cellular proteins and nucleic acids (Guzel-Seydim, Greene, & Seydim, 2004). Ozone can be applied in a gaseous or aqueous form on fresh produce. Gaseous ozone is commonly released into the storage atmosphere to achieve extended produce shelf-life and decelerated ripeness, and it can also be applied into a treatment chamber before storage (Horvitz & Cantalejo, 2014). Since high levels of ozone gas is toxic to humans, the concentration of ozone in the treatment chamber should be maintained low and monitored regularly (Glowacz & Rees, 2016). A previous study reported that 5 ppm ozone gas can inactivate 2.2 log *E. coli* on the surface of tomatoes after 3 min in a treatment chamber (Bermúdez-Aguirre & Barbosa-Cánovas, 2013). The aqueous form of ozone, namely, ozonated water, is more often used before storage to sanitizing produce wash water during postharvest processing (Glowacz & Rees, 2016). Considering ozone gas is barely soluble in water, many experimental studies have found that continuously ozonated water is more effective in reducing microbial loads on fresh produce comparing to pre-ozonated water. For instance, one study found that after immerging bell peppers or fresh-cut lettuce in 0.5 ppm pre-ozonated water for 15 min, the reduction of aerobic mesophiles on these vegetable samples were always less than 0.5 log. However, when the samples were fully dipped in the same concentration of continuously ozonated water, the microbial loads on both types of vegetables were reduced by nearly 2 logs (Alexopoulos et al., 2013). Therefore, the investment for generation and injection equipment, as well as the maintenance could be costly (Glowacz & Rees, 2016).

Electrolyzed oxidizing water (EOW) has been considered as another potential substitute for chlorine-based sanitizers (Pietrysiak et al., 2019). It can be easily generated through the electrolysis of sodium chloride solution in an electrolysis generator with an anode side and a cathode side separated by a membrane in the middle. The active agents of EOW such as hypochlorous acid and sodium hypochlorite are produced at the anode side of the device. The EOW at this side has a pH less than 2.5 and oxidation reductive potential (ORP) higher than 1000 mV, which are mainly responsible for the antimicrobial property of this sanitizer (Huang, Hung, Hsu, Huang, & Hwang, 2008; Park, Alexander, Taylor, Costa, & Kang, 2009). Given the fact that a relatively low pH can be corrosive to the produce packing equipment and facility, recent studies have explored the efficacies of low acidic or neutral EOW in reducing microbial contamination on fresh produce (Calvo et al., 2019; Ding et al., 2015; Machado et al., 2016). One laboratory study found that when fresh peaches were washed in the electrolyzed water with an active agent concentration of 165 ppm (pH 6.7, ORP 775 mV) for 5 min, the total mesophilic aerobes count, as well as the yeasts and molds count was reduced by more than 2 logs; the equivalent result was observed when the authors changed the sanitizer to 100 ppm of sodium hypochlorite (Calvo et al., 2019). However, EOW has only been studied at the experimental level. More studies on the efficacy of this sanitizer in fresh produce washing should be done on a larger scale, for instance, in a simulated produce packing house.

References

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

Alexopoulos, A., Plessas, S., Ceciu, S., Lazar, V., Mantzourani, I., Voidarou, C., Stavropoulou, E., Bezirtzoglou, E. (2013). Evaluation of ozone efficacy on the reduction of microbial population of fresh cut lettuce (*Lactuca sativa*) and green bell pepper (Capsicum annuum). *Food Control, 30*(2), 491-496. https://doi.org/10.1016/j.foodcont.2012.09.018

Allen, K. (2014, August 4). Stone fruit recall widens on possible *Listeria* outbreak. [news]. Retrieved from https://www.cnbc.com/2014/08/04/stone-fruit-recall-widens-on-possible-listeria-outbreak.html. Accessed 19 April 2021.

Andrews, A. J. (2018). What are the different varieties of peaches? SFGATE. https://homeguides.sfgate.com/different-varieties-peaches-42398.html. Accessed 19 April 2021.

Anon. 1962. pH values of food products. *Food Engineering*. 34(3): 98-99

Aubert, C., & Chalot, G. (2020). Physicochemical characteristics, vitamin C, and polyphenolic composition of four European commercial blood-flesh peach cultivars (*Prunus persica* L. Batsch). *Journal of Food Composition and Analysis, 86*. https://doi.org/10.1016/j.jfca.2019.103337

Balaguero, A. N., Sreedharan, A., & Schneider, K. R. (2015). Effect of overhead spray and brush roller treatment on the survival of *Pectobacterium* and *Salmonella* on tomato surfaces. *Journal of Food Protection, 78*(1), 51-56. https://doi.org/10.4315/0362-028X.JFP-14-254

Banach, J. L., Sampers, I., Van Haute, S., & van der Fels-Klerx, H. J. (2015). Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. *International Journal of Environmental Research and Public Health, 12*(8), 8658-8677. https://doi.org/10.3390/ijerph120808658

Barrera, M. J., Blenkinsop, R., & Warriner, K. (2012). The effect of different processing parameters on the efficacy of commercial post-harvest washing of minimally processed spinach and shredded lettuce. *Food Control, 25*(2), 745-751. https://doi.org/10.1016/j.foodcont.2011.12.013

Bartz, F. E., Lickness, J. S., Heredia, N., Fabiszewski de Aceituno, A., Newman, K. L., Hodge, D. W., Jaykus, L-A., García, S., & Leon, J. S. (2017). Contamination of fresh produce by microbial indicators on farms and in packing facilities: elucidation of environmental routes. *Applied and Environmental Microbiology, 83*(11), e02984-02916. https://doi.org/10.1128/AEM.02984-16

Bayoudh, S., Othmane, A., Bettaieb, F., Bakhrouf, A., Ouada, H. B., & Ponsonnet, L. (2006). Quantification of the adhesion free energy between bacteria and hydrophobic and hydrophilic substrata. *Materials Science and Engineering: C, 26*(2), 300-305. https://doi.org/10.1016/j.msec.2005.10.045

Benarde, M. A., Snow, W. B., Olivieri, V. P., & Davidson, B. (1967). Kinetics and mechanism of bacterial disinfection by chlorine dioxide. *Applied microbiology, 15*(2), 257-265.

Bento, C., Gonçalves, A. C., Silva, B., & Silva, L. R. (2020). Peach (*Prunus Persica*): phytochemicals and health benefits. *Food Reviews International*, 1-32. https://doi.org/10.1080/87559129.2020.1837861

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2013). Disinfection of selected vegetables under nonthermal treatments: chlorine, acid citric, ultraviolet light and ozone. *Food Control, 29*(1), 82-90. https://doi.org/10.1016/j.foodcont.2012.05.073

Berne, C., Ducret, A., Hardy, G. G., & Brun, Y. V. (2015). Adhesins involved in attachment to abiotic surfaces by Gram-gegative bacteria. *Microbiology Spectrum, 3*(4). https://doi.org/10.1128/microbiolspec.MB-0018-2015

Berne, C., Ellison, C. K., Ducret, A., & Brun, Y. V. (2018). Bacterial adhesion at the single-cell level. *Nature Reviews Microbiology, 16*(10), 166+. Retrieved from https://link.gale.com/apps/doc/A572946261/HRCA?u=uga&sid=HRCA&xid=0929d675. Accessed 19 April 2021.

Boehm A. B., & Sassoubre L. M. (2014) Enterococci as indicators of environmental fecal contamination. In: Gilmore, M. S., Clewell, D. B., Ike Y, et al., editors. Enterococci*: From Commensals to Leading Causes of Drug Resistant Infection.* Boston: Massachusetts Eye and Ear Infirmary

Borges, C. A., Cardozo, M. V., Beraldo, L. G., Oliveira, E. S., Maluta, R. P., Barboza, K. B., Werther, K., & Avila, F. A. (2017). Wild birds and urban pigeons as reservoirs for diarrheagenic *Escherichia coli* with zoonotic potential. *Journal of Microbiology, 55*(5), 344-348. https://doi.org/10.1007/s12275-017-6523-3

Bors, W., & Saran, M. (1987). Radical scavenging by flavonoid antioxidants. *Free Radical Research Communications, 2*(4-6), 289-294. https://doi.org/10.3109/10715768709065294

Brickell, C. D., Alexander, C., Cubey, J. J., David, J. C., Hoffman, M. H. A., Leslie, A. C., Malécot, V., Jin, X., et al. (Eds.). (2009). International code of nomenclature for cultivated plants. *Scripta Horticulturae* (8th ed.). International Society of Horticultural Science. 10: 1–184.

Buchanan, R. L., & Oni, R. (2012). Use of microbiological indicators for assessing hygiene controls for the manufacture of powdered infant formula. *Journal of Food Protection, 75*(5), 989-997. https://doi.org/10.4315/0362-028X.JFP-11-532

Byrne, D. H., Raseira, M. B., Bassi, D., Piagnani, M. C., Gasic, K., Reighard, G. L., Moreno, M. A., & Pérez, S. (2012). Peach. In *Fruit Breeding* (pp. 505-569). Springer.

Cai, Y., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants of the *Amaranthaceae*. *Journal of Agricultural and Food Chemistry, 51*(8), 2288-2294. https://doi.org/10.1021/jf030045u

Calvo, H., Redondo, D., Remón, S., Venturini, M. E., & Arias, E. (2019). Efficacy of electrolyzed water, chlorine dioxide and photocatalysis for disinfection and removal of pesticide residues from stone fruit. *Postharvest Biology and Technology, 148*, 22-31. https://doi.org/10.1016/j.postharvbio.2018.10.009

Campbell, O. E., Merwin, I. A., & Padilla-Zakour, O. I. (2011). Nutritional quality of New York peaches and apricots. *New York Fruit Quarterly, 19*(4), 12-16.

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in Microbiology, 10*(2667). https://doi.org/10.3389/fmicb.2019.02667

Casamali, B., van Iersel, M. W., & Chavez, D. J. (2021). Nitrogen partitioning in young “Julyprince” peach trees grown with different irrigation and fertilization practices in the Southeastern United States. *Agronomy, 11*(2). https://doi.org/10.3390/agronomy11020350

CDC. (2018). National Outbreak Reporting System (NORS). NORS dashboard. Retrieved from https://wwwn.cdc.gov/norsdashboard/. Accessed 19 April 2021.

CDC. (2020). Outbreak of *Salmonella* Enteritidis infections linked to peaches. Retrieved from https://www.cdc.gov/salmonella/enteritidis-08-20/index.html. Accessed 19 April 2021.

Chen, Y., Burall, L. S., Luo, Y., Timme, R., Melka, D., Muruvanda, T., Payne, J., Wang, C., Kastanis, G., Maounounen-Laasri, A., De Jesus, A. J., Curry, P. E., Stones, R., K'Aluoch, O., Liu, E., Salter, M., Hammack, T. S., Evans, P. S., Parish, M., Allard, M. W., Datta, A., Strain, E. A., & Brown, E. W., (2016). *Listeria monocytogenes* in stone fruits linked to a multistate outbreak: enumeration of cells and whole-genome sequencing. *Applied and Environmental Microbiology, 82*(24), 7030-7040. https://doi.org/10.1128/aem.01486-16.

Chen, Y., Evans, P., Hammack, T. S., Brown, E. W., & Macarisin, D. (2016). Internalization of *Listeria monocytogenes* in whole avocado. *Journal of Food Protection, 79*(8), 1440-1445. https://doi.org/10.4315/0362-028X.JFP-16-075

Chung, K. C., & Goepfert, J. M. (1970). Growth of *Salmonella* at low pH. *Journal of Food Science, 35*(3), 326-328. https://doi.org/10.1111/j.1365-2621.1970.tb12176.x

Collignon, S., & Korsten, L. (2010). Attachment and colonization by *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteri*ca subsp. *enterica* serovar Typhimurium, and *Staphylococcus aureus* on stone fruit surfaces and survival through a simulated commercial export chain. *Journal of Food Protection, 73*(7), 1247-1256. https://doi.org/10.4315/0362-028X-73.7.1247

Contreras-Soto, M. B., Medrano-Felix, J. A., Valdez-Torres, B., Chaidez, C., & Castro-Del Campo, N. (2019). Chlorine dioxide: an evaluation based on a microbial decay approach during mango packing process. *International Journal of Environmental Health Research*, 1-12. https://doi.org/10.1080/09603123.2019.1670785

Crisosto, C. H., & Valero, D. (2008). 22. Harvesting and postharvest handling of peaches for the fresh market. *The Peach: Botany, Production and Uses, CAB International, Wallingford*, 575-596.

Cronquist, A. (1981). *Integrated system of classification of flowering plants.* New York: Columbia University Press.

Cui, J., Lian, Y., Zhao, C., Du, H., Han, Y., Gao, W., Xiao, H. and Zheng, J. (2019). Dietary fibers from fruits and vegetables and their health benefits via modulation of gut microbiota. *Comprehensive Reviews in Food Science and Food Safety, 18*(5), 1514-1532. https://doi.org/10.1111/1541-4337.12489

Dabbou, S., Maatallah, S., Castagna, A., Guizani, M., Sghaeir, W., Hajlaoui, H., & Ranieri, A. (2017). Carotenoids, phenolic profile, mineral content and antioxidant properties in flesh and peel of *Prunus persica* fruits during two maturation stages. *Plant Foods Human Nutrition, 72*(1), 103-110. https://doi.org/10.1007/s11130-016-0585-y

Dahl, W. J., Agro, N. C., Eliasson, Å. M., Mialki, K. L., Olivera, J. D., Rusch, C. T., & Young, C. N. (2017). Health benefits of fiber fermentation. *Journal of the American College of Nutrition, 36*(2), 127-136. https://doi.org/10.1080/07315724.2016.1188737

De Jesus, A. J., Sheth, I., Kwon, H. J., Gao, Z., Palmer, J., Hur, M., Hammack, T. S., Macarisin, D., & Chen, Y. (2020). Survival of a serotype 4b strain and a serotype 1/2a strain of *Listeria monocytogenes*, isolated from a stone fruit outbreak investigation, on whole stone fruit at 4 °C. *International Journal of Food Microbiology, 334*, 108801. https://doi.org/10.1016/j.ijfoodmicro.2020.108801

Ding, T., Ge, Z., Shi, J., Xu, Y.-T., Jones, C. L., & Liu, D.-H. (2015). Impact of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads and quality of fresh fruits. *LWT - Food Science and Technology, 60*(2, Part 2), 1195-1199. https://doi.org/10.1016/j.lwt.2014.09.012

Dukan, S. A. M., & Touati, D. (1996). Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. *Journal of Bacteriology, 178*(21), 6145-6150.

Dunne, W. M. (2002). Bacterial adhesion: seen any good biofilms lately? *Clinical Microbiology Reviews, 15*(2), 155. https://doi.org/10.1128/CMR.15.2.155-166.2002

Duvenage, S., & Korsten, L. (2017). Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer. *Food Control, 78*, 374-382. https://doi.org/10.1016/j.foodcont.2017.03.003

FAO. (2020). Food and agriculture data. FAOSTAT. Retrieved from http://www.fao.org/faostat/en/. Accessed 19 April 2021.

Faust, M., & Timon, B. (1995). Origin and dissemination of peach. *Horticultural Reviews, 17*, 331-379.

Feng, P., Weagant, S. D., Grant, M. A., & Burkhardt, W. (2002). BAM: Enumeration of *Escherichia coli* and the coliform bacteria. *Bacteriological analytical manual*, *13*.

Gereffi, S., Sreedharan, A., & Schneider, K. R. (2015). Control of *Salmonella* cross-contamination between green round tomatoes in a model flume system. *Journal of Food Protection, 78*(7), 1280-1287. https://doi.org/10.4315/0362-028X.JFP-14-524

Gil, M. I., Selma, M. V., Lopez-Galvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology, 134*(1-2), 37-45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., & Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry, 50*(17), 4976-4982. https://doi.org/10.1021/jf020136b

Glowacz, M., & Rees, D. (2016). The practicality of using ozone with fruit and vegetables. *Journal of the Science of Food and Agriculture, 96*(14), 4637-4643. https://doi.org/10.1002/jsfa.7763

Gomba, A., Chidamba, L., & Korsten, L. (2017). Viable microbial loads on citrus carpoplane during packhouse processing and survival of foodborne pathogens in reconstituted postharvest fungicides. *Journal of Food Safety, 37*(4). https://doi.org/10.1111/jfs.12357

Gómez-López, V. M., Lannoo, A.-S., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control, 42*, 132-138. https://doi.org/10.1016/j.foodcont.2014.01.034

Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: a review. *Trends in Food Science & Technology, 20*(1), 17-26. https://doi.org/10.1016/j.tifs.2008.09.005

Goulter, R. M., Gentle, I. R., & Dykes, G. A. (2009). Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Letter in Applied Microbiology, 49*(1), 1-7. https://doi.org/10.1111/j.1472-765X.2009.02591.x

Guzel-Seydim, Z. B., Greene, A. K., & Seydim, A. C. (2004). Use of ozone in the food industry. *LWT - Food Science and Technology, 37*(4), 453-460. https://doi.org/10.1016/j.lwt.2003.10.014

Hans, M., Shah, M. A., & Bansal, R. (2020). Peach. In *Antioxidants in Fruits: Properties and Health Benefits* (pp. 449-459). Springer.

Holvoet, K., Jacxsens, L., Sampers, I., & Uyttendaele, M. (2012). Insight into the prevalence and distribution of microbial contamination to evaluate water management in the fresh produce processing industry. *Journal of Food Protection, 75*(4), 671-681. https://doi.org/10.4315/0362-028X.JFP-11-175

Horvitz, S., & Cantalejo, M. J. (2014). Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Review Food Science Nutrition, 54*(3), 312-339. https://doi.org/10.1080/10408398.2011.584353

Huang, Y.-R., Hung, Y.-C., Hsu, S.-Y., Huang, Y.-W., & Hwang, D.-F. (2008). Application of electrolyzed water in the food industry. *Food Control, 19*(4), 329-345. https://doi.org/10.1016/j.foodcont.2007.08.012

Jackson, B.R., Salter, M., Tarr, C., Conrad, A., Harvey, E., Steinbock, L., Saupe, A., Sorenson, A., Katz, L., Stroika, S., Jackson, K. A., Carleton, H., Kucerova, Z., Melka, D., Strain, E., Parish, M., & Mody, R. K. (2015). *Listeriosis* associated with stone fruit-United States, 2014. (2015). *Morbidity and mortality weekly report(MMWR),* 64 (10).

Jamshidi-Kia, F., Wibowo, J. P., Elachouri, M., Masumi, R., Salehifard-Jouneghani, A., Abolhasanzadeh, Z., & Lorigooini, Z. (2020). Battle between plants as antioxidants with free radicals in human body. *Journal of Herbmed Pharmacology, 9*(3), 191-199.

Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., & Bresta, P. (2020). Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research, 31*(1), 1-12.

Khelissa, S. O., Abdallah, M., Jama, C., Faille, C., & Chihib, N.-E. (2017). Bacterial contamination and biofilm formation on abiotic surfaces and strategies to overcome their persistence. *Journal of Material Environmental Science, 8*(9), 3326-3346.

Khelissa, J. S., Lee, M. S., & Kim, J. H. (2020). Recent updates on outbreaks of shiga toxin-producing *Escherichia coli* and its potential reservoirs. *Frontiers in Cellular and Infection Microbiology, 10*, 273. https://doi.org/10.3389/fcimb.2020.00273

Kritchevsky, S. B. (1999). β-Carotene, carotenoids and the prevention of coronary heart disease. *The Journal of Nutrition, 129*(1), 5-8. https://doi.org/10.1093/jn/129.1.5

Lane, D. Jr. (Ed). (n.d.) Elberta peach: the beginning of the Southern peach industry. University of Georgia. Retrieved from https://peaches.caes.uga.edu/history.html. Accessed 19 April 2021.

Lindell, C. A., Steensma, K. M. M., Curtis, P. D., Boulanger, J. R., Carroll, J. E., Burrows, C., Lusch, D. P., Rothwell, N. L., Wieferich, S. L., Henrichs, H. M., Leigh, D. K., Eaton, R. A., & Linz, G. M. (2016). Proportions of bird damage in tree fruits are higher in low-fruit-abundance contexts. *Crop Protection, 90*, 40-48. https://doi.org/10.1016/j.cropro.2016.08.011

Lu, X., Cai, H., Wu, Y., Teng, C., Jiang, C., Zhu, Y., & Jiang, L. (2015). Peach skin effect: a quasi-superhydrophobic state with high adhesive force. *Science Bulletin, 60*(4), 453-459.

Luukkonen, T., & Pehkonen, S. O. (2017). Peracids in water treatment: a critical review. *Critical Reviews in Environmental Science and Technology, 47*(1), 1-39.

Macarisin, D., Wooten, A., De Jesus, A., Hur, M., Bae, S., Patel, J., Evans, P., Brown, E., Hammack, T., & Chen, Y. (2017). Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling. *International Journal of Food Microbiology, 257*, 165-175. https://doi.org/10.1016/j.ijfoodmicro.2017.06.018

Machado, I., Meireles, A., Fulgêncio, R., Mergulhão, F., Simões, M., & Melo, L. F. (2016). Disinfection with neutral electrolyzed oxidizing water to reduce microbial load and to prevent biofilm regrowth in the processing of fresh-cut vegetables. *Food and Bioproducts Processing, 98*, 333-340. https://doi.org/10.1016/j.fbp.2016.02.008

Marcia, V., Luis, C.-Z., David, H. B., David, W. R., & Okie, W. R. (2007). Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. *Journal of the American Society for Horticultural Science, 132*(3), 334-340. https://doi.org/10.21273/JASHS.132.3.334

Mathew, E. N., Muyyarikkandy, M. S., Bedell, C., & Amalaradjou, M. A. (2018). Efficacy of chlorine, chlorine dioxide, and peroxyacetic acid in reducing *Salmonella* contamination in wash water and on mangoes under simulated mango packinghouse washing operations. *Frontiers in Sustainable Food Systems, 2*. https://doi.org/10.3389/fsufs.2018.00018

McCarthy, K., (2020, August 25). Fresh peaches recalled for possible multistate *Salmonella* outbreak, FDA says. [new]. Retrieved from https://abcnews.go.com/GMA/Food/fresh-peaches-recalled-multistate-salmonella-outbreak-fda/story?id=72574132. Accessed 19 April 2021.

Moss, M. O. (2008). Fungi, quality and safety issues in fresh fruits and vegetables. *Journal of Applied Microbiology, 104*(5), 1239-1243. https://doi.org/10.1111/j.1365-2672.2007.03705.x

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

NASS. (2020). Noncitrus Fruits and Nuts 2019 Summary. National Agricultural Statistics Service. U.S Department of Agriculture. Retrieved from https://usda.library.cornell.edu/concern/publications/zs25x846c. Accessed 19 April 2021.

Okie, W. T. (2012). "Everything is peaches down in Georgia": culture and agriculture in the American South. [Doctoral dissertation]. Retrieved from http://gilfind.uga.edu/. Accessed 19 April 2021.

Olsen, S., & Allen, K. (2017). "Farm food safety laws: FSMA vs. GAP". Utah State University Extension. *All Current Publications.* Paper 1750. Retrieved from https://digitalcommons.usu.edu/extension_curall/1750. Accessed 19 April 2021.

Pan, F., Li, X., Carabez, J., Ragosta, G. U. Y., Fernandez, K. L., Wang, E., Thiptara, A., Antaki, E., Atwill, E. R. (2015). Cross-sectional survey of indicator and pathogenic bacteria on vegetables sold from Asian vendors at farmers' markets in Northern California. *Journal of Food Protection, 78*(3), 602-608. https://doi.org/10.4315/0362-028X.JFP-14-095

Pao, S., Kelsey, D. F., & Long Iii, W. (2009). Spray washing of tomatoes with chlorine dioxide to minimize *Salmonella* on inoculated fruit surfaces and cross-contamination from revolving brushes. *Journal of Food Protection, 72*(12), 2448-2452.

Pao, S., Long III, W., Kim, C., & Kelsey, D. K. (2012). 5 Produce washers. In *Decontamination of fresh and minimally processed produce*. John Wiley & Sons, Incorporated.

Parish, M. E. (1998). Coliforms, *Escherichia coli* and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. *Journal of Food Protection, 61*(3), 280-284.

Park, E. J., Alexander, E., Taylor, G. A., Costa, R., & Kang, D. H. (2009). The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. *Food Microbiology, 26*(4), 386-390. https://doi.org/10.1016/j.fm.2008.10.013

Petran, R. L., & Zottola, E. A. (1989). A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science, 54*(2), 458-460. https://doi.org/10.1111/j.1365-2621.1989.tb03105.x

PHAC. (2020) Public Health Notice: Outbreak of *Salmonella* infections linked to peaches imported from the United States. October 15, 2020 - final update. Retrieved from https://www.canada.ca/en/public-health/services/public-health-notices/2020/outbreak-salmonella-infections-peaches-imported-united-states.html. Accessed 19 April 2021.

Pietrysiak, E., Smith, S., & Ganjyal, G. M. (2019). Food safety interventions to control *Listeria monocytogenes* in the fresh apple packing industry: a review. *Comprehensive Reviews in*

Food Science and Food Safety, 18(6), 1705-1726. https://doi.org/10.1111/1541-4337.12496

Prazak, A. M., Murano, E. A., Mercado, I., & Acuff, G. R. (2002). Prevalence of *Listeria monocytogenes* during production and postharvest processing of cabbage. *Journal of Food Protection, 65*(11), 1728-1734. https://doi.org/10.4315/0362-028X-65.11.1728

Rangarajan, A., Bihn, E. A., Gravani, R. B., Scott, D. L., & Pritts, M. P. (2000). Food safety begins on the farm: A Grower's Guide. Retrieved from https://hdl.handle.net/1813/2209. Accessed 19 April 2021.

Reig, G., Iglesias, I., Gatius, F., & Alegre, S. (2013). Antioxidant capacity, quality, and anthocyanin and nutrient contents of several peach cultivars [*Prunus persica* (L.) Batsch] grown in Spain. *Journal of Agricultural and Food Chemistry, 61*(26), 6344-6357. https://doi.org/10.1021/jf401183d

Richardson, S. D., Thruston, A. D., Caughran, T. V., Collette, T. W., & Patterson, K. S. (1998). Chemical by-products of chlorine and alternative disinfectants. *Food technology (Chicago), 52*(4), 58-61.

Rivas, L., Fegan, N., & Dykes, G. A. (2005). Physicochemical properties of Shiga toxigenic *Escherichia coli*. *Journal of Applied Microbiology, 99*(4).

Stavrianakou, S., Liakopoulos, G., Miltiadou, D., Markoglou, A. N., Ziogas, B. N., & Karabourniotis, G. (2010). Antifungal and antibacterial capacity of extracted material from non-glandular and glandular leaf hairs applied at physiological concentrations. *Plant Stress, 4*(1), 25-30.

Stern, K. R. (2006). *Introductory plant biology.* Boston, MA: McGraw-Hill Higher Education.

Suslow, T. (2000). Chlorination in the production and postharvest handling of fresh fruits and vegetables: Chap. 6. Fruit and vegetable processing, in: McLaren, D. A. (Ed.), Use of chlorine based sanitizers and disinfectants in the food manufacturing industry. Food Processing Center at the University of Nebraska, Lincoln NE, pp. 2–15. https://www.siphidaho.org/env/pdf/Chlorination_of_fruits_and_veggies.PDF. Accessed 22 March 2021.

Tokarskyy, O., & Korda, M. (2019). Microbiological comparison of visibly dirty and visibly clean mature green tomatoes before and after treatments with deionized water or chlorine in model overhead spray brush roller system. *Potravinarstvo Slovak Journal of Food Sciences, 13*(1), 779-783. https://doi.org/10.5219/1178

Tortorello, M. L. (2003). Indicator organisms for safety and quality--uses and methods for detection: minireview. *Journal of AOAC International, 86*(6), 1208-1217.

Tuson, H. H., & Weibel, D. B. (2013). Bacteria-surface interactions. *Soft Matter, 9*(18), 4368-4380. https://doi.org/10.1039/C3SM27705D

U.S. FDA. (2015) Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. 80 FR 74353 (Docket No. FDA-2011-N-0921). https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption.

U.S. FDA. (2017). Nutrition information for raw fruits, vegetables, and fish. https://www.fda.gov/food/food-labeling-nutrition/nutrition-information-raw-fruits-vegetables-and-fish

U.S. FDA. (2019). FSMA compliance date. https://www.fda.gov/food/food-safety-modernization-act-fsma/fsma-compliance-dates#Produce_Safety

U.S. FDA. (2020). Outbreak investigation of *Salmonella* Enteritidis: peaches (August 2020). Retrieved from https://www.fda.gov/food/outbreaks-foodborne-illness/outbreak-investigation-salmonella-enteritidis-peaches-august-2020. Accessed 19 April 2021.

USDA. (2019). USDA Good Agricultural Practices Good Handling Practices audit verification checklist. USDA, Agricultural Marketing Service, Specialty Crop Program, Specialty Crop Inspection Division. Retrieved from https://www.ams.usda.gov/services/auditing/gap-ghp/audit. Accessed 19 April 2021.

Van Houdt, R., & Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology, 109*(4), 1117-1131. https://doi.org/10.1111/j.1365-2672.2010.04756.x

Van Pelt, A. E., Quiñones, B., Lofgren, H. L., Bartz, F. E., Newman, K. L., & Leon, J. S. (2018). Low prevalence of human pathogens on fresh produce on farms and in packing facilities: a systematic review. *Frontiers in Public Health, 6*(40). https://doi.org/10.3389/fpubh.2018.00040

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., & Van Camp, J. (2009). Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiology, 26*(8), 882-888.

Whelton, S. P., Hyre, A. D., Pedersen, B., Yi, Y., Whelton, P. K., & He, J. (2005). Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *Journal of Hypertension, 23*(3). Retrieved from https://journals.lww.com/jhypertension/Fulltext/2005/03000/Effect_of_dietary_fiber_intake_on_blood_pressure_.2.aspx. Accessed 19 April 2021.

Wu, V. C. H., & Kim, B. (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiology, 24*(7), 794-800. https://doi.org/10.1016/j.fm.2007.03.010

Yan, R., Gurtler, J. B., Mattheis, J. P., & Fan, X. (2020). Effect of trichome removal and UV-C on populations of *E. coli* O157:H7 and quality of peach fruit. *HortScience, 55*(10), 1626-1631. https://doi.org/10.21273/HORTSCI15231-20

Zambrano-Vaca, C., Zotarelli, L., Migliaccio, K., Beeson, R., Jr., Morgan, K., Chaparro, J., & Olmstead, M. (2018). Irrigation practices for peaches in Florida. Retrieved from https://edis.ifas.ufl.edu/hs1316. Accessed 19 April 2021.

Zhao, T., Doyle, M. P., & Besser, R. E. (1993). Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. *Applied and Environmental Microbiology, 59*(8), 2526-2530. https://doi.org/10.1128/AEM.59.8.2526-2530.1993

Zhao, X., Zhang, W., Yin, X., Su, M., Sun, C., Li, X., & Chen, K. (2015). Phenolic composition and antioxidant properties of different peach [*Prunus persica* (L.) Batsch] cultivars in China. *International journal of molecular sciences, 16*(3), 5762-5778. https://doi.org/10.3390/ijms16035762

Zhu, Y., Wu, F., Trmcic, A., Wang, S., & Warriner, K. (2020). Microbiological status of reusable plastic containers in commercial grower/packer operations and risk of *Salmonella* cross-contamination between containers and cucumbers. *Food Control, 110*, 107021. https://doi.org/10.1016/j.foodcont.2019.107021

CHAPTER 3

HYGIENE STATUS OF FRESH PEACH PACKING LINES IN GEORGIA

Wang, P., Quansah, J. K., Pitts, K. B., and Chen, J. 2020. *LWT - Food Science and Technology*. 110627.

Reprinted here with permission of the publisher.

ABSTRACT

Surface swab samples (n=464) were collected from various sites on 4 fresh peach packing lines in Georgia at 9 am (T1), 12 pm (T2), and 3 pm (T3) in 3 repeated visits per line during the harvest seasons of 2018 and 2019. Each swab sample was collected from a 100 cm^2 area. The collected samples were analyzed for the levels of total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC), as well as the incidences of thermo-tolerant coliforms (TTC) and enterococci (EC). Counts of the three hygiene indicators in the T1 samples were significantly lower ($P<0.05$) than those in the T2 and T3 samples. The incidence of TTC increased from 13.50% in the T1, to 45.83% in the T3, samples, while that of EC from 6.75% to 15.11%. The key areas with greater hygiene indicator counts and higher incidence of TTC and EC were the optical sizer and brushes/rollers inside the washer. Harvest bin had a comparable mean YM count with the brushes/rollers inside the washer. Samples from the manual sorting area had the highest TTC incidence among samples collected from all surveyed sites. The study pinpointed the most heavily contaminated areas on fresh peach packing lines.

Keywords: Hygiene, indicator organisms, fresh produce, peach, packing line

3.1. Introduction

Foodborne outbreaks linked to fresh produce consumption have increased significantly in recent years. According to the data reported by the U.S. Food and Drug Administration (U.S. FDA), 17,212 illnesses, 2,083 hospitalizations, and 69 deaths were caused by the consumption of over 20 different types of fresh produce, including sprouts, leafy greens, melons, berries, herbs, cucumbers, apples, stone fruits, *etc*., from 1996 to 2014 (D'Lima & Vierk, 2011; Merriweather, Cloyd, & Gubernot, 2015). Commodities that have been repeatedly involved in outbreaks of human gastrointestinal infections such as leafy greens (Jongman & Korsten, 2018), vegetable sprouts (Yang et al., 2013) and melons (Aguayo, Allende, & Artés, 2003) have attracted a lot of research attention, while little research has been directed to the microbial safety of stone fruits (Chen et al., 2016).

Peach (*Prunus persica*), as one of the stone fruits, has been previously linked to a multistate outbreak and product recalls over the past few years (Chen et al., 2016; U.S. FDA, 2014, 2019, 2020a, 2020b). However, the source of pathogen contamination for the outbreak and recalls was never identified. Thus, it is critical for the fresh peach industry to understand the potential risk factors associated with peach production and postharvest handling and to take necessary precautions to fully comply with the "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption", as known as, the Final Rules of Produce Safety under the Food Safety Modernization Act (U.S. FDA, 2015).

Foodborne pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, Hepatitis A, and Norovirus have been found in various produce packing facilities (Van Pelt et al., 2018). The origin of these pathogens is always difficult to identify due to the complexity of the product contact surface, plant-specific sanitation practice, and hygiene regime in individual packing facilities. Although certain disinfection treatments are regularly used, the mitigation might not be entirely effective to eliminate produce-borne pathogens (Burnett & Beuchat, 2001). In addition, different produce commodities have different packing operations and equipment design. To date, only a limited number of studies related to peach packing facilities have been conducted. A study in South Africa examined 38 surface samples from sorting and packing lines with no detailed information given on the packing facilities involved (Duvenage & Korsten, 2017). while another study focused only on a particular piece of equipment, automatic sorting system on a stone fruit packing line (Williamson et al., 2018). Thus, additional research is needed to identify the potential risk factors that may affect the microbial safety of fresh peach in packinghouse operations. This study aimed at identifying the critical sanitation control points of fresh peach packing lines (PLs) by quantifying the level of hygiene, as well as the incidence of pathogen indicators.

3.2. Material and methods

3.2.1. Sampling strategy

Four fresh peach PLs in Georgia were surveyed in this study during the harvest seasons of 2018 and 2019. Each packing facility was randomly visited on three different packing days over the two harvest seasons. Fourteen sites on each PL as shown in Table 3-1 were selected for sampling based on the functionality of packing equipment. Samples were collected from each PL at three sampling times on a packing day: at 9 am before packing (T1), during lunch break at noon (T2), and in the mid-afternoon break at 3 pm (T3). The amount of fruits packed between the two adjacent breaks was approximately 61, 235 kg.

A sterile environmental sampling sponge (3.8 × 7.6 × 1.6 cm; Nelson Jameson Inc, Marshfield, WI USA) moistened with 25 ml Dey-Engley neutralizing broth (Becton Dickinson, Sparks, MD USA) in an 18 oz. Whirl-Pak® bag (Nasco, Fort Atkinson, WI USA) was used to swab a 100 cm^2 area using a commercial template (3M™, St. Paul, MN USA) with 10 horizontal, and 10 vertical, strikes. Samples collected from non-flat surfaces were collected within the 100 cm^2 window using the same sampling technique. Detailed measurements of these surfaces were taken to calculate the actual surface areas swabbed. The swab samples were stored in portable coolers at 4 °C after collection and during transportation and analyzed within 24 h.

3.2.2. Microbiological testing

Sampling sponges in Whirl-Pak® bags were hand massaged for 1 min before enumeration. Total aerobes (TA) and total coliforms (TC) in 0.1 ml sponge rinsate were enumerated on tryptic soy agar and MacConkey agar, respectively, at 37 °C for 24 h; total yeasts and molds (YM) were enumerated on acidified potato dextrose agar (pH 3.5) at 25 °C for 72 h; presumptive colonies of thermo-tolerant coliforms (TTC) and enterococci (EC) were selected on MacConkey agar at 44.5 °C and m-*Enterococcus* agar at 37 °C, respectively with 24 h of incubation. Presumptive TTC colonies were confirmed by growth on triple sugar iron slants at 37 °C for 24 h and in EC broth (bio-WORLD, Dublin, OH USA) with Durham Tube (6 x 50 mm, Kimble Chase®, Vineland, NJ USA) at 44.5 °C for 48 h. Salt tolerant enterococci were confirmed in brain heart infusion broth with 6.5% sodium chloride at 37 °C for 24 h. All microbiological media without specific notation were purchased from Becton, Dickinson, and Company.

3.2.3. Data analysis

Significant differences in mean TA, YM, and TC counts across different sampling sites, from different PLs and at different sampling times were fit in a general linear model with a split-plot arrangement and tested by Fisher's least significant difference tests, including a random blocking factor of "visit", fixed whole plot factor "packing line" and two subplots fixed factors "sampling time" and "sampling site". The SAS University Edition (SAS Institute, Cary, NC USA) was used for data analysis. The percentage of samples with confirmed TTC and EC presence in the total number of samples collected from different PLs, and at different sampling sites and times were calculated.

3.3. Results

3.3.1. Hygiene indicators

Overall statistical analyses on microbial data collected from a total of 464 swab samples showed that sampling time/year and sample site had significant ($P < 0.05$), and facility visit had insignificant ($P > 0.05$), impacts on the average counts of three hygiene indicators, TA, YM and TC, while the PL effect was only reflected on mean YM counts (Table 3-2). Means of the three hygiene indicators increased overtime during a packing day; the microbial counts in the T1 samples were significantly lower than those in the T2 and T3 samples. On average, site 9 (singulator) and 10 (entrance of the optical sizer) had significantly higher average TA and YM counts than the other sampled sites. Although significantly lower than site 9 and 10, site 11 (exit of the optical sizer) and 4 (brush roller insider the washer) had significantly higher TA counts compared to the remaining sampled sites. Site 4 had the highest average TC counts, followed by site 10 and 9. Mean TA count in samples from PL 2 was significantly higher than the same count in samples collected from PL 3 and PL 1, but not PL 4. Samples from PL 1 had a significantly lower average YM count than the samples from the other three sampled PLs. There were no significant differences ($P > 0.05$) in the average TC counts in samples collected from all four PLs. The mean TA, YM, and TC counts recovered in the 2019 season were significantly lower than those found in the previous season.

3.3.2. Counts of the three hygiene indicators on different sites of individual PLs

Site 10 of PL 1, and site 9, 10, and 11 of PL 2 had significantly higher (*P* < 0.05) TA counts than the other sampled sites of their respective lines (Table 3-3). The TA count on site 4 of PL 3 was the highest, followed by site 14 (harvest bin). The highest TA counts of PL 4 were found on site 4 and 10, followed by site 9 and 11. Site 10 of PL 1, site 10 and 11 of PL 2, site 4, 5 (exit of the washer), 6 (conveyer to the mansual sorting area) and 14 of PL 3, and site 9, 10 and 11 of PL 4 were significantly higher in YM counts compared to other sites on each respective line. The TC counts on site 4 of PL 3 and PL 4 were significantly higher than the other sampled sites, whereas site 10 of PL 1 and PL 2 had the highest TC counts.

3.3.3. Counts of the three hygiene indicators from individual PLs at specific sampling points

Similar to the results of overall statistical analyses, the TA, YM, and TC counts in the T2 and T3 samples of the four PLs were similar, but they were significantly lower (*P* < 0.05) than the counts in the T1 samples, except the YM counts from PL 1 and the TC counts from PL 1 and PL 4 (Table 3-4). The T1 samples of PL 2 had significantly higher, while the T2 and T3 samples of PL 1 had significantly lower TA counts than the same samples from the other PLs. The T1, T2 and T3 samples of PL 1 had the lowest YM counts compared to the same samples from PL 2 to PL 4. The T1 samples of PL 1 had significantly higher TC counts than the ones from other plants. The levels of TC count in the T2 samples from the four PLs were not significantly different (*P* > 0.05), and a similar observation was noticed with the T3 samples from PL 1, PL 2, and PL 4.

3.3.4. Counts of three hygiene indicators from different sites at different sampling times

T1 samples from site 4, 9 and 10, and T3 samples from site 9, 10, and 11 had significantly higher (*P* < 0.05) TA counts compared to the T1 or T3 samples from other sampled sites (Table 3-5). YM counts were significantly higher in the T1 samples from site 9 and 10, and T3 samples from site 10. T2 sample from site 9 had the highest TA and YM counts. The T1 samples from site 4, and T2 samples from site 5, 9, and 10 had the highest TC counts. The TC counts in most of the T3 samples from all 14 were statistically similar (*P* > 0.05).

Consistent with the results of overall statistics, the T1 samples had significantly lower (*P* < 0.05) TA and YM counts than the T2 and T3 samples, with a few exceptions (Table 3-5): TA counts from site 3 (entrance of the washer), 9, 12 (conveyer belt to the weighing area) and 14 and YM counts from site 3, 9, 10 and 14 were similar among the three sampling points; significant differences in TA counts from site 10 and YM counts from site 11 were only seen between T1 and T3 samples, and T2 and T3 samples at site 4 had significantly lower TA and YM counts than the T1 samples. As the counts of TA and YM, TC counts in the T2 and T3 samples from 5 sites (1 [dumping area], 2 [roller conveyer to the washer], 6, 7 [conveyer belt of the manual sorting area] and 13 [weighing area]) were significantly higher than the counts in the T1 samples; site 3 and 5 had significantly lower TC counts in T1 samples than in T3 or T2 samples, respectively, and counts in the three sets of samples from the remaining 7 sites had similar TC counts.

3.3.4. Incidence of TTC and EC presence

Out of the 464 samples, 156 (33.62%) tested positive for TTC (Table 3-6). The incidence of TTC positive samples from each PL ranged from 24.18 to 42.06%. The occurrence of TTC in samples collected from the manual sorting area (site 7, 52.94%), brush roller inside the washer (site 5, 50.00% and site 4, 47.06%), and optical sizer (site 9, 46.15% and site 10, 42.42%) were higher than the rest of the sites evaluated. As a comparison, the harvest bin (site 14) had a relatively lower incidence of 11.76%. The incidence of TTC positive samples at T2 and T3 were over three times higher than the one found at T1. Among the evaluated PLs, PL 3 had a relatively lower incidence of TTC.

Sixty-three samples (13.58%) tested positive for EC among samples tested in the study, and PL 3 and 2 had the highest, and lowest incidence, respectively (Table 3-6). The top five EC positive areas included sites 10, 4, 2, 3, and 9, while the incidence of EC positive samples at the manual sorting area (site 6, 7, and 8 [conveyer belt to the optical sizer]) was much lower, close to 6%. The percentages of EC positive samples collected at T2 and T3 were twice as higher as samples collected at T1.

3.4. Discussion

The four fresh peach packing facilities involved in this study all have post-operation cleaning and sanitation interventions. Three of the participating PLs undergo a daily routine of dry cleaning, wet cleaning with a cleaner and physical scrubbing, water rinsing, sanitizing, and air drying (K. B. Pitts, personal communication, November 15, 2019). The remaining facility, PL3, is, nevertheless, only dry cleaned daily, combined with a weekly deep cleaning and sanitation routine as described above. The different sanitation routine used by the latter PL did not seem to significantly influence its hygiene status, because samples from this PL did not have significantly higher ($P > 0.05$) average TA and YM counts in the T1 samples compared to the T1 samples of the other three PLs (Table 3-4).

The current study found that the hygiene status of the four participating PLs varied, as reflected by the mean TA or YM counts recovered from various surfaces of the facilities (Table 3-2). The four packing facilities all use quaternary ammonium-based sanitizers (Meyer

Laboratory, Blue Springs, MO USA; National Chemical Laboratories®, Philadelphia, PA USA), but with different cleaners. The cleaning agent used by one of the PLs contains sodium dodecylbenzene sulfonate (Decco US Post-Harvest, Monrovia, CA USA), while the active ingredients in the cleaning agents used by other PLs include sodium silicate (with potassium hydroxide), tetrapotassium pyrophosphate and undisclosed ingredients for heavy-duty cleaning and degreasing purpose (Meyer Laboratories, Blue Springs, MO USA). In addition to the type of cleaning agents used, other factors such as the quality of water used for cleaning and rinsing (Marriott, Schilling, & Gravaniet, 2018), sanitation operation practice (Rodriguez-Caturla et al., 2012), employee hygiene (Todd, Greig, Bartleson, & Michaels, 2009), and insect and animal access to the facility (Blazar, Allard, & Lienau, 2011; Orozco et al., 2008) may also affect the hygiene status of fresh peach PLs.

A TA count of lower than 2 log CFU/cm^2 on a freshly cleaned and sanitized food contact surface is a commonly accepted hygiene standard (Holah, 1999). The average TA count of 2.29 log CFU/cm^2 from the T1 sample of this study (Table 3-2) was slightly higher than the generally acceptable hygiene standard. Similarly, the 0.53 log CFU/cm^2 average TC count in the T1 sample (Table 3-2) was also higher than the reference TC count of 2.50 CFU/cm^2 (0.40 log CFU/cm^2) recommended by Moore & Griffith (2002). The average TA and TC counts observed in the present study were arbitrarily elevated by the counts recovered from a few hard-to-reach sites of surveyed PLs, *e.g.* the average TA and TC counts on the washer (site 4) and color sorting areas (sites 9, 10 and 11) averaged 4.00 and 1.23 log CFU/cm^2, respectively, while the average TA and TC counts from the remaining sites were as low as 1.66 and 0.26 log CFU/cm^2, respectively (Table 3-5). These findings highlight the importance of addressing the critical sanitation control points on fresh peach PLs.

Site 4 is the brush roller within the peach washer, which removes trichomes (*i.e.* fuzzes) from the surface of peach. During the washing process, organic matter and bacterial cells carried by fruits can be easily trapped into the densely arranged bristles of brush rollers (Brooks & Flint, 2008), becoming a potential source of cross-contamination between the equipment and passing fruit (Angelo et al., 2017; Gomba, Chidamba, & Korsten, 2016; Pao, Kelsey, & Long, 2009). Chemicals listed in the Code of Federal Regulations (CFR) such as sodium hypochlorite can be used in fruit wash water (21 CFR 173. 315) to limit the risk of microbial contamination in wash water and control cross-contamination between washed and unwashed produce (U.S. FDA, 2018a). Adding sanitizers into wash water has been shown to reduce the level of artificially inoculated *Salmonella* on the surface of packed tomatoes and improve the hygiene status of brush rollers (Pao, Kelsey, & Long, 2009). In the present study, peach washing water was supplemented with 50-100 ppm sodium hypochlorite in majority of the PLs, except PL 3 which used 0.4-1.0 ppm chlorine dioxide instead. This practice might have made the counts of TA and YM in samples collected from site 4 at T2 and T3 significantly lower ($P < 0.05$) than those collected from the same site at T1 (Table 3-5).

The packing facilities of this study follow the same cleaning and sanitizing practice for site 4 after daily packing, which includes spraying a cleaning agent to foam while brush rollers are in motion, flushing off organic debris with portable water using a water hose, spraying a quaternary ammonium-based sanitizer, following by air drying (K. B. Pitts, personal communication, November 15, 2019). However, water hoses have limited accessibility to the bristles on a brush roller to remove all organic residuals, which might affect the efficacy of sanitation treatments (Marriott, Schilling, & Gravani, 2018). As a result, site 4 is one of the key areas on fresh peach PLs that need special attention in terms of cleaning and sanitation treatments.

Another critical sanitation control point on fresh peach PLs identified in this study is the optical sizer (Table 3-5). Similar observations have been made in studies involving blueberry (Gazula et al., 2019a) and mandarin (Izumi, Poubol, Hisa, & Sera, 2008) PLs. The conveyors inside the optical sizer consist of hundreds of rubber rollers and plastic peach holders. This unique structure creates a large number of contact niches for microbial cells and becomes a burden for daily sanitation (Pompermayer & Gaylarde, 2000). In addition, the equipment is water sensitive, wet cleaning is not applicable. A Clean Out of Place procedure is currently recommended by the U.S. FDA before the harvest season to lower microbial loads on the rubber rollers (U.S. FDA, 2018b). Packing facilities involved in this study use dry cleanings, such as vacuuming and sweeping to remove visible dirt and organic matters from the rollers, followed by sanitation with an alcohol-based sanitizer designed specifically for water-sensitive equipment (K. B. Pitts, personal communication, November 15, 2019). The average TA and TC counts at the color sorting area (site 9, 10, and 11) in the T1 samples were 3.24 and 0.61 log CFU/cm^2 in the present study (Table 3-5). Similar mean TA and TC counts of 3.20 and 0.90 log CFU/cm^2 were found in a California peach packing facility by Williamson et al. (2018).

Peach harvest bin had an average YM count of 2.39 log CFU/cm^2 in the present study (Table 3-2). Although significantly lower ($P < 0.05$) than the YM counts at the optical sizer, it was similar to ($P > 0.05$) the same count on brush roller inside the washer. Microbial loads on harvest containers reported in previous studies varied, depending on the type of commodities and geographic regions of the packing facilities involved: the levels of average TA, YM, and TC counts on berry lugs examined in fresh blueberry packing facilities in Georgia were 3.48, 2.70

and 0.99 log CFU/cm^2, respectively (Gazula et al., 2019a), while plastic containers with visualized soil in selected produce packing facilities of British Columbia had median TA, YM and TC counts of 2.81, 1.72, or 1.82 log CFU/cm^2 (Zhu, Wu, Trmcic, Wang, & Warriner, 2020). All except one packing facilities of this study have a hydro cooler that flushes harvest bins with loaded fruits using water containing 50 ppm chlorine (K. B. Pitts, personal communication, November 15, 2019). This study found that samples from the PL without hydrocooling had relatively higher TA and YM counts at site 14 (Table 3-3). It is not known whether the use of chlorinated hydrocooling water is related to the levels of TA and YM counts on the surface of harvest bins.

Physical and chemical properties of surface materials on various sites of the PLs may affect the levels of microbial loads accumulated on them (Arnold & Silvers, 2000; Mafu, Plumety, Deschênes, & Goulet, 2011). Bacterial cells are negatively charged and tend to attach to materials that are hydrophobic with little surface energy or hydrophilic with positive charges (Pawar, Rossaman, & Chen, 2005). Among hydrophobic materials that are normally used in fresh peach PLs, polyurethane has a higher surface energy (51.5 mJ/m^2) than polyvinyl chloride (45.5 mJ/m^2), followed by polyethylene (40.2 mJ/m^2) (Kinloch, 1987; Kuang & Constant, 2015), therefore, the hydrophobicity of these materials increases in this order, making polyethylene the most favorable material among the three for microbial accumulation. Sampled sites such as brush rollers (site 4), rollers inside the optical sizer (site 10 and 11) and harvest bins (site 14) are normally made of polyethylene or high-density polyethylene, which had relatively higher levels of hygiene indicators compared to other hydrophobic surfaces that are usually made of polyurethane and polyvinyl chloride such as the conveyor belts before the weighing area (site 12), and roller beds before/at the manual sorting area (site 6 and 7) (Table 3-2). Hydrophilic material, such as stainless steel, can be even less attractive to microorganisms (Goulter, Gentle, & Dykes, 2009) with the least amount of residual microbial cells after sanitation treatments compared to other fruit contact surface materials commonly used in fresh produce PLs (Gazula et al., 2019b). These previous observations could explain why the weighing area (site 13) had relatively lower microbial counts in the present study (Table 3-2).

The populations of monitored indicator organisms in samples collected from the equipment surface at T2 and T3 were significantly higher ($P < 0.05$) than at T1 (Table 3-2). A similar finding was noticed in a survey involving fresh blueberry PLs (Gazula et al., 2019a). The accumulation of microbial cells overtime on equipment surface has the potential to cause cross-contamination among different batches of products (Gounadaki, Skandamis, Drosinos, & Nychas, 2008; Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003). The results of this study emphasize that fresh produce packing facility should be cleaned and sanitized daily during the harvest season, even though it is not mandated by the Produce Safety Rules (U.S. FDA, 2015).

The incidence of TTC was 3 times higher in samples collected at T3 than at T1 (Table 3-6), which could be attributed to both pre- and post-harvest causes. TTC, an indicator of potential fecal contamination, could be introduced to the packinghouse environment by incoming fruits from the field. Potential sources of fruit contamination at the farm level may include contaminated irrigation water, bird or insect dropping (Alegbeleye, Singleton, & Sant'Ana, 2018; Jay-Russell, 2013), insanitary harvest utensils and workers with poor hygiene and handling practice. The four peach growers involved in this study did not require peach pickers to wear gloves, but bathrooms with handwashing stations were established (K. B. Pitts, personal communication, November 15, 2019). However, failure in following good handwashing practices may result in cross-contamination from human hands to harvested fruits (Beuchat, 1996). At the postharvest stage, water used in hydro cooler has been found to cross-contaminate fresh produce due to the lack of sufficient concentration of sanitizers in the system (Macarisin et al., 2017; Vigneault, Bartz, & Sargent, 2000). In the present study, the highest incidence of TTC was found in the manual sorting area of the PLs (Table 3-6). With intensive human activities at this site, manual sorting rollers may be contaminated by workers with poor personal hygiene or handwashing practices (Park et al., 2013; Bartz et al., 2017) during the physical examination of the fruits for flaws and bruises. Workers frequently touch fruits and non-fruit contact surfaces at this site, which could transfer contaminants from the environment to fruits (U.S. FDA, 2018b).

3.5. Conclusions

This study identified the optical sizer and brushes/rollers inside the washer as the most contaminated sites along fresh peach packing lines. The harvest bins and brush roller within the washer had a comparable YM counts. Manual sorting area had the highest TTC incidence among all examined sites. The levels of hygiene and pathogen indicators on selected PLs surveyed increased overtime during a packing day. These results emphasize the importance of effective daily sanitation and food safety education for facility employees. Future studies are needed to identify the precise source of microbial contamination of, and more effective intervention and technologies to decontaminate, fresh peach packing lines.

References

Aguayo, E., Allende, A., & Artés, F. (2003). Keeping quality and safety of minimal fresh processed melon. *European Food Research Technology, 216,* 494-499. https://doi.org/10.1007/s00217-003-0682-7.

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

Angelo, K. M., Conrad, A. R., Saupe, A., Dragoo, H., West, N., Sorenson, A., Barnes, A., Doyle, M., Beal, J., Jackson, K. A., Stroika, S., Tarr, C., Kucerova, Z., Lance, S., Gould, L. H., Wise, M., & Jackson, B. R. (2017). Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014-2015. *Epidemiology Infection, 145*(5), 848-856. https://doi.org/10.1017/s0950268816003083.

Arnold, J. W., & Silvers, S. (2000). Comparison of poultry processing equipment surfaces for susceptibility to bacterial attachment and biofilm formation. *Poultry Science, 79*(8), 1215-1221. https://doi.org/10.1093/ps/79.8.1215.

Bartz, F. E., Lickness, J. S., Heredia, N., Fabiszewski de Aceituno, A., Newman, K. L., Hodge, D. W., Jaykus, L., Garcia, S., Leon, J. S. (2017). Contamination of fresh produce by microbial indicators on farms and in packing facilities: elucidation of environmental routes. *Applied Environmental Microbiology, 83*(11), e02984-02916. https://doi.org/10.1128/AEM.02984-16.

Beuchat, L. R. (1996). Pathogenic Microorganisms associated with fresh produce. *Journal of Food Protection, 59*(2), 204-216. https://doi.org/10.4315/0362-028X-59.2.204.

Blazar, J., Allard, M., & Lienau, E. K. (2011). Insects as vectors of foodborne pathogenic bacteria. *Terrestrial Arthropod Reviews*, *4*(1), 5-16. https://doi.org/10.1163/187498311X543989.

Brooks, J. D., & Flint, S. H. (2008). Biofilms in the food industry: problems and potential solutions. *International Journal of Food Science and Technology, 43*(12), 2163-2176. https://doi.org/10.1111/j.1365-2621.2008.01839.x.

Burnett, S. L., & Beuchat, L. R. (2001). Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *Journal of Industrial Microbiology and Biotechnology, 27*(2), 104-110. https://doi.org/10.1038/sj.jim.7000199.

Chen, Y., Burall, L. S., Luo, Y., Timme, R., Melka, D., Muruvanda, T., Payne, J., Wang, C., Kastanis, G., Maounounen-Laasri, A., De Jesus, A. J., Curry, P. E., Stones, R., K'Aluoch, O., Liu, E., Salter, M., Hammack, T. S., Evans, P. S., Parish, M., Allard, M. W., Datta, A., Strain, E. A., Brown, E. W. (2016). *Listeria monocytogenes* in stone fruits linked to a multistate outbreak: enumeration of cells and whole-genome sequencing. *Applied and Environmental Microbiology, 82*(24), 7030-7040. https://doi.org/10.1128/aem.01486-16.

D'Lima, C., & Vierk, K. (2011). Memorandum for the record: produce related outbreaks and illnesses 1996 to 2010. U.S. Food and Drug Administration. https://www.regulations.gov/document?D=FDA-2018-D-3631-0037. Accessed 18 September 2020.

Duvenage, S., & Korsten, L. (2017). Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer. *Food Control, 78,* 374-382. https://doi.org/10.1016/j.foodcont.2017.03.003.

Gazula, H., Quansah, J., Allen, R., Scherm, H., Li, C., Takeda, F., & Chen, J. (2019a). Microbial loads on selected fresh blueberry packing lines. *Food Control, 100,* 315-320. https://doi.org/10.1016/j.foodcont.2019.01.032.

Gazula, H., Scherm, H., Li, C., Takeda, F., Wang, P., & Chen, J. (2019b). Ease of biofilm accumulation, and efficacy of sanitizing treatments in removing the biofilms formed, on coupons made of materials commonly used in blueberry packing environment. *Food Control, 104*, 167-173. https://doi.org/10.1016/j.foodcont.2019.04.036.

Gomba, A., Chidamba, L., & Korsten, L. (2016). Prevalence and serovar diversity of *Salmonella* spp. in primary horticultural fruit production environments. *Food Control, 69*, 13-19. https://doi.org/10.1016/j.foodcont.2016.04.026.

Goulter, R. M., Gentle, I. R., & Dykes, G. A. (2009). Issues in determining factors influencing bacterial attachment: a review using the attachment of *Escherichia coli* to abiotic surfaces as an example. *Letters in Appllied Microbiology, 49*(1), 1-7. https://doi.org/10.1111/j.1472-765X.2009.02591.x.

Gounadaki, A. S., Skandamis, P. N., Drosinos, E. H., & Nychas, G. -J. E. (2008). Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. *Food Microbiology, 25*(2), 313-323. https://doi.org/10.1016/j.fm.2007.10.001.

Holah, J. T. (Ed.). (1999). *Effective microbiological sampling of food processing environments*. Chipping Campden, UK: Campden & Chorleywood Food Research Association.

Izumi, H., Poubol, J., Hisa, K., & Sera, K. (2008). Potential sources of microbial contamination of satsuma mandarin fruit in Japan, from production through packing shed. *Journal of Food Protection, 71*(3), 530-538. https://doi.org/10.4315/0362-028x-71.3.530.

Jay-Russell, M. (2013). What is the risk from wild animals in food-borne pathogen contamination of plants? *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 8*(40). https://doi.org/10.1079/PAVSNNR20138040.

Jongman, M., & Korsten, L. (2018). Irrigation water quality and microbial safety of leafy greens in different vegetable production systems: A review. *Food Reviews International, 34*(4), 308-328. https://doi.org/10.1080/87559129.2017.1289385.

Kinloch, A. J. (1987). *Adhesion and adhesives: science and technology.* New York, NY USA: Springer. https://doi.org/10.1007/978-94-015-7764-9.

Kuang, P., & Constant, K. (2015). Increased wettability and surface free energy of polyurethane by ultraviolet ozone treatment. In M. Aliofkhazraei (Ed.), *Wetting and Wettability*. London, UK: IntechOpen. https://doi.org/10.5772/60798.

Kusumaningrum, H. D., Riboldi, G., Hazeleger, W. C., & Beumer, R. R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal Food Microbiology, 85*(3), 227-236. https://doi.org/10.1016/S0168-1605(02)00540-8.

Macarisin, D., Wooten, A., De Jesus, A., Hur, M., Bae, S., Patel, J., Evans, P., Brown, E., Hammack, T., & Chen, Y. (2017). Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling. *International Journal Food Microbiology, 257*, 165-175. https://doi.org/10.1016/j.ijfoodmicro.2017.06.018.

Mafu, A. A., Plumety, C., Deschênes, L., & Goulet, J. (2011). Adhesion of pathogenic bacteria to food contact surfaces: influence of pH of culture. *International Journal Microbiology, 2011,* 10. https://doi.org/10.1155/2011/972494.

Marriott, N. G., Schilling, M. W., & Gravani, R. B. (2018). *Principles of Food Sanitation.* (6th ed.). New York, NY USA: Springer. https://doi.org/10.1007/978-3-319-67166-6.

Merriweather, S., Cloyd, T.C., & Gubernot, D. (2015). Memorandum to the file: 2011-2014 produce related outbreaks and illnesses. U.S Food and Drug Administration. https://www.regulations.gov/document?D=FDA-2018-D-3631-0102. Accessed 18 September 2020.

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

Orozco, R. L., Iturriaga, M.H., Tamplin, M.L., Fratamico, P.M., Call, J.E., Luchansky, J.B., & Escartin, E.F. (2008). Animal and environmental impact on the presence and distribution of *Salmonella* and *Escherichia coli* in hydroponic tomato greenhouses. *Journal of Food Protection*, 71, 676-683. https://doi.org/10.4315/0362-028X-71.4.676.

Pao, S., Kelsey, D. F., & Long, W., 3rd. (2009). Spray washing of tomatoes with chlorine dioxide to minimize *Salmonella* on inoculated fruit surfaces and cross-contamination from revolving brushes. *Journal of Food Protection, 72*(12), 2448-2452. https://doi.org/10.4315/0362-028x-72.12.2448.

Park, S., Navratil, S., Gregory, A., Bauer, A., Srinath, I., Jun, M., Szonyi, B., Nightingale, K., Anciso, J., Ivanek, R. (2013). Generic *Escherichia coli* contamination of spinach at the preharvest stage: effects of farm management and environmental factors. *Applied and Environmental Microbiology, 79*(14), 4347-4358. https://doi.org/10.1128/aem.00474-13.

Pawar, D. M., Rossman, M. L., & Chen, J. (2005). Role of curli fimbriae in mediating the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces. *Journal of Applied Microbiology, 99*(2), 418-425. https://doi.org/10.1111/j.1365-2672.2005.02499.x.

Pompermayer, D. M. C., & Gaylarde, C. C. (2000). The influence of temperature on the adhesion of mixed cultures of *Staphylococcus aureus* and *Escherichia coli* to polypropylene. *Food Microbiology, 17*(4), 361-365. https://doi.org/10.1006/fmic.1999.0291.

Rodriguez-Caturla, M. Y., Valero, A., Carrasco, E., Posada, G. D., Garcia-Gimeno, R. M., & Zurera, G. (2012). Evaluation of hygiene practices and microbiological status of ready-to-eat vegetable salads in Spanish school canteens. *Journal of Science of Food and Agriculture, 92*(11), 2332-2340. https://doi.org/10.1002/jsfa.5634.

Todd, E. C., Greig, J. D., Bartleson, C. A., & Michaels, B. S. (2009). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. *Journal of Food Protection, 72*(1), 202-219. https://doi.org/10.4315/0362-028x-72.1.202.

U.S. FDA. (2014). Recall - firm press release: Wawona Packing Co. expands its voluntary recall of fresh, whole peaches, plums, nectarines, and pluots because of possible health risk. https://wayback.archive-it.org/7993/20170406101206/https://www.fda.gov/Safety/Recalls/ArchiveRecalls/2013/ucm407600.htm. Accessed 18 September 2020.

U.S. FDA. (2015). Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. 80 FR 74353 (Docket No. FDA-2011-N-0921). https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption. Accessed 18 September 2020.

U.S. FDA. (2018a). Guide to minimize food safety hazards of fresh-cut produce: draft guidance for industry; availability. 83 FR 53197 (Docket No. FDA-2018-D-3583). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-guide-minimize-food-safety-hazards-fresh-cut-produce. Accessed 18 September 2020.

U.S. FDA. (2018b). Standards for the growing, harvesting, packing, and holding of produce for human consumption; draft guidance for industry; availability. 83 FR 53196 (Docket No. FDA–2018–D–3631). https://www.federalregister.gov/documents/2018/10/22/2018-23006/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption-draft. Accessed 18 September 2020.

U.S. FDA. (2019). Company Announcement: Jac. Vandenberg, Inc. recalls fresh peaches, fresh nectarines and fresh plums because they may be contaminated with *Listeria monocytogenes*. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/jac-vandenberg-inc-recalls-fresh-peaches-fresh-nectarines-and-fresh-plums-because-they-may-be. Accessed 18 September 2020.

U.S. FDA. (2020a). Company Announcement: Prima® Wawona Recalls Bagged Peaches Due to Possible Salmonella Risk. https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/primar-wawona-recalls-bagged-peaches-due-possible-salmonella-risk. Accessed 18 September 2020.

U.S. FDA. (2020b). Company Announcement: Aldi Voluntarily Recalls Assorted Peaches from Wawona Packing Company LLC Due to Possible *Salmonella* Enteritidis Contamination.

https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/aldi-voluntarily-recalls-assorted-peaches-wawona-packing-company-llc-due-possible-salmonella. Accessed 18 September 2020.

Van Pelt, A. E., Quinones, B., Lofgren, H. L., Bartz, F. E., Newman, K. L., & Leon, J. S. (2018). Low prevalence of human pathogens on fresh produce on farms and in packing facilities: a systematic review. *Frontiers in Public Health, 6*, 40. https://doi.org/10.3389/fpubh.2018.00040.

Vigneault, C., Bartz, J. A., & Sargent, S. A. (2000). Postharvest decay risk associated with hydrocooling tomatoes. *Plant Diseases, 84*(12), 1314-1318. https://doi.org/10.1094/PDIS.2000.84.12.1314.

Williamson, K., Pao, S., Dormedy, E., Phillips, T., Nikolich, G., & Li, L. (2018). Microbial evaluation of automated sorting systems in stone fruit packinghouses during peach packing. *International Journal of Food Microbiology, 285,* 98-102. https://doi.org/10.1016/j.ijfoodmicro.2018.07.024.

Yang, Y., Meier, F., Ann Lo, J., Yuan, W., Lee Pei Sze, V., Chung, H. -J., & Yuk, H. -G. (2013). Overview of recent events in the microbiological safety of sprouts and new intervention technologies. *Comprehensive Reviews in Food Science and Safety, 12*(3), 265-280. https://doi.org/10.1111/1541-4337.12010.

Zhu, Y., Wu, F., Trmcic, A., Wang, S., & Warriner, K. (2020). Microbiological status of reusable plastic containers in commercial grower/packer operations and risk of *Salmonella* cross-contamination between containers and cucumbers. *Food Control, 110*, 107021. https://doi.org/10.1016/j.foodcont.2019.107021.

Table 3-1 Selected sample sites on fresh peach packing lines

No.	**Site**
1	Dumping area
2	Roller conveyor to the washer
3	The entrance of the washer
4	Brush roller inside the washer
5	The exit of the washer
6	Roller conveyor to the manual sorting area
7	Roller conveyor of the manual sorting area
8	Conveyor belt to the optical sizer
9	Singulator
10	The entrance of the optical sizer
11	The exit of the optical sizer
12	Conveyor belt to the weighing area
13	Weighing area
14	Harvest bin

Site 9, 10, and 11: optical sorting area

Table 3-2 Overall mean TA, YM, and TC counts in samples collected at different sampling times and sites, and from different packing lines

	Overall mean population (log CFU/cm^2)		
	TA	YM	TC
Sampling time			
T1 (n=163)	2.29^{B*}	1.83^{B}	0.53^{B}
T2 (n=162)	3.20^{A}	2.40^{A}	1.09^{A}
T3 (n=139)	3.29^{A}	2.43^{A}	1.24^{A}
Sampling site			
Site 1 (n=34)	2.50^{DE}	1.74^{EF}	0.86^{CDEF}
Site 2 (n=34)	2.49^{DE}	1.69^{EF}	0.64^{EFG}
Site 3 (n=34)	2.85^{CD}	2.05^{DE}	0.69^{DEFG}
Site 4 (n=34)	3.72^{B}	2.48^{C}	1.69^{A}
Site 5 (n=34)	2.88^{CD}	1.93^{EF}	1.09^{CD}
Site 6 (n=34)	2.58^{CD}	1.63^{FG}	0.89^{CDEF}
Site 7 (n=34)	2.60^{CD}	1.60^{FG}	1.07^{CDE}
Site 8 (n=34)	2.06^{EF}	1.19^{H}	0.79^{CDEF}
Site 9 (n=26)	4.50^{A}	4.25^{A}	1.22^{BC}
Site 10 (n=33)	4.61^{A}	4.41^{A}	1.54^{AB}
Site 11 (n=32)	3.86^{B}	3.79^{B}	1.07^{CDE}
Site 12 (n=33)	1.65^{F}	1.08^{H}	0.29^{G}
Site 13 (n=34)	1.87^{F}	1.31^{GH}	0.68^{DEFG}
Site 14 (n=34)	2.96^{C}	2.39^{CD}	0.58^{FG}
PL			
PL 1 (n=121)	2.58^{B}	1.48^{B}	1.14^{A}
PL 2 (n=126)	3.26^{A}	2.76^{A}	0.94^{A}
PL 3 (n=91)	2.70^{B}	2.23^{A}	0.67^{A}
PL 4 (n=126)	3.01^{AB}	2.35^{A}	0.87^{A}
Year			
2018 (n=315)	3.13^{A}	2.35^{A}	1.12^{A}
2019 (n=149)	2.44^{B}	1.92^{B}	0.73^{B}

TA: total aerobic counts; YM: total yeast and mold counts; TC: total coliform counts.

T1: samples collected at 9 am; T2: samples collected at noon; T3: samples collected at 3 pm.

PL: packing line.

The detection limit is generally 0.10 log CFU/cm^2 with a few exceptions due to the sampling areas of certain non-flat surfaces (0.01 – 0.24 log CFU/cm^2).

* The results followed by different letters within the same comparative variable (sampling time, sample site, or packing line) in the same column were significantly different ($P < 0.05$).

Table 3-3 Mean TA, YM, and TC counts in samples collected from different sites of individual fresh peach packing lines

	Mean population (log CFU/cm^2)											
	TA				YM				TC			
Site	PL 1	PL 2	PL 3	P L 4	PL 1	PL 2	PL 3	PL 4	PL 1	PL 2	PL 3	P L 4
Site 1	2.29^{CDE*}	3.00^{BC}	2.03^{GH}	2.56^{D}	1.43^{DE}	2.35^{CD}	1.43^{EF}	1.68^{DE}	0.88^{BCDE}	1.14^{ABCDE}	0.13^{F}	1.06^{BCD}
Site 2	1.88^{EF}	2.74^{BC}	2.74^{DEF}	2.65^{D}	0.64^{FGH}	2.44^{CD}	2.02^{CD}	1.71^{D}	0.57^{CDE}	0.83^{BCDEF}	0.32^{EF}	0.75^{CDE}
Site 3	2.12^{DE}	2.85^{BC}	3.37^{BC}	3.19^{C}	0.90^{EFG}	2.49^{C}	2.58^{B}	2.34^{C}	0.54^{DE}	0.43^{DEF}	0.55^{CDEF}	1.21^{BC}
Site 4	3.57^{B}	2.36^{CD}	4.30^{A}	4.77^{A}	1.90^{CD}	1.42^{E}	3.22^{A}	3.56^{B}	1.90^{AB}	1.08^{ABCDE}	2.16^{A}	1.77^{A}
Site 5	3.17^{BC}	3.05^{B}	3.04^{CD}	2.29^{DE}	1.44^{DE}	2.26^{CD}	2.94^{A}	1.32^{EFG}	1.66^{AB}	0.65^{CDEF}	1.19^{B}	0.90^{BCD}
Site 6	2.39^{CDE}	3.16^{B}	3.01^{CDE}	1.86 ^{EF}	0.59^{FGH}	2.21^{CD}	3.22^{A}	0.86^{G}	0.97^{BCDE}	1.23^{ABCD}	0.51^{CDEF}	0.73^{CDEF}
Site 7	2.43^{CDE}	3.28^{B}	2.41^{EFG}	2.25^{DE}	0.63^{FGH}	2.48^{CD}	1.66^{DEF}	1.65^{E}	1.61^{ABC}	1.15^{ABCDE}	0.73^{BCDE}	0.69^{DEF}
Site 8	2.38^{CDE}	1.86^{D}	2.24^{FG}	1.81^{F}	1.10^{EF}	0.95^{E}	1.94^{DE}	0.95^{FG}	1.47^{ABCD}	0.43^{DEF}	0.46^{DEF}	0.69^{DEF}
Site 9	3.52^{B}	5.29^{A}	N/A**	4.59^{AB}	3.40^{B}	4.77^{B}	N/A	4.47^{A}	1.37^{ABCD}	1.06^{ABCDE}	N/A	1.25^{B}
Site 10	4.61^{A}	5.44^{A}	2.95^{CDE}	5.07^{A}	4.16^{A}	5.58^{A}	2.53^{BC}	4.92^{A}	2.18^{A}	1.82^{A}	1.04^{BC}	1.07^{BCD}
Site 11	2.95^{CDE}	5.52^{A}	1.80^{GH}	4.52^{B}	2.18^{C}	5.84^{A}	1.54^{DEF}	4.75^{A}	1.44^{ABCD}	1.67^{AB}	0.30^{EF}	0.66^{DEF}
Site 12	0.82^{G}	1.76^{D}	1.61^{H}	2.32^{DE}	0.11^{H}	1.42^{E}	1.31^{F}	1.41^{EF}	0.29^{E}	0.37^{EF}	0.03^{F}	0.38^{EF}
Site 13	1.07^{FG}	2.70^{BC}	1.85^{GH}	1.86^{EF}	0.36^{GH}	2.39^{CD}	1.42^{EF}	1.08^{FG}	0.18^{E}	1.35^{ABC}	0.36^{EF}	0.74^{CDEF}
Site 14	3.21^{BC}	2.65^{BC}	3.69 ^{AB}	2.46^{D}	2.43^{C}	1.99^{D}	3.14^{A}	2.18^{CD}	1.23^{ABCD}	0.02^{F}	0.94^{BCD}	0.27^{F}

TA: total aerobic counts; YM: total yeast and mold counts; TC: total coliform counts.

PL: packing line.

* A different letter in the same column shows a significant difference ($P < 0.05$) in TA, YM, or TC counts at a specific sample site from the counts at other sites sampled within the same plant.

** PL 3 does not have site 9, a single period (.) was used when conducting the statistical analysis in SAS.

Table 3-4 Mean TA, YM, and TC counts in samples collected from individual PLs at different sampling times

Sampling time	Mean population (log CFU/cm^2)											
	TA				YM				TC			
	PL 1	PL 2	PL 3	PL 4	PL 1	PL 2	PL 3	PL4	PL 1	PL 2	PL 3	PL 4
T1	2.11^{B*b*}	2.75^{Ba}	2.04^{Bb}	2.24^{Bb}	1.37^{Ac}	2.39^{Ba}	1.70^{Bbc}	1.85^{Bb}	1.08^{Aa}	0.33^{Bb}	0.26^{Bb}	0.43^{Cb}
T2	2.80^{Ab}	3.49^{Aa}	3.13^{Aa}	3.33^{Aa}	1.49^{Ac}	2.90^{Aa}	2.65^{Aab}	2.52^{Ab}	1.15^{Aa}	1.21^{Aa}	1.08^{Aa}	0.94^{Ba}
T3	2.84^{Ab}	3.54^{Aa}	3.36^{Aa}	3.47^{Aa}	1.58^{Ac}	2.98^{Aa}	2.55^{Ab}	2.69^{Aab}	1.20^{Aa}	1.28^{Aa}	N/A***	1.24^{Aa}

TA: total aerobic counts; YM: total yeast and mold counts; TC: total coliform counts.

T1: samples collected at 9 am; T2: Samples collected at noon; T3: samples collected at 3 pm.

PL: packing line.

*Uppercase letters compare the significance of the differences in TA, YM, and TC counts in samples collected at different sampling times within each plant.

**Lowercase letters show the significance of the differences in TC, YM, or TC counts in samples collected from different plants at a specific sampling time.

***Samples were unavailable due to the early ending of the packing days in the 2nd and 3rd visits.

Table 3-5 Mean TA, YM, and TC counts in samples collected from various sites at different sampling times

	Mean population (log CFU/cm^2)								
	TA			YM			TC		
Site	T1	T2	T3	T1	T2	T3	T1	T2	T3
Site 1	$1.36^{DEF*b**}$	2.89^{EFGa}	3.39^{Ba}	0.88^{EFb}	2.20^{CDa}	2.21^{Ca}	0.01^{Cb}	1.19^{ABa}	1.56^{Aa}
Site 2	1.61^{CDEFb}	2.77^{EFGa}	3.19^{BCa}	1.11^{EFb}	1.95^{CDEFa}	2.07^{CDa}	0.00^{Cb}	0.79^{BCa}	1.31^{ABa}
Site 3	2.37^{BCa}	3.04^{EFGa}	3.21^{BCa}	1.77^{Da}	2.16^{CDEa}	2.25^{Ca}	0.06^{Cb}	0.82^{BCab}	1.36^{Aa}
Site 4	4.29^{Aa}	3.40^{CDEb}	3.41^{Bb}	2.95^{BCa}	2.42^{Cb}	2.01^{CDEb}	2.16^{Aa}	1.38^{ABa}	1.47^{Aa}
Site 5	1.94^{CDb}	3.61^{CDa}	3.12^{BCa}	1.46^{DEb}	2.32^{CDa}	2.04^{CDa}	0.57^{BCb}	1.58^{Aa}	1.13^{ABab}
Site 6	1.57^{CDEFb}	3.32^{CDEFa}	2.91^{BCa}	1.24^{DEb}	1.89^{CDEFa}	1.80^{CDEa}	0.26^{Cb}	1.17^{ABa}	1.36^{Aa}
Site 7	1.81^{CDEb}	2.98^{DEFGa}	3.10^{BCa}	0.91^{EFb}	2.00^{CDEFa}	1.96^{CDEa}	0.50^{BCb}	1.32^{ABa}	1.51^{Aa}
Site 8	0.97^{EFb}	2.69^{FGa}	2.63^{CDa}	0.61^{Fb}	1.47^{EFa}	1.56^{DEa}	0.43^{BCa}	0.94^{ABCa}	1.08^{ABa}
Site 9	4.19^{Aa}	4.97^{Aa}	4.40^{Aa}	4.15^{Aa}	4.73^{Aa}	3.91^{Ba}	0.85^{BCa}	1.56^{Aa}	1.31^{ABa}
Site 10	4.28^{Ab}	4.54^{ABab}	5.08^{Aa}	4.18^{Aa}	4.27^{ABa}	4.84^{Aa}	1.30^{ABa}	1.55^{Aa}	1.85^{Aa}
Site 11	3.24^{Bb}	4.02^{BCa}	4.38^{Aa}	3.47^{Bb}	3.87^{Bab}	4.07^{Ba}	0.61^{BCa}	1.11^{ABa}	1.62^{Aa}
Site 12	1.17^{DEFa}	1.81^{Ha}	1.99^{Da}	0.52^{Fb}	1.31^{Fa}	1.41^{Ea}	0.01^{Ca}	0.41^{Ca}	0.49^{BCa}
Site 13	0.86^{Fb}	2.34^{GHa}	2.53^{CDa}	0.57^{Fb}	1.64^{DEFa}	1.80^{CDEa}	0.07^{Cb}	0.95^{ABCa}	1.14^{ABa}
Site 14	2.89^{Ba}	3.13^{DEFa}	2.85^{BCa}	2.49^{Ca}	2.47^{Ca}	2.18^{CDa}	0.66^{BCa}	0.79^{BCa}	0.20^{Ca}

TA: total aerobic counts; YM: total yeast and mold counts; TC: total coliform counts.

T1: samples collected at 9 am; T2: Samples collected at noon; T3: samples collected at 3 pm.

*Uppercase letters in the same column compare the significance of the differences in TA, YM, and TC counts among different sample sites at each sampling time.

**Lowercase letters show the significance of differences in TA, YM, or TC counts among three sampling times at the same site.

Table 3-6 Incidence of TTC and EC in samples collected from various sites of 4 fresh peach packing lines.

	Number of positive samples	Number of samples per unit	Percentage (%)	Number of positive samples	Number of samples per unit	Percentage (%)
		TTC			EC	
PL						
PL 1	39	121	32.23	14	121	11.57
PL 2	53	126	42.06	7	126	5.56
PL 3	22	91	24.18	21	91	23.08
PL 4	42	126	33.33	21	126	16.67
Sampling site						
Site 1	12	34	35.29	6	34	17.65
Site 2	12	34	35.29	8	34	23.53
Site 3	10	34	29.41	8	34	23.53
Site 4	16	34	47.06	9	34	26.47
Site 5	17	34	50.00	2	34	5.88
Site 6	12	34	35.29	2	34	5.88
Site 7	18	34	52.94	2	34	5.88
Site 8	11	34	32.35	2	34	5.88
Site 9	12	26	46.15	5	26	19.23
Site 10	14	33	42.42	9	33	27.27
Site 11	10	32	31.25	4	32	12.50
Site 12	1	33	3.03	1	33	3.03
Site 13	7	34	20.59	2	34	5.88
Site 14	4	34	11.76	3	34	8.82
Sampling time						
T1	22	163	13.50	11	163	6.75
T2	71	162	43.83	31	162	19.14
T3	63	139	45.32	21	139	15.11
Total	156	464	33.62	63	464	13.58

TTC: Thermo-tolerant coliforms; EC: Enterococci.

PL: Packing line.

T1: samples collected at 9 am; T2: Samples collected at noon; T3: samples collected at 3 pm.

CHAPTER 4

MICROBIAL LOADS ON FRESH PEACH AND GLOVES WORN BY PEACH PACKERS IN SELECTED PACKING FACILITIES IN GEORGIA

Wang, P., Pitts, K. B., Chavaz, D., and Chen, J. To be submitted to *LWT - Food Science and Technology.*

ABSTRACT

Duplicate peach samples were collected from the dumping and weighing areas of four fresh peach packing lines in Georgia at 0, 3, and 6 h into the packing process on 3 random packing days per operation in the summers of 2018 and 2019, along with duplicate glove samples from peach sorters and packers at the manual sorting and packing areas. The samples were analyzed for total aerobes (TA), total yeasts and molds (YM), and total coliforms (TC), as well as the incidence of thermotolerant coliforms (TTC) and enterococci (EC). Not affected by sampling times, peach samples from the weighing area had significantly higher (P<0.05) levels of TA, YM, and TC counts and >5 or 3 times higher incidence of TTC or EC than samples from the dumping area. The levels of TA, YM, and TC on gloves from peach sorters and packers were similar, but they were significantly higher at the 3 and 6 h sampling points than the 0 h sampling point. Among analyzed glove samples, 39.39% and 7.58% tested positive for TTC or EC. This study indicated that the packing process itself, as well as the sorters and packers involved, may be important sources of fresh peach contamination.

Keywords: Indicator organisms, fresh produce, peach, gloves, fresh produce packing line

4.1. Introduction

The commercial fresh produce packing process involves sorting, grading, and weighing and some operations may also require washing/rinsing (U.S. FDA, 2015) to reduce surface contaminants and achieve better produce quality. The process has been deemed as one of the key steps influencing the microbial safety of fresh produce, according to the Produce Safety Rule of the Food Safety Modernization Act (FSMA). Studies have linked the elevated microbial loads on fresh produce to contact with contaminated packing equipment (Ilic, Odomeru, & LeJeune, 2008; Izumi, Poubol, Hisa, & Sera, 2008) and postharvest water (Castillo et al., 2004; Gagliardi, Millner, Lester, & Ingram, 2003), or employees with poor agricultural practices (Heredia et al., 2016). Other studies have reported that when sufficient sanitizers were used and their concentrations were properly maintained in the produce wash water, the levels of microbial contaminations on packed produce could be significantly reduced, although the contaminants could not be eliminated (De et al., 2018; Materon, Martinez-Garcia, & McDonald, 2007). In some facilities, however, the packing process did not seem to have profoundly improved the microbial quality of fresh produce, and the microbial loads on packed fruits had numerically, but insignificantly, higher ($P > 0.05$) microbial loads than unpacked fruits (Quansah et al., 2019).

Peaches harvested from the field are usually covered with a dense layer of trichomes which are considered as part of the plant defense mechanisms, preventing pathogens and other pollutants from reaching the peach epidermis (Karabourniotis, Liakopoulos, Nikolopoulos, & Bresta, 2019). A laboratory study illustrated the removal of trichomes can cause micro-damages to the epidermis and increases the polarity of the peach surface which consequently becomes relatively more hydrophilic and more attractive to microbial contamination from the environment

(Fernández et al., 2011). However, the microbial quality of peaches from commercial packing operations with the washing/rinsing step in place have not been explored previously.

The fresh peach packing process relies heavily on human handling such as sorting and weighing, where human hands may have direct contact with the surface of the peach. To minimize the potential transmission of human pathogens from contaminated hands onto produce surface (Conover & Gibson, 2016), peach sorters and packers are required to properly wash their hands before entering the working station (U.S. FDA, 2018), and gloves are sometimes required by either the clients or packing facilities to achieve an additional layer of protection (Montville, Chen, & Schaffner, 2001). However, gloves may occasionally be damaged during a prolonged packing process or intensive production activities (Paulson, 2002). Both intact and damaged gloves can facilitate the cross-contamination among fresh produce (Qi, He, Beuchat, Zhang, & Deng, 2020) between contaminated hands and contacted produce (Jensen, Danyluk, Harris, & Schaffner, 2017). Therefore, investigating microbial buildups on the surface of gloves overtime becomes pivotal as an attempt to explore the best practices for glove-wearing during the fresh peach packing process.

The objective of this study was to examine the microbial quality of fresh peaches in selected commercial packing operations and the microbial status of gloves worn by peach packers.

4.2. Material and methods

4.2.1. Sample collection

In the harvest seasons of 2018 and 2019, four fresh peach packing operations (coded as M, N, P, and Q) in Georgia were visited on 3 different packing days at each operation. On an 8-hour operation day, duplicate fresh peach samples (5 peaches/sample) were collected at 0, 3, and

6 h of the packing process from the dumping and weighing areas of the packing lines. Collected peach samples were placed in sterile sample bags (Fisher Scientific, Pittsburgh, PA USA).

Two glove samples with 3 pairs per sample were collected from peach sorters at the manual sorting area and packers at the weighing area after 3 h and 6 h into the packing process, and 2 samples of unused gloves were collected from the glove boxes at the beginning of the packing process (0 h). The glove samples were placed in sterile sample bags (Fisher Scientific). Both peach and glove samples were kept at 4 °C in a portable cooler (Katy, TX USA) during transportation to the laboratory and stored at refrigeration condition (4 °C) till analysis on the following day.

The length of daily packing time in a facility usually depends on the amount of available fruits harvested on a particular day. For this exact reason, packing operation P concluded its daily packing after 3 h during the second visit and after 45 min in the third visit. Therefore, fruit and glove samples at the 6 h sampling point in the second visit, and the 3 h and 6 h sampling points in the third visit, were not available.

4.2.2. Sample processing

Three peaches from each original fruit sample were randomly selected and placed in a sterile stomacher bag specified above, and the average weights per sample at the dumping and weighing areas were 531.32 g and 530.50 g, respectively. After adding 200 ml phosphate-buffered saline (PBS; pH 7.4), the bag was sealed and rotated ont an orbital shaker (Lab-line Instruments, Melrose, IL USA) at 225 rpm for 30 min, with 15 min for each side of the bag. The samples were subsequently hand massaged for 1 min.

A knot was tied at the wrist area of each glove in a collected sample to seal its inner surface. This process was handled aseptically by wearing a pair of sterile gloves. Three pairs of gloves with tied knots were placed in a sterile bag with 100 ml PBS. The gloves in the sample bags were hand massaged for 30 s, stomached (Seward Laboratory, Bohemia, New York, USA) at normal speed for 30 s, and hand massaged for another 30 s before analysis.

Rinsates of both peach and glove samples were serially diluted when necessary and analyzed for the levels of total aerobes (TA) on tryptic soy agar plates and total coliforms (TC) on MacConkey plates at 37 °C for 24 h, and total yeasts and molds (YM) on acidified potato dextrose agar (using tartaric acid to pH 3.5) at 25 °C for 48-72 h. Presumptive colonies of thermotolerant coliforms (TTC) and enterococci (EC) were selected on MacConkey plates at 44.5 °C for 18-22 h and on m-*Enterococcus* agar plates at 37 °C for up to 48 h, respectively. Further biochemical tests were conducted by inoculating a loopful of presumptive TTC culture on triple sugar iron agar slants (TSI) and incubating at 37 °C and in EC broth at 44.5 °C. Selected presumptive EC colonies were confirmed in brain heart infusion broth with 6.5% sodium chlorite at 37 °C under 24 h incubation. The microbiological media used in this study were from Becton, Dickinson, and Company (Sparks, MD USA), except for the EC broth that was purchased from bio-WORLD (Dublin, OH USA). The detection limits of the assay for peach samples ranged from 0.10 to 0.64 log CFU/g based on the weights of peach samples. The detection limit of the assay for glove sample was 1.92 log CFU/glove.

4.2.3. Statistical analysis

The means of TA, YM, and TC counts on fresh peach and glove samples were fit in two separate general linear models and tested for significant differences over sampling time by Fisher's least significant difference tests, at different sampling locations and packing operations using SAS University Edition (SAS Institute, Cary, NC USA). The incidences of TTC and EC presence from fresh peaches and glove samples were calculated using the number of positive samples divided by the total numbers of samples collected from different packing operations, sample locations, or at different sampling times.

4.3. Results

4.3.1 Peach samples

Based on the statistical test results in Table 4-1, the effect of packing operation was a significant factor ($P < 0.05$) influencing the TA and YM counts, but not the TC count. Although there were no significant differences ($P > 0.05$) among the mean TA and TC counts on samples collected at different time intervals, the average counts of all 3 hygiene indicators on samples collected at the weighting area were significantly higher than those collected at the dumping area (Table 4-2). Operation N and Q had similar mean TA counts which were significantly lower than the average TA count from operation M. In comparison, operation P had the highest average TA count, and the samples from this operation also had an average YM which was significantly higher than the same indicator counts from the other 3 operations. The same trends were observed when the TA and YM counts on fruit were analyzed based on the results from the dumping area of individual packing operations (Table 4-3), except that the differences in the average TA counts between the samples from Operation Q and M were not significant. The two counts on peach samples collected at the weighing area of operation P were significantly higher than other operations involved in the study, except for the TA counts on samples from operation M.

Most of the peach samples collected from the dumping area had a significantly lower ($P < 0.05$) level of the 3 indicators than the samples from the weighing area (Table 4-3). However, the mean TA count on peach samples from operation P and the mean TC counts from operations

P and Q were not statistically different (*P* > 0.05) at different sampling locations. The YM counts on peach samples collected at the dumping area was significantly higher than the ones collected at the weighing area of operation P.

Fifty-eight fruit samples tested positive for TTC (43.94%), but only 4 out of the 132 samples were positive for EC (3.03%) (Table 4-4). Operation N had the highest TTC incidence, followed by operations P and Q, then M; EC was only isolated from operation M and P. The incidences of TTC presence on fruit samples collected at the three different sampling points ranged from 42.50 to 45.45% with less than 3% differences between any two of the sampling points. Two samples were positive for EC on samples collected after 3 h into packing (4.55%), while only one sample tested positive for EC among samples collected after 0 (2.08%) and 6 (2.50%) h into packing. The percentage of samples positive for TTC from the weighing area (74.24%) was more than 5 times higher than the samples collected at the dumping area (13.64%). Similarly, the number of samples tested positive for EC at the weighing area was more than doubled compared to the number of positive samples at the dumping area, although the incidences were both less than 5%.

4.3.2. Glove samples

Among all 3 effects tested, the levels of TA, YM, and TC on gloves were only affected ($P < 0.05$) by sampling time (Table 4-5). Although the counts of all three indicators increased significantly in samples collected after 3 and 6 h, compared to 0 h, into the packing process, no significant differences ($P > 0.05$) were observed on glove samples collected from sorters and packers (Table 4-6). The highest and lowest TA counts on gloves were found in operation M and P, respectively, and the mean YM count on gloves from operation N was significantly higher than the same count on samples from operation M.

More than one-third of the glove samples (26/66) tested positive for TTC, but only 7.58% (5/66) tested positive for EC (Table 4-7). A higher incidence of TTC presence was noticed in operations N, followed by Q, P, and M. Positive EC samples were only detected in operations M and Q. Similar to the trend of the three hygiene indicators (Table 4-6), a higher incidence of TTC presence was found in samples collected after 3 and 6 h into the packing process, compared to the lower incidences from samples collected at the beginning of the packing. Only samples collected at the 6 h sampling point tested positive for EC (25%). Differences in the incidences of TTC on glove samples collected from the sorting *vs*. packing areas were less than 9%, while the incidences of EC positive glove samples collected from the two areas differed by less than 3%.

4.4. Discussion

In the present study, the levels of TA, YM, and TC counts on peach samples collected from the weighing area were 0.79, 0.54, or 0.92 log CFU/g higher than the respective counts on samples collected from the dumping areas (Table 4-2). Similar to these results, a study taking place in a satsuma packing shed found 0.90 log CFU/g more mesophilic and fungi counts on fruit collected at the packing shed compared to those collected in the field (Izumi, Poubol, Hisa, & Sera, 2008). Heredia et al. (2016) reported that the TC counts on cantaloupes collected in a packing environment in Mexico were 0.34 - 0.91 log CFU/fruit higher than the samples collected during transport. The increase in microbial loads on produce during packing could be due to the failure in maintaining a sufficient concentration of sanitizers in wash water (Castillo et al., 2004; Gagliardi, Millner, Lester, & Ingram, 2003), improper produce handling practice, and poor hand hygiene (Bartz et al., 2017), as well as cross-contamination from equipment surface in the packing environment (Ilic, Odomeru, & LeJeune, 2008).

Different from the samples of other three operations, those from operation P had similar average TA and TC counts from the dumping and weighing areas (*P* > 0.05), and the average YM counts on samples from the weighing area were even significantly lower (*P* < 0.05) compared to the counts on samples from the dumping area (Table 4-3). It is not known whether the smaller sample size of operation P (Table 4-2) played a role in the observed phenomena. What is known, however, is that the four packing operations used different sanitizers to decontaminate their fruit. Operation P included a maximum of 1 ppm chlorine dioxide in peach wash water, while the rest of the operations all used chlorine as the added sanitizer in wash water; 50 ppm by operation N and Q, and 50 - 100 ppm by operation M (K. B. Pitts, personal communication, November 15, 2019). Both chemicals rely on the high oxidation ability to achieve the bacterial disinfection. The oxidation power of chlorine dioxide could be 2.5 times more than the same molar concentration of chlorine (Benarde, Snow, Olivieri, & Davidson, 1967), but contact time between the sanitizer and fresh produce may also influence the efficacy of the washing step (Praeger, Herppich, & Hassenberg, 2018). Previous research showed that treatment with 1 ppm chlorine dioxide for 1 min decreased the artificially-inoculated yeasts and molds on fresh blueberries by only 0.25 log CFU/g and other pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* Typhimurium by less than 0.15 log CFU/g (Wu & Kim, 2007). In a separate study, treating fresh lettuce with 100 ppm chlorine for 1 min reduced the level of *Escherichia coli* O157:H7 by 0.7 log CFU/g (López-Gálvez, Gil, Truchado, Selma, & Allende, 2010). These results suggest that the disinfecting effects of chlorine dioxide or chlorine in peach wash water used by each of the 4 peach operations, might be very limited.

It was noticed that peach samples collected from the dumping area of operation P had the highest ($P < 0.05$) average TA and YM counts, as well as the highest level ($P > 0.05$) TC count compared to the respective counts on samples collected from the same area at other operations (Table 4-3). Apart from the possible varietal differences of peaches packed in different operations, the choice of the adopted cooling process could play a role in affecting the microbial loads on peach samples upon arriving at the dumping areas. Different types of cooling systems have been used by fresh produce packers to slow down the enzymatic activities of plant cells and spoilage microorganisms to achieve prolonged shelf life and product quality (Fellows, 2009). In this study, peaches from operation P were subjected to air cooling in a cold room, while in other operations, peaches went through a hydro-cooling system which contained water with 50-100 ppm chlorine (K. B. Pitts, personal communication, November 15, 2019). The lower levels of hygiene indicators found on peach samples collected at the dumping areas of operation M, N, and Q could be attributed to the disinfecting effect of chlorinated hydro-cooling water. A recent study found that when submerging *Salmonella*-inoculated blueberries into a simulated hydro-cooling system with water containing 150 ppm chlorine at 2 °C with constant agitation for 6 min, more than 4 log CFU/g reduction in *Salmonella* population could be achieved (De et al., 2019). Another study concluded that hydro-cooling was superior to air cooling in controlling inoculated *Salmonella* on strawberries and could reduce the population of the pathogen to below the detectable level (Sreedharan, Tokarskyy, Sargent, & Schneider, 2015). Furthermore, hydro-cooling was thought to be 15 times more efficient than air cooling in reducing the internal temperature of fresh produce (Boyette, Estes, & Rubin, 1992). Previous studies have, nevertheless, reported that if the concentration of the sanitizer in water is not sufficient to inactivate pathogens, hydro-cooling may facilitate the internalization of foodborne pathogens, *e.g. Listeria monocytogenes*, into fresh produce such as cantaloupes (Macarisin et al., 2017) and avocados (Chen, Evans, Hammack, Brown, & Macarisin, 2016) through the surface stem scars. Therefore, maintaining the proper concentration of sanitizers in hydro-cooling water is critical when this type of system is used in a peach packing operation.

Sorters and packers in the packing operations involved in the present study were required to change gloves every 3 hours. Interestingly, the findings of this study revealed that gloves collected at the 3 and 6 h sampling points had approximately similar levels of microbial contamination (Table 4-6). Montville and Schaffner (2003) stated that the inoculum level of bacteria on produce can significantly affect their transfer rate from produce to the surface of hands/gloves. In the current study, the levels of all three indicators on fruit passing the packing lines did not change significantly over time (Table 4-2), thus, it was reasonable for the gloves to contain similar levels of TA, YM, and TC counts after the first, and the second 3-h intervals on a packing day. Microbial buildup on gloves during produce packing has not been explored previously in an industrial setting. But in a related study in a commercial bell pepper packinghouse, it was found that after a 3-h of packing period, the TC counts on 50% of the bare hands of pepper sorters and handlers increased from the initial 1.8 log CFU/hand to 3.0 log CFU per hand, although on 20% of the sampled hands, the count decreased to the non-detectable level (Soto-Beltran, Campo, Campos-Sauceda, Aven-Bustillos, & Chaidez, 2015). The authors attributed the reduction of TC counts on packers' hands to the residual hand sanitizer of copper sulfate and transfer of TC to other contact surfaces (Soto-Beltran et al., 2015).

The levels of all three indicators on glove samples were not significantly affected ($P > 0.05$) by the type of peach handlers, sorters *vs*. packers (Table 4-5); furthermore, similar numbers of TTC/EC positive samples were identified from the two sources (Table 4-6). These observations are consistent with the findings of a commercial bell pepper packing line study where out of the 88 samples analyzed, the number of coliform positive samples from the sorters (n = 23) and packers (n = 22) varied only by 1 sample after a 3 h of packing period (Soto-Beltran et al., 2015). Since the sorters and packers in the surveyed packing facilities are all assigned for a single, specific task, microbial loads on glove surfaces from both groups of workers should be closely linked to the same batch of fruit, if good agricultural and hygiene practices are followed and no other source of contamination is involved. If produce sorters and packers are assigned with multiple responsibilities, the microorganisms from other sources, such as equipment surfaces, machine frameworks, and packaging containers, could all end up on their hands or gloves. Some of these surfaces may not be cleaned as often as produce-contact surfaces, the risk of microbial transmission could be greater. Therefore, limited activities must be assigned to each handler to slow down the microbial buildup on handlers' hands or gloves and to reduce the risk of cross-contamination (Montville, Chen, & Schaffner, 2001; Paulson, 2002). The authors realized that the number of glove samples examined in this study could be higher, future research with a large sample size should be conducted to confirm the finding of this study.

4.5. Conclusions

Not affected by sampling time, peach samples from the weighing area had significantly higher ($P < 0.05$) average TA, YM, and TC counts and higher incidences of TTC and EC presence, compared to the samples collected from the dumping area, suggesting that the role of the packing process in decontaminating fresh fruit is very limited and should be improved. The levels of TA, YM, and TC on gloves of peach sorters and packers were similar, but they were significantly higher at the 3 and 6 h sampling points comparing to the 0 h sampling point. This study indicates that the packing process and the sorters and packers involved are among the key elements in maintaining the microbial safety of peach detained for the fresh market.

References

Bartz, F. E., Lickness, J. S., Heredia, N., de Aceituno, A. F., Newman, K. L., Hodge, D. W., Jaykus, L.-A., García, S., & Leon, J. S. (2017). Contamination of fresh produce by microbial indicators on farms and in packing facilities: elucidation of environmental routes. *Applied and Environmental Microbiology, 83*(11), e02984-02916.

Benarde, M. A., Snow, W. B., Olivieri, V. P., & Davidson, B. (1967). Kinetics and mechanism of bacterial disinfection by chlorine dioxide. *Applied microbiology, 15*(2), 257-265.

Boyette, M., Estes, E., & Rubin, A. R. (1992). Hydrocooling. Postharvest technology series. North Carolina Cooperative Extension. Retrieved from https://content.ces.ncsu.edu/hydrocooling. Accessed 19 April 2021.

Castillo, A., Mercado, I., Lucia, L. M., Martinez-Ruiz, Y., De Leon, J. P., Murano, E. A., & Acuff, G. R. (2004). *Salmonella* contamination during production of cantaloupe: a binational study. *Journal of Food Protection, 67*(4), 713-720.

Chen, Y., Evans, P., Hammack, T. S., Brown, E. W., & Macarisin, D. (2016). Internalization of *Listeria monocytogenes* in whole avocado. *Journal of Food Protection, 79*(8), 1440-1445.

Conover, D. M., & Gibson, K. E. (2016). A review of methods for the evaluation of handwashing efficacy. *Food Control, 63*, 53-64.

De, J., Li, Y., Sreedharan, A., Schneider, R. G., Gutierrez, A., Jubair, M., Danyluk, M. D., & Schneider, K. R. (2018). A three-year survey of Florida packinghouses to determine microbial loads on pre-and post-processed tomatoes. *Food Control, 86*, 383-388.

De, J., Sreedharan, A., Li, Y., Gutierrez, A., Brecht, J. K., Sargent, S. A., & Schneider, K. R. (2019). Comparing the efficacy of postharvest cooling methods to enhance fruit quality and reduce *Salmonella* in artificially inoculated Southern highbush blueberry. *HortTechnology, 29*(3), 314-319.

Fernández, V., Khayet, M., Montero-Prado, P., Heredia-Guerrero, J. A., Liakopoulos, G., Karabourniotis, G., Del Río, V., Domínguez, E., Tacchini, I., Nerín, C., Val, J., & Heredia, A. (2011). New insights into the properties of pubescent surfaces: peach fruit as a model. *Plant Physiology, 156*(4), 2098-2108. doi:10.1104/pp.111.176305.

Fellows, P. J. (Ed.). (2009). 2. Raw material preparation. In *Food Processing Technology: Principles and Practice* (pp. 99). Boca Raton, FL: CRC Press.

Gagliardi, J. V., Millner, P. D., Lester, G., & Ingram, D. (2003). On-farm and postharvest processing sources of bacterial contamination to melon rinds. *Journal of Food Protection, 66*(1), 82-87. doi:10.4315/0362-028X-66.1.82.

Heredia, N., Caballero, C., Cardenas, C., Molina, K., Garcia, R., Solis, L., Burrowes, V., Bartz, F. E., de Aceituno, A. F., & Jaykus, L.-A. (2016). Microbial indicator profiling of fresh produce and environmental samples from farms and packing facilities in northern Mexico. *Journal of Food Protection, 79*(7), 1197-1209.

Ilic, S., Odomeru, J., & LeJeune, J. T. (2008). Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. *Journal of Food Protection, 71*(12), 2398-2403. doi:10.4315/0362-028x-71.12.2398.

Izumi, H., Poubol, J., Hisa, K., & Sera, K. (2008). Potential sources of microbial contamination of satsuma mandarin fruit in Japan, from production through packing shed. *Journal of Food Protection, 71*(3), 530-538. doi:10.4315/0362-028x-71.3.530.

Jensen, D. A., Danyluk, M. D., Harris, L. J., & Schaffner, D. W. (2017). Quantifying bacterial cross-contamination rates between fresh-cut produce and hands. *Journal of Food Protection, 80*(2), 213-219. doi:10.4315/0362-028X.JFP-16-240.

Jimenez, M., Siller, J. H., Valdez, J. B., Carrillo, A., & Chaidez, C. (2007). Bidirectional *Salmonella enterica* serovar Typhimurium transfer between bare/glove hands and green bell pepper and its interruption. *International Journal of Environmental Health Research, 17*(5), 381-388. doi:10.1080/09603120701372664.

Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., & Bresta, P. (2019). Protective and defensive roles of non-glandular trichomes against multiple stresses: structure-function coordination. *Journal of Forestry Research*, 1-12.

López-Gálvez, F., Gil, M. I., Truchado, P., Selma, M. V., & Allende, A. (2010). Cross-contamination of fresh-cut lettuce after a short-term exposure during pre-washing cannot be controlled after subsequent washing with chlorine dioxide or sodium hypochlorite. *Food Microbiology, 27*(2), 199-204. doi:10.1016/j.fm.2009.09.009.

Materon, L. A., Martinez-Garcia, M., & McDonald, V. (2007). Identification of sources of microbial pathogens on cantaloupe rinds from pre-harvest to post-harvest operations. *World Journal of Microbiology and Biotechnology, 23*(9), 1281-1287.

Macarisin, D., Wooten, A., De Jesus, A., Hur, M., Bae, S., Patel, J., Evans, P., Brown, E., Hammack, T., Chen, Y. (2017). Internalization of *Listeria monocytogenes* in cantaloupes during dump tank washing and hydrocooling. *International journal of food microbiology, 257*, 165-175.

Montville, R., Chen, Y., & Schaffner, D. W. (2001). Glove barriers to bacterial cross-contamination between hands to food. *Journal of Food Protection, 64*(6), 845-849.

Montville, R., & Schaffner, D. W. (2003). Inoculum size influences bacterial cross contamination between surfaces. *Applied and Environmental Microbiology, 69*(12), 7188-7193.

Paulson, D. S. (2002). 17: Handwashing, gloving, and disease transmission by the food preparer. In Paulson, D. S. (Ed.), *Handbook of Topical Antimicrobials: Industrial Applications in Consumer Products and Pharmaceuticals* (pp. 255-270). New York City, NY: Marcel Dekker.

Praeger, U., Herppich, W. B., & Hassenberg, K. (2018). Aqueous chlorine dioxide treatment of horticultural produce: Effects on microbial safety and produce quality-A review. *Critical Reviews in Food Science and Nutrition, 58*(2), 318-333. doi:10.1080/10408398.2016.1169157.

Quansah, J. K., Gazula, H., Holland, R., Scherm, H., Li, C., Takeda, F., & Chen, J. (2019). Microbial quality of blueberries for the fresh market. *Food Control, 100*, 92-96. doi:10.1016/j.foodcont.2018.12.034.

Qi, Y., He, Y., Beuchat, L. R., Zhang, W., & Deng, X. (2020). Glove-mediated transfer of *Listeria monocytogenes* on fresh-cut cantaloupe. *Food Microbiology, 88*, 103396.

Sreedharan, A., Tokarskyy, O., Sargent, S., & Schneider, K. R. (2015). Survival of *Salmonella* spp. on surface-inoculated forced-air cooled and hydrocooled intact strawberries, and in strawberry puree. *Food Control, 51*, 244-250. doi:https://doi.org/10.1016/j.foodcont.2014.11.042

Soto-Beltran, M., Campo, C. N., Campos-Sauceda, J., Aven-Bustillos, R., & Chaidez, C. (2015). Prevalence of *Salmonella*, *Escherichia coli* and coliforms on bell pepper from the field to the packing process. *African Journal of Microbiology Research, 9*(10), 718-724.

Tortorello, M. L. (2003). Indicator organisms for safety and quality--uses and methods for detection: minireview. *Journal of AOAC International, 86*(6), 1208-1217. http://europepmc.org/article/MED/14979704.

U.S. FDA. (2015) Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. 80 FR 74353 (Docket No. FDA-2011-N-0921). Retrieved from https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption. Accessed 19 April 2021.

U.S. FDA. (2018) Standards for the growing, harvesting, packing, and holding of produce for human consumption; draft guidance for industry; availability. 83 FR 53196 (Docket No. FDA–2018–D–3631). Retrieved from https://www.federalregister.gov/documents/2018/10/22/2018-23006/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption-draft. Accessed 19 April 2021.

Wu, V. C. H., & Kim, B. (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiology, 24*(7), 794-800. doi:https://doi.org/10.1016/j.fm.2007.03.010.

Table 4-1 The results of the significance test for the effects of packing operation, sampling time, and sampling location on the levels of hygiene indicators on peach samples

Indicators	Effect	DF	Type III SS	Mean Square	F Value	Pr > F
TA	Packing operation	3	12.04	4.01	10.78	0.0079
	Sampling time	2	0.33	0.16	0.29	0.75
	Sample location	1	17.68	17.68	31.56	<.0001
YM	Packing operation	3	21.93	7.31	37.32	0.0003
	Sampling time	2	0.30	0.15	0.51	0.60
	Sample location	1	7.52	7.52	25.81	<.0001
TC	Packing operation	3	0.57	0.19	1.04	0.49
	Sampling time	2	1.61	0.80	2.08	0.13
	Sample location	1	15.43	15.43	40.07	<.0001

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

DF: degrees of freedom, the number of degrees of freedom in this model is 25.

The number of degrees of freedom associated with this model errors is 106.

Type III SS: Type III sum of square

Pr > F: *P* value, the tested effect is significant when the *P* value is less than 0.05.

Table 4-2 Total aerobe, total yeast and mold, and total coliform counts on peach samples collected from different packing operations, at different sampling times and locations (n=132)

Fruit samples	TA	YM	TC
	log CFU/g		
Packing operation			
M (n = 36)	3.36^{B}	2.30^{B}	1.19^{A}
N (n = 36)	2.83^{C}	2.27^{B}	0.98^{A}
P (n = 24)	3.95^{A}	3.41^{A}	1.37^{A}
Q (n = 36)	2.94^{C}	2.18^{B}	1.07^{A}
Sampling time			
0 h (n = 48)	3.30^{A}	2.61^{A}	1.30^{A}
3 h (n = 44)	3.15^{A}	2.49^{A}	1.01^{A}
6 h (n = 40)	3.15^{A}	2.25^{B}	1.01^{A}
Sampling location			
Dumping area (n = 66)	2.81^{B}	2.19^{B}	0.66^{B}
Weighing area (n = 66)	3.60^{A}	2.73^{A}	1.58^{A}

TA: total aerobes; YM: yeasts and molds; TC: total coliforms; the 4 packing operations were coded as M, N, P, and Q.

Results with different letters were significantly ($P < 0.05$) different within each factor (packing operation, sampling time, or sample location).

Table 4-3 Total aerobe, total yeast and mold, and total coliform counts on peach samples collected at different sample locations and packing operations

Indicators	Sampling location	Packing operation			
		M (n=36)	N (n=36)	P (n=24)	Q (n=36)
TA	Dumping area	2.97^{Bb}	2.18^{Bc}	3.94^{Aa}	2.53^{Bbc}
	Weighing area	3.75^{Aab}	3.48^{Abc}	3.95^{Aa}	3.35^{Ac}
YM	Dumping area	2.08^{Bb}	1.68^{Bb}	3.55^{Aa}	1.93^{Bb}
	Weighing area	2.52^{Ac}	2.87^{Ab}	3.28^{Ba}	2.43^{Ac}
TC	Dumping area	0.42^{Ba}	0.49^{Ba}	1.01^{Aa}	0.91^{Aa}
	Weighing area	1.97^{Aa}	1.48^{Abc}	1.73^{Aab}	1.23^{Ac}

TA: total aerobes; YM: yeasts and molds; TC: total coliforms; the 4 packing operations were coded as M, N, P, and Q.

Results of each hygiene indicator (TA, YM, or TC) followed by different uppercase letters were significantly different ($P < 0.05$) within the same operation; results with different lowercase letters were significantly different among the 4 packing operations.

Table 4-4 Incidences of thermotolerant coliforms and enterococci on peach samples collected from different packing operations at different sampling times and locations

Peach samples	TTC			EC		
	No. of positive samples	No. of samples per unit	Percentage (%)	No. of positive samples	No. of samples per unit	Percentage (%)
Packing operation						
M	13	36	36.11	3	36	8.33
N	20	36	55.56	0	36	0.00
P	10	24	41.67	1	24	4.17
Q	15	36	41.67	0	36	0.00
Sampling time						
0 h	21	48	43.75	1	48	2.08
3 h	20	44	45.45	2	44	4.55
6 h	17	40	42.50	1	40	2.50
Sampling location						
Dumping area	9	66	13.64	1	66	1.52
Weighing area	49	66	74.24	3	66	4.55
Total	58	132	43.94	4	132	3.03

TTC: Thermotolerant coliforms; EC: Enterococci; The 4 packing operations were coded as M, N, P, and Q.

Table 4-5 Results of significance test for the effects of packing operation, sampling time, and peach handler on the levels of hygiene indicators on glove samples

Indicators	Effect	DF	Type III SS	Mean Square	F Value	Pr > F
TA	Packing operation	3	0.91	0.30	0.32	0.81
	Sampling time	2	246.28	123.14	155.94	<.0001
	Peach handler	1	0.20	0.20	0.26	0.61
YM	Packing operation	3	17.79	5.93	4.00	0.070
	Sampling time	2	192.67	96.33	227.64	<.0001
	Peach handler	1	0.07	0.07	0.16	0.69
TC	Packing operation	3	1.45	0.48	0.32	0.81
	Sampling time	2	61.31	30.65	37.37	<.0001
	Peach handler	1	0.59	0.59	0.72	0.41

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

DF: degrees of freedom, the number of degrees of freedom in this model is 25.

The number of degrees of freedom associated with this model errors is 40.

Type III SS: Type III sum of square

Pr > F: *P* value, the tested effect is significant when the *P* value is less than 0.05.

Table 4-6 Levels of total aerobes, total yeasts and molds, and total coliforms on glove samples collected from peach handlers at different packing operations and sampling times (n=66)

Glove samples	TA	YM	TC
	log CFU/glove		
Packing operation			
M (n = 18)	4.03^{A}	2.25^{B}	2.24^{A}
N (n = 18)	3.88^{AB}	3.44^{A}	2.07^{A}
P (n = 12)	3.05^{B}	2.88^{AB}	0.73^{A}
Q (n = 18)	3.71^{AB}	2.83^{AB}	1.78^{A}
Sampling time			
0 h (n = 24)	1.02^{B}	0.54^{B}	0.14^{B}
3 h (n = 22)	5.15^{A}	4.10^{A}	2.68^{A}
6 h (n = 20)	5.40^{A}	4.23^{A}	3.15^{A}
Peach handler			
Sorter (n = 32)	3.74^{A}	2.82^{A}	1.91^{A}
Packer (n = 34)	3.70^{A}	2.87^{A}	1.78^{A}

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms; The 4 packing operations were coded as M, N, P, and Q; Sorter: gloves from peach handlers at the manual sorting area; Packer: gloves from peach handlers at weighing area.

The results with different uppercase letters within the same factor (packing operation, sampling time, or peach handler) were significantly ($P < 0.05$) different.

Table 4-7 Incidences of TTC and EC on glove samples collected from peach handlers at different packing operations and sampling times

Glove samples	TTC			EC		
	No. of positive samples	No. of samples per unit	Percentage (%)	No. of positive samples	No. of samples per unit	Percentage (%)
Packing operation						
M	4	18	22.22	3	18	16.67
N	11	18	61.11	0	18	0.00
P	3	12	25.00	0	12	0.00
Q	8	18	44.44	2	18	11.11
Sampling time						
0 h	1	24	4.17	0	24	0.00
3 h	14	22	63.64	0	22	0.00
6 h	11	20	55.00	5	20	25.00
Peach handler						
Sorter	14	32	43.75	2	32	6.25
Packer	12	34	35.29	3	34	8.82
Total	26	66	39.39	5	66	7.58

TTC: Thermotolerant coliforms; EC: Enterococci; The 4 packing operations were coded as M, N, P, and Q; Sorter: gloves from peach handlers at manual sorting area; Packer: gloves from peach handlers at weighing area

CHAPTER 5

EFFICACY OF COMMERCIAL OVERHEAD WASHING AND WAXING SYSTEMS ON THE MICROBIOLOGICAL QUALITY OF FRESH PEACHES

Wang, P., Pitts, K. B., & Chen, J. 2021. *Food Microbiology*, 97, 103761.

Reprinted here with permission of the publisher.

ABSTRACT

Overhead spray washing and waxing systems (WWS) are used commercially to reduce the risk of microbial contamination and improve the quality of fresh produce during packing. This study evaluated the microbiological quality of overhead spray water and spent peach wash water, as well as fresh peaches before and after they pass the WWS. Pre- and post-washed/waxed peach samples (n=192) and overhead spray water and spent peach wash water samples (n=54) were collected several times over the course of a processing day in three packing facilities located in the state of Georgia. Populations of total aerobes (TA), yeasts and molds (YM), and coliforms (TC) and the presence of thermotolerant coliforms (TTC) and enterococci (EC) were measured in collected samples. The average TA and TC counts and the incidences of TTC and EC were significantly higher ($P < 0.05$) on peach samples collected after the WWS compared to those collected before the WWS. Counts and incidences of TA, YM, and TC in spent peach wash water were significantly higher than in the overhead spray water where neither TTC nor EC was detected. Results suggest that the commercial washing and waxing systems had little effect in improving the microbiological quality of fresh peaches.

5.1. Introduction

Foodborne pathogens and spoilage microorganisms can be introduced to fresh produce during production from contaminated irrigation water, soil and manure, animal shedding and feces, harvesting tools and containers, as well as produce handlers (Alegbeleye et al., 2018). These organisms are subsequently carried over to fresh produce packing facilities. Some of the disinfection treatments that are commonly used to improve the microbiological quality of processed food such as pasteurization do not apply to raw agricultural commodities because they may alter the nature of the products (U.S. FDA, 2015). Washing fresh produce with chemical sanitizers during postharvest packing is, nevertheless a common and economical approach to mitigate the potential risk of microbial contamination (Yoon and Lee, 2018).

Washing fresh produce in a dump tank with added sanitizers is usually used in produce packing and minimal processing facilities (Castro-Ibáñez et al., 2016; De et al., 2018; Duffy et al., 2005; Ilic et al., 2008; Newman et al., 2017). However, limited disinfection efficacy of these systems has been reported in numerous studies (Murray et al., 2018; Yoon and Lee, 2018; Zhou et al., 2014). Cross-contamination between different batches of products could occur if water quality and the concentration of sanitizers are not maintained and properly monitored (Banach et al., 2017). Overhead washing systems have therefore been adopted as an alternative in packing facilities of various fresh produce such as tomatoes, bell peppers, peaches, and grapefruits. (Balaguero et al., 2015; Soto-Beltran et al., 2015; Danyluk, et al., 2019). The system consists of overhead nozzles and revolving brush beds. The nozzles spray water containing sanitizers to the fresh produce to be washed, while the revolving brush beds remove dirt, leaves, and other contaminants from the product surface (Pao et al., 2012). Overhead washing systems have been found more effective in reducing bacterial populations on produce surfaces than systems consisting of wash tanks operated with similar sanitizer concentrations and contact times (Chang and Schneider, 2012; Balaguero et al., 2015). However, detailed studies on the efficacies of overhead washing systems in improving the microbiological quality of fresh produce in commercial packing facilities are currently limited (Danyluk et al., 2019). The risk of microbial contamination associated with the overhead washing system is, nevertheless of great concern to the growers who have adopted the system in their packing operations.

Overhead spray washing systems are usually integrated with wax sprayers (WWS) in the fresh peach packing operations of Georgia. The peach surface is covered with a dense layer of waxy trichomes (LaRue and Johnson, 1989). The brush rollers inside the WWS facilitate the removal of the natural wax and possibly pesticide residues during washing (Dorsey and Potter, 1932). Subsequently, an artificial wax coating is applied to the peach surface to create a shiny and attractive appearance and to slow down fruit respiration and dehydration during storage (Baldwin et al., 2011). Fungicides are occasionally added to the wax to control spoilage and extend the shelf-life of the products (Baldwin et al., 2011).

In the present study, we determined the efficacy of WWS, in three commercial packing facilities of Georgia in decontaminating fresh peaches by 1). determining the microbial populations on the surface of fresh peaches collected before and after the washing/waxing process, and 2). assessing the microbiological quality of overhead spray water and spent peach wash water collected at different sampling times over the course of a packing day.

5.2. Material and methods

5.2.1. Fresh peach packing facilities involved in the study

Three fresh peach packing facilities (F1, F2, and F3) in Georgia were involved in the current study. The throughputs of the three facilities ranged from 9, 071 to 20, 411 kg per hour.

The WWS on the three packing lines had a similar design. Fresh peaches are cooled by either forced air or non-circulated well water. The temperature of the well water was *ca*. 1 °C, whereas peach wash water was at ambient temperature.

Peaches from the dumping area were conveyed on brush rollers inside the WWS, sprayed by the overhead spray water with added chlorine, dried by blowing air and coated with wax containing fungicide by the wax coating sprayers. The chlorine concentration in the overhead spray water at F2 and F3 was 50 ppm, while a wider range of chlorine concentrations from 50 to 100 ppm was used by F1 (Personal communication, 2019). The wax (Prima Fresh® 220, Pace International, Rochester, MN USA) and fungicides were automatically mixed and injected into the spray bars based on the demand of daily production. According to the facility managers, 0.60 oz. or 0.80 oz. of Scholar® SC fungicide (Syngenta, Greensboro, NC USA) was used by F2 and F3, respectively, and 2.05 oz. of PacRite FDL (Pace International, Wapato, WA) was used by F1 to treat 4, 536 kg of peaches. The total residence time of a peach from the entrance to the exit of the WWS was estimated as 20 s.

5.2.2. Collection and evaluation of fresh peach samples

Eight independent samples, each containing 3 peaches, were randomly collected before and after the WWS every 2 h immediately after packing started (0 h) till the end of a packing day (6 h) in three fresh peach packing facilities (F1, F2, and F3) in Georgia in the summer of 2019. The average weights of the peach samples collected before and after the WWS were 349.85 and 338.20 g, respectively. Two 40-quart Igloo iceless thermoelectric coolers (Katy, TX USA) were used to maintain collected samples at 4 °C during transportation to the laboratory. The peach samples were stored at 4 °C until analysis on the following day.

Each peach sample in a sterile polyethylene sample bag (Fisher Scientific, Pittsburgh, PA USA) was rinsed with 200 ml phosphate buffer saline (PBS, pH 7.4) on a platform shaker (Lab-line Instruments, Melrose, IL USA) at 225 rpm for 30 min, with 15 min for each side of the sample bag. Rinsed samples were subsequently massaged by hand for 1 min. The rinsates were serially diluted in PBS when necessary and spread plated on different microbiological media. The counts of total aerobes (TA) and total coliforms (TC) were determined using tryptic soy agar and MacConkey agar, respectively at 37 °C for 24 h, and those of total yeasts and molds (YM) on potato dextrose agar acidified with tartaric acid to pH 3.5 at 25 °C for 48 – 72 h. Presumptive enterococci (EC) and thermotolerant coliforms (TTC) were isolated using the m-*Enterococcus* agar and MacConkey agar and incubation at 37 °C for 24 – 48 h and 44.5 °C for 18 – 22 h, respectively. Isolated EC were subsequently grown in brain heart infusion broth with 6.5% sodium chloride and TTC on triple sugar iron slant and EC broth at 37 °C for 24 – 48 h (bio-WORLD, Dublin, OH USA). The microbiological media used in this study were purchased from Becton, Dickinson, and Company (Sparks, MD USA), except for the EC broth, the supplier of which has been noted above.

5.2.3. Collection and evaluation of overhead spray water and spent peach wash water samples

In the peach harvest seasons of 2018 and 2019, the three packing facilities described in Section 5.2.1 were each randomly visited three times. Three independent samples of overhead spray water and spent peach wash water (1,000 ml each) were collected after 0, 3, and 6 h into the packing process at each facility. Each sample was placed in a 1-liter sterile HDPE wide-mouth bottle (Thermo Fisher Scientific, Waltham, MA USA). The overhead spray water samples were collected from the nozzles of the WWS, whereas spent wash water at the catchments located at bottom of the WWS.

All collected samples were handled, transported, and stored under the conditions described in Section 5.2.2. Counts of TA, YM, and TC, as well as the incidences of TTC and EC in the collected samples were determined using the methodology described above. Water samples (up to 100 ml) with non-detectable microorganisms (<1 CFU/ml) by the plate count assay were concentrated using a vacuum membrane filtration system which contained a sterile mixed ester cellulose water-testing filter membrane with 47 mm in diameter and 0.45 µm in pore size (Fisherbrand®, Pittsburgh, PA USA). The membranes were placed on the same microbiological media and incubated under the same conditions described above.

5.2.4. Statistical analysis

Results of peach and water sampling were fitted into separated models in SAS University Edition (SAS Institute, Cary, NC USA). Counts of TA, YM, and TC from peach samples were analyzed in a Proc Mixed model with the fixed effects of sampling facility, sampling time, and sampling location. The random error term was created by the 8 replicates collected at each location. The significance of all fixed effects was tested by the Kenward-Roger approximation. Counts of TA, YM, and TC from water samples were arranged into a split-plot experimental design creased by the Proc GLM model and tested by Fisher's least significant difference tests. The packing facility was considered the whole plot effect, sampling time and sampling location were the subplot effects. The randomization of the water sampling model was the 3 repeated visits to each packing facility, while the interaction of visit and packing facility effect was the whole plot error term for this model. The incidences of TTC and EC presence were reported as the percentage of positive samples in the total number of samples analyzed within each factor.

5.3. Results and discussion

5.3.1. Microbiological quality of fresh peach samples

Results of statistical analysis revealed that participating packing facility was a significant factor ($P < 0.05$) influencing the levels of all microbial indicators evaluated in the study (Table 5-1). On average, peach samples collected from F2 had significantly higher ($P < 0.05$) mean TA and YM counts than F3 which in turn had significantly higher TA and YM counts than F1 (Table 5-2). Significantly different TC counts were seen among the three packing facilities with F1 had the highest counts, following by F2 and F3.

Sampling time also significantly ($P < 0.05$) affected the mean TA and TC counts but not the YM counts ($P > 0.05$) (Table 5-1). The mean TA count from samples collected at the 0 h sampling point was significantly lower than samples collected at the other three intervals (Table 5-2). Samples collected at the 2 h sampling point had a significantly higher YM count than those collected at 0 and 4 h sampling points, a significantly lower TC count than those from the 0 h sample point, and a significantly higher TC count than samples collected at the 4 h sampling point. Samples collected after the WWS had significantly higher average TA and TC counts but a similar YM count compared to the samples collected before the WWS.

When averaging the microbial loads from the three packing facilities (Table 5-3), the mean TA counts in samples collected after the WWS were significantly higher ($P < 0.05$) at all sampling points compared to the samples collected before the WWS, however, differences in the microbial loads between the two sets of samples became smaller as the length of packing time increased. A similar observation was, however, not made with the YM and TC counts. Mean YM counts were significantly higher at 0 h but significantly lower at 4 and 6 h in samples collected after the WWS compared to those collected before the WWS. Mean TC counts in samples collected after the WWS were also significantly higher than those in samples collected before the WWS.

Average TA, YM, and TC counts in samples collected after the WWS of individual packing facilities were significantly higher ($P < 0.05$) compared to the counts from the samples collected before the WWS, except that the average YM counts in samples collected from F1 and F3 (Table 5-4). Samples from F2 had the highest increase in average TA and YM counts and the lowest increase in average TC count between the two sets of samples. Samples from F1 had the lowest average TA and YM count increase and the highest average TC count increases between the two sets of samples.

Among the 192 tested peach samples (Table 5-5), 88 were positive for TTC (45.83%). The highest incidence of TTC (54.69%) was found in samples from F1, followed by those from F2 (46.88%) and F3 (35.94%). More than 50% of the samples tested positive for TTC at the 0 and 6 h sampling points, while incidences were relatively lower (both at 35.42%) in the two middle sampling points. The positive rate of TTC in peach samples (67.71%) was more than doubled in samples collected after the WWS than those collected before the WWS (23.96%). As for EC, only 10 samples were confirmed positive in the total of 192 samples collected (5.21%, Table 5-5). Samples from F1 had the highest incidence of positive samples, followed by those from F3 and F2. The incidence of EC was the lowest at the beginning of the packing day compared to the rest of the sampling points. EC was not detected from samples collected before the WWS, while 10.42% of samples collected after the WWS tested positive for EC.

5.3.2. Microbiological quality of overheard spray water and spent peach wash water

The packing facility had a significant influence ($P < 0.05$) on the levels of all three microbial indicators in tested water samples (Table 5-6). Water samples from F1 and F2 had similar ($P > 0.05$) levels of the three microbial indicators which were significantly lower than the levels of microbial loads in samples from F3 (Table 5-7). The average TC counts at the 0 h sampling point were significantly higher than the counts in samples collected at the 6 h sampling point (Table 5-7). The levels of all three indicators were significantly higher in spent peach wash water than in the overhead spray water which had a mean TA and YM count of less than 5 CFU/100 ml and a non-detectable mean TC count (< 0.01 CFU/100 ml).

Among the 54 water samples tested, TTC has only been detected in 10 spent peach wash water samples (37.04%), 9 from F3 (50%), and 1 from F1 (5.56%) (Table 5-8). At the 0 h sampling point, TTC was found in 4 samples (22.22%), while 3 samples each were found at the other two time points (16.67%). With an overall incidence of 9.26%, all 5 EC positive samples were from spent peach wash water in F3 (27.78%). EC was not detected at the beginning of the packing day but was found in 16.67 and 11.11% of the samples collected at the 3 h and 6 h sampling points, respectively.

5.3.3. Relevance and significance of the research findings

The average counts of TA and TC and incidences of TTC and EC presence on peach samples collected after the WWS were significantly higher ($P < 0.05$) than those collected before the WWS in the present study (Table 5-2). One of the possible sources for the additional microbial load on washed/waxed peach samples is the overhead spray water. However, the three packing facilities involved in the study regularly monitored the chlorine concentrations and pH levels of overhead spray water (Personal communication, 2019). The low TA and YM counts and the non-detectable TC, TTC, and EC counts in the overhead spray water samples (Tables 5-7 and 5-8) suggest that the monitoring programs were effective in maintaining the microbiological quality of overhead spray water. In comparison, spent peach wash water had significantly higher levels of TA, YM, and TC counts (Table 5-7) and higher incidences of TTC and EC presence (Table 5-8), which suggest that the revolving brush beds could be the source of contamination and the sanitizer added to peach wash water might be insufficient to completely inactivate the microorganisms in the WWS.

The brush rollers are enclosed inside the WWS on a commercial peach packing line, it is, therefore hard to reach, and deeply clean and sanitation are impossible without completely dissembling the equipment. Insufficient sanitation of brush rollers from the previous production day could result in the retention of microorganisms within the brush bristles (Wang et al., 2012). According to the results of a previous survey of our laboratory where various sites of four different fresh peach packing lines were surveyed and microbial loads enumerated, brush rollers had an average of 4.29 log CFU/cm^2 of TA, 2.95 log CFU/cm^2 of YM, and 2.16 log CFU/cm^2 of TC in the early morning hours before packing started (Wang et al., 2021). The estimated contact time between fresh peach and brush beds during packing was estimated at *ca*. 20 s (Personal communication, 2019). The microbial cells on brush beds could be transferred to packed products. During fruit passage through the WWS, simultaneous fruit to fruit cross-contamination is also likely to occur. These arguments are supported by some of the observations of the current and several previous studies. In the current study, EC was not detected from peach samples collected before the WWS, but 10.42% of the peach samples collected after the WWS tested positive (Table 5-5). TTC was detected in 23.96% of the peach samples collected before the WWS, and the incidence increased to 67.71% in samples collected after the WWS. A study performed by another laboratory demonstrated that contact with brush rollers artificially inoculated with 6.9 log CFU/cm^3 of *Salmonella* for 10 s added 5.7 ± 0.1 log CFU/cm^2 of *Salmonella* cells to tomato surface (Pao et al., 2009). In a previous study performed in a pilot-scale tomato packing experiment, Wang and Ryser (2014) found that after the first batch of *Salmonella*-inoculated tomatoes going through brush rollers, 8% of the subsequent tomatoes were contaminated with the pathogen. These findings emphasized the necessity of daily cleaning and sanitizing of brush rollers during the production season.

The WWS reduces microbial contamination on fresh peaches through the combination of mechanical forces of spraying, brushing and flushing, sanitizer disinfection, and fungicide action (Pao et al., 2009; Baldwin et al., 2011). In the current study, the YM counts on peaches collected after the WWS were only 0.03 log CFU/g lower than those on fruits collected before the WWS, and a similar decrease in the average TA and TC counts was not even observed (Table 5-2). These observations could be attributed to the insufficient treatment time and sanitizer concentrations used by the three packing facilities. In a pilot plant study, overhead spray with water alone or water containing 50 ppm sodium hypochlorite for 15 s removed a similar number of *Salmonella* cells from the tomato surface (Chang and Schneider, 2012). However, when the spray time increased to 30 s, the *Salmonella* counts on chlorine-treated tomato surfaces was significantly lower ($P < 0.05$) compared to the water-treated control samples. As stated previously, the chlorine concentration used in overhead spray water at F2 and F3 was 50 ppm, while a wider range of chlorine concentrations from 50 to 100 ppm was used by F1. A fresh peach passed through the WWS in about 20 s, the actual spray washing time inside the WWS could be even shorter. To improve the efficacy of WWS, extending the length of spray time and/or increasing the concertation of chlorine in peach wash water might be among the feasible alternatives for the current practices. Extending the length of spray time could be accomplished by adjusting the speed of peach conveyor beds. Increasing the concentration of sanitizer in overhead spray water is feasible since chlorine at concentrations up to 75-150 ppm is normally used in produce wash water (Suslow, 2000), although the actual concentration of a commercial chlorine-based product should not exceed the maximum level specified on its label registered with the U.S. Environmental Protection Agency.

It is worth mentioning that although the levels of TA and TC counts were significantly higher on samples collected after than before the washing/waxing process (Table 5-2), the differences in the two sets of counts were relatively smaller at the 4 h and 6 h sampling points compared to samples collected at the earlier sampling intervals (Table 5-3). The averages of YM counts on samples collected after the WWS at the 4 and 6 h sampling points were even slightly lower than those collected before the WWS (Table 5-2). It was noticed in the current study that the WWS was usually running during the lunch break between the 2 h and 4 h sampling points for *ca*. 15-30 min without passing fruits. This practice may have sanitized the brush rollers to a certain degree, making fruit samples collected before and after the WWS at these two sampling points had narrower differences in microbial loads. These results suggest that additional mitigation measures other than post-operational sanitation could be adopted to further improve the hygiene status of brush rollers. For instance, a pre-operational running of the WWS may potentially improve the hygiene status of brush rollers and reduce the level of microbial carry-over to incoming peaches.

In addition to chlorine, Scholar® SC or PacRite FDL fungicide (Greensboro, NC USA) was added into wax coating sprayers by the three packers involved in this study (Personal communication, 2019). The active ingredients of the products are fludioxonil which can effectively control Rhizopus rot and gray mold, common fungal contaminations associated with postharvest peach (Förster et al., 2007). In addition to this fungicide, chlorine also has antifungal activities. A previous study showed that dipping inoculated fresh tomatoes into 100 ppm chlorine for 1 min reduced the YM counts by 0.70 log CFU/g (May and Fickak, 2003). In the present study, the YM was the only microbial indicator that did not increase significantly after peach samples passed through the WWS (Table 5-2), this result could be attributed to the synergic effects of fungicide and chlorine in the WWS.

5.4. Conclusions

The WWS had limited effect in decontaminating passing fruit according to the results of this study. Mean counts of TA and TC, as well as the incidences of TTC and EC, were significantly higher ($P < 0.05$) on peach samples collected after than before the WWS; although opposite observation in YM counts at most of the sampling points were noticed. The water monitoring program of the three facilities was effective as no TC, TTC, and EC were detected in the overhead spray water. The study suggests that additional sanitation mitigation should be considered to further improve the hygiene status of brush rollers inside the WWS, subsequently the microbiological quality of fresh peaches.

References

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

Balaguero, A. N., Sreedharan, A., Schneider, K. R., 2015. Effect of overhead spray and brush roller treatment on the survival of *Pectobacterium* and *Salmonella* on tomato surfaces. J. Food Prot. 78, 51-56. https://doi.org/10.4315/0362-028X.JFP-14-254.

Baldwin, E. A., Hagenmaier, R., Bai, J., 2011. Edible coatings and films to improve food quality. CRC Press., Boca Raton FL.

Banach, J., Van Bokhorst-van de Veen, H., Van Overbeek, L., Van der Zouwen, P., Van der Fels-Klerx, H., Groot, M. N., 2017. The efficacy of chemical sanitizers on the reduction of *Salmonella* Typhimurium and *Escherichia coli* affected by bacterial cell history and water quality. Food Control. 81, 137-146. https://doi.org/10.1016/j.foodcont.2017.05.044.

Castro-Ibáñez, I., López-Gálvez, F., Gil, M. I., Allende, A., 2016. Identification of sampling points suitable for the detection of microbial contamination in fresh-cut processing lines. Food Control. 59, 841-848. https://doi.org/10.1016/j.foodcont.2015.07.004.

Chang, A. S., Schneider, K. R., 2012. Evaluation of overhead spray-applied sanitizers for the reduction of *Salmonella* on tomato surfaces. J. Food Sci. 77, M65-69. https://doi.org/10.1111/j.1750-3841.2011.02486.x.

Danyluk, M. D., Friedrich, L. M., Dunn, L. L., Zhang, J., & Ritenour, M. A., 2019. Reduction of *Escherichia coli*, as a surrogate for *Salmonella* spp., on the surface of grapefruit during various packingline processes. Food Microbiol. 78, 188-193. https://doi.org/10.1016/j.fm.2018.10.014

De, J., Li, Y., Sreedharan, A., Schneider, R. G., Gutierrez, A., Jubair, M., Danyluk, M. D., Schneider, K. R., 2018. A three-year survey of Florida packinghouses to determine microbial loads on pre-and post-processed tomatoes. Food Control. 86, 383-388. https://doi.org/10.1016/j.foodcont.2017.11.036.

Dorsey, M. J., Potter, J. S., 1932. A study of the structure of the skin and pubescence of the peach in relation to brushing. University of Illinois Agricultural Experiment Station, Urbana IL.

Duffy, E. A., Lucia, L. M., Kells, J. M., Castillo, A., Pillai, S. D., Acuff, G. R., 2005. Concentrations of *Escherichia coli* and genetic diversity and antibiotic resistance profiling of *Salmonella* isolated from irrigation water, packing shed equipment, and fresh produce in Texas. J. Food Prot. 68, 70-79. https://doi.org/10.4315/0362-028X-68.1.70.

Förster, H., Driever, G. F., Thompson, D. C., Adaskaveg, J. E., 2007. Postharvest decay management for stone fruit crops in California using the "reduced-risk" fungicides fludioxonil and fenhexamid. Plant Dis. 91, 209-215. https://doi.org/10.1094/PDIS-91-2-0209.

Ilic, S., Odomeru, J., LeJeune, J. T., 2008. Coliforms and prevalence of *Escherichia coli* and foodborne pathogens on minimally processed spinach in two packing plants. J. Food Prot. 71, 2398-2403. https://doi.org/10.4315/0362-028X-71.12.2398.

LaRue, J. H., Johnson, R. S., 1989. Peaches, plums, and nectarines: Growing and handling for fresh market. UCANR Publications, Oakland CA.

May, B. K., Fickak, A., 2003. The efficacy of chlorinated water treatments in minimizing yeast and mold growth in fresh and semi-dried tomatoes. Dry. Technol. 21, 1127-1135. https://doi.org/10.1081/DRT-120021879.

Murray, K., Aldossari, H., Wu, F., Warriner, K., 2018. Dynamic changes in free-chlorine levels within a commercial post-harvest wash and prevention of cross-contamination between shredded lettuce batches. Food Control. 85, 127-134. https://doi.org/10.1016/j.foodcont.2017.09.029.

Newman, K. L., Bartz, F. E., Johnston, L., Moe, C. L., Jaykus, L.-A., Leon, J. S., 2017. Microbial load of fresh produce and paired equipment surfaces in packing facilities near the U.S. and Mexico border. J. Food Prot. 80, 582-589. https://doi.org/10.4315/0362-028X.JFP-16-365.

Pao, S., Kelsey, D., Long, W., 2009. Spray washing of tomatoes with chlorine dioxide to minimize *Salmonella* on inoculated fruit surfaces and cross-contamination from revolving brushes. J. Food Prot. 72, 2448-2452. https://doi.org/10.4315/0362-028X-72.12.2448.

Pao, S., Long Iii, W., Kim, C., Kelsey, D. F., 2012. 5 Produce washers, in: Gómez-López, V. M. (Ed.), Decontamination of fresh and minimally processed produce. John Wiley & Sons, Inc., Hoboken NJ. pp. 87-103. https://doi.org/10.1002/9781118229187.ch5.

Soto-Beltran, M., Campo, C. N., Campos-Sauceda, J., Aven-Bustillos, R., Chaidez, C., 2015. Prevalence of *Salmonella*, *Escherichia coli* and coliforms on bell pepper from the field to the packing process. Afr. J. Microbiol. Res. 9, 718-724. https://doi.org/10.5897/AJMR2014.7341.

Suslow, T., 2000. Chlorination in the production and postharvest handling of fresh fruits and vegetables: Chap. 6. Fruit and vegetable processing, in: McLaren, D. A. (Ed.), Use of Chlorine Based Sanitizers and Disinfectants in the Food Manufacturing Industry. Food Processing Center at the University of Nebraska, Lincoln NE, pp. 2–15.

https://www.siphidaho.org/env/pdf/Chlorination_of_fruits_and_veggies.PDF (accessed 17 August 2020).

U.S. FDA (U.S. Food and Drug Administration), 2015. Standards for the growing, harvesting, packing, and holding of produce for human consumption; final rule. 80 FR 74353 (Docket No. FDA-2011-N-0921). https://www.federalregister.gov/documents/2015/11/27/2015-28159/standards-for-the-growing-harvesting-packing-and-holding-of-produce-for-human-consumption (accessed 17 August 2020).

Wang, H. Q., Davidson, G., Ryser, E. T., 2012. Microbial cross-contamination of tomatoes during washing with a peroxyacetic acid based sanitizer in a commercial packinghouse. Abstr. Annu. Meet. IAFP, Providence RI. https://iafp.confex.com/iafp/2012/webprogram/Paper2816.html (accessed 17 August 2020).

Wang, P., Quansah, J. K., Pitts, K. B., & Chen, J. 2021. Hygiene status of fresh peach packing lines in Georgia. LWT – Food Sci. Technol. 139:110627. https://doi.org/10.1016/j.lwt.2020.110627.

Wang, H., Ryser, E. T., 2014. *Salmonella* transfer during pilot plant scale washing and roller conveying of tomatoes. J. Food Prot. 77, 380-387. https://doi.org/10.4315/0362-028X.JFP-13-314.

Yoon, J.-H., Lee, S.-Y., 2018. Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. Crit. Rev. Food Sci. Nutr. 58, 3189-3208. https://doi.org/10.1080/10408398.2017.1354813.

Zhou, B., Luo, Y., Turner, E. R., Wang, Q., Schneider, K. R., 2014. Evaluation of current industry practices for maintaining tomato dump tank water quality during packinghouse operations. J. Food Process. Pres. 38, 2201-2208. https://doi.org/10.1111/jfpp.12200.

Table 5-1 Type III tests for fixed effects by the statistical model of peach sampling (α=0.05)

Indicator	Effect	DF	F Value	Pr > F
TA	Packing facility	2	53.4	<.0001
	Sampling time	3	4.67	0.0037
	Sample site	1	125.72	<.0001
YM	Packing facility	2	20.85	<.0001
	Sampling time	3	2.38	0.072
	Sample site	1	0.45	0.50
TC	Packing facility	2	58.51	<.0001
	Sampling time	3	15.73	<.0001
	Sample site	1	159.58	<.0001

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms
DF: Degree of freedom
The number of degrees of freedom associated with this model errors is 161.
Pr > F: *P* value, reflects the significance of the effect; when the value in this column is smaller than 0.05, it is a significant effect.

Table 5-2 Average counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected from the washing and waxing system (WWS) at different sampling points and sites in different packing facilities (n=192)

Peach samples collected at the WWS	Mean population (log CFU/g)		
	TA	YM	TC
Of different packing facilities			
F1 (n=64)	2.91±0.05^{C}	2.94±0.05^{C}	1.48±0.12^{A}
F2 (n=64)	3.53±0.09^{A}	3.29±0.05^{A}	1.03±0.08^{B}
F3 (n=64)	3.23±0.07^{B}	3.12±0.03^{B}	0.70±0.07^{C}
At different sampling points			
0 h (n=48)	3.08±0.09^{B}	3.07±0.07^{B}	1.36±0.13^{A}
2 h (n=48)	3.28±0.09^{A}	3.21±0.05^{A}	1.10±0.12^{B}
4 h (n=48)	3.22±0.07^{A}	3.06±0.05^{B}	0.79±0.11^{C}
6 h (n=48)	3.32±0.10^{A}	3.11±0.05^{AB}	1.04±0.10^{B}
At different sample sites			
After the WWS (n=96)	3.50±0.05^{A}	3.10±0.04^{A}	1.44±0.08^{A}
Before the WWS (n=96)	2.95±0.06^{B}	3.13±0.04^{A}	0.70±0.07^{B}

Mean population: mean ± standard error of the mean

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

F1-F3: packing facilities

The 0 h samples were collected immediately after packing started.

The results followed by different letters within the same effect (packing facility, sampling time, or sample site) were significantly different ($P < 0.05$).

Table 5-3 Mean counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected before and after the washing and waxing system (WWS) at different sampling points

Indicator	Sample site	Mean population (log CFU/g)			
		0 h	2 h	4 h	6 h
TA	After the WWS	3.55±0.05^{A}	3.61±0.07^{A}	3.38±0.05^{A}	3.45±0.10^{A}
	Before the WWS	2.60±0.08^{B}	2.96±0.09^{B}	3.05±0.08^{B}	3.19±0.09^{B}
	Difference	0.95	0.65	0.33	0.26
YM	After the WWS	3.24±0.08^{A}	3.21±0.05^{A}	2.98±0.05^{B}	2.97±0.05^{B}
	Before the WWS	2.90±0.06^{B}	3.21±0.05^{A}	3.15±0.04^{A}	3.26±0.04^{A}
	Difference	0.34	0.00	-0.17	-0.29
TC	After the WWS	1.76±0.12^{A}	1.67±0.11^{A}	0.96±0.08^{A}	1.37±0.08^{A}
	Before the WWS	0.96±0.11^{B}	0.52±0.07^{B}	0.62±0.12^{B}	0.70±0.09^{B}
	Difference	0.80	1.15	0.34	0.67

Mean population: mean ± standard error of the mean

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

Difference: counts collect after the WWS minus those collected before the WWS.

The 0 h samples were collected immediately after packing started.

Means of TA, YM, or TC followed by different letters within the same sampling time were significantly different ($P < 0.05$) from each other.

Table 5-4 Mean counts of total aerobes, total yeasts and molds, and total coliforms on peach samples collected before and after the washing and waxing system (WWS) in different packing facilities

Indicator	Sample site	Mean population (log CFU/g)		
		F1	F2	F3
TA	After the WWS	3.06 ± 0.04^{A}	3.95 ± 0.03^{A}	3.49 ± 0.05^{A}
	Before the WWS	2.77 ± 0.06^{B}	3.11 ± 0.09^{B}	2.97 ± 0.06^{B}
	Difference	0.29	0.84	0.52
YM	After the WWS	2.82 ± 0.05^{B}	3.43 ± 0.04^{A}	3.04 ± 0.03^{B}
	Before the WWS	3.05 ± 0.04^{A}	3.14 ± 0.06^{B}	3.19 ± 0.03^{A}
	Difference	-0.23	0.29	-0.15
TC	After the WWS	2.20 ± 0.08^{A}	1.16 ± 0.06^{A}	0.97 ± 0.06^{A}
	Before the WWS	0.76 ± 0.09^{B}	0.91 ± 0.10^{B}	0.43 ± 0.07^{B}
	Difference	1.44	0.25	0.54

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

F1-F3: packing facilities

Difference: counts collected after the WWS minus those collected before the WWS.

Means of TA, YM, or TC counts followed by different letters within the same packing facility were significantly different ($P < 0.05$).

Table 5-5 Incidences of TTC and EC on peach samples collected from the washing and waxing system (WWS) at different sampling points and sample sites in different packing facilities

Peach samples collected at the WWS	No. of positive samples	No. of samples per unit	Percentage (%)	No. of positive samples	No. of samples per unit	Percentage (%)
	TTC			EC		
Of different packing facilities						
F1	35	64	54.69	7	64	10.94
F2	30	64	46.88	1	64	1.56
F3	23	64	35.94	2	64	3.13
At different sampling points						
0 h	27	48	56.25	1	48	2.08
2 h	17	48	35.42	3	48	6.25
4 h	17	48	35.42	3	48	6.25
6 h	27	48	56.25	3	48	6.25
At different sample sites						
After the WWS	65	96	67.71	10	96	10.42
Before the WWS	23	96	23.96	0	96	0.00
Total	88	192	45.83	10	192	5.21

TTC: Thermo-tolerant coliforms
EC: Enterococci
F1-F3: packing facilities
The 0 h samples were collected right after packing started.

Table 5-6 Type III tests for fixed effects by the statistical model of water sampling (α=0.05)

Indicator	Effect	DF	Type III SS	Mean Square	F Value	Pr > F
TA	Packing facility	2	45.22	22.61	15.22	0.0135
	Sampling time	2	0.83	0.42	0.99	0.3828
	Sample site	1	115.60	115.60	273.67	<.0001
YM	Packing facility	2	25.34	12.67	43.82	0.0019
	Sampling time	2	0.10	0.05	0.14	0.8712
	Sample site	1	68.77	68.77	186.44	<.0001
TC	Packing facility	2	22.78	11.39	82.17	0.012
	Sampling time	2	0.70	0.35	2.32	0.1256
	Sample site	1	12.66	12.66	84.14	<.0001

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms
DF: degrees of freedom, the number of degrees of freedom in this model is 24.
The number of degrees of freedom associated with this model errors is 99.
Type III SS: Type III sum of square
Pr > F: *P* value, reflects the significance of the effect; when the value in this column is smaller than 0.05, it is a significant effect.

Table 5-7 Average counts of total aerobes, total yeasts and molds, and total coliforms in overhead spray water and spent peach wash water at different sampling points and sample sites in different packing facilities (n=54)

Water samples collected at the WWS	Mean population (log CFU/100 ml)		
	TA	YM	TC
Of different packing facilities			
F1 (n=18)	1.12±0.28^{B}	0.75±0.23^{B}	0.06±0.05^{B}
F2 (n=18)	0.59±0.24^{B}	0.60±0.21^{B}	ND^{B}
F3 (n=18)	2.74±0.65^{A}	2.13±0.49^{A}	1.72±0.43^{A}
At different sampling points			
0 h (n=18)	1.60±0.52^{A}	1.18±0.37^{A}	0.77±0.38^{A}
3 h (n=18)	1.53±0.46^{A}	1.20±0.38^{A}	0.58±0.31^{AB}
6 h (n=18)	1.31±0.46^{A}	1.10±0.36^{A}	0.43±0.23^{B}
At different sites			
Overhead spray water (n=27)	0.02±0.01^{B}	0.03±0.03^{B}	ND^{B}
Spent peach wash water (n=27)	2.95±0.39^{A}	2.29±0.30^{A}	1.19±0.32^{A}

Mean population: mean ± standard error of the mean

TA: total aerobes; YM: total yeasts and molds; TC: total coliforms

F1-F3: packing facilities

The results with different letters within each effect (packing facility, sampling time, and sample type) were tested significantly different ($P < 0.05$).

ND: The detection limit is 1 CFU/100 ml.

Table 5-8 Incidences of TTC and EC in water samples collected at various sampling points and sample sites in different packing facilities

Water samples collected at the WWS	No. of positive samples	No. of samples per unit	Percentage (%)	No. of positive samples	No. of samples per unit	Percentage (%)
		TTC			EC	
Of different packing facilities						
F1	1	18	5.56	0	18	0.00
F2	0	18	0.00	0	18	0.00
F3	9	18	50.00	5	18	27.78
At different sampling points						
0 h	4	18	22.22	0	18	0.00
3 h	3	18	16.67	3	18	16.67
6 h	3	18	16.67	2	18	11.11
At different sample sites						
Overhead spray water	0	27	0.00	0	27	0.00
Spent peach wash water	10	27	37.04	5	27	18.52
Total	10	54	18.52	5	54	9.26

TTC: Thermo-tolerant coliforms; EC: Enterococci

F1-F3: packing facilities

The 0 h samples were collected immediately after packing started.

CHAPTER 6

CHARACTERIZATION OF GENERIC *ESCHERICHIA COLI* ISOLATED FROM FRESH PEACH PACKING ENVIRONMENTS

6.1. Introduction

Escherichia coli is an important member of the *Enterobacteriaceae*, and most strains of *E. coli* are harmless to the healthy population. However, some members of *E. coli* are pathogenic and may cause severe foodborne illnesses. These *E. coli* are categorized into different virogroups, *i.e.,* enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC), a subset of Shiga-toxin producing *E. coli* (STEC) (U.S. FDA, 2012). Virulence genes in each group of pathogenic *E. coli* have been identified (Hornes, Wasteson, & Olsvik, 1991; Oswald et al., 2000; Pass, Odedra, & Batt, 2000; Ratchtrachenchai, Subpasu, & Ito, 1997; Sethabutr, Venkatesan, Murphy, Eampokalap, Hoge, & Echeverria, 1993; Tamanai-Shacoori, & Jolivet-Gougeon, 1994; Yamasaki et al., 1996). Oligonucleotide primers for multiplex PCR assays developed by Toma et al. (2003) have been widely used to determine the presence/absence of 6 virulence genes in *E. coli* isolated from a variety of sources such as human feces (Canata et al., 2016), surface water (Akter et al., 2013; Ndlovu, Le Roux, Khan, & Khan, 2015; Widmer et al., 2013), raw milk cheese (Bernini, Sgarbi, Bove, Gatti, & Neviani, 2010; Zago et al., 2007), seafood (Kambire, Adingra, Yao, & Koffi-Nevry, 2017), and fresh produce (Feng et al., 2014).

Apart from bacterial virulence genes, one other critical characteristic of enteric bacterial pathogens is the expression of bacterial adhesins that are involved in not only bacterial pathogenicity but also their survival outside animal hosts (Nagy et al., 2015). Genes responsible for adhesin expression have been identified in *E. coli* O157:H7 and other serotypes of *E. coli* (Low et al., 2006b), and these genes are critically important for the initial bacterial attachment to biotic and abiotic surfaces, and the formation of the secondary structure of biofilms (Berne, Ducret, Hardy, & Brun, 2015). Pathogen cells, once introduced into the fresh produce packing/processing environment, can survive and multiply and form biofilms in niches where sanitation measures are lacking or inaccessible (Srey, Jahid, & Ha, 2013).

Consequently, bacterial cells embedded in mature biofilms on these niches may disperse, further contaminating the environment (Sauer, Camper, Ehrlich, Costerton, & Davies, 2002) and increasing the risk of microbial contamination of fresh produce. Due to the lack of killing steps, contaminated fresh produce can be an effective vehicle in transmitting foodborne illness among consumers.

The objectives of this study were to determine the distribution of selected virulence, as well as putative adhesin, genes among a few generic *E. coli* isolated from the fresh peach packing environments.

6.2. Material and methods

6.2.1. Bacterial strains used in the study

Generic *E. coli* (n = 7) previously isolated from fruit, equipment surface, glove, and peach wash water samples in fresh peach packing environments were preserved at -20 °C prior to the current study. The isolates were resuscitated on tryptic soy agar (Becton, Dickinson and

Company, Sparks, MD USA) plates at 37 °C overnight and sub-cultured under the same condition on the following day.

6.2.2. Characterization of the generic E. coli *isolates*

6.2.2.1. DNA extraction

A loopful of each resuscitated bacterial culture was transferred into 1 ml tryptic soy broth (Becton, Dickinson, and Company) in a 1.5 ml Eppendorf tube (ThermoFisher Scientific, Waltham, MA USA) to grow overnight at 37 °C, followed by centrifugation at 10,000 *g* for 10 min. After discarding the supernatant, the bacterial cell pellet was washed with 1 ml distilled water and then centrifuged for 10 min under the same condition described above. This process was repeated twice to thoroughly wash off the broth residue from bacterial cells. Sterile distilled water in the amount of 100 µl was then added to the Eppendorf tube after the final wash and suspended bacterial cells were heated in a boiling water bath for 10 min, followed by immediate cooling on ice. The total cellular DNA in the supernatant, obtained by centrifugation under the same conditions described previously, was stored at 4 °C before use. *E. coli* O157:H7 F4546 was used as a positive control for the identification of the putative adhesin genes.

6.2.2.2. PCR amplification and data analysis

A 25 µl PCR mix was prepared, including 2.5 µl a DNA template, 1 µM primers, 1 mM deoxynucleoside triphosphates, 0.5 U *Taq* polymerase, and 2.5 µl of 10x PCR buffer (ThermoFisher Scientific). Primers used to amplify the virulence, and putative adhesin, genes were listed in Tables 6-1 and 6-2, respectively based on the studies of Toma et al. (2003) or Low et al. (2006b). PCR reactions were conducted in a DNA thermal cycler 480 (Perkin Elmer, Norwalk, CT USA) with a 5 min initial denaturation cycle at 94 °C, followed by 30 cycles of 1 min denaturation at 92 °C, 1 min annealing at 52 °C, and 45 s extension at 72 °C. The final extension step was at 72 °C for 10 min.

PCR products were loaded on 1% agarose (ThermoFisher Scientific) gel in Tris-borate-EDTA buffer (pH 8.0) and separated using a gel electrophoresis system (ThermoFisher Scientific). After staining the gel with 1% ethidium bromide (MilliporeSigma, St. Louis, MO USA), PCR amplicons were visualized under the UV trans-illuminator in the Gel Doc System 2000 (Bio-Rad 183 Laboratories, Hercules, CA USA). The presence and absence of the 6 typical virulence genes and 14 putative adhesin genes in each generic *E. coli* isolate were reported, along with the prevalence of each gene in the total number of generic *E. coli* isolates used in the study.

6.3. Results

The six virulence genes of pathogenic *E. coli* were not detected in any of the generic *E. coli* isolates used in the study. However, all the 7 isolates tested positive for loc 3, 5, 7, and 14, while none of them tested positive for loc 2 and 9 (Table 6-3). The incidence of loc 11 presence was the second highest (71.43%) among the evaluated putative adhesin genes, followed by loc 8 (57.14%), and loc 4 and 10 (42.86%). Loc 1, 6, 12, and 13 were only detected in one of the generic *E. coli* isolates (14.29%). Each isolate carried at least 5 putative adhesin genes (35.71%) tested in the study, but not more than 9 of them (64.29%). Isolates from the fruit sample and one of the glove samples were found to be positive for 7 or more putative adhesin genes (50%) tested, while isolates from the equipment surface only carried 5 or 6 putative adhesin genes (35.71% or 42.86%) per isolate. None of the isolates had the same number of putative adhesin genes evaluated in the study.

6.4. Discussions

The oligonucleotide primers used in the study targeted 6 different virulence genes of diarrheagenic *E. coli* (Table 6-1). The *eae* gene encodes an adhesin, known as intimin, which facilitates bacterial attachment to host epithelial cells, forming the typical attaching and effacing lesions (Nataro & Kaper, 1998). The intamin is a critical virulence factor for EPEC and EHEC, a subgroup of STEC capable of causing illness in humans (Blanco et al., 2005). STEC is known to produce potent cytotoxins, referred to as Shiga toxins (STX) which are encoded by the *stx1*, *stx 2,* or related variant genes (Nataro & Kaper, 1998). In addition to cytotoxins, *E. coli* also produced two enterotoxins, heat liable toxin (LT) and heat-stable toxin (ST) which are encoded by *elt* and *est*, respectively. They are the key virulence factors of ETEC. The invasion-associated plasmid antigen gene, *ipaH*, can be found on the chromosome or plasmid of EIEC (Venkatesan, Buysse & Kopecko, 1989). The primers from the *aggR* target the pathotype EAEC, as it encodes the essential transcriptional activator AggR which regulates the expression of the aggregative adherence fimbriae genes in EAEC (Harrington, Dudley, & Nataro, 2006; Nataro, Yikang, Yingkang & Walker, 1994).

The generic *E. coli* used in the present study tested negative for all selected virulence genes. The explanation for this observed phenomenon could be multiple folds. First, the presence of pathogenic *E. coli* in the environment is usually sporadic (Devleesschauwer et al., 2019) and at a very low level (Van Pelt et al., 2018), and the chances to find them from food processing/packing environment could be even lower. Secondly, the virulence genes were screened by the PCR assays using short oligonucleotide primers. Owing to the specificity of each primer, any variation on the DNA template, for instance, partial deletion, would lead to false-negative results (Bekal et al., 2003; Johnson & Stell, 2000; Schmidt et al., 1995).

The current study only used PCR assays to characterize the generic *E. coli*. In addition to the molecular test, some phenotypic tests such as tissue culture assays (*e.g.* HEp-2 adherence assay) or animal tests (*e.g.* rabbit ligated ileal loop assay) could help further characterize the *E. coli* isolates (Nataro & Kaper, 1998).

In the present study, loc 3 (*sfm*), 5 (*ycb*), 7 (*curli*), and 14 (*fim*) were detected in all 7 generic *E. coli* isolates, followed by loc 11 (*yra*) and loc 8 (*F9*) (Table 6-3). In the study of Low et al. (2006b) where the same 14 putative adhesin genes were screened among different serotypes of EHEC and EPEC, these 6 genes were also present more prevalently than the other examined adhesin genes. Loc 7 encodes curli, one of the well-characterized adhesins found on the cells of numerous enteric bacteria (Evans, & Chapman, 2014), and this adhesin has been proven to be one of the key factors of bacteria initial attachment and biofilm formation on biotic and abiotic surfaces (Leech, Golub, Allan, Simmons, & Overton, 2020; Nagy et al., 2015). Loc 14 encodes type 1 fimbriae, the most classic chaperone-usher pathway adhesin (Berne, Ducret, Hardy, & Brun, 2015; Larsonneur, et al., 2016), which is widely conserved in *Enterobacteriaceae* (Low et al., 2006b). It is believed that type 1 fimbriae are essential for *E. coli* to achieve a stable attachment to abiotic surfaces (Pratt & Kolter, 1998). Another putative adhesin gene, *sfm* was found, in one study, in *E. coli* K-12, but its sequence was not conserved in *Klebsiella pneumoniae* or several common serovars of *Salmonella* (McClelland, et al., 2000). Korea, Badouraly, Prevost, Ghigo, & Beloin (2010) reported that without type 1 fimbriae, the *ycb* in *E. coli* K-12 was express and promoted biofilm formation on polyvinyl chloride and polystyrene surfaces at 37 and 30 °C. In addition, about half of the isolates of this study tested positive for loc 8 (F9), which was also frequently found in the EHEC and EPEC tested by Low et al. (2006b). In a different study, Low et al. (2006a) observed that the expression level of F9 fimbriae at 28 °C was higher than at 37 °C, and since F9 fimbriae could facilitate the formation of biofilm (Ulett, Mabbett, Fung, Webb, & Schembri, 2007), it could be extrapolated that the expression of F9 fimbriae could promote bacteria adapting to the environment outside of human or animal hosts.

Putative fimbriae genes *yeh* and *yad* were not found in any isolates tested in the current study (Table 6-3). The negative test results could be caused by the absence of the genes or the variation in the target DNA sequences, as the oligonucleotide primers were derived from an *E. coli* O157:H7 strain. Ravan and Amandadi (2015) proposed that the *yeh* gene could be used as a marker for the detection of *E. coli* O157:H7, as so far it was only detected in this serotype. Similarly, in the study conducted by Low et al. (2006b), *yeh* was detected in the only *E. coli* O157:H7 isolate among all the 20 EHEC and EPEC isolates screened. Spurbeck et al. (2011) reported that *yad* was more prevalent in UPEC isolates than in human commensal *E. coli* isolates.

Putative fimbriae genes *lpf*, *stc*, and *sfa* were only detected once among all the isolates tested in the study (Table 6-3). According to the results of Low et al. (2006b), these three genes were solely present in *E. coli* O157:H7 not in *E. coli* K-12. Nevertheless, the gene for long polar fimbriae (*lpf*) on loc12 and loc13 were also found in most *E. coli* virogroups (EPEC, EAEC, ETEC, EIEC, STEC), as well as in non-pathogenic *E. coli* by Toma, Higa, Iyoda, Rivas, & Iwanaga (2006), and the fimbriae were involved in the adherence and colonization of *Salmonella* Typhimurium and *E. coli* O157:H7 on host cells (Baumler, Tsolis & Heffron, 1996; Low et al., 2006b). The rest of the two putative adhesin genes, *ybg* and *stf,* were each detected in 3 isolates in the current study. Two of these genes were detected in isolates with serotypes O157 and O145 in the study of Low et al. (2006b). Wurpel, Beatson, Totsika, Petty, & Schembri (2013) found that Ybg fimbriae were expressed by both diarrheagenic and commensal *E. coli* strains. However, information about the specific function of these two genes is minimal in the current body of literature.

Considering the putative genes screened were quite diversely distributed among the tested isolates in the current study, their roles in conferring the abilities of bacterial cells in biofilm formation on peach and the surface of peach packing equipment surface should be verified in future studies.

6.5. Conclusions

Selected virulence genes were not detected in the generic *E. coli* isolated from fresh peach packing environments. Putative adhesin genes, *sfmA*, *ycbQ*, *csgA*, and *fimA*, were found in all tested isolates. The number of adhesin genes varied in the tested isolates, ranging from 5 to 9 genes per isolate. This study expanded our knowledge on the potential virulence and surface attachment potentials of generic *E. coli* isolated from the fresh peach packing environments.

References

Akter, S., Islam, M., Afreen, K. S., Azmuda, N., Khan, S. I., & Birkeland, N. K. (2013). Prevalence and distribution of different diarrhoeagenic *Escherichia coli* virulotypes in major water bodies in Bangladesh. *Epidemiology and Infection, 141*(12), 2516-2525. https://doi.org/10.1017/s0950268813000320

Baumler, A.J., Tsolis, R.M., and Heffron, F. (1996) The *lpf* fimbrial operon mediates adhesion of *Salmonella* Typhimurium to murine Peyer's patches. *Proceedings of the National Academy of Sciences of the United States of America*. 93, 279–283.

Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J., & Harel, J. (2003). Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. *Journal of clinical microbiology*, *41*(5), 2113–2125. https://doi.org/10.1128/jcm.41.5.2113-2125.2003

Berne, C., Ducret, A., Hardy, G. G., & Brun, Y. V. (2015). Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. *Microbiology spectrum*, *3*(4), 10.1128/microbiolspec.MB-0018-2015. https://doi.org/10.1128/microbiolspec.MB-0018-2015

Bernini, V., Sgarbi, E., Bove, C. G., Gatti, M., & Neviani, E. (2010). A polyphasic approach to detect enterotoxigenic *Staphylococcus aureus* and diarrheagenic *Escherichia coli* in raw milk Italian cheeses by multiplex PCR. *Journal of Food Protection, 73*(12), 2281-2284. https://doi.org/10.4315/0362-028X-73.12.2281

Blanco, M., Schumacher, S., Tasara, T., Zweifel, C., Blanco, J. E., Dahbi, G., Blanco, J., & Stephan, R. (2005). Serotypes, intimin variants and other virulence factors of *eae* positive *Escherichia coli* strains isolated from healthy cattle in Switzerland. Identification of a new intimin variant gene (*eae-eta2*). *BMC microbiology*, *5*, 23. https://doi.org/10.1186/1471-2180-5-23

Canata, M. G., Navarro, R., Velázquez, G., Rivelli, S., Rodríguez, F., Céspedes, A., Espínola, C., Canese, J., & Guillén, R. (2016). Molecular characterization of virulence factors of *Escherichia coli* isolates obtained from feces of children with gastroenteritis at the Institute for Social Welfare Central Hospital in 2012. *Pediatría (Asunción)*, *43*(1), 13-17.

Devleesschauwer, B., Pires, S. M., Young, I., Gill, A., Majowicz, S. E., & study team. (2019). Associating sporadic, foodborne illness caused by Shiga toxin-producing *Escherichia coli* with specific foods: a systematic review and meta-analysis of case-control studies. *Epidemiology and infection*, *147*, e235. https://doi.org/10.1017/S0950268819001183

Feng, Y., Li, G., Lv, X., Xu, Y., Wu, Q., Shi, C., Li, Q., Yang, B., Wang, X., Xi, M., & Xia, X. (2014). Prevalence, distribution, and diversity of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* in kiwifruit orchards and processing plants. *Foodborne Pathogens and Disease, 11*(10), 782-790. https://doi.org/10.1089/fpd.2014.1759

Evans, M. L., & Chapman, M. R. (2014). Curli biogenesis: order out of disorder. *Biochimica et Biophysica Acta*, *1843*(8), 1551–1558. https://doi.org/10.1016/j.bbamcr.2013.09.010

Harrington, S. M., Dudley, E. G., & Nataro, J. P. (2006). Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiology Letter, 254*(1), 12-18. doi:10.1111/j.1574-6968.2005.00005.x

Hornes, E., Y. Wasteson, and Ø. Olsvik. (1991). Detection of *Escherichia coli* heat-stable enterotoxin genes in pig stool specimens by an immobilized, colorimetric, nested polymerase chain reaction. *Journal of Clinical Microbiology*. *29*, 2375–2379.

Johnson, J. R., & Stell, A. L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *The Journal of Infectious Diseases, 181*(1), 261-272. https://doi.org/10.1086/315217

Kambire, O., Adingra, A. A., Yao, K. M., & Koffi-Nevry, R. (2017). Prevalence of virulence genes associated with diarrheagenic pathotypes of *Escherichia coli* isolates from water, sediment, fish, and crab in Aby Lagoon, Côte d'Ivoire. *International Journal of Microbiology, 2017*, 9532170. https://doi.org/10.1155/2017/9532170

Korea, C. G., Badouraly, R., Prevost, M. C., Ghigo, J. M., & Beloin, C. (2010). *Escherichia coli* K-12 possesses multiple cryptic but functional chaperone-usher fimbriae with distinct surface specificities. *Environmental Microbiology, 12*(7), 1957-1977. https://doi.org/10.1111/j.1462-2920.2010.02202.x

Larsonneur, F., Martin, F.A., Mallet, A., Martinez-Gil, M., Semetey, V., Ghigo, J.-M. and Beloin, C. (2016), Functional analysis of *Escherichia coli* Yad fimbriae reveals their potential role in environmental persistence. *Environmental Microbiology*, *18*, 5228-5248. https://doi.org/10.1111/1462-2920.13559

Leech, J., Golub, S., Allan, W., Simmons, M. J. H., & Overton, T. W. (2020). Non-pathogenic *Escherichia coli* biofilms: effects of growth conditions and surface properties on structure and curli gene expression. *Archives of Microbiology, 202*(6), 1517-1527. doi:10.1007/s00203-020-01864-5

Low, A. S., Dziva, F., Torres, A. G., Martinez, J. L., Rosser, T., Naylor, S., Spears, K., Holden, N., Mahajan, A., Findlay, J., Sales, J., Smith, D. G., Low, J. C., Stevens, M. P., & Gally, D. L. (2006a). Cloning, expression, and characterization of fimbrial operon F9 from enterohemorrhagic *Escherichia coli* O157:H7. *Infection and Immunity*, *74*(4), 2233–2244. https://doi.org/10.1128/IAI.74.4.2233-2244.2006

Low, A. S., Holden, N., Rosser, T., Roe, A. J., Constantinidou, C., Hobman, J. L., Smith D. G. E., Low, J. C., & Gally, D. L. (2006b). Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic *Escherichia coli* O157:H7. *Environmental Microbiology, 8*(6), 1033-1047. https://doi.org/10.1111/j.1462-2920.2006.00995.x

McClelland, M., Florea, L., Sanderson, K., Clifton, S. W., Parkhill, J., Churcher, C., Dougan, G., Wilson, R. K., & Miller, W. (2000). Comparison of the *Escherichia coli* K-12 genome with sampled genomes of a *Klebsiella pneumoniae* and three *Salmonella enterica* serovars, Typhimurium, Typhi and Paratyphi. *Nucleic Acids Research*, *28*(24), 4974–4986. https://doi.org/10.1093/nar/28.24.4974

Nagy, A., Mowery, J., Bauchan, G. R., Wang, L., Nichols-Russell, L., & Nou, X. (2015). Role of extracellular structures of *Escherichia coli* O157:H7 in initial attachment to biotic and abiotic surfaces. *Applied and Environmental Microbiology*, *81*(14), 4720-4727.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews, 11*(1), 142. https://doi.org/10.1128/CMR.11.1.142

Nataro, J. P., Yikang, D., Yingkang, D., & Walker, K. (1994). AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. *Journal of Bacteriology*, *176*(15), 4691–4699. https://doi.org/10.1128/jb.176.15.4691-4699.1994

Ndlovu, T., Le Roux, M., Khan, W., & Khan, S. (2015). Co-Detection of virulent *Escherichia coli* genes in surface water sources. *PLOS ONE, 10*(2), e0116808. https://doi.org/10.1371/journal.pone.0116808

Oswald, E., H. Schmidt, S. Morabito, H. Karch, O. Marche`s, and A. Caprioli. (2000). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. *Infection and Immunity*. *68*, 64–71.

Pass, M. A., R. Odedra, and R. M. Batt. (2000). Multiplex PCRs for identification of *Escherichia coli* virulence genes. *Journal of Clinical Microbiology*. *38*, 2001–2004.

Pratt, L. A., & Kolter, R. (1998). Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis, and type I pili. *Molecular Microbiology*, *30*(2), 285-293.

Ratchtrachenchai, O. A., S. Subpasu, and K. Ito. (1997). Investigation on enteroaggregative *Escherichia coli* infection by multiplex PCR. *Bulletin of the Department of Medical Sciences*. 39:211–220.

Ravan, H., & Amandadi, M. (2015). Analysis of *yeh* fimbrial gene cluster in *Escherichia coli* O157:H7 in order to find a genetic marker for this serotype. *Current Microbiology, 71*(2), 274-282. https://doi.org/10.1007/s00284-015-0842-6

Sauer, K., Camper, A. K., Ehrlich, G. D., Costerton, J. W., & Davies, D. G. (2002). Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. *Journal of Bacteriology, 184*(4), 1140-1154. https://doi.org/10.1128/jb.184.4.1140-1154.2002

Schmidt, H., Knop, C., Franke, S., Aleksic, S., Heesemann, J., & Karch, H. (1995). Development of PCR for screening of enteroaggregative *Escherichia coli*. *Journal of Clinical Microbiology*, *33*(3), 701-705.

Sethabutr, O., M. Venkatesan, G. S. Murphy, B. Eampokalap, C. W. Hoge, and P. Echeverria. (1993). Detection of *Shigellae* and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. *Journal of Infectious Diseases*. *167*, 458–461.

Spurbeck, R. R., Stapleton, A. E., Johnson, J. R., Walk, S. T., Hooton, T. M., & Mobley, H. L. (2011). Fimbrial profiles predict virulence of uropathogenic *Escherichia coli* strains: contribution of *ygi* and *yad* fimbriae. *Infection and Immunty, 79*(12), 4753-4763. https://doi.org/10.1128/iai.05621-11

Srey, S., Jahid, I. K., & Ha, S.-D. (2013). Biofilm formation in food industries: A food safety concern. *Food Control, 31*(2), 572-585. https://doi.org/10.1016/j.foodcont.2012.12.001

Tamanai-Shacoori, Z., and A. Jolivet-Gougeon. (1994). Detection of enterotoxigenic *Escherichia coli* in water by polymerase chain reaction amplification and hybridization. *Canadian Journal of Microbiology*. *40*, 243–249.

Toma, C., Higa, N., Iyoda, S., Rivas, M., & Iwanaga, M. (2006). The long polar fimbriae genes identified in Shiga toxin-producing *Escherichia coli* are present in other diarrheagenic *E. coli* and in the standard *E. coli* collection of reference (ECOR) strains. *Research in Microbiology, 157*(2), 153-161. https://doi.org/10.1016/j.resmic.2005.06.009

Toma, C., Lu, Y., Higa, N., Nakasone, N., Chinen, I., Baschkier, A., Rivas, M., & Iwanaga, M. (2003). Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. *Journal of Clinical Microbiology*, *41*(6), 2669–2671. https://doi.org/10.1128/jcm.41.6.2669-2671.2003

Ulett, G. C., Mabbett, A. N., Fung, K. C., Webb, R. I., & Schembri, M. A. (2007). The role of F9 fimbriae of uropathogenic *Escherichia coli* in biofilm formation. *Microbiology, 153*(7), 2321-2331. https://doi.org/10.1099/mic.0.2006/004648-0

U.S. FDA. (2012). Pathogenic *E. coli* group. In *Bad Bug Book, Foodborne pathogenic microorganisms and natural toxins*. Second Edition. pp. 69-81.

Van Pelt, A. E., Quinones, B., Lofgren, H. L., Bartz, F. E., Newman, K. L., & Leon, J. S. (2018). Low prevalence of human pathogens on fresh produce on farms and in packing facilities: a systematic review. *Frontier in Public Health, 6*, 40. https://doi.org/10.3389/fpubh.2018.00040

Venkatesan, M. M., Buysse, J. M., & Kopecko, D. J. (1989). Use of Shigella flexneri ipaC and ipaH gene sequences for the general identification of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Journal of Clinical Microbiology*, *27*(12), 2687–2691.

Yamasaki, S., Z. Lin, H. Shirai, A. Terai, Y. Oku, H. Ito, M. Ohmura, T. Karasawa, T. Tsukamoto, H. Kurazono, and Y. Takeda. (1996). Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay, and polymerase chain reaction. *Microbiology and Immunology*. *40*, 345–352.

Widmer, K., Van Ha, N. T., Vinitnantharat, S., Sthiannopkao, S., Wangsaatmaja, S., Prasetiati, M. A., Thanh, N. C., Thepnoo, K., Sutadian, A. D., Thao H. T. T., Fapyane D., San, V., Vital, P., & Hur, H. G. (2013). Prevalence of *Escherichia coli* in surface waters of Southeast Asian cities. *World Journal of Microbiology Biotechnology, 29*(11), 2115-2124. https://doi.org/10.1007/s11274-013-1376-3

Wurpel, D. J., Beatson, S. A., Totsika, M., Petty, N. K., & Schembri, M. A. (2013). Chaperone-usher fimbriae of *Escherichia coli*. *PLOS ONE, 8*(1), e52835. https://doi.org/10.1371/journal.pone.0052835

Zago, M., Bonvini, B., Platero, A. M. M., Mucchetti, G., Carminati, D., & Giraffa, G. (2007). Characterisation of *Escherichia coli* isolated from raw milk cheeses. *Annals of Microbiology, 57*(1), 49.

Table 6-1 Oligonucleotide primers (Toma et al., 2003) used for the screening of virulence genes in the generic *E. coli* isolated from fresh peach packing facilities.

Primer	**Target genes**	**Primer sequence**	Amplicon size (bp)
SK1	*eae*	CCCGAATTCGGCACAAGCATAAGC	881
SK2		CCCGGATCCGTCTCGCCAGTATTCG	
VT com-u	*stx*	GAGCGAAATAATTTATATGTG	518
VT com-d		TGATGATGGCAATTCAGTAT	
AL 65	*est*	TTAATAGCACCCGGTACAAGCAGG	147
AL 125		CCTGACTCTTCAAAAGAGAAAATTAC	
LTL	*elt*	TCTCTATGTGCATACGGAGC	322
LTR		CCATACTGATTGCCGCAAT	
Ipa III	*ipaH*	GTTCCTTGACCGCCTTTCCGATACCGTC	619
ipa IV		GCCGGTCAGCCACCCTCTGAGAGTAC	
aggRks1	*aggR*	GTATACACAAAAGAAGGAAGC	254
aggRkas2		ACAGAATCGTCAGCATCAGC	

Table 6-2 Putative adhesin gene subunits and primers derived from *E. coli* O157:H7 Sakai strain (Low et al., 2006b)

Primer	Protein	Target gene*	Sequence
Loc 1	Stc	*stc*A	5′-CGACAACGTTGATGTTTAGC 3′-GCCTTTTGTAACAGGATTGC
Loc 2	Yad	*yad*N	5′-GGTATGCATAGCGTTACC 3′-CTGCTGGCAAATCTTATGC
Loc 3	Sfm	*sfm*A	5′-GCGGTACAATTCACTTTGAAGG 3′-CATTTGCTTGCCCTGCTGATGC
Loc 4	Ybg	*ybg*D	5′-GCCATATCTCTACTATTCGC 3′-GTTATCCATCTGTTCCATCC
Loc 5	Ycb	*ycb*Q	5′-CTGTGGTATGTGCAACGTCC 3′-CCCCGTAGCGATATAATCAAC
Loc 6	Sfa	*sfa*A	5′-CCTACAGTCACTTTTCAGGG 3′-GATTAATTAGAGGTAGCTCAGG
Loc 7	Csg	*csg*A	5′-CTTCATTTAATCAGGCAGCC 3′-GAGTACCATACTGTGTAATATTTGC
Loc 8	F9	*fim*A	5′-GGTGATGAATCAGTAACGACC 3′-GTGCCATCAATCAAGTCGG
Loc 9	Yeh	*yeh*D	5′-CACCATGTACATTTGTCGC 3′-CAGTACGTCACTGCTATCTCC
Loc 10	Stf	*stf*G	5′-GCTGCAACAATGGTAATGGG 3′-GTAATCTGGAAGGTCGTGTTGGC
Loc 11	Yra	*yra*H	5′-CTTTTCGCAGGTAATGCCG 3′-GATTTCGGATGCTTCAACG
Loc 12	Lpf	*lpf*A	5′-GTGGTATCGCAATCTTCC 3′-GGTAAAGTAGAGAACCG
Loc 13	Lpf	*lpf*A	5′-GATTGTAGGAGCATTAGCG 3′-CTATCGATCTGACTCAATGCC
Loc 14	Fim	*fim*A	5′-GTCGTTGCTGCCAATGTTTGC 3′-GAAATGTAGCGAAGTAGAGCC

The molecular sizes of amplified PCR products are 300-500 bp.

*The name of each putative fimbrial gene listed in this table is referred to the working name in the paper of Low et al. (2006b).

Table 6-3 Distribution and incidence of putative adhesin genes among the generic *E. coli* isolates included in the study

		Adhesin genes (loc)														**Incidence (%)**
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Sample source	ID															
E. coli O157:H7	F4546	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Fruit	1212	-	-	+	-	+	-	+	+	-	+	+	+	+	+	64.29
Glove-1	1238	+	-	+	+	+	+	+	+	-	+	-	-	-	+	64.29
	1243	-	-	+	-	+	-	+	+	-	+	+	-	-	+	50.00
Glove-2	2470	-	-	+	-	+	-	+	+	-	-	+	-	-	+	42.86
Surface-1	2561	-	-	+	-	+	-	+	-	-	-	+	-	-	+	35.71
Surface-2	2901	-	-	+	+	+	-	+	-	-	-	+	-	-	+	42.86
	2902	-	-	+	+	+	-	+	-	-	-	-	-	-	+	35.71
Incidence (%)		14.29	0	100	42.86	100	14.29	100	57.14	0	42.86	71.43	14.29	14.29	100	

CHAPTER 7

CONCLUSIONS

The main research findings of this dissertation are as follows.

1) Levels of hygiene and pathogen indicators on selected packing lines surveyed in the present study increased overtime during a packing day. Higher levels of TA, YM, and TC counts and higher incidences of TTC and EC were observed on optical sizers and brushes/rollers inside the washer compared to other sites sampled. Harvest bins had a comparable YM count to the brush rollers within the washer. Samples from the manual sorting area had the highest TTC incidence among all collected samples compared to other sites sampled. This study identified the most heavily contaminated areas of the fresh peach packing process and emphasizes the necessity of daily cleaning and sanitation after packing.

2) Not affected by sampling time, peach samples from the weighing area had significantly higher ($P < 0.05$) average TA, YM, and TC counts and higher incidences of TTC and EC presence, compared to the samples collected from the dumping area, suggesting that the role of the packing process in decontaminating fresh fruit is very limited and should be improved. The levels of TA, YM, and TC on gloves of peach sorters and packers were similar, but they were significantly higher at the 3 and 6 h sampling points comparing to the 0 h sampling point. This study indicates that the packing process and the sorters and packers involved are among the key elements in maintaining the microbial safety of peach detained for the fresh market.

3) The WWS played a limited role in the decontamination of passing fruits according to the results of this study. Mean counts of TA and TC, as well as the incidences of TTC and EC presence, were significantly higher ($P < 0.05$) on peach samples collected after than before the WWS; although opposite observation in YM counts at the majority of the sampling points were noticed. The water monitoring program of the three facilities was effective as no TC, TTC, and EC were detected in the incoming peach wash water. This study suggests that additional sanitation mitigation should be considered to further improve the hygiene status of brush rollers inside the WWS, subsequently the microbial quality of fresh fruit.

4) Selected virulence genes were not detected in the generic *E. coli* isolated from fresh peach packing environments. Putative adhesin genes, *sfmA*, *ycbQ*, *csgA*, and *fimA* were found in all tested isolates. The number of adhesin genes varied in the tested isolates, ranging from 5 to 9 genes per isolate. This study expanded our knowledge on the potential virulence and surface attachment potentials of generic *E. coli* isolated from the fresh peach packing environments.