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Evaluation of Microbial Safety and Quality of Louisiana Strawberries after Flooding

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EVALUATION OF THE MICROBIAL SAFETY AND QUALITY OF LOUISIANA STRAWBERRIES AFTER FLOODING

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Master of Science

in

The School of Nutrition and Food Sciences

by

Shifa Shiraz
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# TABLE OF CONTENTS

ACKNOWLEDGMENTS........................................................................................................... ii

LIST OF FIGURES.................................................................................................................. v

ABSTRACT .............................................................................................................................. vi

CHAPTER 1. INTRODUCTION................................................................................................. 1
   Introduction ...................................................................................................................... 1
   References .................................................................................................................... 3

CHAPTER 2. LITERATURE REVIEW...................................................................................... 5
   History of Strawberries ................................................................................................. 5
   World Strawberry Production .................................................................................... 6
   Louisiana Strawberry Industry .................................................................................. 8
   Flooding in Louisiana ................................................................................................. 9
   Challenges for Strawberry Growers ........................................................................... 11
   Microbial Safety ......................................................................................................... 12
   Sources of Contamination .......................................................................................... 12
   Foodborne Pathogens Related to Fresh Produce ....................................................... 13
   Food and Drug Administration – Food Safety Modernization Act ......................... 18
   Quality of Fresh Produce after Flooding .................................................................... 21
   Color .............................................................................................................................. 22
   Texture .......................................................................................................................... 22
   References .................................................................................................................... 23

CHAPTER 3. MICROBIAL SAFETY OF STRAWBERRIES AFTER FLOODING ............. 28
   Materials and Methods ............................................................................................... 28
   Results and Discussion ............................................................................................... 32
   References .................................................................................................................... 41

CHAPTER 4. QUALITIES OF STRAWBERRIES AFTER FLOODING .......................... 43
   Materials and Methods ............................................................................................... 43
   Results and Discussion ............................................................................................... 46
   References .................................................................................................................... 59

CHAPTER 5. CONCLUSION ................................................................................................. 61

APPENDIX: LSU AGCENTER BOTANIC GARDENS ....................................................... 63

VITA ....................................................................................................................................... 66
LIST OF TABLES

Table 1. Influence of flooding on indicator bacteria population on mature strawberries during shelf life at 4°C. ..........................................................34

Table 2. Influence of flooding on indicator bacteria population on immature strawberries during shelf life at 4°C. ..........................................................36

Table 3. Influence of flooding on indicator bacteria population in soil during specific sampling times..........................................................38

Table 4. Influence of flooding on indicator bacteria population on foliage at harvest.........................39

Table 5. Influence of flooding on E. coli O157:H7 and Listeria monocytogenes population on mature and immature strawberries, soil, and foliage at harvest. .................................................40

Table 6. Influence of flooding on yeast and mold counts of mature strawberries during shelf life at 4°C. .......................................................................................48

Table 7. Influence of flooding on yeast and mold counts of immature strawberries during shelf life at 4°C................................................................................50

Table 8. Influence of flooding on color of mature strawberries during shelf life at 4°C. .............54

Table 9. Influence of flooding on color of immature strawberries during shelf life at 4°C........56

Table 10. Influence of flooding on texture of mature strawberries during shelf life at 4°C. ....58

Table 11. Influence of flooding on texture of immature strawberries during shelf life at 4°C....58
LIST OF FIGURES

Figure 1. 2016 Louisiana Flooded Strawberry Field Photographer Unknown ..........................10
Figure 2. Field Experimental Design..................................................................................29
Figure 3. Field Experimental Design..................................................................................44
Figure 4. Strawberries Growing in Hanging Baskets Prior to Flooding .........................63
Figure 5. Field Setup. .........................................................................................................63
Figure 6. Low Flooding Low Contamination (LFLC) .......................................................64
Figure 7. Low Flooding High Contamination (LFHC) .......................................................64
Figure 8. High Flooding Low Contamination (HFLC) .......................................................65
Figure 9. High Flooding High Contamination (HFHC) .......................................................65
ABSTRACT

Rainfall and flooding have impacted the strawberry industry in Louisiana. Floodwater is an ideal medium for microbiological growth and may cause contamination of soil, agricultural water, and fresh produce with foodborne pathogens. This research aimed to evaluate the microbial safety and quality of strawberries after flooding. Three strains of generic *Escherichia coli* were used to establish a baseline population of approximately $10^6$ CFU/L (high contamination) and $10^2$ CFU/L (low contamination) in floodwater. Five raised beds were filled with water to simulate a flooding event. Simulated floodwater was mixed with cow manure and spiked with generic *E. coli* then applied to strawberry plants. Treatments included High Flooding High Contamination (HFHC), High Flooding Low Contamination (HFLC), Low Flooding High Contamination (LFHC) and Low Flooding Low Contamination (LFLC). One bed served as the control (C). Strawberry plants were flooded for 4 h and sampled at the time of harvest and during shelf life at 4°C for 48, 96, and 144 h. Soil samples were collected on site for one week. The population of foodborne pathogens and microbial indicators was evaluated. Strawberry quality (yeast and mold count, color, and texture) was also evaluated. Results indicated that the presence of *E. coli O157:H7* and *Listeria monocytogenes* in strawberries, soil, and foliage was not detected. Additionally, generic *E. coli* was not detected (<10 CFU/g) in strawberry fruit or in foliage samples. In soil samples, generic *E. coli* was higher in HFHC samples (1.61 log CFU/g) compared to HFLC samples (1.09 log CFU/g) at harvest. However, generic *E. coli* was not detected after 96 h (<10 CFU/g) in soil samples. Significant levels of coliform were present in the strawberry fruit and soil at 0, 48, 96, and 144 h in all treatment beds. Yeast and mold were detected in all samples but with no clear trend throughout shelf life across all floodwater treatments. Moreover, results did not indicate clear correlation among flooding and
contamination levels and color and texture change. This study provides local growers science-based information to understand potential effects of flooding on the microbial safety and quality of fresh produce.
CHAPTER 1. INTRODUCTION

Introduction

Strawberries are produced throughout the United States; with California, Florida and Oregon being the top three in terms of total acres grown. Louisiana is one of the contributing states, together with New York, Michigan, Washington, North Carolina and Ohio (USDA National Agricultural Statistics Service, 2016). Strawberries are the official state fruit of Louisiana (LA Department of State, 2016). Louisiana’s primary strawberry producing region includes Tangipahoa and Livingston parishes, where approximately 400-500 acres of strawberries are grown (Schloemann, 2005). As a leading producer of strawberries, Tangipahoa parish annually generates millions of dollars in revenue towards the local economy. In 2014, the Louisiana strawberry industry contained 81 growers who produced more than 367 acres of strawberries for a gross farm value of about $23.7 million (Louisiana Ag Summary, 2014). Each year in April, the town of Ponchatoula puts on the state’s largest strawberry festival to remark the significance of this crop.

Perishable commodities, such as strawberries, are susceptible to excessive water exposure. Unfortunately, Louisiana annually receives 60 inches of rainfall a year (US Climate Data, 2017). During the last century extreme rainfall events and flooding have increased and are expected to continue. Rainfall coupled with recent flooding incidents has impacted the Louisiana strawberry industry. In March 2016, an accumulation of more than 16 inches of rainfall in a two-day period led to severe flooding in southeast of Louisiana (Yan & Flores, 2016). This resulted in staggering loss of strawberry production just one month prior to the annual strawberry festival, and caused decrease in revenue to the local economy (Wold, 2016). Due to the heavy rainfall, the fields of the strawberry submerged underwater, which not only interrupted the peak harvest
period, but also raised a health concerns to the public. Five months later in August 2016, Louisiana received 6.9 trillion gallons of rainfall within one week. This was known as “The Great Flood”, one of the worst disasters in U.S. history (Yan & Flores, 2016). This historic flooding caused $3.8 billion in residential property damages out of which $1.3 billion damages were in the Livingston Parish and $1.0 billion in East Baton Rouge Parish (Terrell, 2016). As for the state’s agricultural industry, the LSU AgCenter estimated losses of over $110 million (McClure, 2016). According to the LSU AgCenter state vegetable specialist Kathryn Fontenot (personal communication), strawberry producers in Livingston parish were greatly affected by this flood. Some growers lost fields and homes and others were unable to replant the following season, as damaged areas were not repaired.

Microbial Contamination in floodwater can cause adverse effects in human health. Floodwater can serve as a perfect medium for bacteria, viruses, protozoa, and helminthes, some of which are able to adopt a form that could resist living conditions (World Health Organization, 2004). Particularly, if the agricultural field is adjacent to a livestock farm, industrial areas or untreated sewage and wastewater, then floodwater can intermingle to cause possible sources of bacterial contamination in crops (Miraglia et al., 2009). Contaminated floodwater may lead to the contamination of the produce, soil and plants with foodborne pathogens such as E. coli O157:H7 and Listeria monocytogenes (Taylor et al., 2011). Exposure to higher levels of pathogens in floodwater raises public health concern (EPA, 2001). In 2001, a study conducted during a flooding event identified an increased rate of gastrointestinal illness due to flooding in U.S. (Salvato et al., 2003). Even if the crop is not completely submerged during flooding, there may still be microbial contamination of the edible portion of the crop (FDA, 2009b). Fresh produce grown in flooded fields may serve as a potential channel for pathogenic microorganisms.
FDA states that if the edible portion of a crop has been exposed to floodwaters, it is considered adulterated, and should not enter human food channels. There is no practical method to recondition the edible portion of a crop to provide reasonable assurance of human food safety (FDA, 2009b). For crops where floodwaters did not contact the edible portions of the crops, the growers should evaluate the safety of the crops for human consumption on a case-by-case basis for possible adulteration (FDA, 2009b). However, strawberry growers are challenged to make such decision due to lack of time, training, and resources.

This study aimed to assess the microbial safety and quality of strawberries that have come into direct contact and not in direct contact with floodwater immediately after flooding and during shelf life 4°C. Different growth stages of strawberries were investigated. Foliage and soil samples were also analyzed for potential microbial contamination.

**References**


FDA. (2009b). A Notice from the Food and Drug Administration to Growers, Food Manufacturers, Food Warehouse Managers, and Transporters of Food Products About the Safety of Food Affected by Hurricanes, Flooding, and Power Outages.


CHAPTER 2. LITERATURE REVIEW

History of Strawberries

One of consumers’ favorite fruit in the United States is the sweet, delicious and nutritious strawberry, with annual per person consumption ranging to about eight pounds per year (ERS, 2016). Strawberries are consumed year-round both fresh and as ingredients in other value added products such as ice cream, salads, yogurt, desserts, jams and jellies. The U.S. is the major producer and consumer of strawberries. Due to this reason strawberries are the fourth most valuable fruit crop produced in the U.S., after grapes, apples and oranges (ERS, 2016). The economic value of strawberries produced is second only to the values of apples produced in the nation. Since the 1970’s strawberry production has increased annually with record high yields recorded in 1993 (Bertelsen, 1995). Consumer demand has led to national strawberry production doubling in the past 20 years. The strawberry industry had an estimated value of $2.4 billion in 2012 making the U.S a world leader in strawberry production (Agricultural Marketing Resource Center, 2016). Today strawberries are the fourth highest ranked U.S. fruit in terms of value of production (Boriss et al., 2006).

Discovering the exact history and ancestry of strawberries is difficult because there are numerous cultivars that are similar in appearance and by far distant in origin. However, it is believed that the history of strawberries goes back as far as the Romans and the Greeks, who cultivated the berries in their garden and used it for medical purposes, and served it in feasts as a delicacy (Boriss et al., 2006). In the first century, roman poets Virgil and Ovid referenced strawberries in poetic works. New England gardeners in Virginia have cultivated strawberry plants since the 16th century. The most current commercial strawberries are crossbreeds of the large and aromatic “Fragaria chiloensis” a native to the regions of the Pacific slope from Alaska.
Thereafter, in the 20th century, the California industry derived a variety from a Massachusetts seedling (Boriss et al., 2006). There are multiple philosophies as to how strawberries were named. In A.D. 900 Anglo-Saxons called it a “hay berry” because it ripened at the same time hay was harvested. In the nineteenth century, children threaded the plants into straw and offered them for sale (Darrow, 1966). The cultivation of strawberries became common among royalty as it became a common garden plant, ornament and a table delicacy.

In 1843, Ohio growers were the first to ship strawberries and shipping has continued as a regular practice for this highly perishable fruit (Whidden et al., 2012). The ability to ship has led to increased markets and popularity of strawberries, playing an important role in the development of the strawberry industry, new plant breeding programs and developing better cultural systems. Commercial strawberry production grew in America during the nineteenth century (Huang, 2013). The U.S. strawberry industry is mostly located in the southern and coastal areas of California, with strawberry production mostly on the east coast near large cities where strawberry production is best suited for moderate climates with warmer days and low humidity (USDA, 2004).

World Strawberry Production

The U.S. is the world’s largest strawberry producer and supplier. Thereafter the following highest producing countries are Turkey, Spain, Egypt, Korea, Mexico, and Poland (Wu, Guan, & Whidden, 2012). Frequent import and export of strawberries have been documented. Two countries, Mexico and Canada are two major suppliers of fresh market strawberries to the U.S. The mainstream of all America’s fresh, frozen, and preserved strawberry imports come from Mexico. Imported fresh strawberries from Mexico constituted about 99.7% of the imported market in 2014, along with Canada bringing in just less than 1%. Mexico and Chile supplied
82.1% and 5.4% respectively for the frozen strawberry imports (Agricultural Marketing Resource Center, 2016). Considering prepared or preserved strawberry imports, Mexico supplied 21.5%, followed by Canada producing 15.8%, and with France distributing 14.1% (Agricultural Marketing Resource Center, 2016). Meanwhile, most of the fresh, frozen and preserved strawberry exports from U.S. were consumed by Canada. In 2014, Canada received 83% of the fresh strawberry exports, which then was followed by Mexico receiving 9%. Of the frozen strawberry exports, Canada received 42% and Japan received 30%. As for the preserved strawberry exports, Canada received 24%, followed by Mexico receiving 22%, and South Korea receiving 15% (USDA ERS - Data By Commodity, n.d.; Huang, 2013)

**Strawberry Production in the United States**

The demand for strawberries and the growth of the industry is projected to increase in the U.S. for many years. The U.S. produced three billion pounds of strawberries valued at approximately $2.9 billion in the 2014-2015 plant and harvest year (Perez, Ferreira, & Minor, 2017). Strawberry production and fresh strawberry consumption on the fresh market has boosted the U.S. economy. Fresh market strawberry imports have grown rapidly due to an increase amount of fresh strawberry consumption in the U.S. In the 1970's fresh consumption of strawberries accounted for approximately 60 percent of total strawberry consumption with continued increases in consumption rates through the mid- 1980’s (ERS, 2013). Fresh strawberry per capita usage in the U.S represents more than 80 percent (value of $2.6 billion) of the total strawberry production (Perez & Gustavo, 2004). Today more than 86 percent of fresh strawberries are consumed in households (ERS, 2016). Most U.S. citizens purchase fresh strawberries from farmers market and grocery stores. Processed strawberries accounted for the remaining 19 percent, valued at nearly $241.8 million (USDA National Agricultural Statistics
Service, 2016). Processed strawberries can be found in the forms of jellies, jams, pies and other desserts.

California, the largest strawberry producing state in the U.S produces over ninety one percent of the entire strawberry crop (NASS-USDA, 2016). Florida is the second largest strawberry producing state, which produces the majority of the domestic winter strawberry crop, which delivers roughly fifteen percent of strawberries to the U.S market (Perez & Baldwin, 2011; NASS-USDA, 2016). The third largest profitable strawberry producing state is Oregon, providing between two and five percent of the nation’s strawberries. In 2016, strawberries were harvested from about 52,500 acres located in 10 different states, California (37,900 acres), Florida (10,700 acres) and the other contributing states incudes 4,495 acres from Oregon, North Carolina, Washington, New York, Michigan, Pennsylvania, Wisconsin, and Ohio combined (NASS-USDA, 2016).

**Louisiana Strawberry Industry**

Although the production is not as large as the aforementioned states, the heritage of Louisiana strawberries originated when the Italian farmers began to grow strawberries on the North shore of Lake Pontchartrain in the late 1800s. In the early 90’s a strawberry farmer named Robert L. Cloud from Independence Louisiana produced the ‘Klondyke’ cultivar, which was a more durable variety for shipping. This particular variety of Klondyke strawberries was the standard in the U.S. for more than 30 years, which thereafter put Louisiana on the strawberry map (Thompson & Thompson, n.d.). The strawberry industry began in Independence, Louisiana in 1876. Most of the Louisiana strawberries were sold commercially. Growers distribute the strawberries to grocery store warehouses, wholesalers, fruit stands, farmers markets and roadside stands. Most of the early grown strawberries carried premium prices and contributed in high
returns for Louisiana crops averaging to 1,600 flats/acre and yielding to 2,000+ flats/acre (Hinson & Bruchhaus, 2005; AgCenter, 2004).

**Flooding in Louisiana**

Flooding are considered as the most common weather related natural disasters, which can occur for various reasons, such as long-lasting rainfall, rainfall with intense thunderstorms, dams or levees break or when waves come onto the shore (FDA, 2011b). Some of the deadliest natural disasters in the American history were the Hurricane of 1900 in Galveston, Texas, 1972 dam failure in Buffalo Creek, West Virginia, 1976 flash flood in Colorado’s Big Thompson Canyon and the Great Flood of 1993, excessive rainfall in the Mississippi River (Zimmerman, 2015). As a consequence of global warming, flooding could become a more widespread and inevitable problem.

Unfortunately, flooding is not uncommon in Louisiana. In 2005, Hurricane Katrina was a deadly and destructive hurricane that struck the U.S. Katrina was one of the costliest storms in U.S. history causing $125 billion in estimated economic damages in New Orleans, Louisiana (Zimmerman, 2015). The Louisiana fruit and vegetable producers also encountered damages from Hurricane Rita and Tropical Storm Gustav. The next natural disaster to affect Louisiana fruit and vegetable production was the Louisiana flooding in 2016. The 2016 flood was termed a “historic, unprecedented flooding event” (Wold, 2016). Prolonged rainfall in the southern parts of Louisiana resulted in catastrophic flooding, which submerged thousands of houses, businesses and agricultural fields. In most areas the rainfall rates were up to 2 to 3 inches an hour, exceeding nearly 2 feet in some areas remaining stationary. Total accumulation of rainfall peaked at 31.39 inches in Watson, northeast of Baton Rouge, dumping nearly 7.1 trillion gallons of water in Lake Pontchartrain (Wold, 2016). The floodwaters in Louisiana have impacted nurseries, the cattle
industry, and strawberry farms in the area (Ag Summary, 2016). Ponchatoula is known as home of the annual strawberry festival and heart of the strawberry farms. More than 13 inches of rainfall in Ponchatoula turned the strawberry fields into a lake as shown in figure 1. The flooding delayed and reduced strawberry productivity and caused decrease in revenue to the local economy.

Figure 1. 2016 Louisiana Flooded Strawberry Field Photographer Unknown

There are two categories of flooding which can occur after a heavy rainstorm. The first is when fields become flooded with rainwater and a pool of water remains on the surface of the soil. This type of flooding can decrease yield and kill crops but does not result in contamination of produce with chemicals or human pathogens. This first category of flooding is not recognized as flooding by the Food and Drug Administration (FDA). The second category of flooding is more severe and occurs when there is a runoff from surface waters such as rivers, lakes, or
streams overflow and in turn run into the field. These type of floodwaters are likely to contain chemical and biological contaminants that may be harmful to the health of humans and animals (Produce Safety Alliance, 2011).

**Challenges for Strawberry Growers**

Growers face numerous government regulations and compliance requirements on agricultural production and the environment. In order to protect workers, consumers, and the environment, there are rules and standards strawberry producers must obey. This includes the agricultural worker protection standard (WPS) adhering to water management, fumigation management and food safety. Abiding to these requirements has increased the workload of production and cost to the growers (EPA, 2015). In recent years, substantial fresh strawberry imports from Mexico have created great challenges to the U.S domestic industry. The rise in large import volume from Mexico potentially threatens strawberry growers by weakening the market prices, squeezing the market share and profit margins. Strawberry imports from Mexico accounted for about 95% of total imported strawberries in the U.S. market and it has gradually increased four times between 2004-2014 creating tremendous pressure on the growers (Suh, et al., 2017).

In addition to financial issues, strawberry producers also experience multiple challenges producing the crop. Uncertainty of the weather conditions and unsuitable growing conditions are challenging to the agricultural sector. Strawberries are sensitive to climate change. Therefore, creating a favorable environment establishes an increase yield production of the crop. Extreme weather and unusually high and low temperatures may disrupt strawberry production and create significant stress on the crop, limiting yield potential (AgCenter, 2012). For example, in Louisiana, heavy rainfall throughout many northern states caused excessive flooding along the
Red and Sabine rivers to disrupt agricultural production and in turn making it very challenging to the growers to handle (AgCenter, 2012). Other factors such as disease, insect and weed pressure affect production costs. Producers must recoup funds from pesticides as well as the labor used to apply products to mitigate pest damage.

**Microbial Safety**

Microbial safety of fresh fruits and vegetables is a major concern as these products are often consumed raw. There are many types and sources of contamination.

**Sources of Contamination**

Contamination of fresh fruit crops can occur in multiple forms including physical contamination such as broken glass from tractor lights, chemical contamination such as over application of pesticides and biological contamination such as human pathogens. Microbial contamination, unlike contamination from soil, other debris, or plant pathogens is not always visible to the naked eye and therefore is often overlooked by producers and consumers. The U.S. Food and Drug Administration (FDA) conducted a rigorous quantitative produce risk assessment to study the contamination of fresh produce during growth, harvest, processing, transportation, retail, and preparation for consumption. It identified farms, flood, soil and contaminated irrigation as major routes of microbial contamination (Oryang et al., 2014). Domestic animal farms are considered as a potential pathogen source, where bacterial or viral strains are responsible for foodborne illness outbreaks. These pathogens gets in contact to the food products with the help of floodwaters and run-off waters (Oryang et al., 2014). Fecal material from cattle, soil and other inputs such as sewage overflow introduce enteropathogens directly to watercourses, especially during rainy seasons due to the potential of contamination from flooding. Environmental controls such as soil saturation, rainfall duration and rainfall intensity
had the largest influence in foodborne pathogens (Martinez et al., 2014). In 2011, in U.S. an outbreak with *Escherichia coli* O157:H7 was caused by consumption of strawberries which were contaminated on the field by deer feces, causing 15 cases and out of which 2 were deadly (Knudsen et al., 2001). This outbreak emphasizes problems concerning deer feces as the source of contamination and focuses on problems concerning with locally grown produce. Produce contamination by wildlife, accompanied with rainfall events can be an economic loss to growers and to the agricultural industry (Laidler et al., 2013).

**Foodborne Pathogens Related to Fresh Produce**

Pathogenic bacteria that are mainly associated with produce outbreak are known to be *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*, which are transported into the environment by animal host. In the U.S. the primary pathogens linked to produce related foodborne outbreaks were known to be 29% *Salmonella* and 13% *Escherichia coli* O157:H7 (Park et al., 2013).

Furthermore, certain kinds of *E. coli* cause disease by producing Shiga toxin. These Shiga toxin-producing *Escherichia coli* (STEC) are defined as *E. coli* having Shiga toxin gene stx1, stx2 or both, thus the hemolytic uremic syndrome (HUS) is more related with the Shiga toxin 2 by itself or by both toxins (Possé et al., 2007). The most identified STEC in North America is *E. coli* O157:H7 because it is the most common serotype that threatens the public health (CDC, 2014). The Shiga toxins produced by *E. coli* O157:H7 are an A-B5 bacterial exotoxin where the A subunit of the toxin injures the adenine base halting protein synthesis in target cells and the five B subunits bind to the cellular receptor, globotriaosylceramide (Possé et al., 2007).

The *E.coli* O157 strain is mainly characterized due to its sole production of large amount of toxins that cause severe damage to the coating of the intestine (CDC, 2014). Hemorrhagic
colitis or bloody diarrhea caused by the *E. coli* O157:H7 can progress to potentially fatal hemolytic uremic syndrome. The minimum infection dose is under 100 cells, but the FDA reports that 10 cells could cause the illness causing severe abdominal pain and diarrhea which starts as watery but becomes grossly bloody (Feng et al., 2017). In some cases, symptoms vary for different forms of illness and mainly include vomiting, low-grade fever, chills, dehydration, and discomfort. The duration of this illness starts from 8 hours to 9 days after consuming contaminated food and can last from 6 hours up to 19 days (FSPCA, 2014). Majority of Shiga toxin-producing *E. coli* strains that are associated with severe human disease are implicated in foodborne outbreaks causing the potentially life threatening hemorrhagic colitis and HUS (Delbeke et al., 2015). In addition, a collection of the other STEC serotypes with the highest pathogenic potential causing diarrhea in humans includes O26, O103, O111, and O145 (Delbeke et al., 2015).

Multiple outbreaks show that the microbial safety of fresh produce should not be overlooked, since foodborne illness in fresh produces can affect both the consumers and the growers. *E. coli* O157:H7 outbreaks occurred with bagged spinach in 2006, strawberries and romaine lettuce in 2011 (Manfreda & De Cesare, 2016). As for the spinach outbreak the cases were reported in 26 states with 199 people ill, 102 (51%) were hospitalized and 31 (16%) developed HUS and 3 deaths (Wendel et al., 2009). The outbreak was somewhat related to the presence of wild pigs on the ranch and surface waterways were contaminated from the runoff getting into the groundwater (Wendel et al., 2009). Similarly, an outbreak of *E. coli* O157:H7 was linked to eating fresh strawberries, which implicated deer feces as the source of contamination. There were 15 cases identified out of which 6 cases were hospitalized, 4 cases developed HUS and 2 cases died with HUS. These strawberries were locally grown in Oregon
and sold to buyers who in turn distributed them to roadside stands and farmers market (Laidler et al., 2013). In 2017, CDC and the U.S. Food and Drug Administration investigated a multistate outbreak of STEC O157:H7 infections linked to leafy greens. Reports concluded that 25 people were infected with the outbreak from 15 states, resulting in 9 hospitalizations, 2 with HUS and 1 death in California (CDC, 2018).

*Listeria monocytogenes* is another important foodborne pathogen that is a significant concern to human health. *L. monocytogenes* is a gram-positive, non-spore forming, facultative anaerobic rod that grows between -0.4 and 50°C (Farber & Peterkin, 1991). It is abundant in the environment and widely present in soil, water, vegetation, livestock feces and vegetation irrigated with contaminated water. This bacterium sheds through the feces of infected animals and human hosts (Heaton & Jones, 2008). Moreover, it is difficult to eliminate or counteract *Listeria* contamination in fresh produce during the postharvest stage, possibly leading to enteric infection. Produce contamination is considered as a serious human health problem because produce is often consumed raw or subjected to minimal processing. Contamination is higher in root vegetables and this is due to increased contact with the soil. Contaminated floodwater products of sewage treatments to agricultural fields carry *L. monocytogenes* which has the ability to survive and multiply in diverse habitats and therefore causes infection in a variety of animal species and humans (Jung et al., 2014). It is therefore important to identify and control *L. monocytogenes* in produce contamination at the pre-harvest levels to help reduce human health risk (Heaton & Jones, 2008).

Likewise, listeriosis is a life-threatening infection caused by eating food contaminated with *L. monocytogenes*. People who are at high risk for listeriosis include pregnant women, their newborns, adults 65 and older, and the people with weakened immune system. Symptoms start 1
to 4 weeks after eating food contaminated with *Listeria* and include headache, stiff neck, confusion, loss of balance, and convulsions in addition to fever and muscle ache. The infection can be severe ranging from a mild illness lasting few weeks to a severe illness lasting to several months (Farber & Peterkin, 1991). In the 1990’s, *Listeria* outbreaks were primarily linked to deli meats and hot dogs. Now, *Listeria* outbreaks are associated with dairy products and produce and have traced recent outbreaks to soft cheeses, celery, sprouts, cantaloupe, and ice cream (CDC, 2016). In 2015, CDC reported a *L. monocytogenes* multistate outbreak linked to Blue Bell Creameries ice cream with total of 10 cases infected with several strains of *Listeria* from four different states, causing 10 hospitalization and 3 deaths (CDC, 2016). Moreover, berries of any sort have rarely been associated with outbreaks caused by this bacterium. There is very low risk associated with fresh or frozen strawberry consumption due to their naturally low pH, however a multistate outbreak of foodborne hepatitis A associated with commercially frozen strawberries was reported (Knudsen et al., 2001). The contamination of the berries was thought to have taken place by contact with infected harvesters or contaminated irrigation water (FDA, 2016).

*Salmonella* species are gram-negative, rod-shaped, facultative, non-spore forming bacteria that grow at 41-117°F and at pH 4.2 (Chung, 1970). Most of the *Salmonella* strains are considered to be potential human pathogens and some of these strains including *S. typhimurium*, *S. enteriditis*, *S. newport*, *S. heidelberg* and *S. javiana* are associated with foodborne illness (FSIS, 2012). *Salmonella* is a leading bacterial cause of food poisoning in the U.S. (Scallan et al., 2011). Food poisoning is a major cause of gastroenteritis, resulting in unpleasant symptoms such as diarrhea, abdominal cramps, fever, headache, nausea, and vomiting which begin 12 to 72 hours after ingestion of contaminated food or water (Scallan et al., 2011). The illness usually lasts 4 to 7 days, and most healthy people recover without treatment. However, in cases when the
infection may spread from intestines to the blood stream and to other areas of the body, fatal illness and death may result among children, elderly, and people with weakened immune systems if not promptly treated with antibiotics. The mechanism of the pathogenic *Salmonella* lies in their ability to invade and replicate in the host cells. The infective dosage can be as little as 15-20 cells depending on the age and health of the individual and the strength of the microorganism serotype (Scallan et al., 2011).

On the other hand, enteric fevers also known as typhoid fever are the severe forms of salmonellosis caused by *S. typhi* and *S. paratyphi*. The symptoms of enteric fever include fever, anorexia, headache, and constipation in humans, infecting various organs and then leading to injuries. For most forms of salmonellosis, the fatality rate is less than 1% but higher for typhoid fever (FSIS, 2012). From the food-manufacturing standpoint, *Salmonella* infection is of major concern in raw poultry, swine, and ready-to-eat products such as fruits and vegetables. Therefore, fresh produce that is eaten raw is increasingly recognized as a vehicle for transmission of pathogenic *Salmonella* species. Because wild animals are reservoirs of *Salmonella* in the agricultural production environment, they may contaminate fresh produce on the field directly or by contaminated agricultural water (Ceuppens et al., 2015).

Several outbreaks associated with *Salmonella* illustrate that the microbial safety of fresh produce should not be overlooked. A multistate outbreak strain of *Salmonella enterica* serotype Saintpaul associated with peppers was reported in 2008 (Behravesh et al., 2011). Cases of the illness with the outbreak strain expanded to 43 states, the District of Columbia and Canada, and particularly with high incidence rates in New Mexico and Texas. Among the 1500 cases, 21% were hospitalized and 2 died (Behravesh et al., 2011). Another outbreak of *Salmonella* infection associated with garden cucumber imported from Mexico and distributed by Andrew &
Williamson Fresh Produce was reported in 2015. Similarly, in the periods 2007–2011 in Europe, leafy greens eaten raw as salads were involved in seven salmonellosis outbreaks which involved 268 human cases in total (Da Silva Felício et al., 2015). Moreover, as reported by FDA from 1996 to 2008, 82 foodborne outbreaks were related with fresh produce and the principal pathogens involved as listed in order were *Salmonella enterica* (47.1%), norovirus (22.4%), *E. coli* O157:H7 (5.9%), *Campylobacter jejuni* (3.5%), and *Shigella sonnei* (2.4%) (Scallan et al., 2011).

Most of the common waterborne bacterial pathogens identified during outbreaks were noted to be after extreme water events, like flooding and heavy rainfall. Moreover, these annual foodborne cases will increase with a higher occurrence of flooding caused due to heavy rainfall and climate change (Manfreda & De Cesare, 2016). There can be potential sources of pathogen contamination in soil, wildlife feces, agricultural water, fungicide and insecticides or domestic animals during pre-harvest. Research conducted under field conditions has shown that enteric pathogens survive longer in agricultural waters and in soil (Jung et al., 2014).

**Food and Drug Administration – Food Safety Modernization Act**

The FDA has been charged with creating a law to minimize human pathogens in crops for both human and animal consumption with an underlying goal of preventing rather than reacting to foodborne illness outbreaks. Therefore, the Food safety Modernization Act (FSMA) was created to regulate on farm production practices related to foods human and animals consume. For the purposes of this study, we are solely focusing on the portion of FSMA that regulate human food products. Because most foodborne pathogens can be killed when heated, the food products that FSMA regulates are those that are primarily consumed raw, which includes most fruit and vegetable crops. There are many ways in which fruit and vegetable crops
can be contaminated with foodborne pathogens. Flooding, a potential source of contamination of strawberries is the focus of this paper.

Fruit and vegetable crops may pose a food safety risk after a flooding event. These catastrophic events can impact crops and other food commodities that are exposed to floodwaters, which can be considered adulterated and not suitable for human consumption. For these reasons, in the U.S. FSMA requires the FDA to better protect the public health by establishing science-based minimal standards for safe production and harvesting of raw fruits and vegetables (Gerrity et al., 2013). These standards are necessary to strengthen the food safety system, by minimizing the risk to human health, including death. The FSMA regulated science-based minimal standards focus mainly on evaluating microbiological hazards, because most of the outbreaks have been associated due to the presence of foodborne pathogens (FDA, 2011).

One of the production practices that the FSMA produce safety rule regulates and imposes standard parameters is on the quality of water used in agriculture. To do this, FSMA requires producers to monitor for the presence of generic *E. coli*, which can indicate the presence of fecal contamination (FDA, 2015). The FDA’s FSMA Produce Safety Rule requires producers to use agricultural water that falls below the numerical criteria of generic *E. coli* for the use of agricultural water, which is directly used for growing crops and for those consumed raw. Based on the FSMA rule, the water used for pre-harvest practices on fruits and vegetables should meet the following specifications; regarding levels of generic *E. coli* based on two values, the Geometric Mean (GM) <126 Colony Forming Units (CFU) generic *E. coli* per 100 mL of water and Statistical Threshold Value (STV) <410 Colony Forming Units (CFU) generic *E. coli* per 100 mL of water (FDA, 2015). Here, the STV values define the water quality based on the
amount of *E. coli* levels due to adverse conditions such as rainfall or a high river stage, that can wash waste into rivers and canals (FDA, 2015).

There is no known contamination level of floodwaters as many factors in the environment can affect contamination rates. Microbial safety of fresh produce after hurricanes and floods may have a persistent and a potentially hazardous impact on crops. Crops may be submerged in floodwater that have been exposed to contaminants or even subjected to mold or other pathogen growth. Therefore the major concerns for crop safety are microbial contamination and possible uptake of chemical heavy metals and mold contamination in the plant (FDA, 2011b). The different types of human pathogens potentially in floodwater that would affect produce include bacteria (*E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella*), viruses (hepatitis A virus, norovirus) and parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*) (Jung et al., 2014). The sources of these pathogens and microbes are transferred from farms to plate through floodwater, contaminated agricultural water, soil, etc.

It is the responsibility of growers, who produce and sell these crops to assure the safety of flood-affected food crops for human consumption. According to the guideline of FDA, “If the edible portion of a crop is exposed to floodwaters, it is considered adulterated under section 402(a)(4) (21 U.S.C. 342(a)(4)) of the Federal Food, Drug, and Cosmetic Act and should not enter human food channels.” There is no practical method of reconditioning the edible portion of a crop, which will provide a reasonable assurance of human food safety. So, FDA recommends that these crops be disposed and kept separate from crops that have not been flood damaged to avoid adulterating "clean" crops (FDA, 2011b ; FDA 2009b).“For crops that were in or near flooded areas but where floodwaters did not contact the edible portions of the crops,” FDA also states: “the growers should evaluate the safety of the crops for human consumption on a case-by-
case basis for possible adulteration” (FDA, 2011b; FDA 2009b), which left growers to make a difficult decision, especially those with limited resources.

Quality of Fresh Produce after Flooding

Fresh fruits and vegetables are considered to be of highly perishable commodities because of their living nature and their naturally spoilage factors. Some of the desirable changes associated with postharvest are the development of the sweetness, color and flavor that will last just for few days. At the same time, when undesirable changes take place like water loss, shrinkage, cell wall degradation, softening, over ripening, disease attack, rotting and in turn this would decrease shelf life of fresh produce (Johnston et al., 2005). If not supervised all these changes can ultimately affect the quality of fresh produce and thus deteriorate the quality of fresh produce. It is important to reduce these changes in fresh produce in order to increases the shelf life, marketing period of fresh produce and maintains quality during postharvest handling. An estimation of about 20% of all fruits and vegetables produced are lost each year due to spoilage (Johnston et al., 2005).

Spoilage microorganisms can be introduced on the seed of the crop, during growth of the crop field, during harvesting and postharvest handling, or during storage and distribution (Ahmad & Siddiqui, 2015). Yeasts and molds can tolerate acidity and therefore are associated with the spoilage of acidic foods such as fruits. Yeasts can grow in the range of pH 3–10 and molds can grow in the range of pH 2-11 but favors an acidic pH. In addition, yeasts have a slightly higher growth rate than molds and are responsible for off-flavors and off-odors (Barth et al., 2009).

Molds produce spores on fresh fruits and vegetables or animal matter as a furry coating associated with dampness. Mold spoilage of fresh fruits is caused by species of Penicillium,
Phytophthora, Alternaria, Botrytis (Ahmad & Siddiqui, 2015). The symptoms include noticeable growth, rots and discoloration; such as blue, gray, brown mold and botrytis. Yeasts and mold populations have been reported in most types of fresh-cut fruits and vegetables and visible molds in unpalatable fresh fruits, such as strawberry, honeydew, pineapple, and cantaloupe (Barth et al., 2009). To maintain maximum shelf life in strawberries, they are usually harvested directly into retail containers and rapidly cooled before distribution. They are not washed because small amounts of moisture can result in grey mold due to the growth of “Botrytis cinerea”. However, because of flooding, excessive water is introduced to the strawberry, which may become a significant quality concern.

Color

Color is an important factor in the perception of strawberry fruit quality. Color and appearance attract the consumer to a product and can support in impulse purchases (Barrett, Beaulieu, & Shewfelt, 2010). There are three indicators $L^* \ a^* \ b^*$ to determine color measurements. The $L^*$ value measures the degree of lightness (0 indicates black color and 100 indicates white color). A positive $a^*$ value represents redness and a negative $a^*$ value represents greenness. A positive $b^*$ value represents yellowness and a negative $b^*$ value represents blueness.

Texture

Texture is a critical quality attribute in the consumer acceptability of strawberries. The texture or firmness of the strawberry fruit changes during ripening and maturation. Textural parameters such as turgidity and firmness are observed with the sense of touch by the hand or in the mouth by chewing (Barrett, Beaulieu, & Shewfelt, 2010). Shear strength is used to measure the firmness of strawberry fruit and is an empirical indicator of force.
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CHAPTER 3. MICROBIAL SAFETY OF STRAWBERRIES AFTER FLOODING

Materials and Methods

Strawberry Production

Strawberries plants were planted and maintained at the LSU AgCenter Botanic Gardens at Burden using standard growing procedures in Louisiana (Fontenot, et al., 2014). Strawberries were planted in October and were managed through March. Three bareroot ‘St. Festival’ strawberry plants were planted in Rose medium purchased in bulk at Cleggs Nursery on Siegen Lane in Baton Rouge, Louisiana. Strawberries were fertilized with Peters 20-20-20 at a rate of 200 parts per million (ppm) of nitrogen (N) four times prior to harvest. Strawberries were manually irrigated for the first 3 weeks to allow roots to establish. After root establishment, strawberries were irrigated automatically using drip emitters (Rain Bird, Azusa, CA) for 15 minutes per day, one emitter per three strawberry plants. Between planting and the first harvest, strawberry plants were sprayed twice with Quadris Top (Syngenta Crop Protection, St Gabriel, LA) and Pristine WG (BASF Corporation, Geismar, LA) fungicides at rates listed in the Southern Region Small Fruit Consortium Production Manuals (SRSFC, 2017). The presence of Botrytis cinerea, gray mold was observed before the harvest. Along with fungicide application, strawberries exhibiting gray mold symptoms were removed from the plants and discarded.

Inoculum Preparation

In this study, three strains of generic E. coli (ATCC 23716, 25922 and 11775) were used as indicators for fecal contamination and stored at -80°C until use. Ten µL of each strain was transferred to fresh Tryptic Soy Broth (TSB) (Hardy Diagnostics, Santa Maria, CA) and incubated at 37°C for 18 h. Ten µL of each strain was then transferred again to fresh TSB and
incubated at 37°C for 24 h to generate the culture of approximately $10^9$ CFU/mL. To prepare the cocktail inoculum, equal volume of each bacterial strain was diluted and mixed to establish the baseline of approximately $10^6$ or $10^2$ CFU/mL for the high or low contamination level, respectively.

**Field Setup: Heavy Flooding (HF) and Light Flooding (LF)**

Five raised beds were constructed on site. The raised beds were 4 feet wide and 8 feet long and 24 inches tall. The raised beds were constructed with treated lumber and lined with black visqueen to hold floodwater. Five raised beds were flooded as shown in Figure 1.

![Field Experimental Design](image)

Figure 2. Field Experimental Design.

Control group strawberries were flooded with Baton Rouge municipal water. Cow manure was stirred into floodwater in treatment groups to simulate the organic load of floodwater. High Flooding High Contamination (HFHC) strawberries were flooded with 12 inches of manure water spiked with $10^6$ CFU/L of generic *E. coli*. High Flooding Low Contamination (HFLC) strawberries were flooded with 12 inches of manure water spiked with $10^2$ CFU/L of generic *E. coli*. Low Flooding High Contamination (LFHC) strawberries were flooded with 8 inches of manure spiked with $10^6$ CFU/L of generic *E. coli*. Low Flooding Low Contamination (LFLC)
strawberries were flooded with 8 inches of manure water spiked with $10^2$ CFU/L of generic *E. coli*. In two beds with high flooding treatments, the floodwater completely submerged the strawberries. While in two beds with low flooding treatments, the floodwater came in contact with the plants but not the berries. After flooding for 4 hours, floodwater was then drained with an electric pump (Xtreme Pump, Thibodaux, LA) and handheld pump (Xtreme Pump, Thibodaux, LA). Samples were taken as described below.

**Objective 1**: Assess the microbial safety of strawberries that come into direct contact and did not come into direct contact with floodwater immediately after flooding and during shelf life. Different growth stages of strawberries were investigated. Foliage and soil samples were also analyzed for potential microbial contamination.

**Strawberry Sample Collection**

Red mature strawberries from HFHC and HFLC raised beds were harvested and placed in labeled Rubbermaid rigid containers. Samples were then transported to the laboratory on ice within one hour, where the strawberries were placed into designated Genpak Secure Seal 1 Qt. clamshell boxes and stored in the refrigeration at 4°C for microbial and quality analysis.

Immature green strawberries were left in the field to become mature and were collected one week after flooding. Immature strawberries allowed to ripe after flooding (will be referred to as immature strawberries) were also transported to the laboratory on ice within one hour and stored in the same manner as mature strawberries. Mature strawberry and immature strawberry samples were analyzed at 0, 48, 96 and 144 hours after harvesting.

**Soil Sample Collection**

Soil samples were collected at 0, 48, 96 and 144 hours after flooding. The 200g of soil were collected at random spots in each raised bed and divided into two separate Nasco™ Whirl-Pak™
Easy-To-Close sterile collection bags in replicates for analysis. Soil samples were transported to the laboratory on ice and were stored in refrigeration at 4°C until microbial analysis.

**Plant Sample Collection**

Plant samples were harvested at the same time the immature strawberries were harvested approximately 1 week after flooding. Enough leaves were collected from random spots to collect 200g of leaves per treatment bed. Samples were divided equally into 2 separate Nasco™ Whirl-Pak™ Easy-To-Close sterile collection bags. Samples were transported to the laboratory on ice and were stored in refrigeration at 4°C until microbial analysis.

**Detection of Foodborne Pathogens**

The presence or absence of foodborne pathogens of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in mature (red berries at the time of harvest) and immature strawberries (green at time of flooding allowed to ripen on the plant), soil, and plants were determined using 3M™ Molecular Detection System (3M Food Safety, St Paul, MN). For sample enrichment, 25 g of each mature, immature strawberries, soil, or plant samples were added to 225 mL of buffered peptone water (3M™ Food Safety, St Paul, MN) and incubated at 41.5 °C for 24 hours. The presence or absence of *E. coli* O157:H7 was analyzed using 3M™ Molecular Detection Assay 2- *E. coli* O157:H7; catalog number MDA2ECO96 (3M™ Food Safety, St Paul, MN). For sample enrichment, 25 g of each mature, immature strawberries, soil, or plant samples were added to 225 mL of Demi-Fraser Broth Base (3M™ Food Safety, St Paul, MN) and incubated at 37 °C for 24 hours. The presence or absence of *Listeria monocytogenes* was analyzed using 3M™ Molecular Detection Assay 2- *Listeria monocytogenes*; catalog number MDA2LMO96 (3M™ Food Safety, St Paul, MN).
Enumeration of Fecal Indicators

To estimate levels of fecal indicators in mature, immature strawberries, soil, or plant samples, a 1:10 dilution of 25 g of samples in 225 mL of 0.1% peptone water was prepared and homogenize using a stomacher for 1 minute to generate a uniform distribution. One mL of each of the mixtures (mature/immature strawberries, soil and plant) was plated on 3M™ Petrifilm™ E. coli/Coliform Count Plates to determine coliforms and E. coli presence, Petrifilms were incubated at 37°C for 48 hours in duplicates to determine colony counts. Results are reported in CFU/g.

Statistical Analysis

All experiments were repeated twice. The data of the microbial load (E. coli, coliforms, yeast and mold) in the mature/immature strawberries, soil and plant was converted into logarithmic units (CFU/g). Microbial safety and quality data were analyzed using the SAS® program PROC GLM by analysis of variance (ANOVA), using a completely randomized design to observe the interaction of the factors between the five treatments (Control, HFHC, HFLC, LFHC and LFLC) and time periods (0, 48, 96 and 144 hours), differences between means were separated using Duncan and difference at ($P \leq 0.05$) were considered to be significant with a level of 95% of confidence.

Results and Discussion

Indicator Bacteria in Mature Strawberries during Shelf Life after Flooding

The results in Table 1 reflect E. coli populations on mature strawberries at the time of harvest and at 48 h increments until 144 h. E. coli was not detected on the mature strawberries after harvest or during shelf life using a detection limit of 10 CFU/g. Knudsen et al., (2001) observed that the population of pathogens such as E. coli O157:H7 on whole strawberries
decreased by 2 log cycles over a 7-day storage period at 4°C. Another study indicated significant reduction in the levels of generic *E. coli* population between two nonpathogenic *E. coli* strains tested on the surface of strawberry fruit stored for 24 hours, wherein the population reductions were 2.31 log CFU/g and 1.05 log CFU/g (Yu et al., 2001). A study by Yu et al., (2001) on whole broccoli, cucumber, and green pepper reported that *E. coli* O157:H7 populations decreased by 1 log CFU/g after 3 days of storage at 4°C with an initial inoculum level of 10^6 CFU/mL. Another study indicated that *E. coli* O157:H7 and *Salmonella* had limited ability to multiply on cut strawberries due to the naturally low pH of 3.2 to 4.1 (Knudsen et al., 2001). Also, the surface of whole strawberry is waxy and dry, which may reduce bacteria survival. The acidic nature of certain fruits has the potential to inactivate pathogens. Pathogens are more effectively recovered at a higher pH because they are protected from potential acid injury (Flessa et al., 2005).
Table 1. Influence of flooding on indicator bacteria population on mature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>LFLC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Coliform Population at Specific Sampling Times

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>1.45±0.29a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>1.14±0.51a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>1.01±0.67a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation
ND= Not detected. Below Detection Limit (10 CFU/g)

(C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination

Sampling time at which indicator bacteria population were measured after flooding

Means in columns with different letters are significant at \( P \leq 0.05 \)

Indicator bacteria are used to detect and estimate the level of fecal contamination and detect the presence of pathogens in water and food. The most commonly used indicator bacteria include total coliforms and fecal coliforms such as generic *E. coli*. In this study, the numbers of coliforms in mature strawberries at the time of harvest and at 48 h increments until 144 h are also presented in Table 1. When strawberry fruits were subjected to treatments LFHC, HFLC and HFHC immediately after flooding (0 h), populations of coliform reached 1.45 log CFU/g, 1.14 log CFU/g, and 1.01 log CFU/g, respectively. However, after 48 h of storage at refrigeration temperature (4°C), the levels of coliforms were below the detection limit in all treatments. This indicates that coliforms in mature strawberry samples were present immediately after flooding when exposed to high contamination levels \( 10^6 \) CFU/L at both low and high flood levels.
Coliform were also present in low contamination levels \((10^2 \text{ CFU/L})\) only when water contacted the strawberries in high flood conditions.

**Indicator Bacteria in Immature Strawberries during Shelf Life after Flooding**

Immature green strawberries at the time of flooding were left in the field to mature. One week after the initial flooding, strawberries that reached acceptable size and color were harvested. These were labeled as “immature strawberries” as they matured after flooding. As shown in Table 2, *E. coli* was not detected in any of the immature strawberries fully submerged in floodwater (high flooding, HF) or in strawberries not contacted by floodwaters (low flooding, LF). Low survival populations of *E. coli* have been reported on strawberry fruit surfaces (Yu et al., 2001), who found decreases in bacterial population on fruit that may be due to lack of nutrients for the bacteria to grow or other competing microflora.
Table 2. Influence of flooding on indicator bacteria population on immature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>ND</td>
<td>ND</td>
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</table>

Coliform Population at Specific Sampling Times

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>2.18±0.40Aa</td>
<td>1.02±0.91Ba</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>ND</td>
<td>1.32±1.15a</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>ND</td>
<td>1.39±0.98Aa</td>
<td>1.11±0.99Aa</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>ND</td>
<td>2.01±0.51a</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>ND</td>
<td>1.82±0.79Aa</td>
<td>ND</td>
<td>1.11±0.97A</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation
ND= Not detected. Below Detection Limit (10 CFU/g)
(C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination

Sampling time at which indicator bacteria population were measured after flooding
Means in columns with different lowercase letters are different at (P≤0.05)
Means in rows with different uppercase letters are different at (P≤0.05)

In the immature strawberries, the coliform population was present in all the floodwater treatments at 48 h as illustrated in Table 2. Significant reduction was observed only in the control samples at 96 h of refrigerated storage (4°C). Although coliform population in the low flood high contamination treatment did not decrease between 48 h and 96 h it was not detected by 144 h. These data indicate that coliform population gradually reduced over time. Immature strawberries subjected to water treatment HFHC were positive for coliforms at 48 h storage period (1.82 log CFU/g) and 144 h (1.11 log CFU/g), in the same treatment. Compared to mature strawberries harvested immediately after flooding, the overall coliform population of the immature strawberries was higher after storage for 48 h in all water treatments and at 96 h in the control.
and LFHC. Several fresh produce studies have indicated coliform population varies in wide ranges. Johnston et al. (2005) found total coliforms on green leaves and herbs ranged from 1.0 to 4.3 log CFU/g. Total coliforms ranged from 2.7 log CFU/g to 8.2 log CFU/g in mixed salad vegetables and the levels of total coliforms on fresh lettuce were up to 5 log CFU/g (Mohammad & Bahreini, 2012).

**Indicator Bacteria in Soil after Flooding**

Soil samples were taken every 48 h from the strawberry field after the floodwater receded. As shown in Table 3, generic *E. coli* (~1 log CFU/g) was only detected in soil subjected to water treatments LFLC, LFHC, HFLC and HFHC but not the control. After 48 h, *E. coli* was detected only in the soil that was flooded with high contamination. The generic *E. coli* level in the HFHC (1.61 log CFU/g) was greater than the levels detected in soil subjected to the HFLC (1.09 log CFU/g) treatment immediately after flooding. Interestingly, generic *E. coli* levels did not change significantly between 0 h (1.61 log CFU/g) and 48 h (1.54 log CFU/g) when subjected to HFHC water treatments but was not detected at 96 h and 144 h. Similarly, the generic *E. coli* levels of soil subjected to the HFLC water treatment was 1.09 log CFU/g at 0 h but was below the detection limit after 48 h and subsequently. The decrease of generic *E. coli* population in the soil may be due to the harsh environmental conditions such as UV and high temperature. A study indicated that higher levels of *E. coli* were present in soil exposed to a higher outside temperature due to climatic change (Holvoet et al., 2014). Also, as presented in Table 3, the level of contamination had a significant effect on the *E. coli* population at high flooding only immediately after harvest. In this study, results imply that even when the edible portion of strawberries did not come in direct contact with floodwaters; there is potential risk for them to pick up contaminants from the soil for up to two days after floodwaters recede if left in
the field. It is important to note that the study was completed without the use of plastic mulch. Most strawberry producers have a plastic barrier between the plants and soil. Further research should be completed using this model.

Table 3. Influence of flooding on indicator bacteria population in soil during specific sampling times.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>1.15±0.22ab</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>1.21±0.78Aab</td>
<td>1.48±0.37Aa</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>1.09±0.54b</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>1.61±0.31Aa</td>
<td>1.54±0.51Aa</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Coliform Population at Specific Sampling Times

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.38±0.18Ab</td>
<td>1.71±1.21ABB</td>
<td>2.04±0.25Ac</td>
<td>1.03±1.13Bc</td>
</tr>
<tr>
<td>LFLC</td>
<td>2.61±0.24Aab</td>
<td>2.22±0.98ABAa</td>
<td>2.37±0.19Aa</td>
<td>1.48±1.00Bbc</td>
</tr>
<tr>
<td>LFHC</td>
<td>2.69±0.18Aa</td>
<td>2.35±0.70Aa</td>
<td>2.31±0.18Aab</td>
<td>1.91±0.16Bab</td>
</tr>
<tr>
<td>HFLC</td>
<td>2.38±0.38Ab</td>
<td>2.21±0.42Aa</td>
<td>2.15±0.25Abc</td>
<td>2.19±0.12Aab</td>
</tr>
<tr>
<td>HFHC</td>
<td>2.82±0.20Aa</td>
<td>2.08±0.49Cab</td>
<td>2.24±0.31BCab</td>
<td>2.44±0.19Ba</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation
ND= Not detected. Below Detection Limit (10 CFU/g)

\(^y\) (C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination

\(^z\) Sampling time at which indicator bacteria population were measured after flooding

Means in columns with different lowercase letters are different at \((P \leq 0.05)\)
Means in rows with different uppercase letters are different at \((P \leq 0.05)\)

Results in Table 3 also depict coliform population in soil at harvest and in 48 h increments after harvest. High levels of coliform were present in all water treatments ranging from 1.03 log CFU/g to 2.82 log CFU/g. However, coliform population decreased during shelf life at the 144 h in the control, LFLC and LFHC water treatments indicating that low and high contamination may not have contributed to soil contamination. Yet, high floodwater levels displayed presence of coliform population even at the 144 h time period (> 1 log CFU/g).
study concluded that flooding could possibly cause contamination of fresh produce if the soil has higher levels of generic *E. coli* and coliform population; and if there was splashing of soil particles onto the berries during a rainfall (Delbeke et al., 2015). But at the same time, soil contamination does not necessarily indicate contamination of the strawberry fruit. Since most strawberries are grown on protected plastic covered rows to assist picking, soil is not the only contributing factor to cause contamination in strawberries (Delbeke et al., 2015). Moreover, it is very unlikely for pathogens to internalize through irrigation water or the soil into the strawberries through the roots with naturally contaminated water and with low numbers of pathogens (Holvoet et al., 2014).

**Strawberry Plant Foliage**

The results in Table 4 depict *E. coli* levels and coliform levels immediately after flooding on the foliage of strawberry plants subjected to all water treatments. The foliage was not positive for generic *E. coli* and very low levels of coliforms were detected (below detection limit -10 CFU/g). Foliage samples showed no significant differences within treatments and time periods (*P*≤0.05).

Table 4. Influence of flooding on indicator bacteria population on foliage at harvest.

<table>
<thead>
<tr>
<th>Treatments(^{y})</th>
<th>0hr(^{z})</th>
<th>0hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation
ND= Not detected. Below Detection Limit (10 CFU/g)
\(^{y}\)(C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination
\(^{z}\)Sampling time
Foodborne Pathogen Detection in Strawberries, Soil, and Plants

Table 5 shows the absence of the foodborne pathogens *E. coli* O157:H7 and *Listeria monocytogenes* in mature and immature strawberries, soil, and foliage immediately after harvesting. Studies conducted on the survival of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on whole and cut strawberries at room and refrigeration temperatures and during frozen storage have no growth observed of these pathogens on the surface of the strawberries, even though all these pathogens are capable to survive (Knudsen et al., 2001; Flessa et al., 2005; Yu et al., 2001). In this study, no *E. coli* O157:H7 or *Listeria monocytogenes* was detected (Table 5) probably because the environment was not contaminated. Also, the naturally low pH of the fruit may have prevented the survival of the aforementioned pathogens. This was observed in studies conducted by Knudsen et al. (2001) where *E. coli* O157:H7 and *Salmonella* had limited ability to multiply on cut strawberries due to the naturally low pH of 3.2 to 4.1.

Table 5. Influence of flooding on *E. coli* O157:H7 and *Listeria monocytogenes* population on mature and immature strawberries, soil, and foliage at harvest.

<table>
<thead>
<tr>
<th>Treatments ^(y)</th>
<th>0hr (^z)</th>
<th>0hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFLC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>LFHC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFLC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HFHC</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND=Not Detected. ND= Not detected. Below Detection Limit (10 CFU/g)
\(^y\)(C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination
\(^z\) Sampling time

Overall, the level of flooding and level of contamination had no definite correlation with the microbial counts in all treated samples. Generic *E. coli* was not detected in the strawberry fruits at all time periods and in soil after 96 h of storage at 4°C. When producers use good
agricultural practices (GAP) to reduce the potential of a food safety outbreak such as combining drip irrigation during strawberry production (Delbeke et al., 2015b) with proper storage temperatures and proper handling of fruit (hand washing), strawberries affected by floods may be free from potential sources of pathogen contamination. Additional studies should be conducted to support this research before an official statement regarding food safety can be pronounced.

References


CHAPTER 4. QUALITIES OF STRAWBERRIES AFTER FLOODING

Materials and Methods

Strawberry Production

Strawberries plants were planted and maintained at the LSU AgCenter Botanic Gardens at Burden using standard growing procedures in Louisiana (Fontenot, et al., 2014). Strawberries were planted in October and were managed through March. Three bareroot ‘St. Festival’ strawberry plants were planted in Rose medium purchased in bulk at Cleggs Nursery on Siegen Lane in Baton Rouge, Louisiana. Strawberries were fertilized with Peters 20-20-20 at a rate of 200 parts per million (ppm) of nitrogen (N) four times prior to harvest. Strawberries were manually irrigated for the first 3 weeks to allow roots to establish. After root establishment, strawberries were irrigated automatically using drip emitters (Rain Bird, Azusa, CA) for 15 minutes per day, one emitter per three strawberry plants. Between planting and the first harvest, strawberry plants were sprayed twice with Quadris Top (Syngenta Crop Protection, St Gabriel, LA) and Pristine WG (BASF Corporation, Geismar, LA) fungicides at rates listed in the Southern Region Small Fruit Consortium Production Manuals (SRSFC, 2017). The presence of Botrytis cinerea, gray mold was observed before the harvest. Along with fungicide application, strawberries exhibiting gray mold symptoms were removed from the plants and discarded.

Inoculum Preparation

In this study, three strains of generic E. coli (ATCC 23716, 25922 and 11775) were used as indicators for fecal contamination and stored at -80°C until use. Ten µL of each strain was transferred to fresh Tryptic Soy Broth (TSB) (Hardy Diagnostics, Santa Maria, CA) and incubated at 37°C for 18 h. Ten µL of each strain was then transferred again to fresh TSB and incubated at 37°C for 24 h to generate the culture of approximately \(10^9\) CFU/mL. To prepare the
cocktail inoculum, equal volume of each bacterial strain was diluted and mixed to establish the baseline of approximately $10^6$ or $10^2$ CFU/mL for the high or low contamination level, respectively.

**Field Setup: Heavy Flooding (HF) and Light Flooding (LF)**

Five raised beds were constructed on site. The raised beds were 4 feet wide and 8 feet long and 24 inches tall. The raised beds were constructed with treated lumber and lined with black visqueen to hold floodwater. Five raised beds were flooded as shown in Figure 2.

![Figure 3. Field Experimental Design.](image)

Control group strawberries were flooded with Baton Rouge municipal water. Cow manure was stirred into floodwater in treatment groups to simulate the organic load of floodwater. High Flooding High Contamination (HFHC) strawberries were flooded with 12 inches of manure water spiked with $10^6$ CFU/L of generic *E. coli*. High Flooding Low Contamination (HFLC) strawberries were flooded with 12 inches of manure water spiked with $10^2$ CFU/L of generic *E. coli*. Low Flooding High Contamination (LFHC) strawberries were flooded with 8 inches of manure spiked with $10^6$ CFU/L of generic *E. coli*. Low Flooding Low Contamination (LFLC) strawberries were flooded with 8 inches of manure water spiked with $10^2$ CFU/L of generic *E. coli*. 

44
coli. In two beds with high flooding treatments, the floodwater completely submerged the strawberries. While in two beds with low flooding treatments, the floodwater came in contact with the plants but not the berries. After flooding for 4 hours, floodwater was then drained with an electric pump (Xtreme Pump, Thibodaux, LA) and handheld pump (Xtreme Pump, Thibodaux, LA). Samples were taken as described below.

**Strawberry Sample Collection**

Red mature strawberries from HFHC and HFLC raised beds were harvested and placed in labeled Rubbermaid rigid containers. Samples were then transported to the laboratory on ice within one hour, where the strawberries were placed into designated Genpak Secure Seal 1 Qt. clamshell boxes and stored in the refrigeration at 4°C for microbial and quality analysis. Immature green strawberries were left in the field to become mature and were collected one week after flooding. Immature strawberries allowed to ripe after flooding (will be referred to as immature strawberries) were also transported to the laboratory on ice within one hour, and stored in the same manner as mature strawberries. Mature strawberry and immature strawberry samples were analyzed at 0, 48, 96 and 144 hours after harvesting.

**Objective 2:** Assess the quality of strawberries that come into direct contact or did not come into direct contact with floodwater immediately after flooding and during shelf life. Different growth stages of strawberries were investigated.

**Quality of Strawberries**

To estimate levels of yeast and mold in mature or immature strawberry fruits, yeast and mold count assessment was determined using 3M™ Petrifilm™ Rapid Yeast and Mold Count Plate. One mL of the homogenized strawberry mixtures were plated on a 3M™ petrifilm and incubated at room temperature for 4-5 days.
Color and texture of the mature and immature strawberry fruits were analyzed at 0, 48, 96 and 144 hours after harvesting. Color of whole strawberries was determined using a color BC-10 Baking Meter (Konica Minolta, Wayne, NJ). Color parameters were quantified in the Hunter L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) color space. Texture of whole strawberries was measured as shear strength using a texture analyzer (TA.XT2i, Texture Technologies, New York, USA). Samples were placed on the TA-91 platform and compression tests were carried out. The maximum force (Fmax) needed to compress the samples was reported.

Statistical Analysis

All experiments were repeated twice. The data of the microbial load (E. coli, coliforms, yeast and mold) in the mature/immature strawberries, soil and plant was converted into logarithmic units (CFU/g). Microbial safety and quality data were analyzed using the SAS® program PROC GLM by analysis of variance (ANOVA), using a completely randomized design to observe the interaction of the factors between the five treatments (Control, HFHC, HFLC, LFHC and LFLC) and time periods (0, 48, 96 and 144 hours), differences between means were separated using Duncan and difference at ($P \leq 0.05$) were considered to be significant with a level of 95% of confidence.

Results and Discussion

Mature Strawberry Fruit Decay

The overall fungal contamination of the tested strawberry fruits is summarized in Table 6 and Table 7. The results in Table 6 illustrate yeast population on the mature strawberry fruit at the time of harvest and at 48 h increments until 144 h after a flooding event. Results indicated presence of yeast on strawberry fruits subjected to all water treatments (C, LFLC, LFHC, HFLC
and HFHC) during all time periods (0, 48, 96 and 144 h) of shelf life. At the time of harvest and at 144 h, HFHC had the highest yeast count, which was not significantly different from some of the treatments. This treatment also had significantly higher yeast count from the rest of the treatments at 96 h. However, there was no clear trend indicating a reduction or increase in yeast presence as a result of floodwater depth (HF, LF) or the level of contamination (HC, LC).

Moreover, there was no clear trend indicating the influence of storage period on yeast presence. As strawberries are a highly perishable fruit, shelf life is usually decreased due to fungal infections, where severity is correlated to cultivation, harvesting, handling, transport, postharvest storage and marketing environments (Barth et al., 2009). Yeasts are common spoilers of fruit, contaminating 78% of blackberry, 55% of blueberry, 75% of raspberry and 77% of strawberry samples. Particularly in strawberries, it has been noticed that the decay produced an off odor (Tournas & Katsoudas, 2005). Yeasts are widely found in nature and the presence of sugars and acids in strawberries creates an ideal water activity ($a_w$ 0.88) and low pH for fungal growth. This happens due to the limitations of bacterial competition as most bacteria prefer near neutral pH (FSIS, 2012). Another reason for noticing higher incidence levels of yeast decay may be due to cutting and packaging even though these were not performed in this study. These factors can result in damaging the cells of the outer skin layers of the vegetables and thereby facilitating entry of organisms such as yeast (Tournas, 2005). Yeast will still naturally grow in strawberries despite contact with floodwater.

This study would have benefited from a dry control to determine if yeast and mold counts were greater as a result of flooding. Moreover, threshold values of yeast population have not been determined (Personal correspondence with LSU AgCenter postharvest horticulture specialist Dr. David Picha).
Table 6. Influence of flooding on yeast and mold counts of mature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Yeast Population at Specific Sampling Times</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>C</td>
<td>LFLC</td>
<td>LFHC</td>
<td>HFLC</td>
</tr>
<tr>
<td><strong>0 h</strong></td>
<td>3.65±0.06Bb</td>
<td>3.69±0.25Ab</td>
<td>3.95±0.04Aa</td>
<td>3.48±0.11Cc</td>
</tr>
<tr>
<td><strong>48 h</strong></td>
<td>3.76±0.12Aab</td>
<td>3.97±0.28Aa</td>
<td>3.93±0.14Aab</td>
<td>3.71±0.37ABab</td>
</tr>
<tr>
<td><strong>96 h</strong></td>
<td>3.82±0.12Ab</td>
<td>3.84±0.25Ab</td>
<td>3.96±0.09Ab</td>
<td>3.78±0.13Ab</td>
</tr>
<tr>
<td><strong>144 h</strong></td>
<td>3.58±0.06Bb</td>
<td>3.78±0.17Aa</td>
<td>3.32±0.22Bc</td>
<td>3.56±0.19BCb</td>
</tr>
<tr>
<td>Treatments</td>
<td>LFLC</td>
<td>LFHC</td>
<td>HFLC</td>
<td>HFHC</td>
</tr>
<tr>
<td><strong>0 h</strong></td>
<td>3.69±0.25Ab</td>
<td>3.95±0.04Aa</td>
<td>3.48±0.11Cc</td>
<td>3.95±0.16ABa</td>
</tr>
<tr>
<td><strong>48 h</strong></td>
<td>3.97±0.28Aa</td>
<td>3.93±0.14Aab</td>
<td>3.71±0.37ABab</td>
<td>3.89±0.11Bab</td>
</tr>
<tr>
<td><strong>96 h</strong></td>
<td>3.84±0.12Ab</td>
<td>3.96±0.09Ab</td>
<td>3.78±0.13Ab</td>
<td>4.19±0.48Aa</td>
</tr>
<tr>
<td><strong>144 h</strong></td>
<td>3.78±0.17Aa</td>
<td>3.32±0.22Bc</td>
<td>3.56±0.19BCb</td>
<td>3.87±0.18Ba</td>
</tr>
</tbody>
</table>

Mold Population at Specific Sampling Times

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 h</th>
<th>48 h</th>
<th>96 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td>3.22±0.34Ab</td>
<td>3.20±0.14Aab</td>
<td>2.96±0.33Bb</td>
<td>3.24±0.16Aa</td>
</tr>
<tr>
<td><strong>LFLC</strong></td>
<td>3.37±0.03Aab</td>
<td>3.37±0.11Aa</td>
<td>3.11±0.31Bab</td>
<td>3.11±0.20Ba</td>
</tr>
<tr>
<td><strong>LFHC</strong></td>
<td>3.35±0.11Aab</td>
<td>3.15±0.20Bb</td>
<td>3.25±0.02ABa</td>
<td>3.11±0.32Ba</td>
</tr>
<tr>
<td><strong>HFLC</strong></td>
<td>3.24±0.18Ab</td>
<td>3.33±0.28Aab</td>
<td>3.21±0.13Aa</td>
<td>3.05±0.50Aa</td>
</tr>
<tr>
<td><strong>HFHC</strong></td>
<td>3.44±0.04Aa</td>
<td>3.29±0.15Bab</td>
<td>3.28±0.10Ba</td>
<td>3.18±0.11Ba</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation

\( ^{\text{\( z\)}} (C)=\text{Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination} \)

\( ^{\text{\( y\)}} \text{Sampling time at which yeast and mold populations were measured after flooding} \)

Means in columns with different lowercase letters are significant at \((P \leq 0.05)\)

Means in rows with different uppercase letters are significant at \((P \leq 0.05)\)

The results in Table 6 also illustrate the mold population on mature strawberry fruit at the time of harvest and at 48 h increments until 144 h after a flooding event and during shelf life. The population of mold was present in all flooding water treatments C, LFLC, LFHC, HFLC and HFHC and during shelf life 0, 48, 96 and 144 h. HFHC had the highest mold count at harvest though it was not significantly different from low flooding treatments. However, there was no clear trend indicating a reduction or increase in mold presence as a result of floodwater depth or the level of contamination. Also, strawberry fruit mold counts decreased in the HFHC treatment at 48 h but did not change between 96 h and 144 h. Mold counts on strawberry fruit in the LFLC flood treatment also decreased at 96 h but did not further decrease at 144 h. In other treatments, there was no clear trend indicating the influence of storage period on mold presence. Molds are
overwhelmingly present in postharvest diseases of several types of fresh cut fruits and vegetables and have resulted in unpalatable fresh-cut fruits such as strawberry, honeydew, pineapple, and cantaloupe (Barth et al., 2009). Similar to yeast, it is also naturally found in the environment and therefore direct contact or in direct contact with floodwater did not affect the mold population on strawberries.

A study outlined that strawberry fruits purchased from the supermarket lasted for periods longer than 3 days but resulted in sporadic growth of gray mold (Riordan et al., 2000). A study by Mohammad & Bahreini (2012) observed similar trends where the presence of yeasts and molds found in mixed fresh-cut vegetable salads was 5.68 log CFU/g and mixed ready-to-eat fresh herbs were at 5.78 log CFU/g. Therefore, it is important to monitor fungal contaminants in fresh vegetables and fruits as some molds produce mycotoxins on produce while certain yeasts and molds can cause infections or allergies (Mohammad & Bahreini, 2012).

**Decay of Immature Strawberries that Matured into a Red Fruit**

Immature green strawberries remained in the field and were sampled one week after flooding, once they had matured into a red colored fruit. The results in Table 7 depict yeast populations on immature strawberries that were allowed to ripen after flooding and during shelf life at 0, 48, 96 and 144 h. The presence of yeast was observed on immature strawberries subjected in all treatments after flooding and during shelf life, ranging from 3.31 log CFU/g to 4.01 log CFU/g (Table 7). HFLC had a significantly higher yeast count at harvest compared to the rest of the treatments. However, there was no clear trend indicating a reduction or increase in yeast presence as a result of floodwater depth or the level of contamination. This treatment also manifested a decreasing yeast count throughout shelf life though it was not significantly different
at 48 h and 96 h. In other treatments, there was no clear trend indicating the influence of storage period on yeast presence.

Table 7. Influence of flooding on yeast and mold counts of immature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yeast Population at Specific Sampling Times</th>
<th>Mold Population at Specific Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0 h</td>
<td>48 h</td>
</tr>
<tr>
<td>C</td>
<td>3.69±0.21AaBc</td>
<td>3.74±0.07Aa</td>
</tr>
<tr>
<td>LFLC</td>
<td>3.81±0.30Ab</td>
<td>3.69±0.16Aa</td>
</tr>
<tr>
<td>LFHC</td>
<td>3.86±0.20Ab</td>
<td>3.73±0.11Aa</td>
</tr>
<tr>
<td>HFLC</td>
<td>4.01±0.08Aa</td>
<td>3.73±0.22Ba</td>
</tr>
<tr>
<td>HFHC</td>
<td>3.88±0.19Aa</td>
<td>3.77±0.12Aa</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation

(C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination

Sampling time at which yeast and mold populations were measured after flooding

Means in columns with different lowercase letters are significant at (P≤0.05)

Means in rows with different uppercase letters are significant at (P≤0.05)

The results in Table 7 also indicate mold population on immature strawberries that were allowed to ripen after flooding at harvest and during shelf life 48 h, 96 h and 144 h. The strawberry fruit was moldy after being exposed to C, LFLC, LFHC, HFLC and HFHC flood treatments at harvest and during shelf life. The incidence of mold decay was observed in variable amounts in each flood treatment and mold presence ranged from 3.35 log CFU/g to 3.45 log CFU/g. Even at shelf life 144 h, the mold growth in the strawberry samples ranged from >3 log CFU/g but less than 4 log CFU/g. At harvest and at 144 h, the low flooding treatments had the highest mold count (LFHC and LFLC, respectively) though these were not significantly different
from some treatments. At harvest, high contamination treatments manifested significantly higher mold counts from the control, indicating that contamination level of floodwater influenced mold incidence at the time of harvest. However, there was no clear trend indicating the influence of storage period on mold growth.

Although contamination of floodwater somehow affected mold presence in immature strawberries in this study, other literature suggest that storage temperature also influences mold growth. Strawberries in this study were stored at 4°C during shelf life. A similar study storing cut pineapples in closed containers at 4°C and 20°C reflected a major quality defects at the higher temperature. Under refrigerated storage temperatures, yeast and mold populations dominated the microflora of fresh-cut pineapple and strawberries (Barth et al., 2009). Berries stored at 10 or 25°C developed mold after 48 h and the mold incidence was 5% when the fruit was held at 0°C and gradually increased to 22% at 10°C and 79% when fruit was stored at 20°C (Barth et al., 2009). Furthermore, regardless if the strawberries were impacted by floodwaters, mold would naturally grow in the fruit.

Data from this study signify that strawberry samples exposed to high and low contamination associated with high and low flooding events and even in control floodwaters exhibited variable levels of yeast and mold growth after flooding and during storage. A study conducted to investigate the microbiological quality of fruits and vegetables illustrated that the yeast and mold counts for fruits ranged from <1.0 to 8.0 log CFU/g, with the majority of samples having 3.0 to 5.0 log CFU /g counts (Badosa et al., 2008). In this study, yeasts had slightly higher counts than molds in both mature and immature strawberries.
Color of Mature Strawberries

Color is an important factor in determining the quality of the strawberry fruit. Results in Table 8 depict changes in surface color of the strawberries at harvest and during shelf life of 48, 96 and 144 h after flooding. There are three indicators to estimate color change. These include $L^*$ value, $a^*$ value and $b^*$ value. The $L^*$ values measures lightness of a fruit. An $L^*$ value of 0 indicates black color (darker) whereas an $L^*$ value of 100 indicates more white color (lighter). The $a^*$ value is a measure of red to green color. A positive $a^*$ value represents more redness whereas a negative $a^*$ value represents more greenness. Finally, the $b^*$ value is a measurement of yellow to blue color. A positive $b^*$ value represents a yellow color whereas a negative $b^*$ value represents more blue color. Results show that among all treatments, the $L^*$ values were not significantly different. Therefore, the $L^*$ value or the lightness or darkness of a berry is not affected by flood levels (LF and HF) or contamination levels (LC and HC). Unlike in this study where no change was observed, several studies did find changes in $L^*$ value of strawberries during storage periods. In contrast to this study, Péneau et al., (2007) showed that $L^*$ value of strawberries decreased during storage and progressively increased in surface darkening (decrease in $L^*$) during storage. Overall, the $L^*$ values of the flooding treatments did not reveal significant changes in the initial color coordinates of the strawberry fruit at the time of harvest or during shelf life.

The red color of the strawberry fruit measured by $a^*$ value indicates the redness to greenness color. Strawberries subjected to treatments with high contamination had the highest redness at harvest and at 144 h (LFHC and HFHC, respectively) though the values were not significantly different from some of the treatments. However, there was no clear trend in the $a^*$ value of the strawberry fruit among treatments and across storage times, indicating that treatment
(flood level or contamination level) or storage time does not affect the redness of the strawberry fruit. In contrast to this study, Cordenunsi et al., (2005) reported an increase in redness as depicted by the increasing anthocyanin content of three different strawberry cultivars during storage. This increase in anthocyanin formation might be explained by the dependency between temperature and storage time (Rahman et al., 2016).

The $b^*$ value indicates the yellowness of the fruit. In this study, there was also no clear trend in the yellowness of the mature strawberry fruits among treatments and across storage periods. Therefore, there is not enough evidence to support a correlation in redness or yellowness of strawberry fruit after flooding.
Table 8. Influence of flooding on color of mature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Color L*</th>
<th>Specific Sampling Timesz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatmentsy</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>49.60±2.38Ba</td>
</tr>
<tr>
<td>LFLC</td>
<td>48.45±1.86Ba</td>
</tr>
<tr>
<td>LFHC</td>
<td>48.30±4.76Aa</td>
</tr>
<tr>
<td>HFLC</td>
<td>50.25±2.41Aa</td>
</tr>
<tr>
<td>HFHC</td>
<td>51.00±4.00Aa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Color a*</th>
<th>Specific Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>17.67±5.42ABa</td>
</tr>
<tr>
<td>LFLC</td>
<td>17.97±6.59Aa</td>
</tr>
<tr>
<td>LFHC</td>
<td>20.85±2.00Aa</td>
</tr>
<tr>
<td>HFLC</td>
<td>18.72±5.41Aa</td>
</tr>
<tr>
<td>HFHC</td>
<td>17.07±5.04Aa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Color b*</th>
<th>Specific Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>11.20±4.37ABb</td>
</tr>
<tr>
<td>LFLC</td>
<td>14.87±4.09ABab</td>
</tr>
<tr>
<td>LFHC</td>
<td>14.42±4.75Aab</td>
</tr>
<tr>
<td>HFLC</td>
<td>11.30±1.39Bb</td>
</tr>
<tr>
<td>HFHC</td>
<td>16.25±2.83Aa</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation
y (C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination
z Sampling time at which color L*(lightness) a* (redness to greenness) b* (yellowness to blueness) were measured after flooding
Means in columns with different lowercase letters are significant at (P≤0.05)
Means in rows with different uppercase letters are significant at (P≤0.05)

**Color of Immature Strawberries that Matured into a Red Fruit**

Results in Table 9 depict changes in surface color of the immature strawberries that became a red fruit at harvest and during shelf life 48, 96 and 144 h, given by L* a* b*. At 48 h after flooding, the L* value of LFLC strawberry samples was higher (lighter color) than berries exposed to C, LFHC, HFLC and HFHC water treatments. However, there was no evident trend
in the change in lightness in the immature strawberries across treatments and during storage. In contrast to the present study, Nunes et al., (2006) reported that the $L^*$ value tended to decrease either during development in the field or during storage as a darker color naturally develops when strawberry fruit ripens.

Across all treatments, the red color of the strawberry fruit measured by the $a^*$ value and the yellowness measured by the $b^*$ value increased from the time of harvest until 48 h, except for the $b^*$ value of LFLC (Table 9). However, there was no clear trend in the $a^*$ value and $b^*$ value of immature strawberry fruit among treatments and across storage times, indicating that treatment (flood level or contamination level) or storage time does not affect its redness and yellowness.
Table 9. Influence of flooding on color of immature strawberries during shelf life at 4°C.

<table>
<thead>
<tr>
<th>Color L*</th>
<th>Specific Sampling Times$^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments$^y$</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>56.10±6.64Aa</td>
</tr>
<tr>
<td>LFLC</td>
<td>52.22±2.49Aa</td>
</tr>
<tr>
<td>LFHC</td>
<td>55.80±4.51Aa</td>
</tr>
<tr>
<td>HFLC</td>
<td>58.35±5.80Aa</td>
</tr>
<tr>
<td>HFHC</td>
<td>52.50±6.16Aa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Color a*</th>
<th>Specific Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>6.45±3.94Bc</td>
</tr>
<tr>
<td>LFLC</td>
<td>18.25±4.88Aa</td>
</tr>
<tr>
<td>LFHC</td>
<td>9.62±5.39Cc</td>
</tr>
<tr>
<td>HFLC</td>
<td>12.22±10.31Ac</td>
</tr>
<tr>
<td>HFHC</td>
<td>13.15±9.93Aab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Color b*</th>
<th>Specific Sampling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>0 h</td>
</tr>
<tr>
<td>C</td>
<td>12.75±5.88Aa</td>
</tr>
<tr>
<td>LFLC</td>
<td>11.38±2.06Ba</td>
</tr>
<tr>
<td>LFHC</td>
<td>13.98±3.35Aa</td>
</tr>
<tr>
<td>HFLC</td>
<td>15.90±2.94Aa</td>
</tr>
<tr>
<td>HFHC</td>
<td>12.48±5.14Aa</td>
</tr>
</tbody>
</table>

Values are the average log CFU/g ± standard deviation

$^y$ (C)=Control, (LFLC)=Low Flooding Low Contamination, (LFHC)=Low Flooding High Contamination, (HFLC)=High Flooding Low Contamination, (HFHC)=High Flooding High Contamination

$^z$ Samples were conducted at the following time points: (L)* (lightness), (a*) (redness to greenness), (b*) (yellowness to blueness) were measured after flooding

Means in columns with different lowercase letters are significant at ($P\leq0.05$)
Means in rows with different uppercase letters are significant at ($P\leq0.05$)

Overall, at harvest, immature strawberries had higher $L^*$ values (lighter color) and lower $a^*$ values (lower redness) compared to mature strawberries. Mature strawberries generally became lighter from the time of harvest until 144 h of shelf life, while immature strawberries generally became darker. In addition, mature strawberries showed a generally decreasing trend in redness from harvest to 144 h of shelf life, and this trend was in contrary to that for immature
strawberries. These signify that the flooding treatments resulted in desirable darkness and redness in immature strawberries at 144 h of shelf life.

**Firmness of Mature and Immature Strawberries**

Texture of fresh fruit and vegetables is an essential quality attribute when it comes to consumer acceptability. Strawberries are considered as a soft fruit and suffer a rapid loss of firmness during the ripening stage. This quality contributes to its short postharvest life (Collins & Perkins-Veazie (1993). In this study, mature strawberry firmness was variable immediately after flooding and throughout shelf life at all tested hours among water treatments (Table 10). At 144 h, mature strawberries subjected to high flooding treatments had significantly lower texture values compared to the other treatments, signifying that high flooding may have negatively affected the texture of these strawberries. Nevertheless, no clear trend was observed in the texture of both mature (Table 10) and immature (Table 11) strawberries among treatments and across sampling times.

In both mature and immature strawberries subjected to all treatments, variation may be a result of testing multiple berries at each time. Nunes & Emond (1999) observed that the firmness of strawberries decreased during storage, regardless of the storage temperature (Brecht et al., 2003). Flooding, contamination, and storage temperature may all affect firmness but with no evident trend in this study.
Yeast and mold growth, color, and firmness of strawberries are all quality factors that influence shelf life and consumer willingness to purchase strawberries. This study provided no conclusive data to indicate that flooded strawberries were more susceptible to postharvest decay than those that were not flooded. Postharvest handling of strawberries is a major factor that affects the fruits’ microbiological and organoleptic qualities.
References


CHAPTER 5. CONCLUSION

This study assessed the microbial safety and quality of strawberries subjected to high floodwater (HF) and low floodwater (LF) and in high and low contamination levels (HC or LC) immediately after flooding (0 h) and during shelf life at 48 h, 96 h, and 144 h at 4°C. Results demonstrated generic E. coli was not detected in the mature or immature strawberries at harvest and during shelf life, implying that flooding depths and contamination levels did not show any definite correlation with generic E. coli counts on the strawberries in all treatments. In the mature strawberries, coliforms were detected immediately after flooding in LFHC, HFLC and HFHC but no significant difference was observed among the treatments. On the other hand, coliforms were present in the immature strawberries in all treatments at 48 h. However, they were detected in reduced amounts in the control and LFHC at 96 h and again in reduced amounts in the HFHC treatment at 144 h. Generic E. coli was detected in some soil samples. Soil samples subjected to HFHC had significantly higher levels of generic E. coli compared to HFLC immediately after flooding, but there was no detection of E. coli in soil in all flooding treatments at 96 h and 144 h. Coliforms were present in soil subjected to all flooding samples at all time periods. However, there was no presence of indicator bacteria in the foliage in all treatments at harvest. Most importantly, E. coli O157:H7 and Listeria monocytogenes were not detected in the mature and immature strawberries, soil, or foliage at harvest. In this study, the quality parameters of strawberries such as yeast and mold count, color, and texture were not correlated with flooding depths or contamination levels at harvest and during shelf life.

Overall, results from this study indicated that the strawberries were not affected by generic E. coli or coliforms after being subjected to a simulated 4 h flooding. The soil was found to have generic E. coli immediately after flooding and up to 48 h of storage. However, generic E.
coli was not detected after 96 h in soil samples. However, additional studies need to be conducted before making an official statement on the relationship between potential foodborne pathogens and flooded strawberry fields. Further research will also be required to determine if flood conditions affect the presence of yeast and molds, color, and texture of strawberries.
APPENDIX: LSU AGCENTER BOTANIC GARDENS

Figure 4. Strawberries Growing in Hanging Baskets Prior to Flooding

Figure 5. Field Setup.
Figure 6. Low Flooding Low Contamination (LFLC)

Figure 7. Low Flooding High Contamination (LFHC)
Figure 8. High Flooding Low Contamination (HFLC)

Figure 9. High Flooding High Contamination (HFHC)
VITA

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