MICROBIAL INDICATOR LOADS ON BLUEBERRIES AFFECTED BY HARVESTING METHODS AND SUITABILITY OF SELECTED FOOD-GRADE MATERIALS FOR THE MODIFICATION OF OVER-THE-ROW MACHINE HARVESTERS

by

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(Under the Direction of JINRU CHEN)

ABSTRACT

This study determined the microbial quality of fresh blueberries harvested by different methods (hands *vs.* machines) and the efficacy of selected sanitizers on surfaces for fruit-catching surfaces of modified over-the-row blueberry harvester machine. Enumeration of total aerobes, total yeasts and molds, total coliforms, and incidence of fecal coliforms and enterococci were conducted for collected blueberries. White silicone, neoprene, ethylene propylene diene monomer (EPDM), red silicone, and plexiglass surfaces were incubated with different mixtures of fecal coliforms and then treated with different sanitizers. Developed biofilms and efficacy of sanitizers were quantified with CV assay. A significant difference ($P<0.05$) was observed in microbial loads from machine-harvested blueberries. Neoprene showed the highest biofilm formations, and sodium hypochlorite was the least effective in removing accumulated biofilms whereas dish detergent was the most effective, followed by AlpetD2 and peracetic acid. Results highlight the importance of sanitizing machine harvester with increased concentrations and contact times.

INDEX WORDS: Blueberry, Machine harvester, Fruit quality, Biofilm, Sanitizers, Food-contact surfaces

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DEDICATION

Thank you to my parents for their unconditional love and support. Their undying support and belief in my abilities have made the last few years possible. I would like to thank my sister for being my best friend, and my friends for encouragement.

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CHAPTER 1

INTRODUCTION

Increased consumer recognition of health benefits linked to the consumption of berry fruits has led to the large quantity of blueberry production in the United States as well as in the world over the last 20 years (Kalt et al., 2019). In 2019, blueberry production in the United States ranked first with 680.7 million pounds among all other countries in the world (USDA, 2020).

Blueberries, like other ready-to-eat fresh produce that is usually consumed raw, generally do not go through any washing steps until market display as they are susceptible to the growth of molds and yeasts when exposed to moisture. However, they have the potential for foodborne illness due to microbiological contamination. Few outbreaks potentially linked to the consumption of blueberries have been associated with *Salmonella* and hepatitis A (Bozkurt, Phan-Thien, Ogtrop, Bell, & McConchieet, 2020; Calder et al., 2003; Carstens, Salazar, & Darkohet, 2019). The growth of berry consumption per capita globally has generated the consideration of the established food security in the blueberry production and industry during harvesting, processing, and packing (Danyluk, Friedrich, & Ehsaniet, 2008; Gazula et al., 2019; Oliveira, Rodrigues, & Teixeira, 2019).

In the blueberry industry, blueberry harvesting is usually performed by hand pickers in the early and main season, and over-the-row (OTR) mechanical harvesters in the later seasons (Sargent, Takeda, Williamson, & Berryet, 2020; Takeda, 2018). Blueberry growers in the major production areas are gradually getting more interested in adopting OTR harvesters to increase the production of fresh blueberry; reasons also include increasing labor cost, decreasing the cost per pound of blueberries, and escalating production demands (Gallardo & Zilberman, 2016; Ni, Li, Jiang, & Takeda, 2020). Over the years, there have been attempts to modify the fruit-catching surface of OTR mechanical harvesters to a soft surface to create a quality of fresh blueberries comparable to handpicking (DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Takeda, 2018).

Biofilm formations on food-contact surfaces are a problem in the food industry as biofilms can be resistive against antimicrobial and cleaning agents. Uncleaned or contaminated mechanical harvester along with harvesting tools could introduce microorganisms or pathogen transmission to blueberries during harvesting in the field (Friedrich, Spann, McEgan, Ebel, & Danyluk, 2009). Those microorganisms may lead to biofilm formation when they are not completely removed from food-contact surfaces. This potential problem highlights the need for adequate hygiene practice of cleaning and sanitation on the mechanical harvester, especially fruit-catching surfaces.

This research aims to determine the potential food safety risks associated with conventional and new machinery of blueberry harvesting methods.

The main goals of this study were:

1. To examine the microbial quality of fresh blueberries harvested by different methods (hands *vs.* machines)
2. To determine the effectiveness of sanitizers against microorganisms and developed biofilms by fecal coliforms on different surfaces potentially used in the fruits catching surfaces of blueberry machinery harvester.

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CHAPTER 2

LITERATURE REVIEW

The blueberry production

Blueberries are perennial and deciduous shrubs contingent upon species, cultivar, and height from 1 to 3.5 m (Brennan et al., 2014; Camp, 1945). Blueberry fruit has an indigo color with more light or dark colors of fruits and hairless roots which are in heavy soils due to their sensibility to too much water (Williamson, Krewer, Pavlis, & Mainland, 2006). Relatively small amounts of nutrient sources are required for blueberries compared to other common fruit trees and grow well in soils under low pH conditions (4.0 – 5.5) (Brennan et al., 2014).

Of the blueberry productions, most cultivars in commercial are northern highbush *(Vacinnium corymbosum)* blueberry, southern highbush blueberry *(Vacinnium darowii)*, rabbiteye blueberry (*Vacinnium virgatum* Aiton), lowbush blueberry (*V. angustifolium* Aiton), and European bilberry (*V. myrtillus* L.) in the nationwide (Gallardo et al., 2018). Highbush blueberries are geographically dispersed and widely planted not only over the United States but also in other countries such as Chile, Canada, Argentina, New Zealand, and China (Strik & Yarborough, 2005). Among the northern high bush blueberries, Duke, Legacy, Draper, and Liberty are usually cultivated in the west area while Farthing, Rebel, Emerald, Star, and Brightwell are in the Southeast area of the United States (Gallardo et al., 2018).

Blueberry consumption in the United States has been steadily increasing in the past few decades, primarily due to increased consumer awareness of the health benefits associated with blueberry consumption. Large quantities of blueberry are produced on a global scale to meet the increasing consumer demands (Kalt et al. 2019; Szajdek & Borowska, 2008; FAO, 2019). The United States has led the worldwide acreage and ranked first in blueberry production in the last three decades (Retamales & Hancock, 2018). The production area of blueberry in North America has expanded by almost double from 53,420 acres in 2007 to 102,700 acres in 2019 (USDA, 2020). In 2019, the production of blueberries recorded the highest with 309 million kilograms in the United States (USDA, 2020). Washington state produced over 32 million and 74 million kilograms of blueberries, recording the largest blueberry producing area in the world in 2010 and 2019, respectively (Olmstead & Finn, 2014; FAO, 2019; USDA 2020). It was the third-largest harvested area in the states of Washington with 16,700 acres following the state of Georgia and Michigan (USDA, 2020).

In the light of benefits associated with fresh blueberry consumption, the price of fresh blueberries per kilogram has been slightly increased for the last three years about 16% from $0.71 in 2017 to $0.82 in 2019 in Washington while the price of processed blueberries per kilogram has been decreased by 24% from $0.33 in 2017 to $0.25 in 2019 (USDA, 2020). This trend has an agreement with the state of Georgia, Michigan, and Oregon for the last three years. Nowadays, fresh blueberries for the fresh market are harvested by both hand pickers and over-the-row (OTR) mechanical harvester to relieve increasing labor cost and to decreasing the blueberry cost per pound (Takeda, 2018).

Nutritional benefits of blueberries consumption

The composition of a fresh intact blueberry is mostly water (84%), carbohydrates (9.7%), proteins (0.6%), and fat (0.4%) and they serve as a good source of dietary fiber that accounts for 3 – 3.5% of the fruit weight (Michalska & Łysiak, 2015). Blueberry has one of the highest levels of anthocyanins among common fruits and even among berries fruits (Kalt et al., 2019; Wu et al., 2006). It is commonly known that the consumption of blueberries gives the human body multiple nutritional and clinical benefits. Polyphenol compounds and flavones in blueberries potentially have functions of antioxidant and anti-inflammatory upon daily intake (Joseph, Edirisinghe, & Burton-Freeman, 2014). Multiple studies confirmed that polyphenols are abundant in blueberries up to 304 mg/100g of total fresh blueberries weight (up to 0.3%) (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002), depending on conditions such as cultivar (Kalt et al., 2001), and maturity (Kalt et al., 2003), and selection of analytical methods (Barnes, Nguyen, Shen, & Schug, 2009). Over the last decades, many researchers have observed the association between the consumption of blueberries with the prevention of disease risks including cardiovascular disease, type 2 diabetes mellitus, cognitive decline, as well as obesity, and hypertension (Jennings, Welch, Spector, Macgregor, & Cassidy, 2014; Letenneur, Proust-Lima, Le Gouge, Dartigues, & Barberger-Gateau, 2007; Shi, Loftus, McAinch, & Su, 2017).

Hands harvesting *vs.* machine harvesting

Blueberries destined for the fresh market are mainly harvested by hand pickers during the early harvest season (Szajdek & Borowska, 2008). Hand-picking provides high-quality fruits with better firmness and longer postharvest shelf-life. However, the lack of worker availability and an increase in labor costs are major constant constraints for berry growers (US FDA, 2017). Mechanized harvest equipment is used later in the main or late harvest season for the processed and frozen markets blueberries, especially for growers producing larger volumes of blueberries (Gallardo et al., 2018; Michalska & Łysiak, 2015; Funt, Wall, & Scheerens, 1999). Commercial over-the row (OTR) machine harvester has, thus, been developed, which can alleviate human labor costs by 85% in addition to providing increased labor efficiency (Brown et al. 1996; Brown, 1983).

Current conventional OTR harvesters have fruit catching plates composed of plexiglass surfaces. Besides the development of OTR harvester machines, Takeda and others (2017) reported a semi-mechanical harvester could be considered as a more efficient harvesting system by reducing worker loading and fatigue which can be a burden to hand-pickers or hand-held shaking device. Nevertheless, due to challenges for worker's fatigue from repetitive vibrations, an increase in labor costs, and shortage of worker availabilities, development in the OTR machine harvesting system for fresh market fruits is likely inevitable for major blueberry producers (Olmstead & Finn, 2014). Even though the cost for initial investment employing a machine harvester might be a burden to small, mid-size blueberry growers, OTR mechanical harvester seems to provide better profits among different harvesting methods in future years (Gallardo, Lu, & Zilberman, 2019).

The flow of blueberries during machine harvesting

Blueberries go through four different parts of a conventional OTR machine: plastic beating bar, fruits catching plate, harvester tunnel, and conveyor belt during machinic harvesting (Li, Yu, Takeda, & Krewer, 2013). Figure 3.1 shows a diagram of a blueberry machine harvester. Firstly, the vibration of plastic beating bars separates the fruits which are comprised of two major columns. Fruits start to be taken off from the bushes, and separated fruits fall onto each side of the plastic-like fruit catching plates. The fruits are then moved by the conveyor belts to small rubber buckets at the rear of the machine. Another Conveyor belt at the back of the machine lifts the small rubber buckets with harvested fruits to the top of the machine harvester. Plant debris or leaves are removed by air-blowers before lifted blueberries fall into harvest lugs on top of the machine harvester.

Potential microbiological safety risk associated with fruits, production, and harvest

The surge of interests in the nutritional and health benefits of blueberries along with raised consumption also has promoted an interest in food safety concerns such as microbial quality and potential public health risks related to the consumption of blueberries (Oliveira, Rodrigues, & Teixeira, 2019). Because soft fruits like fresh blueberries are susceptible to the growth of molds and yeasts when exposed to moisture, they are usually not washed, or they are minimally processed upon fresh markets and consumers (Oliveira, Rodrigues, & Teixeira, 2019). Fresh market blueberries usually could not be easily decontaminated before a fresh market once exposed to contaminants; susceptible to cross-contamination caused by microbial spoilage, viral, or pathogenic organisms via harvesting equipment, food handler, or water (Concha-Meyer, Eifert, Williams, Marcy, & Welbaum, 2014).

Several sources including fecally polluted irrigation water, infected food handler, un-cleaned machine harvester, failure of proper sanitation in a facility and during transportation could potentially lead to contamination on harvested blueberries at any steps before sold (Bozkurt, Phan-Thien, Ogtrop, Bell, & McConchie, 2020; Calder et al., 2003). Blueberries have been associated with foodborne illness outbreaks associated with *Salmonella enterica serovars Muenchen* and *Newport* in the United States and Hepatitis A in New Zealand, respectively (Calder et al., 2003; Miller, Rigdon, Robinson, Hedberg, & Smith, 2013). Calder and others (2003) and Palumbo, Harris, and Danyluk (2013) reported that one of the possible contaminations related to the outbreak in New Zealand was likely to an infected food handler.

Moreover, in 1984, fresh blueberries were potentially associated with an outbreak of *Listeria monocytogenes* in the state of Connecticut (Oliveira, Rodrigues, & Teixeira, 2019; Ryser & Marth, 1999).

Less contact time of fresh fruits with hand pickers might be beneficial for lower microbial contaminants on fresh blueberries by operating a mechanical harvester. On the other hand, the implementation of a mechanical harvester could increase the chance of microbial contamination on fresh blueberries if the fruits-contact surfaces of the harvester are not thoroughly cleaned and sanitized (US FDA, 2017; WHO, 2011). Several researchers have emphasized the importance of proper maintenance of harvesting equipment along with cleaning and sanitation, especially surfaces that come into contact with fruits such as fruit-catching plates, conveyor belts, fruit buckets, or harvester lugs to avoid cross-contamination of the fruits (Brackett, 1992; DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Stafne & Allen, 2019). Additionally, growers need to check those surfaces of the machine harvester regularly whether mechanical harvesters or harvesting equipment are cleaned and sanitized well before and after each harvest. Some harvester surfaces may include catch plates, conveyors, and walls, or lugs (a plastic container), and they are likely to have a low level of hygiene condition for some of the indicator bacteria (Stafne & Allen, 2019).

Monitoring microbial loads of fresh blueberries and microbial indicators

The microbial quality of blueberries for the fresh market should be controlled at the pre-harvest stage, during harvesting in the field. Potential contaminants such as manure, water, plant-debris, and soil are likely to affect the microbial loads of fresh blueberries (Alegbeleye et al., 2018; Tournas & Katsoudas, 2005). However, the monitoring for the microbial quality of freshly harvested berries are not legally established, and therefore, less information about the prevalence of foodborne pathogens in blueberries are available (Oliveira, Rodrigues, & Teixeira, 2019). Naturally presented microbial loads in the low level, un-inform distribution of pathogenic bacteria, and insufficient sampling plan may lead to failure to detect quantifying or qualifying pathogenic contaminations (Buchanan & Oni, 2012). Common qualitative testing for pathogenic organisms can be time-consuming, pricy, and difficult to handle. Accordingly, widely accepted indicator organisms such as total aerobic counts, fecal coliform*, enterococcus* can be used to evaluate the potential presence of pathogenic organisms (International Commission on Microbiological Specifications for Foods (ICMSF), 2018). Moreover, since a single application of indicator is not likely to represent the relative abundance of all pathogens or any other harmful organisms, multiple applications in addition to an alternative to the conventional approaches should be used to achieve more comprehensive results on fecal contaminations and its correspondence with pathogenic organisms (Savichtcheva & Okabe, 2006).

Total aerobic counts

Total aerobes (TA) counts indicate general cleanness such as sanitary quality, the current good manufacturing practice (GMP), and levels of spoilage microorganisms for cooked or uncooked food metrics (Buchanan & Oni, 2012; Costa Dias et al., 2012). Although TA counts do not represent the poor safety associated with the presence of pathogens, notable high counts of TA counts may be responsible for potential health hazards (U.S. National Research Council Subcommittee on Microbiological Criteria, 1985) and unacceptable flavor and shelf-life of soft fruits like blueberries (Kim et al., 2011). When TA counts are notably high, additional microbiological tests are recommended to confirm the presence of pathogens (U.S. National

Research Council Subcommittee on Microbiological Criteria, 1985).

Fecal coliforms

Fecal coliforms known as thermotolerance coliforms are rod-shaped, gram-negative, non-spore, oxidase negative, and facultatively anaerobic (U.S. FDA-BAM, 2002, chap.4). The fecal coliforms were brought up due to the question on the association of fecal contamination with some coliforms which can present naturally in environmental samples. They are commonly used as indicators of fecal contamination in raw foods or composite samples to evaluate the sanitary quality of foods. They can grow and produce acid as well as gas by fermenting lactose at 44.5 ± 0.2 °C or 45.5 ± 0.2 °C within 48 h, rather a high incubation temperature in comparison to other indicator microorganisms (Buchanan & Oni, 2012; U.S. FDA-BAM, 2002, chap. 4). The presence of fecal coliforms may indicate the potential presence of pathogenic bacteria, viruses, and protozoans although they are not harmful themselves.

Enterococci

Enterococci is one of the fecal coliforms that indicate fecal contamination, not from fecal origins. They are gram-positive, more difficult to isolate and identity, and rather a high incidence in fresh vegetables than coliforms (Jay, Loessner, & Golden, 2005). Enterococci were also used as hygiene indicator parameters for fresh produce (Bartz et al., 2017), or even for fresh blueberries collected in a field (Macori et al., 2018). Macori and other researchers (2018) observed that the mean count of *Enterobacteriaceae* and *E. coli* were less than 10 CFU/g in all fresh blueberry samples collected in an open field.

Development of biofilm

One of the most common ways for microorganisms to survive is attaching on surfaces or establishing communities of bacteria or higher organisms, which can adhere to a surface irreversibly (Watnick & Kolter, 1999). This strategy for microorganisms is called biofilm. Bacteria may begin to develop biofilm formation if they are not removed thoroughly even after cleaning and sanitation on food-contact surfaces such as machinery harvester or harvesting equipment. The ability of adhesion to the surfaces includes characteristic of attaching surfaces, gene expression related to biofilm formation, cellular components in the bacteria, temperature, roughness of surfaces, interaction of bacteria-bacteria, cell surface charge, and hydrophobicity of bacteria (Bonaventura et al., 2008; Dickson & Koohmaraie, 1989; Donlan, 2002). The biofilm formation is commonly composed of four main steps; attachment, formation, development, and irreversible attachment (Donlan, 2002). The first attachment starts with approaching the surfaces by absorbing organic or inorganic materials to look for a suitable place to settle down on surfaces. Some motile bacteria may lead to this initial attachment to the surfaces. The initial reversible attachment is incorporated with the van der Waals, electrostatic forces, and hydrophobic interaction with a surface (Chmielewski & Frank, 2003). Following the weak reversible attachment, microbial cells then continue to grow and maybe aggregated irreversibly on surfaces by extracellular-polymeric-substance (EPS) as well as covalent bonding, hydrophobic, and physicochemical interactions (Carniello Carniello, Peterson, Mei, & Busscher, 2018). The irreversibly attached bacteria combined with other substances become like hard plastic, normally difficult to remove without physically scrubbing (Marriott et al., 2018). Several studies demonstrated that the irreversible attachment may occur from twenty minutes at 4 to 20 °C (Gilbert, Evans, Duguid, & Brown, 1991; Lundén, Miettinen, Autio, & Korkeala, 2000).

Fruit catching surfaces of a machine harvester

Plexiglass (called fish-scale) materials are currently used for fruit catching surfaces of conventional blueberry machine harvester but they are too hard when soft blueberries fall onto the surfaces. To reduce blueberry quality lost by mechanical harvesting, several attempts have been made by replacing the hard surfaces on the OTR machine harvester with softer materials (Gallardo, Zilberman, Lu, & Jung, 2019). Yu et al. (2012) reported that modification of hard fruit catching plates on blueberry machine harvester with a soft cushioning material significantly reduced the impacts on harvested blueberries. The softer catching surface seems to play a vital role for mechanically harvested blueberries after detached from the bush by falling onto the softer plates or interior of the harvester with fewer impacts which may prevent the invasion of microbial contaminants (DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Xu, Takeda, Krewer, & Li, 2015). The major modification of the OTR prototype, therefore, has been made on the fruit catching surfaces by covering plexiglass plates with softer rubber-like materials.

Biofilm removal and control

Surfaces that come in contact with fresh produce or fruits should be cleaned and sanitized properly and regularly. Once bacteria cells attach to surfaces or food contact surfaces irreversibly, it is hard to remove them due to firm layers of organisms, resulting in requiring some physical forces practice like scraping, pressure washing, rubbing, and application of enzymes, sanitizers, detergents, or heat (Donlan, 2002; Sinde & Carballo, 2000).

The aim of cleaning in the food industry is to lower the food contact surface tension of water and remove soil and plant debris, and residue. This is contributed to the loosening, detaching, and suspension of debris so that suspended debris is rinsed with water (Marriott et al., 2018). On the other hand, sanitation in the food industry aims to keep at minimum growth of food spoilage microorganisms or foodborne pathogens and maintain a clean environment (Marriott et al., 2018).

Sodium hypochlorite

Sodium hypochlorite is also known as sanitizers containing active chlorine, is the most common sanitizing agent and used for both sanitation of fresh produce and food-contact surfaces as well as a disinfectant of surfaces (Chaidez, Lopez, & Castro-del Campo, 2007). It is commonly used in the food industry due to its inexpensive cost, high efficiency, and ease of application in low concentration. Sodium hypochlorite is effective in the removal of large, complex carbohydrate molecules into small parts which are more soluble molecules and easy to diffuse in cleaning reactions (Marriott et al., 2018). The effectiveness of sodium hypochlorite is usually elevated by warm temperature and pH value lower than 7.5 (Stopforth, Samelis, Sofos, Kendall, & Smithet, 2002).

However, organic matter which usually present in fresh produce and fruits can neutralize hypochlorite and active hypochlorous acid since they competitively react with the organic materials. The sanitizer solutions with reduced activity would poorly penetrate biofilms, making the sanitizer inactive (Ramos, Miller, Brandão, Teixeira, & Silva, 2013; Stewart et al., 2001). On top of that, diluted sodium hypochlorite solutions or corresponded cleaners should be utilized because they easily lose their stability during storage.

Peracetic Acid (PAA)

Peracetic acid (PAA), an acid-based sanitizer, can be easily dissolved into oxygen, water, and acetic acid in an aqueous solution (Kitis, 2004). Unlike sodium hypochlorite sanitizers, they are relatively stable and tolerant of organic materials. It is also recognized that PAA is non-toxic and has low environmental hazards (da Costa, Rodgher, Daniel, & Espíndola, 2014; Lemmer et al., 2017). For these reasons, PAA has been introduced as the promising alternative agent of chlorine sanitizers. However, despite these advantages, the application of PAA is still questionable because it inactivates enzymes that are accountable for the discoloration and cause odor on food contact surfaces when used at high concentration (Kašková, Ondrašovičová, Vargová, Ondrašovič, & Venglovský, 2007). According to a study by da Silva Meira, de Medeiros Barbosa, Alves Aguiar Athayde, de Siqueira-Júnior, & de Souza (2012), PAA (30 mg/L) might not be effective in removing mature biofilms on food contact surfaces while other researchers found out that PAA could be comparable to chlorine sanitizer in destroying bacteria against fecal coliform, *Escherichia* coli, and *Salmonella* sp. at the concentration between 0.5 to 4.0 mg/L (Veschetti et al., 2003).

Quaternary ammonium compounds (Quats)

Quaternary ammonium compounds (Quats) are active ingredients of commercial sanitizer which have been used to disinfect and sanitize household settings and food/non-food contact surfaces (Buffet-Bataillon, Tattevin, Bonnaure-Mallet, & Jolivet-Gougeon, 2012; Marriott et al., 2018). Quats are cationic compounds that include NH_4^+ structure and are odorless, colorless, low-toxicity, and non-corrosive to common metal surfaces at recommended doses. Furthermore, they are officially approved by the US Food and Drug Administration to applying on equipment that direct contact on food-contact surfaces at 200mg/L doses, while they are not approved for directly contacting fresh produce because of the n-alkyl, dimethyl benzyl ammonium chloride ingredients (21CFR172.165; FDA,2005).

Quats are likely to effective against gram-positive and negative, molds, and yeast (Buffet-Bataillon, Tattevin, Bonnaure-Mallet, & Jolivet-Gougeon, 2012; Du, Danyluk, & Harris, 2010), but ineffective against the human norovirus on stainless coupons (Bolton et al., 2013). Du and other researchers (2010) observed the reduction of *Salmonella* in the inoculated almond dust almost 7 log CFU/g. In the same study, they also found that the higher presence of the isopropanol showed a significant reduction of *Salmonella* in inoculated dust. And then, they agreed that the 200 ppm of Quats with isopropanol was an effective sanitizer against *Salmonella* even in the presence of a high concentration of organic materials as opposed to the sodium hypochlorite.

Effect of the sanitizers in preventing biofilm formation

Cleaning and sanitizing agents are usually used to reduce the community of biofilms on surfaces of harvesting equipment or packing facilities. However, several researchers found that conventional cleaning and sanitation may fail to eradicate *Salmonella* which could remain on plexiglass surfaces and equipment (Arguello, Carvajal, Collazos, García-Feliz, & Rubio, 2012). Corcoran and others (2014) observed that 500 ppm of sodium hypochlorite was not effective against an established biofilm developed for 2 days and 7 days on tested surfaces. On the other hand, Hua, Korany, El-Shinawy, and Zhu (2019) observed that treatments with 400 ppm Quats reduced 3.0 – 3.7 log CFU/coupon (1.5 x 0.75 cm) *L. monocytogenes* biofilms on tested surfaces at 5 min exposure; Treatment with 200 ppm PAA reduced 4.0 – 4.5 log CFU/coupon *L. monocytogenes* biofilms in the same condition. Rubber surfaces had more resistance than other tested surfaces with PAA and Quats against *L. monocytogenes* biofilms. What they concluded was that surface material had more impact on the efficacies of Quats, but less influence on those of PAA. Additionally, several studies showed that dish soap or Sodium dodecyl sulfate (SDS) destroyed biofilms better than other acidic agents on various surfaces (Redelman et al., 2012; Shelley, Kaiser, Shelley, Williams, & Kramer, 2013; Antoniou & Frank, 2005).

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CHAPTER 3

MICROBIOLOGICAL QUALITY OF FRESH BLUEBERRIES HARVESTED BY DIFFERENT METHODS

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Abstract

More growers are using over-the-row machines to harvest blueberries for the fresh market. This study aimed to assess the microbial quality of fresh blueberries harvested by different methods. Samples (n=336) of 'Draper' and 'Liberty' blueberries, harvested by a conventional, and a modified, over-the-row mechanical harvester, ungloved but sanitized hands, and hands wearing sterilized gloves, were collected from a blueberry farm in the Pacific Northwest at 9 AM, 12 PM and 3 PM on four different harvest days in 2019. Eight replicates of each sample (25 g) were collected at each sampling point and transported under refrigeration temperature. The average microbial counts on harvested blueberries were low; with total aerobic counts (TA) ranging from 1.88 to 1.91 log CFU/g, total yeast and mold (YM) counts from 3.66 to 3.67 log CFU/g, and total coliform (TC) counts from 0.35 to 0.24 log CFU/g on 'Draper' and 'Liberty' blueberries, respectively. Microbial loads on the blueberries of two different cultivars were statistically similar ($P \geq 0.05$). TA counts on fruits harvested at different time points were also similar, except that; the samples collected at 12 PM and 3 PM had significantly lower ($P < 0.05$) YM counts than the 9 AM samples. The 3 PM samples had significantly lower TC counts. Blueberries harvested by the conventional and modified OTR harvesters were significantly higher in TA, but not YM counts than those harvested by the other two methods. Fruits harvested by the modified OTR harvester had also significantly higher TC counts. Seven fecal coliform isolates (2.38%) were recovered from samples collected from the modified OTR mechanical harvester, but no enterococci were detected. Results suggest that the microbial quality of fresh blueberries is affected by the harvesting method, highlighting the importance of cleaning and sanitizing machine harvesters and selecting materials to modify the conventional machine harvesters.

1. Introduction

Blueberry consumption in the United States has been steadily increasing in the past few decades, primarily due to the increased consumer awareness of the health benefits associated with blueberry consumption. A large quantity of blueberries is produced on a global scale to meet the increasing consumers' demands (Kalt et al. 2019; Szajdek & Borowska, 2008; FAO, 2019). The United States ranked first in blueberry production in the last three decades. Washington state produced over 31million kg of blueberries, being the largest blueberry producer in the world in 2010 (Olmstead & Finn, 2014; FAO, 2019).

Blueberries in the fresh market are mainly harvested by hand pickers during the early harvest season (Szajdek & Borowska, 2008). Hand-picking provides high-quality fruits with better firmness and postharvest shelf-life. However, the lack of workers' availability and an increase in labor costs are major constraints for berry growers (US FDA, 2017). Commercial over-the-row (OTR) machine harvesters can alleviate human labor costs by 85% in addition to providing increased labor efficiency (Brown et al. 1996; Brown, 1983).

Blueberries go through four different parts of an OTR machine, *i.e.* plastic beating bar, fruits catch plate, harvester tunnel, and conveyor belt, during machinic harvesting (Li, Yu, Takeda, & Krewer, 2013). The vibration of plastic beating bars separates the fruits from the bushes, and separated fruits fall onto both sides of the catching plates. The fruits are then moved by conveyor belts to the small buckets at the rear of the machine. The convey belts lift the small buckets with harvested fruits to the top of the harvester. Plant debris or leaves are removed by air-blowers before lifted blueberries fall into harvest lugs on top of the harvester.

Despite numerous advantages, the OTR harvesters have been found to produce low-quality fruits with increased bruising and reduced firmness compared to hand-harvested fruits

(Takeda et al. 2017). Studies have found that the hard surfaces of machine harvesters such as fruit shaking beater rods, plastic catching plates-also known as fish scales- creates significant damages to harvested blueberries (Li, Yu, Takeda, & Krewer, 2013; Yu et al., 2012). As a result, berries harvested by the OTR mechanical harvester reduced their shelf-lives (Takeda et al., 2008). Several attempts have been made by replacing the hard surfaces on OTR machine harvester with softer materials to produce higher-quality fresh blueberries comparable to hand-picking (Gallardo, Zilberman, Lu, & Jung, 2019).

The currently available modified OTR prototype is partially modified from previous works mainly on the fruits catching plates (DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Sargent, Takeda, Williamson, & Berry, 2020). The major modification of the OTR prototype is, therefore, made on the fruit catching surfaces by covering plexiglass of fish-scale with neoprene rubber-sheets, which are softer food-contact surface materials.

The softer surfaces of neoprene rubber-sheets seem to play a vital role for mechanically harvested blueberries after detached from the bush by falling onto the softer plates or interior of the harvester with fewer impacts which may be prevented from the invasion of microbial contaminants inside the fruits (DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Xu, Takeda, Krewer, & Li, 2015). Yu et al. (2012) found out that modification of hard catch plates on blueberry machine harvester with a soft cushioning material significantly reduced the impacts on harvested blueberries. However, it is not known whether the modifications will adversely affect the microbial quality of harvested fruits.

In a study by Astill, Minor, & Thornsbury (2019), only 54.4% of surveyed growers cleaned their machine harvesters daily, and 2.1% of the growers never cleaned their harvesting machines. Furthermore, 11.6% of surveyed growers reported that they never had a sanitizing schedule for harvesting machines, while 40.0% of them had a daily sanitation routine, and 15.6% had a weekly sanitation schedule (Astill, Minor, & Thornsbury, 2019). Growers in their study tended to clean machine harvester more frequently than sanitizing. More availability of machine harvesting may increase exposures of microbial contaminants to food-contact surfaces.

The overall purpose of this study was to evaluate the microbial quality of fresh blueberries harvested by different methods, namely standard OTR machine harvester, a modified machine harvester prototype with softer catching surfaces, ungloved but cleaned and sanitized hands, and hands wearing sterile gloves.

2. Materials and methods

2.1. Main differences between a conventional OTR harvester and a modified OTR prototype

In the current study, the modified OTR machine prototype has three major differences in comparison to the conventional OTR machine harvesters (Sargent, Takeda, Williamson, & Berry, 2020); (1) each top side of plexiglass fruit-catching plates hollowed in the center was covered with soft neoprene rubber-sheets with rigid stainless-steel rings on each side of harvester prototype (*e.g.*, 'soft catching plate'), (2) canvas-fabric materials were installed to cover intermediate frames over each side of plastic conveyor belts and partly wells of the harvester tunnel, (3) neoprene rubber-sheets were also added on the front and rear wheel side-wells of modified harvester tunnel and in the middle of the tunnel side of the beating bar in the tunnel.

2.2. Sampling plan and collection

Blueberry samples (n = 336) of cultivars 'Draper' and 'Liberty' were collected at three different intervals, at 9 am, 12 pm, and 3 pm at two different locations of a blueberry farm in the

Pacific Northwest of the United States in the harvest season of 2019. At each sampling point, eight replicate bags of blueberry samples were collected by different harvesting methods: namely ungloved, but cleaned and sanitized hands, standard over-the-row (OTR) machine harvester, or modified OTR prototype (Figure 3.1). Samples collected by hands wearing sterile nitrile gloves (Thermo fisher scientific, Pittsburg, PA, USA) served as controls. Hand-picked blueberry samples were randomly collected from different locations of various blueberry bushes, while berries harvested with conventional, and modified OTR prototype were randomly collected from a stackable berry lug per one bag at the edge of the field. Collected blueberries were placed in sterile whirl-pak bags (Nasco, Fort Atkinson, WI, USA) and stored in a cooler (Rubbermaid; Newell Brands Inc, Atlanta, GA, USA) with ice packs (VWR, Lutterworth, UK) in the field and during the transportation for sample processing in a local laboratory in Lynden, WA, the USA under aseptic conditions.

2.3. Sample processing and transportation

Each blueberry sample (25 g) in the sterile whirl-pak bag was homogenized (Stomacher 80, Seward Ltd, UK) in 50 ml of sterile 0.2 M phosphate-buffered saline (PBS, pH 7.4) for 1 min at normal speed (230 rpm). The resulting homogenate in a volume of 7.5 ml was transferred to a 15-ml conical centrifuge tube containing 2.5 ml of 60% glycerol to make the final glycerol concentration of 15% (vol/vol). Each sample was vigorously mixed by shaking with hands and then stored at -20 °C before being transported overnight by an aircraft to Atlanta, GA, followed by ground transportation to our laboratory in Griffin, GA in an insulated polystyrene foam container (Polar Tech 266C Thermo chill insulated carton with foam shipper, 19” x 12” x 16”, Genoa, IL, USA) with 2.27 kg. of dry ice (Lynden ice company LLC, Lynden, WA, USA). The samples were analyzed immediately upon arriving at the laboratory on the University of Georgia Griffin campus.

2.4. Microbial enumeration

The frozen blueberry homogenates described above were thawed at 4 °C upon arriving at our laboratory in Griffin, GA. A hundred micro-milliliters of individual blueberry homogenates were inoculated in duplicate on four different microbiological media, including tryptic soy agar (TSA), potato dextrose agar (PDA) acidified with 10% tartaric acid to pH 3.5, MacConkey agar (MAC), and Enterococcus agar (EA), respectively. All microbial media used in the study were purchased from Becton Dickinson (Franklin Lake, NJ, USA) unless specified. Serial dilution was made only with sterile 0.1 M PBS when expected to have a higher concentration before inoculation onto various microbiological media. The incubation condition for total aerobes (TA), total coliforms (TC), and enterococci (EA) was at 37 °C for 24 to 48 h, for fecal coliforms (FC) at 44.5 °C for 24 h, and yeast and molds (YM) at 25 °C for 48 to 72 h. Colonies of TA on TSA, YM on PDA, TC and presumptive FC on MAC, and presumptive EC on EA were enumerated after incubation, and enumeration results were presented as log colony-forming units per gram of fresh blueberry sample (log CFU/ml).

2.5. Statistical analysis

All microbial counts from four different microbiological media were analyzed with analysis of variance (ANOVA) in SAS (version 9.4, SAS Institute, Cary, NC) using the PROC GLIMMIX procedure (Generalized Linear Mixed Models). Least square means were calculated, and post-hoc mean separation was done using Tukey's test ($P < 0.05$). Differences were considered to be significant if $P < 0.05$. When the interaction proved significant, the SLICE option in the Least Square Means (LSMEANS) statement was used to determine differences among the treatments.

3. Results

The two blueberry cultivars had statistically similar means for TA, YM, and TC counts ($P \geq 0.05$; Table 3.1). In general, the average TA and TC counts on samples of collected blueberries were low; with the mean TA counts of 2.03 and 2.03 log CFU/ml and TC counts of 0.4 to 0.34 log CFU/ml on Draper and Liberty blueberries, respectively. On average, berries collected at the three different time intervals had similar mean counts. However, the 3 pm samples had a similar YM count to the noon samples and a significantly lower ($P < 0.05$) YM counts than the 9 AM samples. The 3 pm samples also had significantly lower TC counts than the 9 am and 12 pm samples.

When the results of microbial enumeration were separated by time, there was no significant difference ($P \geq 0.05$) in the mean TA and TC counts on Draper *vs*. Liberty blueberry samples (Table 3.2). The average microbial loads on samples of Liberty blueberries collected at different time intervals were statistically similar. Draper berry samples collected at the three-time intervals only had similar mean TA and TC counts, however, the 3 pm Draper berry samples had lower mean YM counts than the other 9 am and 12 pm Draper berry samples.

Blueberries harvested by the standard OTR machine harvester and modified OTR prototype had significantly higher ($P < 0.05$) mean TA counts than those harvested by hands with sterile gloves, and ungloved, but cleaned and sanitized hands (Table 3.1). However, the levels of mean YM counts on all collected berries samples were statistically similar ($P \geq 0.05$). Berries samples collected by the modified OTR prototype had significantly higher TC counts than those harvested by the other three different methods. When the results of microbial enumeration on samples harvested by different methods were separated by harvesting time, similar results were observed (Table 3.3). As for the harvest time, samples harvested by ungloved but cleaned and sanitized hands at 3 pm had a greater mean TA count than the 9 am samples, but a lower mean TC count than the 9 and 12 pm samples.

No *enterococci* were detected in all collected samples, whereas 7 fecal coliforms were isolated from berries samples harvested by the modified OTR prototype. The percentage of fecal coliform positive samples was 2.1%. Three out of seven isolates were isolated from the 9 AM samples, and the other three isolates were from noon samples.

4. Discussion

Physical damages on the fruits could vary depending on blueberry cultivars during machine harvesting (Hussein, Fawole, & Opara, 2020). Other researchers compared blueberry cultivars to how much they are physically affected by machine harvesting (Ballington, Mainland, Duke, Draper, & Gallettaet, 1990; Olmstead & Finn, 2014). Those mechanical damages may promote the growth of spoilage or pathogenic bacteria that could lead to microbial contaminations of the fruit. Overall, our results observed a similarity in microbial counts between the 'Draper' and 'Liberty' blueberry cultivars among various harvesting methods used in the study. However, Sargent, Takeda, Williamson, and Berry (2020) showed a different observation that the fruit quality of one cultivar was more susceptible than that of the other cultivar to the machine harvesting regardless of the type of catching surfaces.

Results from this study showed that implementing a modified OTR prototype was not significantly different ($P \geq 0.05$) from the conventional OTR harvester with respect to the microbial quality of blueberries except for the mean TC of berries, and there are similar trends in the TC counts between the conventional and modified OTR machines. Blueberries harvested by conventional and modified prototype OTR harvester had similar mean TA and YM counts in comparison to mechanically harvested strawberries; 2.93 log CFU/g, and 3.58 log CFU/g (Sogvar, Koushesh, & Emamifar, 2016). The variations in total coliforms may be due to the different types of harvester design, surface materials, or frequent exposures to microbial contaminants such as soils or plant debris (Yu et al., 2012). The introduction of a modified OTR prototype would be feasible without scarifying the microbiological quality of fresh berries along with the proper maintenances of the machine harvesters. On the other hand, a study from Michigan observed higher microbial counts than ours on mechanically harvested blueberries with the mean TA, yeast, mold, and coliform; 4.03, 4.32, 4.57, and 1.12 log CFU/g, respectively (Popa, Hanson, Todd, Schilder, & Ryser, 2007) although the overall microbial loads of the berries had a similar trend with our results. Higher microbial counts were also observed by Sales (2013) on fresh blackberries collected from machine harvester; with an average of 4.07, 3.87, and 1.75 log CFU/g for the mean aerobic plate counts, yeasts and molds, and coliforms, respectively. These higher microbial counts of fresh berries harvested by conventional harvesting machines in other studies might be attributed to the degree of microbial contamination influenced by harvesting seasons, temperature, as well as harvesting hygiene practice (Popa, Todd, Schilder, & Ryser, 2007).

Blueberry growers are getting more interested in replacing the berry catching surface materials with softer materials so that fresh blueberries harvested by the OTR machine prototype can be sold in the fresh market at a higher price by maintaining the microbial quality of fruits; in addition to the lack of workers' availability and increase in labor cost for blueberry harvesting (Gallardo et al., 2018; Takeda et al., 2017). The modified OTR prototype used in this study was partially modified from previous works mainly on the fruits catching plates (DeVetter, Yang, Takeda, Korthuis, & Li, 2019; Sargent, Takeda, Williamson, & Berry, 2020). It is revealed that the major modifications to the conventional OTR harvester described above did not affect the overall microbial counts on sampled blueberries. Therefore, the modifications to the conventional OTR harvester would not potentially give a significant difference in the microbial quality of fresh blueberries.

The presence of TA is usually regarded as an index for the hygienic status of fresh produce although they are not usually considered as potential spoilage agents. This study observed that average mean TA counts were significantly higher in blueberries collected from machine harvesting than those from hand harvesting. The results have a similar trend with a study by Danyluk, Friderrich, & Ehsani (2008) who observed that the mean TA counts of fresh orange collected by a machine harvester were slightly higher than those of orange collected by hands. These results may indicate that proper hygiene and sanitation of machine harvester and production practice could allow machine-harvested blueberries to have the microbiological quality comparable to the hands harvested berries. Soft fruits like blueberries are highly susceptible to mechanical damage which prevents fresh berries to be sold in the fresh market. Preventing the contamination of the blueberries from harvester equipment and preserving their hygienic status is essential to the safety of the fresh berries (Machado-Moreira et al., 2019). Overall, microbial counts except for yeasts and molds on blueberries harvested from the OTR harvest machine were significantly higher than those from hands wearing sterile gloves and ungloved sanitized in the current study. However, this different level of hygiene could be ameliorated by enhancing the cleaning and sanitation routine for OTR machine harvester.

The population of indicator microorganisms used in this study such as total aerobic counts, yeasts and molds, total coliform, fecal coliform, and enterococci was expected to provide an informative indication if blueberries have been exposed to risks that could be related to pathogenic or spoilage contamination as the hygiene condition of the harvesting equipment (Buchanan & Oni, 2012). The concept of indicator organisms can be used to monitor the hygienic level and cleaning efficiency of harvesting equipment especially for blueberries having a naturally low level of microbial counts (Morales-Rayas, Griffiths, & Shultz, 2014). Because of constraints to the low microbial detection limit and the quantification of foodborne pathogens on fresh blueberries, applying microbial indicator organisms could be useful to evaluate the overall hygiene degree of fresh blueberries (Bartz et al., 2017).

Our results showed that sampled blueberries had low percentages of fecal coliforms (7 out of 336 samples; 2.1%) and enterococcus (0 out of 336 samples; 0%) incidence on sampled blueberries, and all the positive samples were recovered from mechanically harvested blueberries. The presence of fecal coliforms and enterococcus may indicate the potential presence of pathogenic bacteria, viruses, and protozoans although they are not harmful themselves (FDA-BAM, 2002). Wu, Long, Das, & Dorner (2011) mentioned that the presence of non-enteric indicator such as total and fecal coliforms frequently increases the chance of positive for fecal contamination so that they are likely to have a better association with pathogens than fecal indicators such as *Escherichia* coli or *enterococci*, especially for the low level of fecal contamination. The positive relationship between the presence of *E. coli* and fecal coliforms in alfalfa sprouts at retail was found by Rangel-Vargas and others (2015). In contrast, in the same study, they found that *Salmonella* was not correlated with fecal coliform or *E. coli*. Furthermore, total coliforms can grow in the diverse environment (Won, Schlegel, Schrock, & LeJeune, 2013), and unbalanced distributions in the limited sample size with the low pathogen presence may decrease the accuracy of detection (Denis, Zhang, Leroux, Trudel, & Bietlot, 2016). For these reasons, many concerns regarding the correlation of microbial indicator organisms with the presence of pathogens have been questioned. Therefore, to determine the sanitation level of harvesting equipment and the risk of pathogenic contamination associated with the microbial quality of fresh blueberry, enough number of test samples and combined multiple indicator organisms could be more practicable (Wu, Long, Das, & Dorner, 2011; Savichtcheva & Okabe, 2006).

5. Conclusion

Blueberries in the use of machine harvester are un-touched by workers' hands in comparison to blueberries collected by hand-pickers. We analyzed microbial counts of fresh blueberries to evaluate the effect of different harvesting methods on the microbial quality of fresh berries. This study observed that fresh blueberries harvested by hand pickers showed significantly less microbial counts than those berries harvested by OTR harvester machines ($P < 0.05$). Different levels of microbial quality of fresh blueberries may be attributed to how blueberries are harvested either by hand-pickers or a mechanical harvester. Adoption of the OTR machine increases the need for the proper concentration with regular-basis sanitization practice on blueberry machine harvester, especially for food-contact surfaces such as fruit-catching surface. Therefore, appropriate sanitization protocol and maintenance on the harvesting equipment needs to be emphasized to achieve microbial qualities comparable to hand-picked blueberries.

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Figure 3.1.
The flow of blueberries harvested by a modified machine harvester.

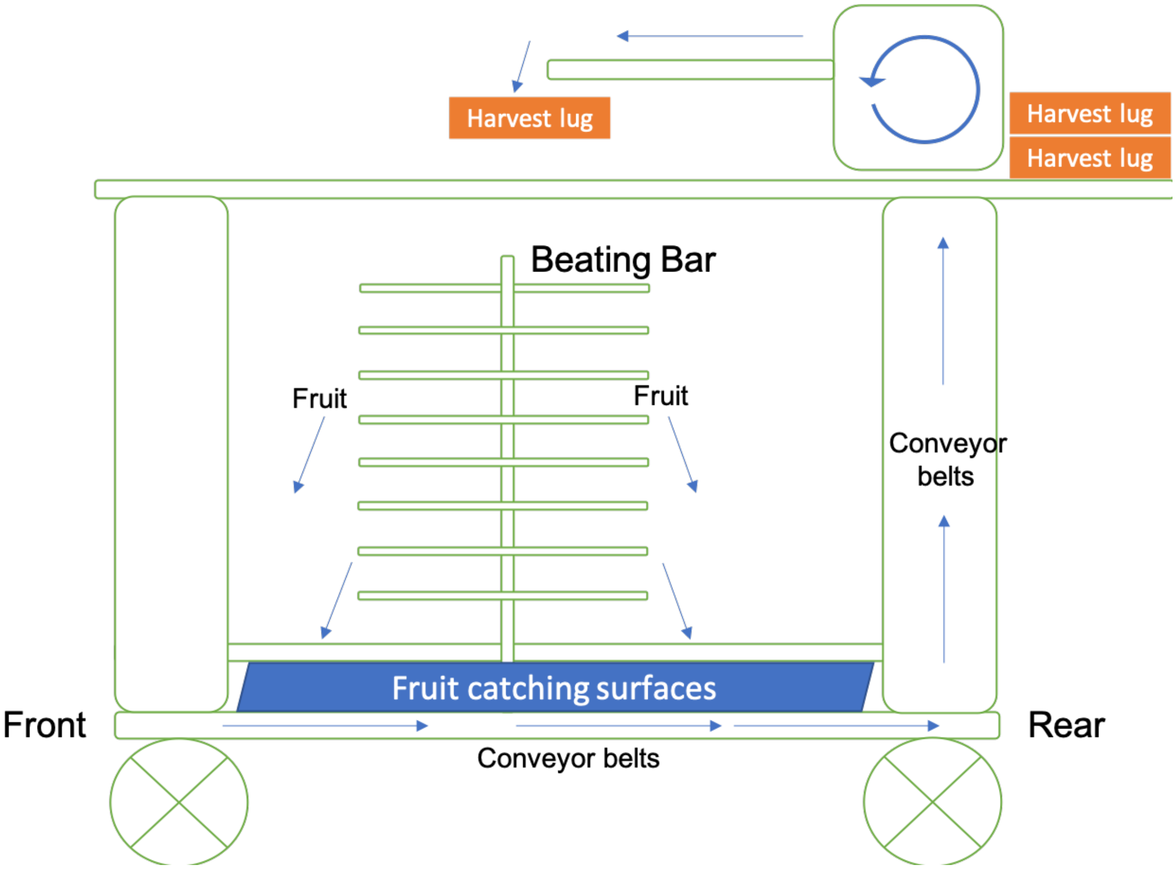

Table 3.1.
Overall mean TA, YM, and TC counts on sampled fresh blueberries by different cultivar, sampling time, and harvesting methods.

	Total aerobic counts	Total yeast and molds	Total coliforms
		log CFU/ml	
Cultivar			
Draper (n = 192)	2.03^{A}	3.87^{A}	0.40^{A}
Liberty (n = 144)	2.03^{A}	3.84^{A}	0.34^{A}
Sampling time			
9 AM (n = 120)	2.10^{A}	3.96^{A}	0.50^{A}
12 PM (n = 120)	2.11^{A}	3.86^{B}	0.39^{A}
3 PM (n = 96)	1.88^{A}	3.74^{B}	0.22^{B}
Harvesting method			
Modified OTR prototype machine harvester (n = 88)	2.45^{A}	3.90^{A}	0.64^{A}
OTR machine harvester (n = 72)	2.22^{A}	3.87^{A}	0.31^{B}
Ungloved sanitized hands (n = 88)	1.79^{B}	3.81^{A}	0.30^{B}
Hands with sterile gloves (n = 88)	1.67^{B}	3.84^{A}	0.23^{B}

* All detection limit for each TA, YM, and TC was 1.12 log CFU/ml.

* Values followed for the different capital letters in each column are significantly different ($P < 0.05$) on the cultivar, sampling time, and harvesting method, respectively.

Table 3.2.
Mean populations of TA, YM, and TC counts (Log CFU/ml) of sampled fresh blueberries by different sampling time and cultivar.

	Draper (n = 192)	Liberty (n = 144)
	log CFU/ml	
Total aerobic counts		
9 AM (n = 120)	2.12^{Aa}	2.09^{Aa}
12 PM (n = 120)	2.12^{Aa}	2.10^{Aa}
3 PM (n = 96)	1.86^{Aa}	1.91^{Aa}
Total yeast and molds		
9 AM (n = 120)	4.02^{Aa}	3.91^{Aa}
12 PM (n = 120)	3.89^{Aa}	3.83^{Aa}
3 PM (n = 96)	3.69^{Ba}	3.79^{Aa}
Total coliforms		
9 AM (n = 120)	0.48^{Aa}	0.52^{Aa}
12 PM (n = 120)	0.41^{Aa}	0.37^{Aa}
3 PM (n = 96)	0.33^{Aa}	0.11^{Ba}

* Values followed for the different capital letters in each column are significantly different ($P < 0.05$) in total aerobes, total yeast and molds, and total coliforms, respectively.
* Values followed for different small letters in each row are significantly different ($P < 0.05$) within the cultivar.

Table 3.3.

Mean populations of TA, YM, and TC counts on sampled fresh blueberries by different harvesting methods at different harvesting time points.

	Modified OTR prototype machine harvester (n = 88)	OTR machine harvester (n = 72)	Ungloved sanitized hands (n = 88)	Hands with sterile gloves (n = 88)
		log CFU/ml		
Total aerobic counts				
9 AM (n = 120)	2.65^{Aa}	2.30^{Aa}	1.66^{Bb}	1.80^{Ab}
12 PM (n = 120)	2.43^{Aa}	2.23^{Ab}	2.04^{Ac}	1.74^{Ac}
3 PM (n = 96)	2..25^{Aa}	2.14^{Aa}	1.67^{Bb}	1.47^{Ab}
Total yeast and molds				
9 AM (n = 120)	4.03^{Aa}	3.96^{Aa}	3.88^{Aa}	3.98^{Aa}
12 PM (n = 120)	3.89^{Ba}	3.88^{Aa}	3.79^{Aa}	3.88^{Ba}
3 PM (n = 96)	3.79^{Ba}	3.75^{Aa}	3.75^{Aa}	3.67^{Ba}
Total coliforms				
9 AM (n = 120)	0.91^{Aa}	0.51^{Ab}	0.27^{Ab}	0.31^{Ab}
12 PM (n = 120)	0.76^{Aa}	0.11^{Ab}	0.44^{Ab}	0.25^{Ab}
3 PM (n = 96)	0.25^{Ba}	0.31^{Aa}	0.20^{Aa}	0.11^{Aa}

* Values followed for the different capital letters in each column are significantly different ($P < 0.05$) in total aerobes, total yeast and molds, and total coliforms, respectively.

* Values followed for the different small letters in each row are significantly different ($P < 0.05$) within the harvesting method.

CHAPTER 4

BIOFILM FORMATION AND EFFECTIVENESS OF SANITIZER ON SELECTED FOOD-GRADE MATERIALS FOR THE MODIFICATION OF OVER-THE-ROW MACHINE HARVESTERS

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Abstract

Blueberry growers have become more interested in the modification of the hard fruit-catching surfaces used in the blueberry over-the-row machine harvester to obtain high-quality fresh fruits and achieve practical sanitation. This study observed biofilm mass on selected surface materials and evaluated the effectiveness of commercial sanitizers against the developed biofilm on the selected surface materials. Five sets of single or mixed fecal coliforms isolated from fruit, gloves, packing line, and machine harvester were incubated with white silicone, neoprene, EPDM, red silicone, and plexiglass coupons for 7 days at 25 °C, and then the surface coupons were treated with different concentrations of sodium hypochlorite, peracetic acid, isopropyl alcohol-based quaternary ammonium compounds (AlpetD2), and dish detergent for 1 min. The developed biofilm and biofilm masses remained on the surface materials after sanitizer treatments were quantified with a 1% crystal violet assay. The overall results indicate that the neoprene surface shows significantly higher biofilm formation than other surface materials ($0.5 \geq P$), and developed biofilms with fecal coliforms retrieved from a harvesting machine have the strongest adhesion compared to other sets of fecal coliforms on the surfaces. The efficacy of treatments on biofilm formed surfaces reveals that sodium hypochlorite was the least effective in removing accumulated biofilms whereas dish detergent was the most effective, followed by AlpetD2 and peracetic acid. Furthermore, the application of sanitizer agents (1 min exposure) at the manufacture's recommended concentration was not effective in removing biofilms on the selected surface materials. The results of this study underline the importance of cleaning and sanitizing machine harvesters with higher concentrations and longer exposure time.

1. Introduction

Over-the-row (OTR) machinery harvesters have usually been utilized to collect blueberries for processes such as freezing, making jams, or juicing due to their unsuitable quality for fresh market (Takeda, 2018). Over the past decades, berry producers have devoted much research and many attempts to improving mechanical harvester to achieve fresh market quality and high harvest capacity (Gallardo et al., 2018; Takeda, Krewer, Li, MacLean, & Olmstead, 2013; Takeda & Peterson, 1999).

The acreage of blueberry production all over the world has expanded remarkably over the last two decades (Brazelton, 2013). In 2019, a total of 18,868.8 ha was harvested for blueberries while the price per kg has been decreased by 0.427 dollars per kg in the United States (USDA, National Agricultural Statistics Service, 2020). Fresh blueberries like other ready-to-eat fresh produce, are mostly consumed raw. However, the increased berry consumption per capita has naturally promoted the consideration of established food safety in blueberry production during harvesting, processing, and packing (Danyluk et al., 2008; Gazula et al., 2019; Oliveira, Rodrigues, & Teixeiraet, 2019).

Contamination of blueberries associated with human pathogen illness could be introduced at any point during harvesting (Morales-Rayas, Griffiths, & Shultz, 2014). During fruit harvesting in the field, soft fruits such as blueberries that come into contact with soil or harvesting equipment need to be handled carefully to avoid contamination and cross-contaminations between fruits and harvesting equipment (Oliveira, Rodrigues, & Teixeira, 2019). According to a survey in 2015/16, 2.1% of growers who participated in a survey never wash their harvesting tools and 11.6% of them never sanitize the harvesting tools (Astill, Minor, Thornsbury, 2019). General hygiene operations in the food industry are sometimes assumed to be effective in dealing with biofilm on food-contact surfaces but even routine cleaning is inadequate (Mettler & Carpentier, 1998; Sinde & Carballo, 2000). Friedrich, Spann, McEgan, Ebel, and Danyluk (2009) demonstrated a potential hygiene risk associated with fruit catching plates of mechanical fruit harvesters as they isolated *E. coli* from some fruit samples that were collected from the fruit catch plates in the field. This finding may indicate that the surfaces of machine harvesters' fruit-catching plates potentially become contaminated and transmit that contamination to the fruits. Blueberries to be sold in the fresh market are usually not washed to remove potential pathogenic organisms. Therefore, proper sanitation practices for blueberry machine harvesters that come into contact with fruit are needed to prevent bacterial colonization (Bower, 2007; Carstens, Salazar, & Darkoh, 2019; Sy, McWatters, & Beuchat, 2005).

Defects in fruit quality are mostly caused during the process of machine harvesting. Blueberries fall onto the catching plate on both sides of the machine harvester, once they are detached from bushes by vibrating plastic beating bars. Plexiglass is currently used for the fruit-catching surface of the OTR machine harvester. Nutritional contents from fruits might be conducive to bacterial attachment and potential biofilm formation of pathogenic bacteria on fruit-catching surfaces (Patel, Sharma, Millner, Calaway, & Singh, 2011). Yu et al., (2012) reported that the greatest impact of bruises on harvested blueberries occurred at the fruit catching plastic plate on the blueberry harvester. Blueberry growers are getting more interested in alternative soft materials on the fruit catching surfaces while researchers need evaluation on bacterial colonization on the fruit-catching plate of the OTR machine harvester. DeVetter, Yang, Takeda, Korthuis, and Yi (2019) pointed out the necessity of providing further food safety examination such as biofilm formation and efficacy of sanitization on the new soft catching surface of OTR harvester.

In the process of seeking alternative materials for the fruits catching surfaces, one way to reduce the bacterial colonization on the fruits catching surfaces of the machine harvester could be to select a suitable sanitizer and usage of it regularly. Sodium hypochlorite or other chlorine-based sanitizers have been universally used due to their effectiveness, affordable price, and ease of use whilst they are corrosive to equipment and sometimes ineffective in the presence of organic matters (Estrela et al., 2002). Subsequently, quaternary ammonium compounds (QACs), peracetic acid (PAA), or iodine have emerged as alternative chemical sanitizing agents for food-contact surfaces of harvesting equipment. However, alternative sanitizing agents may not be effective as sodium hypochlorite on biofilm removal (Rossoni & Gaylarde, 2000; Trachoo & Frank, 2002). The study aims to assess biofilms on potential blueberry-catching surfaces and analyze the effectiveness of selected sanitizer treatments against developed biofilms for modified OTR machine harvesters.

2. Materials and methods

2.1. Bacterial isolates preparation

Confirmed fecal coliform strains retrieved from gloves, fruits, harvesting equipment, and fruit packing line were used in this study. All fecal coliforms were maintained as frozen stock at – 80 °C with Tryptic soy broth (TSB) containing 15% glycerol (Fisher Scientific, Pittsburgh, PA, USA (vol/vol). Selected fecal coliform colonies were purified on MacConkey agar (MAC) agar at 44.5 °C, followed by growing the cultures on tryptic soy agar (TSA) at 37 °C overnight. All purchase of microbial media used in this study were from Becton Dickinson (Franklin Lake, NJ, USA) unless specified.

2.2. Preparation of surfaces materials

Plexiglass currently used as a fruit catching surface on OTR machine harvester and four different types of food-grade surface materials that have the potential to replace the traditional surface materials in a modified OTR prototype machine harvesters were selected for biofilm formation and sanitization study; white silicone, neoprene, EPDM (ethylene propylene diene monomer, 1/8", McMaster, Elmhurst, IL, USA). In addition, red silicone sheets (1/16", McMaster, Elmhurst, IL, USA) were purchased and plexiglass materials of the fruit catching surface were provided by Dr. Takeda. Five different types of surface materials were cut to 2 x 2.5 cm coupons which were cleaned with an alkaline detergent (Sparkleen, Fisher Scientific, Pittsburgh, PA, USA), and washed with tap water, followed by soaking in 75% ethyl alcohol solution. Then, all coupons were rinsed with distilled water and then air-dried. plexiglass coupons were decontaminated with 5% hypochlorite solution for 1 h and then rinsed again for three times with sterile deionized water followed by an air dry in a level II biosafety hood while neoprene, white silicone, EPDM, and red silicone coupons were autoclaved for 15 mins.

2.3. Attachment of biofilm by fecal coliform isolates

Single or mixed fecal coliform(s) previously isolated from fresh fruit, surfaces of packing line, gloves, or machine harvesters were used in this biofilm quantification study; Mix A (Fresh fruit), Mix B (Gloves), Mix C (Packing line), Mix D (Packing line), and Mix E (Machine harvester), respectively. A loop of each fecal coliform culture from frozen stock was transferred to TSA media followed by 24 h of incubation at 37 °C. An enumerated single colony was transferred onto Luria-Bertani with no salt (LBNS) agar media (15 g/L Agar, 10 g/L Tryptone, and 5 g/L Yeast Extract) and incubated at 25 °C overnight. The resulting cultures were then transferred to LBNS broth followed by 18 to 24 h of incubation at 25°C. The total volume of 200 ml was made of 5 ml of each fecal coliform culture or mixture (1:1 ratio or 1:1:1 ratio) with 195 ml of LBNS broth to make 1:40 dilution. Each prepared coupon was submerged in a sterilized glass container (18 cm x 13 cm x 4 cm; Pyrex ware, World Kitchen LLC, Rosemont, IL) containing the 200 ml of LBNS broth cultures to let the fecal coliforms produce biofilm on the coupon surfaces. Two hundred milliliters of LBNS broth with decontaminated five different types of surface coupons were served as control. A single layer of aluminum foil was covered over the glass container to avoid evaporation during the incubation. Test samples with cultures and un-inoculated LBNS broth were incubated at 25 °C for 7 days for biofilm development.

2.4. Quantification of adhered fecal coliform(s) on coupons

Biofilm masses developed by fecal coliform cells on five different types of surface coupons were quantified after 7 days of incubation at 25 °C by using the crystal violet binding assay (Adetunji, Kehinde, Bolatito, & Chen, 2014) with some modifications. Each coupon was washed with 15 ml of sterile deionized water to remove loosely attached cells on both sides of the coupons and then placed in a sterile petri dish. The developed biofilms on each coupon were fixed with 2.5 ml of 95% alcohol (DLI, King of Prussia, PA, USA) for 10 minutes, and an additional 15 mins were allowed for drying coupons after aspirating 95% alcohol solution under a level II biosafety hood. Each coupon fixed with alcohol was stained with 2.5 ml of 1% crystal violet dye (Fisher Scientific, Pittsburgh, PA, USA) for 15 minutes, and then rinsed with tap water until the stains not remained on the coupons. The rinsed coupons were placed under the level II biosafety hood for 30 minutes for each side of the surfaces, and a single dried coupon was transferred in a single sterile petri dish. Five milliliters of acetone: alcohol (80:20, V: V) solution were reacted with each coupon for 10 min to dissolve the crystal violet dye stained on the surface coupons. One milliliter of the dissolved solution was transferred to a cuvette to measure the absorbance at 550 nm using a spectrophotometer (Thermo Electron Corporation, Waltham, MA) with a maximum optical density unit (O.D) value of 3.0. The observed O.D value of coupons from the un-inoculated broth was subtracted from that of coupons from single or mixed broth cultures.

2.5. Biofilm control on surface coupons by commercial sanitizers

Three different commercial sanitizers and one commercial dish soap were selected for their ability to remove biofilm mass developed by single or mixed fecal(s) coliform on selected surfaces coupons; 200 ppm of sodium hypochlorite (pH: 7.0), 200 ppm of PAA sanitizer (1.0 ≥ pH) (peroxyacetic acid-based sanitizer, Spartan chemical company, Inc., Maumee, OH, USA; FDA, 2001), Alpet D2, an isopropanol-based quaternary ammonium compound (IPAQuat), (Best Sanitizers Inc., Penn Valley, CA, USA), and 2% wt/wt a solution of dish soap (Dawn ultra-dishwashing liquid dish soap, Original scent, Proctor & Gamble, Cincinnati, OH, USA). The composition of the AlpetD2 is 58.6% IPA and 150 ppm Quats, and a premixed ready-to-use sanitizer. The treatment time was selected based on the manufacturer's instructions, and fresh working solutions of each sanitizer were directly before each experiment.

All the inoculated coupons with fecal coliforms were incubated for 7 days at 25 °C to develop biofilm formation. After the incubation, all the coupons with developed biofilms were rinsed with 15 ml of sterile deionized water to remove the bacterial cells that were loosely connected with coupon materials. Washed coupons were submerged in each of the prepared four sanitizer solutions for 1 min. All coupons were then immediately immersed in the sterile Dey-Engley neutralizing buffer (Becton Dickinson, Sparks, Maryland, USA) for 10 min to neutralize the sanitizers, followed by washing with deionized water for 5 s. The residual biofilm mass on sanitized coupons was quantified according to the crystal violet binding assay mentioned above.

2.6. Statistical analysis

Every experiment was repeated two times and each coupon sample had a duplicate in a single set of experiments. All absorbance values measured at the 550 nm were analyzed using analysis of variance (ANOVA) in SAS (version 9.4, SAS Institute, Cary, NC) using the PROC GLIMMIX procedure (Generalized Linear Mixed Models). Least square means were calculated, and post-hoc mean separation was done using Tukey's test ($P < 0.05$). When the interaction proved significant, the SLICE option in the Least Square Means (LSMEANS) statement was used to determine differences. Significant differences in biofilms formed by single or mixed fecal coliforms on selected surface materials and the efficacy of various cleaning and sanitizing agents in removing formed biofilms developed by the single or mixture of fecal coliform isolates from various selected coupons were determined in the study.

3. Results

3.1. Adhesions of biofilm to different surface materials with different fecal coliform mixtures

A variety of surface materials and fecal coliforms influenced the biofilm masses after 7 days of incubation. Overall biofilm mass was significantly higher ($p < 0.05$) on neoprene surfaces, followed by plexiglass surfaces which are significantly similar to white silicone, red silicone, and EPDM surfaces in biofilm growth. Furthermore, neoprene surfaces showed the highest biofilm formation among used surfaces when breaking down surfaces into each mixture of fecal coliforms (Table 4.2).

On average, the ability of biofilm formation was significantly higher in Mix E than other fecal coliform mixtures on selected surfaces, followed by Mix B which is significantly similar to other mixture of fecal coliforms; Mix A, Mix C, and Mix D (Table 4.1). Mix E produced more biofilms while Mix C and Mix D produced fewer biofilms on the surfaces of neoprene than on those of the other surfaces (Table 4.2). On the other hand, unlike neoprene surfaces, different fecal coliform mixtures produced statistically similar ($P \geq 0.05$) amounts of biofilms within white silicone, red silicone, EPDM, and plexiglass surfaces (Table 4.2).

3.2. Overall residual biofilms removed on surfaces after sanitizer treatments

In the subsequent experiment, biofilms were developed on white silicone, neoprene, red silicone, EPDM, and plexiglass surfaces, and then remained biofilms on those surfaces were measured after sanitizer treatments to observe their effectiveness. The overall result showed that significantly more amounts of biofilms were remained in neoprene surfaces than other surfaces after sanitizer treatments (including the dish detergent), followed by plexiglass surfaces which showed significantly fewer biofilm removals than the white silicone, red silicone, and EPDM surfaces (Table 4.3). When it comes to the efficacy of sanitizers, although treatment with NaOCl had a more significant effect than treatment with water in removing biofilms, treatment with NaOCl showed the least effectiveness among treatments with PAA, AlpetD2, and dish detergent (Table 4.3). On average, Mix E was the most resistant fecal coliform mixtures against sanitizers on surfaces (Table 4.3).

3.3. Effectiveness of chemical sanitizers against developed biofilms on surfaces

Treatment with the dish detergent, AlpetD2, and PAA was more effective in biofilm removal on the selected surface materials, followed by treatment with NaOCl which was significantly more effective ($P < 0.05$) than water (Table 4.3). Similarly, dish detergent and AlpetD2 treatments were also significantly more effective than other sanitizer treatments in removing biofilms from white silicone and plexiglass surfaces (Table 4.5).

3.4. Influence of different surfaces and fecal coliforms against different sanitizer treatments

In Mix E, neoprene surfaces retained the greatest residual biofilm masses after sanitizer treatments, followed by plexiglass surfaces which retained higher residual biofilms than white silicone, red silicone, and EPDM surfaces (Table 4.4). Similarly, residual biofilm masses on neoprene surfaces and plexiglass surfaces were significantly higher in Mix A than those on white silicone, red silicone, and EPDM surfaces. Table 4.5 also showed that neoprene surfaces retained the greatest amounts of residual biofilms after all sanitizers and water treatments, followed by plexiglass surfaces (Table 5). Similar to overall results, Mix E showed the most resistant fecal coliform mixtures on the surface of neoprene and plexiglass against sanitizer treatments (Table 4.4).

4. Discussion

All single or mixture of fecal coliforms could form biofilms on all tested surfaces. Overall results showed that neoprene surfaces had the highest biofilm formation among the tested surfaces, followed by plexiglass surfaces. Surfaces incubated with fecal coliform mixtures of Mix E isolated from machine harvester and Mix B isolated from gloves showed significantly higher biofilm development than surfaces incubated with those of Mix A isolated from fruit, and Mix C and Mix D isolated from packing line. We chose FDA approved food-grade surface materials as potential alternatives to the plexiglass fruit-catching surface of modified OTR machine harvesters. Different surface types, along with the temperature, nutritional levels, pH, and humidity, contributed to initial attachment and further biofilm formation (Abdallah, Benoliel, Drider, Dhulster, & Chihib, 2014; Kannan et al., 2014).

This study observed that neoprene showed higher cell colonization among rubber surfaces followed by EPDM, white silicone, and red silicone surfaces in stationary biofilm conditions. Gross, Zhao, Mascarenhas, and Wenet (2016) observed similar results that neoprene surfaces demonstrated a higher biofilm attachment than EPDM and silicone surfaces in stationary conditions. Silagyi (2007) supported our results that biofilm was well developed with LBNS broth in neoprene than other tested surfaces. Furthermore, neoprene surfaces retained significantly ($P < 0.05$) higher biofilm mass after 1min exposure of sanitizer treatments than plexiglass, which was significantly higher than white silicone, EPDM, and red silicone surfaces.

On average, fecal coliforms isolated from a machine harvester, Mix E, showed the highest biofilm masses and highest resistance against sanitizers than those isolated from fruit, packing line did on tested surfaces. It is important to point out the source of isolates because contaminated harvesting equipment that contacts with fruit may be able to transfer contaminants or harmful bacteria to fresh produce in the field (Patel, Sharma, Millner, Calaway, & Singh, 2011).

Bacteria tend to be resistant to sanitizers when adhered to solid surfaces compared to the same bacteria in suspension (Houdt & Michiels, 2010)**.** Plexiglass surfaces and four alternatives non-porous, food-contact surfaces were used in the present study to investigate the efficacy of sanitizers against fecal coliform biofilms. Chlorine, a common disinfectant in the food industry, is one of the examples that show less efficacy against attached cells or biofilms than planktonic cells, especially in the presence of organic matter. Furthermore, inactivated cells may remain on sanitized surfaces within the biofilm matrix or EPS, which leads to a stronger resistance against sanitizers (Vickery, Pajkos, & Cossartet, 2004). The efficacy of cleaning surfactants and sanitation could be declined because of the presence of a biofilm matrix or EPS on the treated surfaces (Vickery, Pajkos, & Cossartet, 2004). This study also observed that treatment with AlpetD2 sanitizer was more effective than treatment with NaOCl and water in removing biofilms on tested surfaces. Other researchers found a microbial reduction on *Salmonella* and coliforms as much as 4 log and 6 log CFU/g on contaminated almond dust treated with AlpetD2 (premixture of 58.6% IPA and 200 ppm Quats) for 10 min (Du, Danyluk, & Harris, 2010).

Somers & Wong (2004) observed that dish detergent significantly reduced biofilm on tested surfaces. Our results agreed with their observation that an application of dish detergent was better able to remove biofilms on food-contact surfaces as compared to NaOCl or water treatment. Furthermore, Antoniou & Frank (2005) reported that commercial alkaline cleaner removed most of the biofilm EPS on stainless surfaces with 1 min exposure. Nevertheless, although dish detergent works effectively in removing biofilms, it cannot be relied upon to kill bacteria and biofilms (Simões, Simões, & Vieirab, 2010). Commercial cleaning products alone were not enough to eliminate biofilms of *Pseudomonas* and *Staphylococcus* (Gibson, Taylor, Hall, & Holah, 1999). Other researchers highlighted the importance of applying both cleaning and sanitizing agents to inactivate biofilms on surfaces (Vickery, Ngo, Zou, & Cossart, 2009).

Cleaning and sanitation routines for the food-contact surfaces of machine harvesters could reduce the risk associated with further biofilm maturation. Sanitizers used in the study had different effects on bacterial adhesion to surface materials except for neoprene. Lee & Frank (1991) previously observed the sanitizing of biofilms on contaminated stainless-steel surfaces with 200 ppm chlorine solution for 1 min; our results had similar findings to theirs that 200 ppm of NaOCl solution was ineffective to eliminate biofilm on the contaminated surfaces. Somers & Lee Wong (2004) reported that although food-contact surfaces incubated with *Listeria monocytogenes* for 5 days showed higher biofilm formation than those surfaces incubated for 2 days, the surfaces incubated for 2 days were not easier to clean and sanitize. Increased contact time and the higher sanitizer concentrations (than manufacturer's recommendations) need to be emphasized for more effective biofilm inactivation, although chlorine-based sanitizers could produce harmful disinfectant by-products when high levels of organic materials are present **(**da Costa, Rodgher, Daniel, & Espíndola, 2014; Kim, Pitts, Stewart, Camper, & Yoon, 2008)**.**

5. Conclusion

The more blueberry growers get interested in using the modified machine harvester for blueberries for fresh market, the more important routine cleaning and sanitation practice is to prevent potential microbial contamination. However, bacterial biofilms are of concern in the food industry. The ability of single or multiple fecal coliform cultures to form biofilms on five different surface materials and the efficacy of various sanitizers along with one dish detergent against biofilms were evaluated. Results from this study indicate that fecal coliforms derived from machine harvester could build more biofilms on neoprene surface, and had stronger resistance against sanitizer treatments and dish detergent on test surfaces. Treatments with dish detergent, AlpetD2, and PAA showed significantly higher efficiency in biofilm removals than treatments with NaOCl which has a higher efficiency than treatment with water. Washing with water barely diminished developed biofilms on all variety of surface materials. Biofilm growths with single or mixed fecal coliforms could provide a shielding effect on selected surface materials during the exposure time to sanitizers. Therefore, the use of routine cleaning and sanitation treatments before the biofilm development along with increased contact time and a higher concentration of sanitizer might be an effective practice to inhibit the adhesion of microorganisms on fruit catching surface of the OTR harvester. Further tests regarding fruit quality, food safety, and ergonomic evaluation are recommended to adopt the modified OTR harvester machine commercially for fresh market blueberries.

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Table 4.1

Overall mean O.D of developed biofilms by single or mixtures of fecal coliforms and by different surfaces.

	Developed biofilm mass (O.D at A550 nm)	
Surfaces		
White silicone	0.099	B
Neoprene	0.418	A
EPDM	0.069	B
Red silicone	0.067	B
Plexiglass	0.131	B
Cultures		
Mix A	0.122	B
Mix B	0.164	B
Mix C	0.121	B
Mix D	0.134	B
Mix E	0.243	A

*Mean values within a column that are not followed by the same capital letters are significantly different ($P<0.05$).

Table 4.2
Mean O.D of biofilms on five different surfaces by single or mixtures of fecal coliforms after 7 days of incubation at 25°C.

	Mix A		**Mix B**		**Mix C**		**Mix D**		**Mix E**	
	Remained biofilm mass (O.D at A550 nm)									
Surfaces										
White silicone	0.090	Ba	0.095	Ba	0.092	Ba	0.110	Ba	0.110	Ba
Neoprene	0.229	Ad	0.437	Ab	0.265	Ac	0.363	Ac	0.796	Aa
EPDM	0.074	Ba	0.074	Ba	0.056	Ba	0.054	Ba	0.087	Ba
Red silicone	0.092	Ba	0.064	Ba	0.051	Ba	0.066	Ba	0.062	Ba
Plexiglass	0.126	Ba	0.150	Ba	0.144	Ba	0.080	Ba	0.159	Ba

*Mean values within a column that are not followed by the same capital letter are significantly different (*P*<0.05). Mean values within a row that are not followed by the same lowercase letters are significantly different

Table 4.3

Overall mean O.D of remained biofilms after each sanitizer treatment by different surfaces, a single or mixtures of fecal coliforms, or different sanitizers.

	Remained biofilm mass (O.D at A550 nm)	
Surfaces		
White silicone	0.053	C
Neoprene	0.252	A
EPDM	0.025	C
Red silicone	0.031	C
Plexiglass	0.1	B
Cultures		
Mix A	0.076	B
Mix B	0.1	B
Mix C	0.068	B
Mix D	0.073	B
Mix E	0.144	A
Sanitizers		
Water	0.148	A
PAA	0.082	C
NaOCl	0.099	B
AlpetD2	0.07	C
Dish detergent	0.06	C

*Mean values within a column that are not followed by the same capital letters are significantly different (*P*<0.05).

Table 4.4

Mean O.D value of remained biofilm after sanitizer treatments on a single or mixture of fecal coliforms by five different surfaces.

	White silicone		Neoprene		EPDM		Red silicone		Plexiglass	
	Remained biofilm mass (O.D at A550 nm)									
Cultures										
Mix A	0.042	Ab	0.153	Ca	0.024	Ab	0.038	Ab	0.124	Ba
Mix B	0.067	Ab	0.265	Ba	0.042	Ab	0.038	Ab	0.086	BCb
Mix C	0.05	Ab	0.171	Ca	0.023	Ab	0.025	Ab	0.07	Cb
Mix D	0.053	Abc	0.186	Ca	0.02	Ac	0.033	Ac	0.075	Cb
Mix E	0.053	Ac	0.486	Aa	0.016	Ac	0.02	Ac	0.145	Ab

*Mean values within a column that are not followed by the same capital letter are significantly different ($P<0.05$). Mean values within a row that are not followed by the same lowercase letters are significantly different ($P<0.05$).

Table 4.5
The effectiveness of different sanitizer treatments on different surfaces.

	White silicone		Neoprene		EPDM		Red silicone		Plexiglass	
	Remained biofilm mass (O.D at A550 nm)									
Sanitizers										
Water	0.084	Ac	0.427	Aa	0.033	Ac	0.051	Ac	0.143	Ab
PAA	0.044	Ac	0.203	Ba	0.03	Ac	0.028	Ac	0.107	Ab
NaOCl	0.071	Abc	0.244	Ba	0.032	Ac	0.035	Ac	0.114	Ab
AlpetD2	0.041	Bc	0.196	Ba	0.018	Ac	0.025	Ac	0.073	Bb
Dish detergent	0.026	Bb	0.192	Ba	0.012	Ab	0.016	Ab	0.062	Bb

*Mean values within a column that are not followed by the same capital letter are significantly different (*P*<0.05). Mean values within a row that are not followed by the same lowercase letters are significantly different (*P*<0.05).

CHAPTER 5

CONCLUSIONS

The resulting microbial indicator loads show that different harvesting methods could impact the microbial quality of fruit (hands *vs.* machine). Blueberries harvested by the conventional and modified OTR harvesters were significantly higher than the other two methods in total aerobic counts; likewise, blueberries harvested by modified OTR harvesters were the highest in total coliform and fecal coliform. The microbial indicator results also show that surfaces that come into contact with fruit in the machine harvester could be associated with high microbial counts during machine harvesting due to plant debris or microorganisms. This indicates the significance of routine proper maintenance on machine harvesters, especially food-contact surfaces, to meet a comparable quality of blueberries harvested by hands.

Selected single or a mixture of fecal coliforms, isolated from fruit, packing line environment, gloves, and machine harvester, could build biofilms on all alternative food-grade rubber and plexiglass surfaces used in the study. After 7 days incubation with a single or mixture of fecal coliforms, neoprene surfaces showed the highest biofilm accumulation with fecal coliform mixtures isolated from a machine harvester; likewise, neoprene surfaces retained the highest biofilm masses after treating with cleaning and sanitizing agents for 1 min. On average, dish detergent, AlpetD2, and PAA are significantly similar ($P \geq 0.05$), but significantly more effective than NaOCl in removing biofilms developed on tested surface materials. However, none of them could successfully eliminate biofilms on tested surfaces. This study indicates the significance of determining proper fruit-catching surface (food-grade) materials for a machine harvester and highlights the importance of routine cleaning and sanitizing blueberry machine harvesters. The information generated in the current study will be important for the blueberry industry as it safely transitions to different harvest systems. But, more research is recommended to investigate the hygiene level of harvesting lugs, containers, or tools to prevent microbial hazard on harvested blueberries, and a survey of what kinds of sanitizing agents are currently used for a fruit machine harvester or harvesting tools.