ESTIMATING FOOD WASTE DUE TO FOOD SAFETY RECALLS AND INVESTIGATING WAYS TO MINIMIZE NEGATIVE IMPACTS

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Food Science

by

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January 2021

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ABSTRACT

Estimating Food Waste Due to Food Safety Recalls and Investigating Ways to Minimize Negative Impacts

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For years the issue of food waste has been recognized and quantified; however, food safety issues often go unrecognized as a source of food waste. One objective of this research is to estimate quantities and monetary value of fruits and vegetables implicated in food safety recalls, and thus wasted. Using publicly available data we identified all recalls involving vegetable or fruit commodities contaminated with *Listeria monocytogenes*, pathogenic *E. coli*, or *Salmonella* during 2015-2018. When quantities were provided, monetary value of recalled product was calculated using USDA ERS 2016 average retail prices. Although data limitations only allowed analysis of 17% of the recalls that met the criteria of this study, we estimated an annual loss of 38 million pounds and $61 million in revenue. Overall this shows that food safety issues can result in food waste, therefore mitigation strategies are needed.

There are many ways that produce can become contaminated, however contaminated soils are a potential source of produce contamination and treatments to mitigate this risk while maintaining soil health is lacking. Current biofumigation methods that use glucosinolate hydrolysis products in mustard seed meal to control plant pathogens could also be effective against foodborne pathogens in soil. The purpose of this research is to determine the fate of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in soil treated with *Brassica* spp seed meal and plant material. Seed meals were successful in reducing pathogen concentrations in soil, significant reductions ($p < 0.05$) of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were observed in soil over 72 hours with the addition of 1.0 and 1.5 g of mustard seed meal. Increasing the seed meal concentration did not significantly ($p > 0.05$) increase the observed log reduction for *L. monocytogenes* or *Salmonella*, reductions ranged from 5.6 – 5.9 log CFU/g. However, for *E. coli* O157:H7 seed meal concentration was significant ($p < 0.05$). A 5.7 log CFU/g decrease was observed when 1.5 g of seed meal was used which was larger than 3.5 log CFU/g reduction observed with 1.0 g. Findings suggests that biofumigation with mustard seed meal could

potentially be used to reduce *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* in contaminated soil.

However, the use of plant material was not as successful as the use of the processed seed meals. In soil or in the absence of soil Brassica spp. plant material at 10% 15%, and 75% significantly increased *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* concentrations ($p < 0.05$). The results of these studies support literature indicating Brassica spp. processed plant products, like seed meals or extracts may be a more effective strategy in reducing human pathogen concentrations in contaminated agricultural soils. While the process of Biofumigation using Brassica spp. cover crops has been successful in eliminating plant pests from agricultural soils, due to its low isothiocyanate release efficiency and reactivity in soil organic matter, it may not be sufficient as a soil decontamination method against human pathogens.

Keywords: Food waste, food safety, traceability, fruits and vegetables, *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, Biofumigation, *Brassica* spp.

ACKNOWLEDGMENTS

I would like to thank my committee, Dr. Amanda Lathrop, Dr. Koushik Saha, Dr. Rudi Polloshka and Dr. Jean Davidson for their guidance and support throughout my experience here at Cal Poly. Their involvement has helped me to vastly develop myself and my technical skills, and they have encouraged and enhanced my critical thinking abilities. I would also like to thank Adeline Maykish, Taran Viridi, Chris Lu, Chloe McGovern, Kaleb Roberson, and Emma Sandquist for their help in and out of the lab. I am extremely thankful for my parents, without their unconditional love and support I would not be where I am today. I would like to thank the rest of my family and friends for their emotional support and for always standing in as an audience as I rehearsed presentations. Finally, I would like to express my appreciation for the National Institute of Food and Agriculture, National Needs Graduate Fellowship Program, United States Department of Agriculture for their support, award No. 2017-38420-26767, which funded my graduate fellowship.

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Chapter 1 Literature Review

1.1 Global Food Loss Issue

Annually, 40% of food produced in the United States goes to waste (Buzby et al., 2014). Simultaneously, 14.3 million households reported being food insecure at some point during 2018 (USDA, 2018). The United Nations Food and Agriculture Organization (FAO) estimates that the food currently lost worldwide would be able to feed more than double the amount of people that presently go undernourished. While fruits and vegetables are among the healthiest of foods, they account for the highest amount of post-harvest waste (Buzby et al., 2014). Of all fruits and vegetables produced globally, only 48% is consumed while the other 52% is lost (FAO, 2011). There are many steps along the farm-to-fork continuum where produce can be lost, including agricultural production, post-harvest handling and storage, processing, packaging, distribution, and at the consumer level (Figure 1). Consumers are responsible for roughly 28% of fruits and vegetables that are wasted, justifiably much of the current literature on food waste focuses on the consumer level (Qi &Roe, 2016; Aschemann-Witzel et al., 2015; Neff et al., 2015). However, our understanding of food loss from agricultural production to retail/distribution is minimal, therefore strategies to reduce waste at these levels are limited.

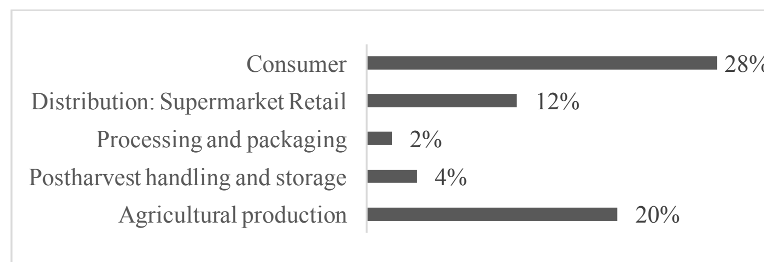

Figure 1 Food losses at each step of the farm to fork continuum for North America/Oceania (FAO, 2011)

Since the issue around food waste has heightened, many definitions of food loss and waste (FLW) have been generated (Table 1). The numerous definitions around FLW create controversy in determining what can be quantified as lost and wasted. Food safety

issues often go unrecognized as a source of food waste. If food is contaminated with a biological, chemical, or physical hazard and must be removed from the food chain to ensure the safety of consumers, is it food waste? The food items that are individually contaminated may fall into an inedible category of food, thus not considered waste. However, in an abundance of caution, food that may not be contaminated is recalled. Due to its potential contamination, this food must be sent to landfills or incinerated, placing it at the bottom of the food recovery hierarchy and leaving no opportunity for rescue (EPA, 2020).

Table 1 Current Food Loss and Waste (FLW) definitions

Organization	Definition: Food Loss	Definition: Food Waste	Source
United States Department of Agriculture (USDA)	Edible amount of food available for human consumption but is not consumed.	Edible item goes unconsumed as a result of human action or inaction and is often the result of a decision made farm-to-fork by businesses, governments, and individual consumers.	Buzby et al., 2014
World Resources Institute (WRI)	Food that spills, spoils, incurs an abnormal reduction in quality such as bruising or wilting, or otherwise gets lost before it reaches the consumer.	Food that is of good quality and fit for human consumption but that does not get consumed because it is discarded—either before or after it spoils	Lipinski et al., 2013
United States Environmental Protection Agency (EPA)	Unused product from the agricultural sector, such as unharvested crops.	Plate waste (i.e., food that has been served but not eaten), spoiled food, or peels and rinds considered inedible that is sent to feed animals, to be composted or anaerobically digested, or to be landfilled or combusted with energy recovery.	U.S. EPA, 2018
The United Nations Food and Agriculture Organization (FAO)	Decrease in the quantity or quality of food resulting from decisions and actions by food suppliers in the chain, excluding retailers, food service providers and consumers.	Decrease in the quantity or quality of food resulting from decisions and actions by retailers, food service providers and consumers.	FAO, 2018

Fruits and vegetables, due to the perishability of these items, have the highest percentage of agricultural production losses in comparison to all other commodities in developed countries (FAO, 2011). Many factors influence product loss in farming that lead to food that is either left unharvested or lost between harvest and distribution. There are some apparent reasons such as weather, pests, plant disease, and labor issues at the field level. However, other factors, like the current status of the market and food safety issues are rarely considered when evaluating product loss. Depending on the market value of products in fields at the time of harvest, retrieving all edible food from fields may not be economically viable for farmers (Dunning et al., 2019). For example, in 2008, the FDA issued a warning to consumers for potential *Salmonella* contamination of certain raw tomatoes, creating a decrease in market demand for all raw tomatoes. A study assessing the economic impact of Georgia tomatoes, not implicated in the outbreak, discovered that even though Georgia tomatoes were safe to consume 32% of tomato acreage went unharvested due to decreased demand (Flanders, 2008). Food products that present a safety hazard, such as human pathogen contamination, can be considered inedible. However, it is essential to include the products that are not necessarily contaminated but removed from the food supply, due to either excessive cautiousness or market responses, in quantifying food loss and waste.

Food loss and waste, no matter the source, has various negative socioeconomic and environmental impacts. Considering all consequences, the cost of food waste globally totals $2.6 trillion per year, with social and environmental costs accounting for $900 and $600 billion, respectively (FAO, 2014). Environmentally, food loss results in the inefficient use of not only the food item itself but also the labor, water, land, energy, agricultural chemicals, and any other inputs used farm to fork. In North America/Oceania, food that is wasted accounts for 35% of freshwater consumption, 31% of cropland, and 30% of fertilizer usage (Kummu et al. 2012). Food loss and waste also contribute directly to climate change. Sent to landfills where it is left to rot, decompose, and release

greenhouse gases into the atmosphere, this organic matter accounting for 16% of all U.S. methane emissions (EPA 2011).

Efforts are focused on bringing awareness to the food loss and waste issue the world faces today and providing approaches to reduce it. Current strategies focus on food redistribution, improving infrastructure, consumer education, new technologies, and market solutions. To comprehensively address food loss and waste, methods to better food safety practices to minimize the risk of contamination should also be considered. Food safety recalls send edible and uncontaminated food to the landfill; this fits all previously discussed definitions of food loss and waste. Fresh produce commodities are particularly problematic, with most commodities grown outdoors and left exposed to physical, chemical, and biological hazards. Aside from concerns at the field level, fresh produce is an undifferentiated product meaning all brands at the retail level are virtually identical to consumers. This makes food safety recalls involving these products even more complex, with products from multiple brands lost due to lengthy traceback investigations or a decrease in market demand overall.

1.2 Impact of Fresh Produce Recalls to Consumers/Industry

Every year there are roughly 3,000 deaths and 128,000 hospitalizations that occur due to foodborne illnesses (Scharff, 2012). While fruits and vegetables are among the healthiest of foods, they frequently act as vectors for foodborne illnesses. From 1998-2013, 972 raw produce outbreaks were reported and lead to 34,674 outbreak-associated illnesses, 2,315 hospitalizations, and 72 deaths (Bennett et al., 2018). This 1998-2013 study also demonstrated that raw produce outbreaks, in comparison to other foods, caused a notably higher number of hospitalizations and deaths. For all food products, particularly fresh produce, *Listeria monocytogenes,* Shiga toxin-producing *E. coli,* and *Salmonella*, are the leading sources for food product recalls due to bacterial pathogen contamination (Page, 2018). Fresh produce and associated foodborne illness outbreaks, along with a threat to public health, present a massive economic burden to the fresh produce industry. A basic model, including costs of hospital services, physician care, and

pharmaceutical costs and enhanced model with an added adjustment for quality of life costs, showed an annual expense of $51 and $78 billion, respectively. *L. monocytogenes* accounting for roughly two billion dollars in both models, pathogenic *E. coli* accounting for approximately 733 and 829 million dollars in the basic and enhanced models, and *Salmonella enterica* accounting for eight and 21 million dollars in the basic and enhanced models. (Scharff, 2012).

The process of recalling fresh produce creates a substantial economic burden on the food industry. Companies involved are responsible for the retrieval and destruction of implicated products, lawsuits and legal fees, and decreased consumer confidence and demand. Depending on the product type involved in large recalls or outbreaks, this event can negatively impact the entire industry or it can only effect the company involved while having positive effects on competitors due increased demand for alternative brands. Fresh produce is problematic because it is an undifferentiated product, this means all brands are essentially identical, making these products easily substitutable but also indistinguishable in the case of a recall or outbreak. So for fresh produce, it is not only the implicated company that experiences the economic burden of food safety recalls and outbreaks. In 2008 the FDA warned consumers to not eat a certain type of raw tomatoes due to potential *Salmonella* contamination. Even though this outbreak was later linked to jalapenos, the FDA's actions cost Florida growers $500 million, Georgia growers $8 million, and restaurant associations $100 million, this example of the economic burden of just a warning for potential contamination can present (Meyerson, 2009).

Another example of the financial burden of food safety issues is the *E. coli* 0157:H7 outbreak involving spinach in 2006. The spinach industry as a whole lost $205.8 million and suffered economically even a year after the outbreak, with spinach sales still down by 10% (Arnade et al., 2009). In this case, consumers avoided spinach altogether, unsure of what is "safe" and shifted to other leafy greens or salads that did not contain spinach. During this outbreak, contaminated spinach from a 2.8-acre plot in central California was able to sicken hundreds of people throughout the United States; which not

only negatively impacted the spinach industry but the fresh produce industry as a whole. Due to the overarching impact of foodborne illness outbreaks involving fresh produce, there is an economic incentive to improve safety standards for the entire industry. When it comes to an undifferentiated product such as fresh produce, it is in the best interest of all parties involved to promote and enforce food safety standards throughout the produce industry. In fact, as a response to the 2006 *E. coli* outbreak involving spinach, California farmers created the California Leafy Green Products Handler Marketing Agreement (LGMA), a commitment to protecting public health. LGMA's collaborative efforts with university and industry scientists, food safety experts, government officials, farmers, shippers, and processors formulated a food safety system. One that is science-based and focuses on reducing potential sources of contamination and assuring the safety of leafy greens grown in California.

Along with the private sector of the food industry, the U.S. government recognizes the adverse socio-economic effects of food safety recalls and has invested increasing amounts of funding, time, and resources in preventing food safety issues. In 2011 the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) was signed into law. FSMA requires a food safety plan, including a hazard analysis, critical limits, monitoring procedures based on scientific evidence, with an overall goal of preventing contamination rather than reacting to it. FSMA updates the FDA's authority to regulate food and better protect public health by granting the FDA: the power to refuse entry of any food into the United States, issue mandatory recalls, and suspend the registration of facilities if their food poses a health risk. With the Produce Safety Rule, the FDA expanded on food safety regulations, providing science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption.

The public health impact on consumers and economic impact on the private and public sectors of the food industry have been driving forces in improving food safety practices. However, food safety issues also generate food waste, leading to various

negative social and environmental impacts that currently cost the world trillions of dollars. If these costs were to be quantified and combined with the economic impact of food safety recalls and outbreaks, it would provide even more of an incentive to improve food safety practices.

1.3 Sources of Contamination along the farm-to-fork continuum

Each step along the farm-to-fork continuum for fresh produce poses a risk for human pathogen contamination and proliferation. Thus, if the product is contaminated early on, there is potential for the pathogens to multiply before making it to the consumer's home, resulting in a higher risk for illness. The contamination of produce depends on a variety of interactions between the product itself, along with its natural microflora, the pathogenic microorganism, and the environment (Solomon, 2009). For pathogens to infect consumers, they must successfully attach, survive, and in some instances grow on fresh produce. The likelihood of this depending on various factors including, temperature, nutrient availability, and interaction with indigenous microflora (Harris et al., 2003; Mandrell, 2009).

1.3.1 Pre-Harvest Contamination

Many produce associated outbreaks are due to contamination at the field level, during growing and harvesting steps. In the field, produce is left exposed and relatively unprotected, without cover or continuous surveillance. Agricultural fields may experience weather fluctuations, for example, high water flow resulting in flooding can transport pathogens over 30 km (Cooley, 2007). The intrusion and fecal shedding by wildlife is also a source of enteric pathogens, one that is random and hard to control. For example, while there was no definitive determination as to the origin of *E. coli* O157:H7 during the 2006 spinach outbreak, the final report identified the presence of feral pigs in and around spinach fields and water sources as a potential risk factor (California Food Emergency Response Team, 2007). Foodborne pathogens such as *L. monocytogenes*, *E. coli* 0157:H7 and *Salmonella* have been shown to both survive and grow in mediums such as water and soil (Alegbeleye et al., 2018; Doyle & Erickson, 2008). So the feces do

not need to come in direct contact with the edible portions in plant to cause illness it can indirectly transfer enteric pathogens to water and soil resulting is subsequent contamination.

In the United States, around 80% of water use goes to agriculture (USDA, 2019). Sources of water for agricultural use, varying in their microbial risk, include groundwater, surface water, and reclaimed wastewater. Groundwater is held underground in aquifers beneath the soil; this water is protected from the outside environment, thus highest in microbiological quality. Surface water is any body of water collected on the surface of the ground, for example, ponds, lakes, and rivers. In places where water is limited, reclaimed wastewater is used for agricultural production after treatment at a wastewater facility. Out of these options for irrigation water, groundwater is most often of the highest microbiological quality unless contaminated with surface water, which is variable in terms of quality. While wastewater has poor quality initially, it goes through an extensive treatment before applied for irrigation (Steele & Odumeru, 2004). There are also various methods of irrigation, including overhead sprinklers, subsurface drips, and furrows. In sprinkler irrigation, water comes in direct contact with edible portions of plants and is of the highest risk for pathogen contamination (Uyttendaele et al., 2015). Studies have also shown that methods of irrigation, or rainfall events, can result in splash transfer of enteric pathogens if the soil is contaminated (Lee et al., 2019; Doyle & Erickson, 2008).

Contaminated soil, via improperly treated soil amendments, is especially of high risk when it comes to crops that are grown in close proximity to the ground, including root crops or leafy green vegetables. During the growing process of these products, edible portions may come in direct contact with soil. If soil is contaminated, plants have shown the ability to internalize enteric pathogens via seedlings and roots (Kutter et al., 2006; Franz et al., 2007; Doyle & Erickson, 2008). A soil amendment is any chemical, biological, or physical matter that is intentionally added to agricultural soils to improve its condition in terms of plant growth and water-holding capacity (PennState Extension, 2019). Whenever soil amendments are applied, it is critical to consider the type of

amendment and the risk it may present to a growing operation. Biological amendments, specifically from animal origin, can present the highest microbial risk if treated improperly by introducing enteric pathogens to agricultural soil where they can survive for extended periods (Jiang et al., 2002; Kim et al., 2009). As mentioned previously, enteric pathogens on fresh produce often originate from animal feces. The presence of human pathogens like *E. coli* 0157:H7 and *Salmonella* is a result of fecal shedding from animals like cattle, poultry, swine, and sheep. However, animal health is not affected by these pathogens in their gastrointestinal tract; therefore, it is difficult to recognize their presence. In the case that agricultural soil becomes contaminated, currently there are no methods to effectively eliminate pathogen presence. This is why methods to treat contaminated soil while also keeping soil health intact need to be explored.

1.3.2 Harvest & Post-Harvest Contamination

Conditions on the surface of undamaged fresh vegetables are not favorable for microbial survival and growth; pathogens do not have enough nutrients to grow or enzymes to break down commodities epidermal wall. However, the survival of these pathogens on plant material significantly increases once physical damage breaks the protective epidermal barrier. Harvest marks the beginning of the lengthy process that brings fresh produce from the fields to the consumer's table, a process that alters the overall physiological state of the product. During harvest, produce is cut from the ground, breaking that protective epidermal barrier and releasing nutrients that can potentially promote the growth of pathogens. Harvesting fresh produce also involves human handling and harvesting equipment, which, if not properly sanitized, may also harbor and transfer human pathogens to the products. The items harvested are also subject to temperature abuse. Commodities are harvested in sections and are not transferred to refrigerated temperatures until the entire section is harvested, up to 90 minutes (Gil et al., 2015).

Following harvest, for minimally processed or fresh-cut vegetables, is the processing step. As previously mentioned, the source of enteric pathogens like *E.*

coli 0157:H7 and *Salmonella* is fecal shedding from animals; therefore, it is critical to avoid cross-contamination if tainted product is brought in from the field. Before and throughout processing, produce is washed and rinsed with water, serving as a source for cross-contamination if not replaced frequently and treated with sanitizing agents properly. In the early 1990s two outbreaks involving *Salmonella* spp. on raw tomatoes, resulting in a total of 258 salmonellosis cases, was determined to be due to contamination of the water bath used by the South Carolina packer (Hedberg et al., 1999). For some fresh produce items, processing includes peeling, cutting, slicing, or shredding, further injuring the protective epidermal barrier and providing an environment more suitable for microbial growth. The machinery used to process the fresh vegetables, if not sanitized correctly, can harbor pathogens and create biofilms on equipment resulting in a source for cross-contamination (Moore et al. 2003; Ryu et al. 2004). During processing, ensuring that wash water and any machinery the product may come in contact with remains sanitary is of the utmost importance to decrease cross-contamination risk.

Once fresh produce is appropriately packaged and stored at refrigerated temperatures, the risk for contamination dramatically decreases. However, during storage, transportation, and distribution the final products are subjected to further physical abuse and fluctuation in temperatures that may promote the microbial growth if the product was contaminated before packaging (Zeng et al., 2014; Mishra et al. 2017; Beuchat, 1996). For human pathogens like *Salmonella* and *E. coli* O157:H7, as little as ten cells is needed to cause infection, therefore, the pathogen if present only needs to survive on produce to be of risk to consumer health (FDA 2012). For these two human pathogens, it is crucial to seek and destroy any contaminated product. However, when it comes to pathogens such as *L. monocytogenes*, with an infective dose likely to be fewer than 1,000 cells, temperature control and availability of nutrients is critical in preventing illness (FDA 2012). Studies have shown that the growth of *L. monocytogenes* is dependent on both temperature and food matrix. Therefore, it is imperative to understand the growth patterns of pathogens like *L. monocytogenes* on a commodity to commodity

basis to reduce the risk for consumers (Carlin & Nguyen, 2008; Lokerse et al., 2016; Li et al., 2002).

1.4 Foodborne pathogens

Foodborne illness and outbreaks have occurred for decades and remains a challenge for consumers, academia, government, and industry. From 2004 to 2017, there were 3,576 foodborne illness outbreaks with a confirmed food vehicle and etiology in the U.S, 11% (391) of these outbreaks associated with fresh produce (Carstens et al., 2019). To provide a safer fresh produce supply, it is important to understand the pathogens commonly associated with foodborne illness as a result of consuming these products. From 1998-2013, *L. monocytogenes*, *E. coli* 0157:H7 and *Salmonella* accounted for 1%, 10%, and 21% of raw produce outbreaks. Of fresh produce recalled during 2004-2013, 91.9% were due to pathogen contamination with *L. monocytogenes, E. coli 0157:H7*, and *Salmonella,* suggesting that fresh produce recalls pose severe hazards to consumer health (Page, 2018). *Recalls for fresh produce items are frequently posted by the FDA, and according to FDA's enforcement reports, there were over 500 recalls involving fresh produce items due to potential contamination with L. monocytogenes, E. coli*, and *Salmonella* during 2015-2018 (FDA, 2020b).

1.4.1 L. monocytogenes

L. monocytogenes is a gram-positive, rod-shaped, non-spore forming, facultative bacteria. It is also one of the leading causes of death from foodborne illness, leading to about 260 deaths a year (CDC, 2020e). It is ubiquitous in the environment and can grow in extreme environmental conditions, including low pH and temperatures below 1°C (FDA 2012). It has 13 serotypes, of these 1/2a, 1/2b, and 4b linked to a majority of foodborne illnesses. *L. monocytogenes* can cause Listeriosis, which can be either non-invasive or invasive. The non-invasive form, after a short incubation period of a few hours to two or three days, may lead to typical symptoms such as nausea, vomiting, and diarrhea. The invasive form has a long incubation period of three days to three months, can lead to septicemia or meningitis, and has an overall fatality rate of 15% to 30% (FDA 2012). As

previously stated, *L. monocytogenes* accounted for only 1% of raw produce outbreaks throughout 1998-2013; however, due to its high fatality rate, it accounted for 54% of all related deaths. The infective dose is not well understood and varies depending on *L. monocytogenes* serotype and the host infected. Currently in place is an FDA compliance policy for the control of *L. monocytogenes*; for ready-to-eat foods that do not support the growth of *L. monocytogenes* the tolerance level is 100 CFU/g and for food products that do support the growth of *L. monocytogenes* there is a zero-tolerance policy (FDA, 2008).

This organism's ability to grow under refrigerated conditions and persist in food-manufacturing environments creates concern to the produce industry as a potential risk of foodborne illness. Contamination with *L. monocytogenes* can occur at any point along the supply chain, but many outbreaks and recalls are due to *L. monocytogenes* presence in packinghouses (Gaul et al., 2013; CDC, 2015; CDC, 2015d; USDA, 2019)*. L. monocytogenes* contamination has more recently been an issue with minimally processed items like bagged salads, with the FDA reporting thirteen salad item recalls due to potential *L. monocytogenes* contamination in 2019 alone (FDA, 2020a). To better understand the threat *L. monocytogenes* poses to consumer health there have been various studies assessing the ability of *L. monocytogenes* to grow on traditional bagged salad ingredients including various lettuces and spinach (Carlin et al., 1994, Farber et al., 1998, Lorkerse et al., 2015, Jacxsens et al.,1999).

Studies assessing the microbiological quality of fresh produce items around the world have shown the presence of *L. monocytogenes* on ready-to-eat items (Zhu et al., 2017). In the U.S. specifically, a study found that a variety of produce items obtained at the retail level were contaminated with a variety of *L. spp., including L. monocytogenes* (Thunberg et al., 2001). Out of the produce obtained various produce types tested positive for *L.* spp., celery (25%), 50% of lettuce (50%), sprouts (41%), 50% of potato (50%), field cress (36%), and watercress (18%). However, out of these sample, *L. monocytogenes* was only confirmed in 2 /4 field cress and 4/4 potato samples that tested positive for *L.* spp. Research conducted by Lianou & Sofos (2007) also demonstrated the

prevalence of *L. monocytogenes* in various produce items found in super markets, restaurants, production sites and retail stores around the world. *L.* spp. are widespread in nature, and therefore commonly found on fresh produce. While most species of *L.* are harmless, the potential for *L. monocytogenes* on ready to eat produce items can be hazardous to public health. *While L. monocytogenes* has not been involved in any recent outbreaks involving fresh produce, during 2010-2016 outbreaks involving *L. monocytogenes* and various produce items have occurred throughout the U.S., sickening over 200 people and resulted in the deaths of 71 people, a mortality rate of about 32 percent (Table 2).

Table 2 *L. monocytogenes* outbreaks 2010 - 2016 involving produce items.

Year	Brand Name	Product	Persons Infected	Hospitalizations	Deaths	Source
2010	Sangar Fresh Cut Produce	Diced Celery	10	10	5	Gaul et al., 2013
2011	Jensen Farms	Whole Cantaloupes	147	143	33	CDC, 2012e
2014	Wholesome Soy Products Inc.	Mung Bean Sprouts	5	5	2	CDC, 2015c
2015	Bidart Bros.	Prepackaged Caramel Apples	35	34	7	CDC, 2015d
2015	Dole	Packaged Salads	19	19	1	CDC, 2016d
2016	CRF Frozen Foods	Frozen Vegetables	9	9	3	CDC, 2016f

1.4.2 *Escherichia coli* O157:H7

E. coli O157:H7 is a Gram-negative, rod-shaped, non-spore forming facultative bacteria. This strain falls into a subset of toxin-producing Shiga-toxigenic *E. coli* (STEC) called enterohemorrhagic *E. coli* (EHEC) (FDA, 2012). There are 200-400 STEC serotypes that are characterized by Shiga toxin production, and the subset EHEC includes the serotypes that lead to severe illnesses. Annually, there are 63,153 and 112,752 foodborne illnesses acquired in the U.S. caused by EHEC O157 and EHEC non-O157, respectively (Scallan et al., 2011). These non-O157 EHEC serotypes, commonly referred to as the "big 6" (O111, O26, O121, O103, O145, O45), are also of public health concern; however, the O157:H7 strain is responsible for a majority of all EHEC infections (FDA, 2012).

It takes as little as 10-100 cells to become infected with *E. coli* O157:H7 and experience symptoms such as severe abdominal cramps and bloody diarrhea (hemorrhagic colitis), which will typically occur three to four days after exposure. About three to seven percent of hemorrhagic colitis cases lead to hemolytic uremic syndrome (HUS), which has a mortality rate of three to five percent (FDA 2012). *E. coli* O157:H7 is found in the intestinal tract of humans and warm-blooded animals, and fecal shedding into water and soil used for agricultural purposes can provide a vector for human pathogens to fresh produce. For example, the *E. coli* O157:H7 strain connected a multistate spinach outbreak in 2006 was isolated from many different possible sources; soil (two samples), river water (two samples), cattle feces (15 samples), and wild pig feces (seven samples) (California Food Emergency Response Team, 2007).

Due to the previously mentioned 2006 outbreak involving spinach, *E. coli* became a significant concern in the fresh produce industry. This outbreak involving bagged spinach left 199 people ill, lead to 102 hospitalizations, 31 developing HUS, and three deaths (CDC, 2006). In the years following this outbreak, EHEC *E. coli* strains continue to be a source of foodborne illness associated with fresh produce (Table 3). Since 2006, the CDC has reported 14 multistate foodborne illness outbreaks involving pathogenic *E.*

coli and fresh produce, leading to 916 infections, 406 hospitalizations, 88 developments of HUS, and 9 deaths. Most frequently implicated in these outbreaks is romaine lettuce, accounting for over half of all infections, hospitalizations, developments of HUS, and deaths. Out of the strains involved in these outbreaks, O157 was the most common and the source of 87% of all illnesses, 93% of hospitalizations, 97% of developments of HUS, and was responsible for all deaths.

Table 3 *E. coli* multistate outbreak information involving fresh produce 2006-2020

ar	Brand Name	Product	*E. coli* Strain	Persons Infected	Hospitalizations	Development of HUS	Deaths	Source
06	Dole	Fresh Spinach	O157:H7	199	102	31	3	CDC, 20
10	Freshway Foods	Shredded Romaine Lettuce	O154	30	12	3	0	CDC, 201
12	Schnuck's	Romaine Lettuce	O157:H7	49	33	3	0	CDC, 201
12	Jimmy John's LLC	Raw Clover Sprouts	O26	29	7	0	0	CDC, 201
12	State Garden	Organic Spinach and Spring Mix	O157:H7	33	13	2	0	CDC, 201
13	Glass Onion Catering	Ready-to-Eat Salads	O157:H7	33	7	2	0	CDC, 201
14	Evergreen Fresh Sprouts, LLC	Raw Clover Sprouts	O121	19	9	0	0	CDC, 20
16	Jack & the Green Sprouts	Alfalfa Sprouts	O157	11	2	0	0	CDC, 201
17	Unknown	Leafy Greens	O157:H7	25	9	2	1	CDC, 201
18	Unknown - Yuma, AZ growing region	Romaine Lettuce	O157:H7	210	96	27	5	CDC, 201
18	Adam Bros. Farming	Romaine Lettuce	O157:H7	62	25	2	0	CDC, 20
19	Unknown - Salinas Valley, CA Growing Region	Romaine Lettuce	O157:H7	167	85	15	0	CDC, 202
19	Fresh Express	Sunflower Crisp Chopped Salad Kits	O157:H7	10	4	1	0	CDC, 202
20	Jimmy John's LLC	Clover Sprouts	O103	39	2	0	0	CDC, 202

1.4.3 *Salmonella* species (spp.)

Salmonella, widely dispersed in nature, originates from livestock, wildlife, domestic pets, humans, pond water sediments, and insects (FDA 2012). It is a gram-negative, rod-shaped, non-spore forming, facultative bacteria. Two species can cause illness in humans, *Salmonella enterica* and *Salmonella bongori*. The two species of *Salmonella* can cause nontyphoidal salmonellosis or typhoid fever. Nontyphoidal salmonellosis has an infective dose as low as one cell, and onset time of 6-72 hours following exposure. Symptoms include nausea, vomiting, abdominal cramps, diarrhea, fever and headache, lasting four to seven days. Typhoid fever, caused by serotypes *S. Typhi* and *S. Paratyphi*, causes a high fever, lethargy, gastrointestinal symptoms, headache, achiness, loss of appetite, rashes, and if left untreated has a mortality rate as high as ten percent.

Historically, *Salmonella* illnesses were associated with foods of animal origin such as meat, poultry, eggs, and dairy products. However, fresh produce has been a source of *Salmonella* illnesses, which may be due to cross-contamination from wildlife, water, soil, or other environmental factors (FDA, 2012). The potential of soil as a vector of contamination of fresh produce, has been shown using tomatoes and spinach plants (Guo et al., 2016, Arthurson et al., 2011). An alternative study investigating the ability of *Salmonella* to persist in soil detected *Salmonella* 332 and 405 days after inoculation in sterilized and non-sterilized soils (You et al., 2006). The long term survival of *Salmonella* in soil and its ability to transfer from soil to produce indicates a potential risk of environmental spread and transmission to ready to eat produce.

While no major outbreaks involving *Salmonella* on leafy green items have occurred, raw sprouts and fruits are often implicated in *Salmonella* outbreaks. The CDC reported seven multistate outbreaks of *Salmonella*, leading to about 600 infections linked to sprouts during 2009-2018 (CDC, 2009; CDC, 2010b; CDC, 2011; CDC, 2015a; CDC, 2016a; CDC 2016b; CDC, 2018a). Since 2013 there have been three major *Salmonella* outbreaks involving cucumbers. Collectively, these outbreaks resulted in 1,266 illnesses, 269 hospitalizations, and seven deaths (Angelo et al., 2014; CDC, 2013b; CDC, 2016e). Melons are also frequently implicated in foodborne illness outbreaks involving *Salmonella*; in 2006, there were 12 outbreaks involving

melons (CDC 2019). A majority of these outbreaks involved cantaloupe; in 2012, the largest outbreak of salmonellosis linked to cantaloupe occurred and resulted in 261 illnesses, 94 hospitalizations, and three deaths (CDC, 2012d). Other fruits, such as papayas and mangoes, have been implicated in *Salmonella* outbreaks (CDC, 2019).

1.5 Control of pathogens – RTE Vegetables

Fresh produce is grown outside; therefore, it is challenging to keep it completely protected from microbial contamination at the field level. Besides growing conditions, fresh produce items, unlike products such as meat, poultry, or dairy, do not undergo a thermal process otherwise known as a kill-step to significantly minimize the risk of contamination. The food industry has developed methods to chemically and physically reduce microbial loads fresh produce items without altering the organoleptic properties, including the use of chlorine, irradiation, chlorine dioxide, ozone, electrostatic sprays. pulsed light, etc.; however, the result of these methods is nowhere near as effective as thermal processing (Goodburn and Wallace, 2013). Considering the fact that the fresh produce industry lacks an effective kill-step, most of the focus in terms of food safety is set on methods to prevent contamination from occurring. In 2011 the Food Safety Modernization Act (FSMA) was signed into law. It mandates the establishment and implementation of a food safety system that includes a hazard analysis and risk-based preventive controls customized for each facility. Under FSMA, the Produce Safety Rule, for the first time, establishes regulations at the field level, with standards for growing, harvesting, packing, and holding fruits, vegetables, mushrooms, and sprouts intended for human consumption (Laborde, 2018). While sources of contamination vary depending on the commodity, there are well-known pre-harvest and post-harvest sources in which similar techniques are used throughout the fresh produce industry to control the risk of human pathogen contamination.

1.5.1 Pre-Harvest Controls

Fresh produce is typically grown and harvested outdoors, exposed to all elements of the uncontrolled environment. Producers must understand and identify steps along with their processing scheme that may introduce, control, or eliminate human pathogens. While growing and harvesting operations vary commodity to commodity, there are a few strategies to minimize

the potential risk of contamination that most fresh produce operations have in common. Before planting fields, the grower needs to become familiar with the location, its land history, and its proximity to areas that may pose a safety risk, such as urban areas and animal operations. When doing a hazard analysis, topographical features must be considered. If topographical maps show that the growing area may be in a hazardous location, biological and physical buffers, such as appropriate distance, mounds, vegetation, and ditches, can be set in place to minimize the risk of pathogen transfer. These buffer zones, along with other methods like removing attractants, scarecrows, and reflective strips can also be used as effective methods to reduce animal activity (Gil et al., 2015).

The FSMA Produce Safety Rule covers biological soil amendments of animal origin. Mandating that soil amendments used for agricultural production must be treated to meet microbial standards, which can be achieved by using a scientifically validated process (CFR 112.55 a & b). Due to sporadic fecal shedding of these enteric pathogens by animal's research efforts have been focused on understanding the effects of environment, diet, and age of ruminants to determine patterns in fecal shedding (Hancock et al., 1997; Harmon et al. 199: Buchko et al., 2000). Although all manures can carry enteric pathogens, it is often used as a soil amendment because it provides many benefits to agricultural soil. Soil amendments are a very cost-effective method to increase soil health, fertility, and water holding capacity, provide nutrients, and manage waste. If treated effectively, the risk of using this nutrient-dense soil amendment decreases. Methods to effectively eliminate pathogenic bacteria from manures have been provided through repeated experimentation (Martens, W & R Böhm. 2009; Weil et al., 2013; Eamens et al., 2001).

Water is essential in growing fresh produce, and thus the quality of water used is an important measure for indicating the risk of produce contamination at the field level. There are various sources of water used for agricultural production surface water, groundwater, or reclaimed water, and the source used for irrigation is often chosen based on availability and cost. Whether the source of water used for agricultural production is from surface water, groundwater, or reclaimed water, it is crucial to protect these sources from pathogen contamination. In the past

generic *E. coli* has been used to indicate pathogen presence in water, however, many studies assessing generic *E. coli* as an indicator for human pathogens in agricultural water have found it to be unreliable (Benjamin et al., 2013; Shelton et al., 2011; Truitt et al., 2018). Therefore, growers must consider alternatives to reducing microbial risk like treating water chemically or physically before use, for example with chlorine, or avoid contact of irrigation water with edible portions of plants (Allende & Monaghan, 2015).

1.5.2 Harvest & Post-Harvest Controls

With fresh produce items like heads of lettuce or romaine, there is no processing step; items are packaged in the fields, cooled, sometimes undergoing test-and-hold for human pathogens, and sent directly to customers. However, for minimally processed items, the risk increases with additional exposure to human contact, wash waters, and physical damage; all having the potential of introducing pathogenic bacteria to the product or potentially increasing the growth potential of these bacteria if the product was previously exposed (Brackett, 1999). The processing environment presents many opportunities for cross contamination, so it is imperative to ensure programs like GMPs and SSOPs are set in place to prevent this from occurring. With multiple products from various regions coming in and out of a processing facility, it is essential to keep the entire processing facility clean and sanitized, especially any surfaces or machinery product comes into contact with, to minimize microbial risk of the final product.

During the processing of ready-to-eat items, washing steps are critical in removing debris and washing produce to improve the final product's microbiological safety. The type of water used during processing and proper management of this water is very important. Post-harvest water is anything that touches produce itself, food contact surfaces, used to make ice or is used for handwashing. Any water used during processing must be potable, meeting the standard of no detectable generic *E. coli* based on a 100 ml sample (CFR 112.151 a & b). Disinfecting agents, like chlorine, which is most widely used, are added to processing water strictly monitored and frequently refreshed. However, the primary use for these sanitizers is not to decontaminate products but rather maintain the microbiological quality of the wash water (Gil et al, 2009).

Therefore, while sanitizers may decrease pathogen populations on fresh produce items, fresh produce processing still lacks an effective kill-step.

In the fresh produce industry prevention is key, if contaminated product is packaged and distributed various factors along the remainder of the farm to fork continuum, including storage temperature, relative humidity, gaseous composition of the atmosphere, nutrient availability, and presence of competitive bacteria or antimicrobial compounds will determine the fate of the surviving pathogens (Doyle and Erickson, 2008). Of these factors, one that the produce industry is well aware of and many efforts go into monitoring is the temperature during storage and transport, maintaining optimality at for fresh produce at 0°C - 5°C (Suslow et al., 2003). Produce is available year-round, depending on season and region, produce can travel great distances to make it to the shelf at the local supermarket. In industry, recording thermometers are frequently used to ensure temperatures are within this optimal range, placed wherever produce is stored or transported, with alarms installed to alert proper personnel if the temperature deviates from this range.

This section highlights the lack of a kill-step in the fresh produce industry and the industries' dependence on preventative measures to ensure consumers' safety. To minimize the contamination of fresh produce, specifically enteric pathogens, mitigation strategies need to be implemented at the field level, which is the source of these contaminants. Providing a safe fresh produce supply starts with the microbiological quality of the agricultural soil in which these plants are grown, and efforts should be focused on identifying approaches to ensure this soil is free of human pathogens that may present a risk to consumers.

1.6 Biofumigation

Cover crops are widely used in farming to promote overall soil health by increasing organic matter and enhancing the biodiversity of the soil microbiome, preventing disease, along with a variety of other benefits. Biofumigation involves the utilization of *Brassica* species as cover crops to control pests, soil-borne disease, and weed management. This biofumigation process can be carried out in a few ways, using *Brassica* species as rotation crops or intercrops, the inclusion of freshly macerated plant material (also referred to as “green manure”) into the soil, or

by the use of processed plant products such as seed meals. There is a plethora of advantages to biofumigation, the improvement of physical and biological soil characteristics, improved soil microbial communities, increased infiltration rate and water holding capacity, reduced wind erosion, nitrogen preservation, and reduced soil compaction (Reddy, 2013). Research on the fumigant properties of the volatile plant chemicals produced during this process has also been extensively studied, revealing its effectiveness in suppressing plant diseases, nematodes, weeds, and insects (Reddy, 2013; Matthiessen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009).

Today, symptoms of soil-borne diseases caused by plant pathogens are a considerable threat to crop production, with an estimated economic loss of 50-75% of the potential yield for many crops (Mokhtar & El-Mougy, 2014). For decades the volatile products of the biofumigation process have been used to control soil-borne plant pathogens. In soils, glucosinolate hydrolysis products can work to control harmful plant pathogens directly, or indirectly by creating an environment that is advantageous to beneficial organisms to create competition. The fungicidal properties of biofumigation have been identified since the 1930s, and have been well studied since then (Walker et al., 1937). The products of Biofumigation have successfully reduced populations of parasitic nematodes and suppress various problematic fungal plant pathogens including, *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., and *Botrytis* spp (Reddy, 2013). However, the literature on biofumigation's efficacy in suppressing bacterial plant pathogens in soils is limited.

Bacterial plant pathogens, while much less common than fungal plant pathogens, can still cause many severe diseases in plants and create an economic burden to the industry (Kennedy & Alcorn, 1980). One that remains problematic is the aerobic and gram-negative bacterium, very similar to *Pseudomonas* spp., called *Ralstonia solanacearum*. This bacterium possesses one of the most generous known host ranges for plant pathogens, with the ability to cause bacterial wilt in about 200 plant species in 33 different plant families (Moorman, 2011). Field experiments using biofumigation with mustard were conducted in Australia during 2003 and 2005; the results of this experiment indicated a reduction in bacterial wilt of tomatoes from 80 to 15% (Kirkegarrd, 2007). Another study in the Philippines showed that incorporating *Brassica* plant matter into soils,

reduced *R. solanacearum* populations in soil 6-15 fold three weeks after incorporation in comparison to control fields (Bayot et al., 2004).

Streptomyces spp., gram-positive bacteria, make up approximately 10% of total soil microbial communities and have been identified as biocontrol agents of plant diseases (Janssen, 2006; Schrey & Tarkka, 2008). Studies have shown that biofumigation processes often increase overall *Streptomyces* spp. and this increase is also considered to play a role in reducing populations of harmful bacteria. Generally, *Streptomyces* spp. are ubiquitous and beneficial in soils; however, there are a few plant pathogenic organisms within the genus (Seipke et al., 2011). One of these being *S. scabiei*, while it not a threat to human health, can directly affect the market value of the product infected and create economic losses in the industry (Hill & Lazarovits, 2005). A study conducted in northern Maine investigated the ability of *Brassica* green manures to control soil-borne potato diseases, one being common scab caused by the gram-positive bacteria *S. scabiei*. This study reported that the use of Indian mustard green manure significantly reduced the incidence and severity of common scab by 25% (Larkin & Griffin, 2007).

It is unclear whether the suppression of these bacteria is directly due to the biologically active compounds released during biofumigation or a secondary effect due to changes in the soil microbial communities. Despite the unknown antimicrobial mode of action, the available literature supports the ability of the biofumigation process to reduce both gram negative and positive pathogenic bacteria populations in soil and their associated disease in plants. The success of these few trials, using biofumigation processes against plant pathogens, indicates the potential for use against human pathogens in soil.

1.7 Glucosinolate containing *Brassica* species

Glucosinolates are b-thioglucoside N-hydroxysulfates with sulfur linked b-D-glucopyranose moiety. There are various types of glucosinolates, which are characterized by a side chain (R). Plant families of the order Capparales, including Tovariaceae, Resedeceae, Capparacea, Moringacea, and Brassicaceae, contain glucosinolates. Due to the economic and agricultural importance of Brassicaceae family, many scientific efforts have been made to understand the effects of their glucosinolate hydrolysis products. The Brassicaceae or Cruciferae

family consists of around 3000 species; of these species hundreds have been investigated and all contain glucosinolates, many commonly incorporated into human diets (Table 1). Many studies have shown the glucosinolate hydrolysis products in cruciferous vegetables possess pesticidal and fumigant effects in soil (Reddy, 2013; Matthiessen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009).

Table 4 Average glucosinolate contents in mg per 100 g of plant matter in commonly known *Brassica* spp.

Common name	**Scientific name**	**Average Glucosinolate (mg/100g)**
Brown Mustard	*Brassica juncea*	4,660
Black Mustard	*Brassica nigra*	4,630
Brussels sprouts	*Brassica oleracea var. gemmifera*	237
Kale	*Brassica oleracea*	108
Turnip	*Brassica campestris*	93
Broccoli	*Brassica oleracea var. italica*	62
Cabbage	*Brassica oleracea var. capitata*	59
Cauliflower	*Brassica oleracea var. botrytis*	43

Source: Bheemreddy & Jeffery, 2006, Ciska et al., 2000

Glucosinolates are secondary metabolites stored in plant tissues, and when intact they do not possess toxicity. Only the biologically active products, produced by glucosinolate hydrolysis or degradation, possess toxicity. It is assumed that the major role of glucosinolates in plant tissue is responses to the external environment and plant defense (Singh, 2017; Bennett & Wallsgrove, 1994). Upon disruption of plant tissues, glucosinolate hydrolysis occurs due to the enzymatic degradation by myrosinase, in the presence of water. Myrosinase, or b--thioglucoside glucohydrolase, an enzyme that is also naturally occurring in plant tissue, is stored in myrosin grains separate from glucosinolates. In plant tissue, interaction between glucosinolates and myrosinase only occurs when cells are damaged. Once myrosinase and glucosinolates are exposed, myrosinase cleaves the sulfur-glucose bond yielding further degradation products alcohols, aldehydes, isothiocyanates, and nitriles. Alternatively, Brassicaceous seeds can be cold-pressed to create seed meal; Brassicaceous seed meals myrosinase and glucosinolates are

preserved and stored together, so all that is needed to activate hydrolysis and formation of enzymatic degradation products is the addition of water. This natural defense system has been exploited to manage plant diseases, nematodes, weeds, and insects through a process known as biofumigation (Reddy, 2013).

1.8 Allyl Isothiocyanate (AITC)

Within glucosinolates found in the *Brassica* species, aliphatic, 3-methylthioalkyl, aromatic and heterocyclic (indole) glucosinolates are of the most commonly studied and understood (Fahey et al., 2001). The three major products of glucosinolate hydrolysis are thiocyanates, nitriles and isothiocyanates. Out of these main byproducts, isothiocyanates are the major inhibitors of microbial activity, and therefore have been extensively studied in comparison to thiocyanates and nitriles. One isothiocyanate in particular, AITC, which is produced by the degradation of the aliphatic glucosinolate prominent in *Brassica* species called sinigrin. AITC when sourced from nature, for example cruciferous vegetables, is “Generally Recognized as Safe” (GRAS) by the FDA (21 CFR 172.S15). While the use of AITC is permitted in food for direct human consumption as a flavoring additive, its use as an antimicrobial is not. This may be due to, in part, that AITC’s antimicrobial mode of action is not well understood. While several studies have investigated AITC’s antimicrobial mode of action against human pathogens in various mediums, a single process has not been identified.

1.8.1 Antimicrobial Activity

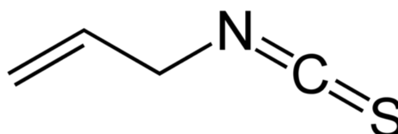

Figure 2 AITC chemical structure

While generally methods of the antimicrobial mode of action vary compound to compound; it may depend on their structural configuration (Gyawali & Ibrahim 2014). AITC contains three double bonds (Figure 2), which may be responsible for its antimicrobial activity

(Dufour et al., 2015). The effectiveness of AITC against bacteria can also be altered, Olaimat & Holley (2013) showed that changes in pH, temperature, and bacterial strain influenced AITC antimicrobial effectiveness in Mueller-Hinton broth. AITC was most effective at neutral pH against *L. monocytogenes, reducing counts by* by 4.14 and 8.45 log_{10} CFU/ml and at acidic pH against *Salmonella* reducing counts by 2.56 and 6.48 log_{10} CFU/ml at 4°C and 10°C respectively. There are various theories as to AITC's antimicrobial mode of action; however, the specific process is unknown. The inhibition of cellular metabolic reactions, damage to the cell membrane, disulfide bond oxidative cleavage in cysteine residues, and reaction with terminal amino groups have all been observed (Lin et al., 2000; Ahn et al., 2001; Luciano & Holley, 2009). AITC has showed to possess antimicrobial effects against both gram-negative and gram-positive bacteria, however the sensitivity of bacterium to AITC is thought to be dependent on strain rather than Gram type (Delaquis & Mazza, 1995).

The idea that bacterial sensitivity to isothiocyanates is dependent on strain is further supported, by a study examining the antimicrobial activity of 10 isothiocyanates and 14 strains of bacteria in culture broth (Wilson et al., 2013). Conclusions were determined by calculating an average antibacterial efficacy index, based on observations of growth delay, reduction in maximum growth rate, and reduction in population size with addition of ITCs. The bacteria studied included *E. coli, Salmonella*, and *L. monocytogenes* strains, and while Gram negative bacteria seemed to be more sensitive overall, AITC sensitivity varied within Gram type. For example, the average antibacterial efficacy index of AITC against the gram negative bacteria *E. coli* and *Salmonella* were 1.3 and 3.8, respectively. While both were considerably higher than the antibacterial efficacy against *L. monocytogenes* (0.4), AITC's antibacterial efficacy was approximately three times more effective against *Salmonella* in comparison to *E. coli*. Borges et al. (2015) assessed the antibacterial activity of AITC against *E. coli* and *L. monocytogenes* in Mueller-Hinton Broth. AITC showed a strong antimicrobial potential against the two bacterium, with minimum inhibitory concentration (MIC) of 100 µg/ml against bacterium and a minimum bactericidal concentration (MBC) of 1,000 and >1,000 µg/ml for *E. coli* and *L. monocytogenes*. This study also explored AITCs antibacterial mode of action by assessing membrane integrity,

intracellular potassium release, physiochemical surface properties and surface charge. The similarities in destruction of cell membranes by AITC demonstrated that the presence of an outer membrane in Gram-negative bacteria did not increase its resistance to AITC. By monitoring hydrophobicity and charge of the bacterial surface, this study showed that regardless of gram type AITC interacts with the bacterial cell surface, changing electrostatic potential and hydrophobicity, thus disrupting cell membrane integrity.

The antibacterial mechanism of AITC, was also investigated against *L. monocytogenes*, *Salmonella* Montevideo and *E. coli* O157:H7 in tryptic soy broth (TSB) at different stages of growth: log, early and late exponential, and stationary (Lin et al., 2000a). Bacteria at each of the stages were used to create bacterial suspensions for each pathogen and growth stage were treated with AITC at 500, 1,000, or 2,500 μg/ml. AITC at 500 μg/ml did not drastically effect bacterial populations, however at 1,000 and 2,500 μg/ml AITC was able to reduce *E. coli* and *Salmonella* counts to non-detectable in less than three hours at all growth stages. *L. monocytogenes* exhibited the greatest resistance to the treatment. While treatment with 2,500 μg/ml AITC resulted in approximately > 4 log reduction at all growth stages, no period of AITC exposure at 2,500 mg/ml completely inhibited growth of *L. monocytogenes*. This study indicates the antibacterial efficacy of AITC at all growth stages against these commonly known foodborne pathogens, showing promise for the use of AITC as an antibacterial agent in food.

1.8.2 AITC on Food Products

Besides studies assessing AITC's antibacterial properties in vitro, studies have also observed its efficacy against human pathogens on food products. Lin et al. (2000b) studied the antimicrobial effects of AITC against *E. coli, Salmonella*, and *L. monocytogenes* on fresh produce. This study assessed the bactericidal activity of AITC against *Salmonella* Montevideo, *E. coli* O157:H7, and *L. monocytogenes* on iceberg lettuce, at both high (10^7 to 10^8 CFU/g) and low (10^3 to 10^4 CFU/g) inoculation levels. At low inoculation levels 400 μl of AITC reduced *E. coli* and *Salmonella* counts to undetectable after two days, and undetectable after four days at high

inoculation levels. Again, *L. monocytogenes* was the most resistant to the treatment and 400 µl of AITC was ineffective at completely eliminating bacterial populations at both low and high inoculation levels. This study also used AITC to achieve 8 and 5 log reductions on tomato skin and stem scars inoculated with *Salmonella* Montevideo, and a 3 log reduction on apple stem scars inoculated with *E. coli* O157:H7. Further research, assessing AITC's antimicrobial effect on tomatoes, observed *Salmonella* and *E. coli* O157:H7 populations on sliced and whole tomatoes treated with AITC vapor at 4, 10, and 25 °C (Obaidat et al., 2016). At the lowest level used, AITC (8.3 µl/liter of air) was able to inactivate *Salmonella* on sliced and whole tomatoes by 3.5 and 2.0 log CFU/ml, at the most effective temperature 10 °C. At 10 °C AITC also inactivated *E. coli* O157:H7 on sliced and whole tomatoes by 3.0 and 1.0 log CFU/ml.

Aside from vegetable products the ability of AITC to reduce pathogen concentrations on fruit has also been observed. In combination with chitosan, AITC reduced *Salmonella* concentrations on whole cantaloupes to undetectable, achieving an overall log reduction of greater than 5 log, along with completely inactivating mold and yeast populations (Chen et al., 2012). Extensive research efforts have also been focused on the use of AITC on food products other than produce items. For example, AITC has been used against several species of bacteria on juices, various meats, cheese, pasta, and sauces (Saladino et al., 2017). The use of AITC as an antimicrobial agent has also been used to develop antimicrobial packaging systems, primarily for meat packaging, to control the growth of human pathogens (Nadarajah et al., 2005; Dias et al., 2013; Chacon et al., 2006; Shin et al., 2010; Park et al., 2012). However, there are limitations to AITC as an antimicrobial on food products and in packaging systems.

The efficacy of AITC as an antimicrobial is compromised with varying pH and temperature; for example, at low temperatures (4-10°C) and alkaline pH (9.0), AITC displayed little to no antimicrobial activity against *L. monocytogenes* and *Salmonella* in broth (Olaimat & Holley, 2013). AITC also has poor water solubility and high volatility, which restricts its use in various food products; however, in meat, these two problems were overcome using microencapsulation (Chacon et al., 2006a). While microencapsulation seemed to be successful in

stabilizing AITC for meat product use, a study assessing microencapsulated AITC on fermented sausage significantly affected sensory attributes (flavor, appearance, and overall impression) (Chacon et al., 2006b). In this study, fermented sausages were treated with 500, 750, and 1,000 ppm AITC; after 40 days, *E. coli* was undetectable with the 500 ppm treatment, an approximate 6 log reduction. While during the sensory evaluation, sausages containing 500 ppm of AITC were considered acceptable by panelists, they also yielded a spicy sensation. The volatility of AITC at low concentrations may be acceptable and even preferred by consumers in meat; however, this will not be the case for all food products.

While AITC has been successfully used to eliminate pathogens like *L. monocytogenes, E. coli*, and S*almonella* in a variety of food products, it is not widely used as an antimicrobial in industry. Along with the various adverse effects of AITC's pungency on sensory attributes of food, its lack of stability in aqueous solutions, and vulnerability to decomposition by reactions with nucleophiles naturally found in food, its use in food systems in limited (Cejpek et al., 2000). Collectively, the literature suggests that AITC can be successful as a food antimicrobial. However, it must be acceptable by the consumer, so the typical flavor profile of the product and how the application of AITC may alter it must be considered.

1.9 Conclusions

Food that goes wasted, and neglects its sole purpose of providing humans with nutrients, makes up around 50% of the current food supply; meanwhile, millions of people are food insecure. While it has never been quantified, every year various food safety recalls and outbreaks send mass amounts of food to landfills. The amount of food waste that occurs each year can be decreased with better mitigation strategies when it comes to food safety issues that can lead to large scale recalls and outbreaks. The food industry is continuously faced with the challenge of providing enough food for growing populations while minimizing waste and ensuring consumer safety. The biofumigation process presents the potential mitigation strategy to suppress human pathogens early on at the field level, thus increasing consumer safety; while also providing beneficial nutrients to agricultural land, increasing sustainability and yields of farming operations.

This research aims to shed light on how food safety issues contribute to the large amounts of food that is wasted annually and the resulting negative socio-economic and environmental impacts. Providing potential mitigation strategies and a monetary value to the fresh produce that is wasted due to food safety issues, in hopes that this will provide an economic incentive to improve current food safety practices.

Chapter 2 Exploring Food Waste Due to Food Safety Recalls

2.1 Introduction

While fruits and vegetables are among the healthiest of foods, they account for the highest amount of post-harvest waste (Buzby, Wells, and Hyman, 2014). The Food and Agriculture Organization of the United Nations (FAO) estimates approximately 45% of all fruits and vegetables produced globally are lost or wasted (FAO, 2012). There are many steps along the farm-to-fork continuum where produce can be wasted, some more apparent and well-studied, including agricultural production, processing, distribution, and at the consumer level (Marion & Matheron, 2014; FAO, 2011; Boys & Rickard. 2019). However, other factors, such as food safety issues, often go unrecognized as a source of food waste (Yiannas, 2018). If food is contaminated with a biological, chemical, or physical hazard and must be removed from the food chain to ensure the safety of consumers, is it food waste? The food items that are individually contaminated may fall into an inedible category of food, thus not considered waste. However, in an abundance of caution, food that may not be contaminated is recalled and is considered a tradeoff for keeping the food supply safe (Gunders, 2017).

Food waste, no matter the source, has various negative socioeconomic and environmental impacts. Measuring the cost of food waste at the global level is very challenging. Yet, FAO estimates the cost of food waste globally totals $2.6 trillion per year, with social and environmental costs accounting for $900 and $600 billion, respectively (FAO, 2014). In the United States food that is wasted is estimated to account for over 30 million acres of cropland, about 4.2 trillion gallons of water, 780 million pounds of pesticides, and 2 billion pounds of fertilizer, and 16% of all U.S. methane emissions (EPA, 2018, Conrad et al., 2018). Justifiably, the food waste issue has gained the attention of the U.S. government. The 2018 Farm Bill was the first to include funding specific to addressing the harmful environmental and socioeconomic impacts of food waste in the United States.; including liability protection for food donors, local composting and food waste reduction plans, and a food loss and waste liaison position to evaluate volumes and costs of food waste (Sandson, 2018).

Food safety issues are also an issue of major importance, foodborne illness and outbreaks have occurred for decades and still remain an ongoing challenge for consumers, industry, government and academia. Every year there are roughly 3,000 deaths and 128,000 hospitalizations that occur due to foodborne illnesses (Scharff, 2012). Produce is particularly problematic, most often grown outdoors and left exposed to physical, chemical, and biological hazards. From 2010 to 2017, there were 1,797 foodborne illness outbreaks in the United States, 12.7% (228) associated with fresh produce (Carstens et al., 2019). Of fresh produce recalled during 2004-2013, 91.9% were due to pathogen contamination with *L. monocytogenes*, *E. coli* 0157:H7, and *Salmonella*, suggesting that these three pathogens are of major concern in the safety of produce (Page, 2018). Food waste and safety have been well studied as two separate issues, however, to our knowledge, the relationship between the two has not yet been explored in depth in the literature.

Produce items are often undifferentiated, meaning all brands at the retail level are essentially identical to consumers. This makes food safety recalls involving such products even more complex, with products from multiple sources wasted due to lengthy trace back investigations or a decrease in market demand overall (Arnade et al., 2009). On average, recalls cost companies $10 million in direct costs; which include notification of involved parties, product retrieval, storage, destruction, and additional labor to carry out these tasks (Tyco Integrated Security, 2012). Aside from direct costs, other expenses due to lawsuits, brand damage, and a loss in sales are also financial consequences companies face due to recalls (Ostroff, 2018). One of the main contributors to food recall occurrence and overall cost is traceability issues within the food industry; including recordkeeping problems, complexity of the supply chain, and the lag time in identifying contaminated products (Tracy, 2017). In some cases, trace back investigations may take weeks or months, and in an abundance of caution companies may end up recalling as much as 50% more product than what is required (CDC, 2020a; Tracy, 2017). It is in the best interest of the government, food industry, and consumers to enhance traceability, and studies have shown we possess the technology to achieve it. Recently, the efficacy of using blockchain technology to improve traceability efforts has been demonstrated; reducing the time to identify the growing farm

for a package of mangoes from over 162 hours to 2.2 seconds (Yiannas, 2018). Especially in the case of produce, which are very perishable items, traceability is crucial to reduce waste.

Currently, the U.S. Food and Drug Administration (FDA) does not have the statutory authority to require electronic record keeping, and all that is required from the industry is "one step forward and one step back" with many smaller companies simply recordkeeping on paper (Biros, 2014; Yiannas, 2018). In July of 2020, Frank Yiannas, now FDA Deputy Commissioner for Food Policy and Response, introduced the New Era of Smarter Food Safety Blueprint, outlining efforts within the next decade to solve the traceability issue using technology to create a more digital and transparent food supply (FDA, 2020d). There are four core elements to this Initiative: tech enabled traceability, smarter tools and approaches for prevention and outbreak response, new business models and retail modernization, and food safety culture (FDA, 2020g). This research sheds light on the contribution of fruit and vegetable recalls and traceability issues to food waste in the United States. Using publicly available data, we estimate the quantity and monetary value of fruits and vegetables wasted due to implications in food safety recalls resulting from potential contamination with *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella* during 2015-2018.

2.2 Methods

When a fruit or vegetable recall occurs, it is posted to the FDA's Recalls, Market Withdraws, and Safety Alerts website (FDA, 2020b). The FDA continues to monitor the recall by obtaining recall status reports (21CFR7.53) from the recall firm and update the public in more detail through Enforcement Reports (FDA, 2020c). To quantify the amount of produce wasted due to food safety issues, we identify all recalls involving vegetable or fruit products contaminated with *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella* posted on the FDA's Archive for Recalls, Market Withdraws, and Safety Alerts website during 2015-2018 (FDA, 2020b). This database provides brand name, product description, product type, recall reason description and company name. In order to determine specific quantities recalled, we perform an advanced search on FDA's Enforcement Report database for each recall identified on the Recalls, Market Withdraws, and Safety Alerts website. The Enforcement Reports provide additional information including,

recalling firm, geographical distribution pattern, a unique recall number, and in some cases product quantity. We use U.S. Department of Agriculture (USDA) 2016 average retail prices to estimate the monetary value of recalled product (USDA ERS, 2018).

In order to properly interpret the FDA data, it is important to understand the limitations of the Recalls, Market Withdraws, and Safety Alerts, and Enforcement Reports databases. According to the FDA regulations for firm-initiated recalls (21CFR7.46) and recall status reports (21CFR7.53), during the event of a recall, specific information like quantities recalled by the recalling firm is only “requested” by the FDA. Considering no information is required according to the regulation, the data includes many instances of missing or aggregated quantities. Overall, there are 430 observations in this data set for recalls of fruit and vegetable products during the study period. Of these observations, only 60% include reported quantities, and only 50% include specific quantities for each item recalled (Table 5). Among the 218 unique recall quantities, the units of measurement are inconsistent, some using convertible measurements including lbs. or oz., but the majority only providing descriptions such as: boxes, packages, cases, trays, kits, bins, packs etc.

Table 5 Count of recall quantities provided through FDA's enforcement report database

	Recall Quantity Provided			
	Unique	Aggregated	Missing	Total
Fruit	34	25	6	65
Vegetable	91	8	104	203
Other	93	9	60	162
	218	42	170	430

Source: U.S. Food and Drug Administration 2020b, 2020c

We categorize the 430 observations into product groups based on items recalled, and assign pathogen type based on the FDA’s Enforcement Reports reason for recall. Some recalls include items that do not fit one specific product group, therefore we create mixed categories. We categorize ready to eat salad kits or mixes as ‘Mix Salad’, any items that included more than one vegetable as ‘Mix Veggies’, any items that include more than one fruit as ‘Mix Fruit’, and any item with both fruit and vegetables as ‘Mix Fruit and Veggies’. According to this data, there are 39 different product groups and 55 companies involved in vegetable and fruit recalls due to potential

contamination with *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella* during 2015-2018 (Table 6).

Table 6 Summary of fruit and vegetable product recalls due to potential contamination with *L. monocytogenes,* pathogenic *E. coli*, or *Salmonella* during 2015-2018

Product	Number of Recalls	Number of Companies Involved	Pathogens Involved
Apples	19	5	*L. monocytogenes, Salmonella,* and/or *E. coli*
Arugula	2	1	*L. monocytogenes*
Beans	24	16	*L. monocytogenes*
Berries	7	6	*L. monocytogenes*
Broccoli	4	4	*L. monocytogenes*
Brussels Sprouts	2	2	*L. monocytogenes*
Carrots	2	2	*L. monocytogenes*
Cauliflower	2	2	*E. coli* O157:H7 and *L. monocytogenes*
Celery	1	1	*L. monocytogenes*
Cherries	1	1	*L. monocytogenes*
Coconut	4	3	*Salmonella*
Coleslaw	6	2	*L. monocytogenes* and *Salmonella*
Collard Greens	1	1	*L. monocytogenes*
Corn	13	9	*L. monocytogenes* and/or *Salmonella*
Cucumber	5	5	*Salmonella*
Garlic	2	2	*Salmonella*
Kale	3	2	*L. monocytogenes*
Leeks	1	1	*L. monocytogenes*
Lettuce	7	3	*L. monocytogenes* and *E. coli* O157:H7
Melons	27	2	*Salmonella*
Micro Greens	2	2	*Salmonella*
Mix Fruit and Veggies	5	2	*L. monocytogenes*
Mix Salad	76	10	*L. monocytogenes* and *Salmonella*
Mix Veggies	104	21	*L. monocytogenes*
Mixed Fruit	27	11	*L. monocytogenes* and *Salmonella*
Mushrooms	16	6	*L. monocytogenes* and/or *Salmonella*
Mustard Greens	1	1	*L. monocytogenes*
Okra	3	1	*L. monocytogenes*
Onions	4	4	*L. monocytogenes*
Peaches	2	2	*L. monocytogenes*
Peas	18	8	*L. monocytogenes*
Peppers	9	5	*L. monocytogenes* and *Salmonella*
Potatoes	2	1	*L. monocytogenes*
Spinach	15	6	*L. monocytogenes* and *Salmonella*
Sprouts	7	3	*E. coli* O157:H7 and *L. monocytogenes*
Squash	3	2	*L. monocytogenes*
Turnips	1	1	*L. monocytogenes*
Yam	1	1	*L. monocytogenes*
Zucchini	1	1	*L. monocytogenes*

2.3 Results

The goal of this paper is to estimate the quantity and monetary value of fruit and vegetable products that are implicated in food safety recalls, and thus wasted, due to potential contamination with *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella*. According to the data, products most frequently implicated are mixed fruit, mix salad, and mix veggies; which together made up 48% of all vegetable and fruit recall instances in this analysis. Often times produce items included in mixes or blends undergo processing, that break the protective epidermal barrier, making product surfaces more favorable for microbial survival and growth (FDA, 2008). The risks associated with produce are apparent; and the Food Safety Modernization Act (FSMA) and Produce Safety Rule establish regulations for the food industry to prevent contamination from occurring (FDA, 2020f).

For all product groups included for further analysis, unique quantities are available for some or all recall instances, allowing an estimation of the quantity and monetary value of product involved. However, there are limitations in the data, and in some instances, we can estimate the quantity recalled whereas in others we cannot, as explained below. For example, the data available for apples includes quantity information in pounds for only 12 out of 19 recalls (Table 7). For two recalls the quantity is provided in number of apples, which we convert to pounds using 182 grams as the average weight of a medium size apple (USDA ARS, 2019). In four instances the descriptions of the items recalled include mixed weights and pack sizes. In this case the pack sizes are averaged to estimate an average case size. For one out of the 19 apple recalls, no quantity information is available, which is a common occurrence throughout the data

Table 7 Quantity and value analysis for Apples

Number of Recalls	Number of Companies Involved	Total Amount Recalled		Pathogens Involved	Notes		
12	4	65,991.85	lbs	*L. monocytogenes*	All quantity information available		
2	2	9,273.49	lbs	*L. monocytogenes, Salmonella,* and/or *E. coli*	Quantity given in apples (convert using average weight 182 g/apple)		
4	3	84,382.06	lbs	*L. monocytogenes*	Average of mixed units given in item description		
1	1	-		*L. monocytogenes*	No units of measurement provided		
Total Quantity		**157,596.41**	**lbs**	**Retail Price (ERS, 2018)**	**$1.62/lb**	**Total Value**	**$254,602.31**

Analysis of other product types present further limitations to the dataset including aggregated totals. For example, the data presents an aggregated total of approximately 3.2 million pounds for 25 out of the 27 melon recalls. Products included in these recalls include watermelon, cantaloupe, and honeydew, therefore we estimate the monetary value of the aggregated total by averaging the per pound prices of these three melon types. Another issue, is the use of “All product in the facility” for quantity recalled. This provides no units or means to estimate quantity or value of the product recalled. However, for 41 fruit recalls involving 5 distinct fruit products, and 32 vegetable recalls involving 9 distinct vegetables products, we gather enough information to estimate product recalled and monetary value (Table 8).

Table 8 Estimated value of fruit and vegetable recalls due to pathogen contamination with *L. monocytogenes,* pathogenic *E. coli*, or *Salmonella* during 2015-2018

Product	Number of Recalls	Number of Companies Involved	Total Amount Recalled		Value of Product Recalled (USD)	Pathogens Involved
Apples	18	4	157,596	lbs	$254,602.31	*L. monocytogenes, Salmonella,* and/or *E. coli*
Beans	9	7	66,326,151	lbs	$110,053,716.22	*L. monocytogenes*
Berries	2	1	351,247	lbs	$1,061,572.28	*L. monocytogenes*
Broccoli	1	1	4,664,738	lbs	$4,113,594.77	*L. monocytogenes*
Brussels Sprouts	1	1	6,960	lbs	$14,196.56	*L. monocytogenes*
Carrots	1	1	1,251,822	lbs	$1,748,277.11	*L. monocytogenes*
Cauliflower	2	2	97,866	lbs	$142,900.56	*E. coli* O157:H7*, L. monocytogenes*
Corn	5	4	33,106,073	lbs	$52,910,018.21	*L. monocytogenes* or *Salmonella*
Cucumber	1	1	17,130	lbs	$21,508.18	*Salmonella*
Kale	1	1	401,928	lbs	$833,417.39	*L. monocytogenes*
Melons	25	1	3,160,648	lbs	$1,752,498.84	*Salmonella*
Peaches	1	1	66,630	lbs	$212,355.19	*L. monocytogenes*
Peas	4	4	39,730,183	lbs	$65,889,850.13	*L. monocytogenes*
Spinach	8	4	2,306,436	lbs	$4,107,955.94	*L. monocytogenes, Salmonella*
		TOTAL	**151,645,408**	**lbs**	**$243,116,463.68**	

According to the data on fruit recalls due to potential contamination with said pathogens, just under 4 million pounds of product was recalled with an estimated retail value of approximately $3.3 million during 2015-2018. Out of 87 fruit recall instances in the initial analysis, only 41 provide enough information to estimate recalled product value. These numbers only represent and estimate less than 50% of all recall instances due to *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella* contamination during this four-year period. The largest recall amount, within the fruit categories, is observed for melons. However, these 25 recall instances were actually a part of one large recall due to a Salmonella Adelaide multi-state outbreak involving melons (FDA, 2018b). This outbreak occurred in 2018 and resulted in 77 cases in nine states and 36 hospitalizations (CDC, 2018a).

Berries demonstrate the second largest total value among fruit analysis, with only two out of the seven recall instances included in analysis. These two recalls alone, result in an estimated value of over one million dollars and only represent 29% of berry recall instances. However, these two berry recalls were part of a *L. monocytogenes* outbreak involving hundreds of vegetable and fruit products (Lamansky, 2016). This outbreak resulted in nine cases and hospitalizations and three deaths (CDC, 2016). The outbreak investigation initiated a recall in May 2016, and included 358 frozen fruit and vegetable products, under 42 brand names, sold in every state in the United States produced since May 2014 (FDA, 2016a). This recall involved all items produced within a two-year span, and for analysis of berries and peaches the only data used to estimate value were related to this recall.

In comparison to fruit, the amount of product recalled and its total retail value for vegetables is estimated to be much larger. Our data analysis indicates that 148 million pounds of product was recalled with an estimated retail value of $240 million, nearly 70 times the value estimated for fruit. Furthermore, this analysis only includes 32 out of the 338 vegetable recalls in the initial analysis, highlighting that this estimation is merely a small fraction of the food waste due to vegetable recalls. The previously discussed large scale frozen produce recall also influenced some larger quantities and total value of vegetable products, and produces the only data

available to estimate the total quantity and retail value of broccoli, brussels sprouts, carrots, and kale.

For vegetables, beans generated the largest estimated value of product recalled during this time period, and accounted for just below 50% of the entire vegetable value estimate. The analysis for the beans category includes seven frozen green bean recalls, one canned green bean recall and one frozen lima bean recall. The frozen green bean and lima bean recalls were both due to the multi-state outbreak of *L. monocytogenes* involving frozen produce in 2016; therefore, these quantities reflect two years' worth of production and justify the large quantities and value under the bean category (CDC, 2016). Frozen spinach was also implicated in this outbreak; the one recall quantity for spinach associated with this large-scale outbreak accounting for approximately 95% of the total quantity of spinach recalled and its retail value. Five of the spinach recalls, without enough information for analysis, were due to a *L. monocytogenes* outbreak involving leafy greens, which resulted in 19 illnesses and hospitalizations and one fatality (FDA, 2016b). For these recalls, the recalling firms report "All product in the facility" for recall quantity; therefore, this multi-state recall could not be included in analysis of estimating quantity and monetary value. This is another example of how the way information is reported to FDA from firms, currently makes it impossible for researchers to estimate the full quantity and value of food waste due to food safety recalls.

An important product group, not included in quantity and value analysis is lettuce. Although lettuce, particularly romaine lettuce, is frequently implicated in foodborne illness outbreaks due to traceability issues targeted product recalls may never occur (CDC, 2020a). In April of 2018 there was an *E. coli* O157:H7 outbreak involving romaine lettuce, resulting in 210 illnesses, 96 hospitalizations, and 5 deaths (CDC, 2018b). While this outbreak did not result in a recall, the FDA advised consumers to avoid all romaine lettuce from the Yuma region (PMA, 2018). Similarly, there was another *E. coli* O157:H7 outbreak involving romaine lettuce that occurred during 2018, resulting in 62 illnesses and 25 hospitalizations (CDC, 2019). Again, romaine was not recalled but the FDA recommended consumers avoid romaine lettuce altogether until further notice (FDA, 2018a). An advisory from the FDA to avoid romaine lettuce altogether or from a

specific region did inevitably generate food waste. However, as food safety outbreaks rather than recalls from specific entities, these occurrences are not included in our estimations.

Overall, for fruits and vegetables included in this analysis, recalls due to potential contamination with *L. monocytogenes*, pathogenic *E. coli*, or *Salmonella* during 2015-2018 result in a loss of nearly 151 million pounds of product and $243 million in retail value. These estimates only account for 17% of all recall instances that meet the criteria for this study, hence it's a significant underestimation. Our inability to include all food recall instances is due to the fact that currently the FDA's Enforcement Reports database lacks information needed for the majority of the food safety recalls. For all product groups in the value analysis, we provide notes for recalls that we're unable to include in the overall estimated quantity (Table 9).

Table 9 Notes by product category for fruit and vegetable recalls not included in value analysis

Product	Notes
Apples	In two instances quantity was given in apples and the average weight of an apple according to the USDA (182 g) was used to estimate overall weight, in four an average of the multiple sizes given was used to estimate overall weight, in one instance no units of measurement provided.
Berries	In four instances no value provided for quantity, in one instance no pricing information available through USDA ERS.
Brussels Sprouts	In one instance no information reported for quantity
Carrots	In one instance no information reported for quantity
Cauliflower	All quantity information available
Corn	In five instances number of cases are reported but no quantity per case, in three instances no information reported for quantity
Cucumber	In 2 instances no units of measurement provided, in one instance mixed sized per container provided, in one instance no units per carton provided
Kale	In two instances no information reported for quantity
Melons	In one instance an aggregated total was given for 25 separate items with either honeydew, watermelon, or cantaloupe the per pound prices of these items were averaged to calculate the value of product, in one instance no unit of measurement was given, in one instance no value provided for quantity
Peaches	In one instance no value given for quantity
Peas	In six instances number of cases are reported but no quantity per case, in one instance no information reported for quantity
Spinach	In five instances recalling firm provided no quantities stating "All product in the facility", in one instance number of cases are reported but no quantity per case, in one instance no information reported for quantity

2.4 Discussion and Conclusions

Historically, food that is disposed or destroyed due to food safety issues has gone unrecognized as food waste and has been excluded from food waste analysis. Our goal with this study is to show the contribution of food safety recalls to food waste. Focusing on fruit and vegetable recalls in the period 2015-2018, we estimate that just 17% (73 out of 430) of the recall instances lead to food waste amounting 151 million pounds of product and $243 million in retail value. This estimation focuses only on food safety recalls due to *L. monocytogenes*, pathogenic *E. coli*, and *Salmonella*, and includes recall instances for which there is sufficient information publicly available through FDA to estimate the quantity of product involved.

There are two limitations to the estimations presented here. First, the estimations of food waste quantity include all recalled product, rather than the quantity actually recovered by the companies involved. Quantities recovered, while available through FDA's Freedom of Information Act, would lead to even more missing data. Second, we estimate the monetary value of the food waste using USDA-ERS retail-level prices, which do include the retailers' mark-up.

To our knowledge, this is the first study addressing the contribution of food safety recalls to food waste. While this study only analyzes a small fraction of food waste due to food safety recalls, it provides justification for the inclusion of food safety issues in future examinations of food waste. Applying a monetary value to the products wasted due to food safety issues should encourage the food industry and policymakers to invest to enhanced food safety; such as strategies to mitigate exposure of food items to human pathogens, and enhancement of the food traceability system to increase the overall accuracy of food recalls.

Finally, it's important to note that many of the recalls posted by the FDA are large scale recalls, involving multiple products with large ranges of production and expiration dates, some even recalling "All product in the facility". This indicates that contaminated product is not effectively targeted in these recalls, and most likely safe and edible product is also recalled in an abundance of caution. The FDA's New Era of Smarter Food Safety, more specifically the recently proposed rule for food traceability, will help the FDA to more effectively and rapidly identify

contaminated product, mitigating foodborne illness outbreaks and minimizing the amounts of edible food sent to landfills (FDA, 2020e).

Chapter 3 Inactivation of Foodborne Pathogens in Agricultural Soil using *Brassica* spp.

3.1 Introduction

While fruits and vegetables are very important components in the human diet, the burden of foodborne illness caused by fresh produce has been ranked fourth among foods linked to foodborne illness (Morris et al., 2011). From 1998-2013, 972 raw produce outbreaks were reported, leading to 34,674 outbreak-associated illnesses, 2,315 hospitalizations, and 72 deaths; notably higher number of hospitalizations and deaths in comparison to other food groups (Bennett et al., 2018). From 2010 to 2017, there were 1,797 foodborne illness outbreaks with a confirmed food vehicle and etiology in the U.S; 12.7% (228) of them associated with fresh produce, increasing in comparison to 9.2% observed during 2004 to 2010 (Carstens et al., 2019). This suggests that outbreaks are occurring more often, identified more frequently, or a combination of both; regardless better strategies are still needed to prevent contamination.

Fresh produce is grown in fields left exposed and relatively unprotected, without cover or continuous surveillance. This provides many opportunities for contamination, including wildlife intrusion, water, soil, improperly treated manure, and human handling (Doyle and Erickson, 2008). Many fresh produce items are grown in close proximity to the ground and edible portions can come in direct contact with soil (Rajwar et al., 2016; Doyle and Erickson, 2008). Pathogen transfer from the soil to the edible portion of the plant may occur via direct contact, splash events, or worker handling; and plants have also shown the ability to internalize enteric pathogens via seedlings and roots (Kutter et al., 2006; Franz et al., 2007; Doyle & Erickson, 2008, Alegbeleye et al., 2018). Frequently, like the recent multistate *E. coli* O157:H7 outbreaks involving romaine lettuce, the identified outbreak strain is traced back to soil samples from the implicated area (FDA, 2020; CDC, 2019; CDC, 2018). Therefore, efforts to reduce the exposure of fresh produce commodities to contaminated soils, and strategies to decontaminate agricultural soils are needed.

Human pathogens, whether naturally present or introduced to agricultural soil, possess the ability to persist in soils for extended periods of time (Alegbeleye et al., 2018). *E. coli* O157:H7 and *Salmonella*, two human pathogens frequently involved in outbreaks implicating fresh produce, are generally introduced to soils by wildlife feces, improperly treated soil amendments,

water, or human contact. However once introduced to soil, studies have observed the survival of *E. coli* O157:H7 for up to 231 days (Jiang et al., 2001), and *Salmonella* for up to 405 days (You et al., 2006). *L. monocytogenes*, ubiquitous in the environment and naturally present in soils, has been observed to survive in soil for up to 6 weeks (Renterghem et al., 1991). While the literature presents a variety of physical, chemical, and biotechnological treatments to inactivate human pathogens in manure; there are no sufficient methods to decontaminate soil once in an agricultural field (Martens & Böhm, 2009).

Only two articles could be found that investigate methods to inactivate foodborne pathogens in soil. These two studies present the use of chloroform fumigation and pyrolysis-generated biochar to significantly reduce *E. coli* O157:H7 in soils (Van Elsas et al., 2007; Gurtler et al., 2014). A majority of the literature on methods for decontaminating soil focuses on the inactivation of plant pathogens; however, some of these methods, like biofumigation, could potentially inactivate foodborne pathogens in soil. While soil decontamination methods can be costly and not practical for large scale application, if they provide additional benefits to the soil it may be more economically feasible (Gurtler, 2017). A commonly used process called biofumigation, involves the utilization of high glucosinolate containing *Brassica* species as cover crops to control pests, soil-borne disease, and weed management.

The biofumigation process not only inactivates plant pathogens in soil, but there is a plethora of advantages to biofumigation. These include the improvement of physical and biological soil characteristics, improved soil microbial communities, increased infiltration rate and water holding capacity, reduced wind erosion, nitrogen preservation, and reduced soil compaction (Reddy, 2013). Research on the fumigant properties of the volatile plant chemicals produced during this process has also been extensively studied, revealing its effectiveness in suppressing plant diseases, nematodes, weeds, and insects (Reddy, 2013; Matthiessen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009). While the literature is limited, biofumigation has also shown the ability to suppress bacterial plant pathogens in soil. This process has been used to significantly reduce concentrations, in one case 6-15 fold in comparison to controls, of Gram-negative bacterium Ralstonia solanacearum, which has the ability to cause bacterial wilt in about 200 plant

species in 33 different plant families (Moorman, 2011; Kirkegarrd, 2007; Bayot et al., 2004). The use of Indian mustard green manures to control common scab caused by the gram-positive bacteria Streptomyces scabiei, resulted in the reduction of incidence and severity of common scab by 25% (Larkin & Griffin, 2007).

It is not for certain the suppression of bacteria during biofumigation is directly due to the biologically active compounds released or a secondary effect due to changes in the soil microbial communities. However, the available literature supports the ability of the biofumigation process to reduce both gram negative and positive pathogenic bacteria populations in soil and their associated disease in plants. Literature also suggests the ability of AITC (AITC), one of the main glucosinolate hydrolysis products, to reduce *E. coli* 0157:H7, *Salmonella*, and *L. monocytogenes* in broths (Olaimat and Holley, 2013), on fresh produce (Obaidat et al., 2016; Lin et al., 2016; Chen et al., 2012), and in antimicrobial packaging systems (Nadarajah et al., 2005; Dias et al., 2013; Chacon et al., 2006; Shin et al., 2010; Park et al., 2012). The success of biofumigation and its glucosinolate hydrolysis byproducts to act as biocide, indicates the potential for use as a treatment for soils contaminated with human pathogens. The objective of this research is to investigate the potential of glucosinolate hydrolysis products, from *Brassica* spp. seed meal and plant matter, to reduce/eliminate concentrations of *E. col*i 0157:H7, *Salmonella*, and *L. monocytogenes* in agricultural soil.

3.2 Materials and Methods

3.2.1 Bacterial pathogen preparation & inoculation

Five strains of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were used in this study (Table 10). All cultures were stored at -70 ℃, in tryptic soy broth (TSB) with 15% glycerol. Frozen cultures of each strain were separately streaked onto tryptic soy agar (TSA) and incubated at 35℃ ± 2℃ for 24 h. A single colony for each strain was transferred to tubes containing 10 mL of TSB and incubated at 35℃ ± 2℃ for 24 h. Strains were pooled to form a cocktail by pathogen type, and this was used as a direct inoculum (~10^8-10^9 CFU/mL). Soil was inoculated with either *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes*. For inoculated soil samples one mL of the pathogen cocktail was added for every 10 g of sterilized soil, achieving a

high inoculation (~10^7-10^8 CFU/mL) in order to observe a reduction of pathogen concentration over time.

Table 10 *Escherichia coli* O157:H7*, Salmonella*, and *L. monocytogenes* strains, isolation information and source

Strain name	Strain number	Isolated from	Source
Escherichia coli O157:H7	NFPA 4211	Odwalla Apple Juice	National Food Lab
Escherichia coli O157:H7	NFPA 4213	Apple Cider Outbreak, Connecticut USA	National Food Lab
Escherichia coli O157:H7	NFPA 4216	Alfalfa Sprout Isolate	National Food Lab
Escherichia coli O157:H7	NFPA 4217	Lettuce Outbreak; Patient Isolate	National Food Lab
Escherichia coli O157:H7	NFPA 4219	Apple Juice Outbreak	National Food Lab
Salmonella anatum	ATCC BAA 1592	Tomato Outbreak; Pennsylvania USA	National Food Lab
Salmonella montevideo	ATCC BAA 710	Clinical Speciman; tomato associated	National Food Lab
Salmonella javiana	ATCC BAA 1593	Human Isolate; linked to fresh roma tomatoes, Pennsylvania USA	National Food Lab
Salmonella oranienburg	NFPA 7201	Alfalfa Sprout Isolate	National Food Lab
Salmonella enteriditis	NFPA 7100	Sprout water isolate	National Food Lab
L. monocytogenes	FSL J1-108	Coleslaw, human, epidemic, Halifax, 1981	ILSI NA (Cornell)
L. monocytogenes	FSL J1-031	Human sporadic case	ILSI NA (Cornell)
L. monocytogenes	R9-5506	Packaged Salad, 2016, multistate US	ILSI NA (Cornell)
L. monocytogenes	R9-5411	Caramel apple	ILSI NA (Cornell)
L. monocytogenes	R9-0506	Cantaloupe 2011	ILSI NA (Cornell)

In examining the effectiveness of glucosinolate hydrolysis products in *Brassica* plant matter only *E. coli* O157:H7 was observed. A single colony for each strain was transferred to tubes containing 10 mL of TSB and incubated at 35℃ ± 2℃ for 24 h. Five milliliters of culture was subjected to centrifugation at 3,000 rpm/rcf for 15 min, which was followed by resuspension of the pellet in 100 µl of TSB and spreading on a TSA plate containing 25 µg/ml of nalidixic acid (NAL). After incubation of the plate at 35°C for 24 h, a large colony was picked and streaked on a 25 µg/ml NAL plate and incubated at 35°C for 24 h. A large colony was picked and further purified by

streaking again on a 25 µg/ml NAL plate. This process was repeated on NAL plates containing 35 and 50 µg/ml. A single colony of resistant strains were transferred to tubes with 20 mL of TSB containing 50 µg/ml nalidixic acid and incubated at 35℃ ± 2℃ for 24 h. Strains were pooled to form a cocktail and centrifuged at 3,000 rpm/rcf for 15 min. Supernatants were discharged and pellets were washed with 40 mL of 0.1 % peptone water. Cells were then resuspended in 40 ml of 0.1% peptone water (~10^8-10^9 CFU/mL). The initial inoculum was diluted with 0.1% peptone water to achieve an inoculum of ~10^7-10^8 CFU/mL for experimentation. Cell concentration of inoculum was confirmed by plating serial dilutions in 0.1% peptone on TSA.

3.2.2 Soil preparation and sterilization

Agricultural soil, silt clay (pH 7.5), was obtained from California Polytechnic State University, San Luis Obispo organic fields and placed into sterile whirl pack bags and stored at room temperature until use. The soil was sifted (mm), added to 10" x 15" instant sealing sterilization pouches s in 50 mg portions, and spread out in a thin layer (1-2 cm) to allow uniform steam penetration. The autoclave bags were placed horizontally into the autoclave separated from each other using racks (4 in) and autoclaved for 60 minutes at 121 ℃ (Trevors, 1996). Following the autoclave process, bags of sterile soil were placed in a drying oven overnight at 67 ℃.

3.2.3 *Brassica* Seed Meal

3.2.3.1 Sample Preparation

Pelletized Pescadero Gold Mustard Seed Meal was obtained through Farm Fuel Inc. Mustard seed meal pellets were blended using a commercial grade blender to form a mustard seed meal powder. Mustard seed meal powder was used to treat sterilized soil, as a control, and inoculated sterilized soil samples at two application rates (10% and 15%). Soil samples and mustard seed meal were added to 250 mL glass bottles, flooded with 20 mL of deionized water, and incubated at 20 °C (Table 11).

Table 11 Soil, seed meal and sterilized DI water mixtures.

Pathogen	Soil (g)	Concentration Seed Meal (g)	DI Water (mL)
Control	10	-	20
Control	10	1.5	20
E. coli O157:H7	10	-	20
E. coli O157:H7	10	1.00	20
E. coli O157:H7	10	1.50	20
Salmonella	10	-	20
Salmonella	10	1.00	20
Salmonella	10	1.50	20
L. monocytogenes	10	-	20
L. monocytogenes	10	1.00	20
L. monocytogenes	10	1.50	20

3.2.3.2 Bacterial Enumeration

Control, *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* samples were tested in duplicate immediately after treatment, and at 24, 48, and 72 h. At each sample time 1 mL was extracted from each 250 mL bottle and serially diluted in 0.1% of peptone water. Bacterial counts were determined using a pour plate method with TSA and incubated at 35℃ ± 2℃ for 18-24 h (Remel; Lexana, KS, USA). Uninoculated control samples will be enumerated for any bacteria.

3.2.4 Brassica Plant Material

3.2.4.1 Sample Preparation

Fresh and packaged brussels sprout (*Brassica* oleracea) and mustard (*Brassica* juncea) samples were obtained from the local supermarket and stored at 4 ℃ and used within 24 hours of purchase. Vegetables were chopped using a commercial grade blender to increase cell disruption. For experiments assessing the potential of *Brassica* plant matter to reduce *E. coli* O157:H7 counts in soil, chopped brussels sprouts were added to contaminated soil (~10^7-10^8 CFU/mL) at two application rates (10 % and 15%) (Table 12). For experiments assessing the potential of *Brassica* plant matter to reduce *E. coli* O157:H7 counts in the absence of soil, plant matter was added to 50 mL of inoculum (~10^5-10^6 CFU/mL) in sterile whirl pack bags at two

application rates, 25 and 50 g (Table 13). Directly after addition of plant matter to inoculum, bags were heat sealed to minimize loss of ITC's due to volatilization.

Table 12 Brussels Sprouts in Contaminated Soil

Pathogen	Soil (g)	Brussels Sprouts (g)	DI Water (mL)
Control	100	-	40
Control	100	10	40
Control	100	15	40
E. coli O157:H7	100	-	40
E. coli O157:H7	100	10	40
E. coli O157:H7	100	15	40

Table 13 Brussels Sprout and Mustard Green Samples in Inoculum

Pathogen	Plant Matter (g)	Inoculum ($10^{5\text{-}6}$)
Control	25	50
Control	50	50
E. coli O157:H7	-	50
E. coli O157:H7	25	50
E. coli O157:H7	50	50

3.2.5 Bacterial Enumeration

Control and *E. coli* O157:H7 samples were tested in duplicate immediately after treatment, and at 2, 10, 24, and 48 h. At each sample time 1 mL was extracted from each sample, serially diluted in 0.1% of peptone water, plated in duplicate on TSA. After incubation at 35℃ for 2 hr plates were overlaid with MacConkey Agar with Sorbitol supplemented with 50 µg/ml of nalidixic acid and incubated at 35℃ ± 2℃ for 18-24 h (Remel; Lexana, KS, USA). Uninoculated control samples were enumerated for any bacteria on MacConkey Agar with Sorbitol supplemented with 50 µg/ml of nalidixic acid.

3.2.6 Statistical Analysis

Seed meal experiments were done in triplicate, soil and plant matter experiments in duplicate, and the plant matter and inoculum experiment were carried out once. Microbial counts were recorded after the specified incubation period, and converted to $\log_{10}$ CFU/ml. A fixed effect test, with round as a random effect, and Tukey pairwise comparison was carried out for each

experiment to determine if the foodborne pathogen presence were significantly different over time and between the mustard seed meal application rate (10% and 15%), or plant matter application rate (50% and 100%) at a 95% confidence interval (a=0.05).

3.3 Results and Discussion

3.3.1 *Brassica* Seed Meal

Initial populations for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 7.51, 6.97, and 7.39 log CFU/ml, respectively. In absence of seed meal, pathogen concentrations significantly increased in soil, with values of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* increasing by 0.6, 1.4, and 0.7 log, respectively over 72 h. Overall, the results show a significant reduction in all pathogens as time increases, with the addition of mustard seed meal ($p < 0.05$).

L. monocytogenes and *Salmonella* were similar in sensitivity to mustard seed meal treatments. When contaminated soil was treated with 1.0 g mustard seed meal *L. monocytogenes* and *Salmonella* counts decreased by 5.8 and 5.7 log CFU/ml over a period of three days, respectively. When contaminated soil was treated with 1.5 g mustard seed *meal L. monocytogenes* and *Salmonella* counts decreased by 6.6 and 6.4 log CFU/ml over a period of three days, respectively (Figure 3) (Figure 4). There was an approximate 1 log difference in *L. monocytogenes* and *Salmonella* reductions when 10% or 15% mustard seed meal treatments were applied, however, this difference was not significant ($p > 0.05$). While there are no studies assessing the effect of mustard seed meal on human pathogens; Oliamat and Holley (2013) investigated the effect of AITC, the glucosinolate hydrolysis byproduct most likely responsible for antibacterial effect, on *L. monocytogenes* and *Salmonella.* At 21 C and pH 7.0 the addition of 200 ppm of AITC reduced *L. monocytogenes* and *Salmonella populations* in broth by 3.67 and 8.30 log CFU/ml after one day, and by 4.31 and 8.88 log CFU/ml after three days (Oliamat and Holley, 2013). In our experiment the release of AITC was not analyzed, however, the log reductions are slightly lower for *Salmonella* and slightly higher for *L. monocytogenes* in comparison to this study. Regardless, these studies show that AITC is successful in achieving significant log reductions of these two foodborne pathogens.

Overall, *E. coli* O157:H7 was more resistant to seed meal application than *L. monocytogenes* and *Salmonella* ($p < 0.05$). However, both seed meal treatments still resulted in significant reductions of *E. coli* O157:H7 concentrations in soil over the three-day incubation period ($p < 0.05$). When contaminated soils were treated with 1.0 g and 1.5 g seed meal, *E. coli* O157:H7 concentrations decreased by 2.54 and 5.56 log CFU/ml after three days, respectively (Figure 5). Unlike the other two pathogens observed in this study, there was a significant difference in *E. coli* O157:H7 reductions between the two seed meal treatments, 1.5 g treatment being more effective in reducing *E. coli* O157:H7 populations in soil ($p < 0.05$). Lin et al (2000) assessed the antibacterial mechanism of AITC against *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7 in broth (pH 7.0) at 37 C. AITC was applied at 500 µg/ml and reduced *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7 concentrations by 0.22, 0.78, and 0.54 log CFU/ml after one hour, respectively (Lin et al., 2000). When AITC was applied at a much higher concentration (2,500 µg/ml) reductions were 5.60, 8.80, and 8.82 log CFU/ml for *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7. This study along with others assessing AITCs antimicrobial activity against foodborne pathogens, observes the antimicrobial effect is generally higher against Gram-negative bacteria opposed to Gram-positive bacteria (Lin et al., 2000; Delaquis & Mazza, 1995; Wilson et al., 2013; Borges et al., 2015).

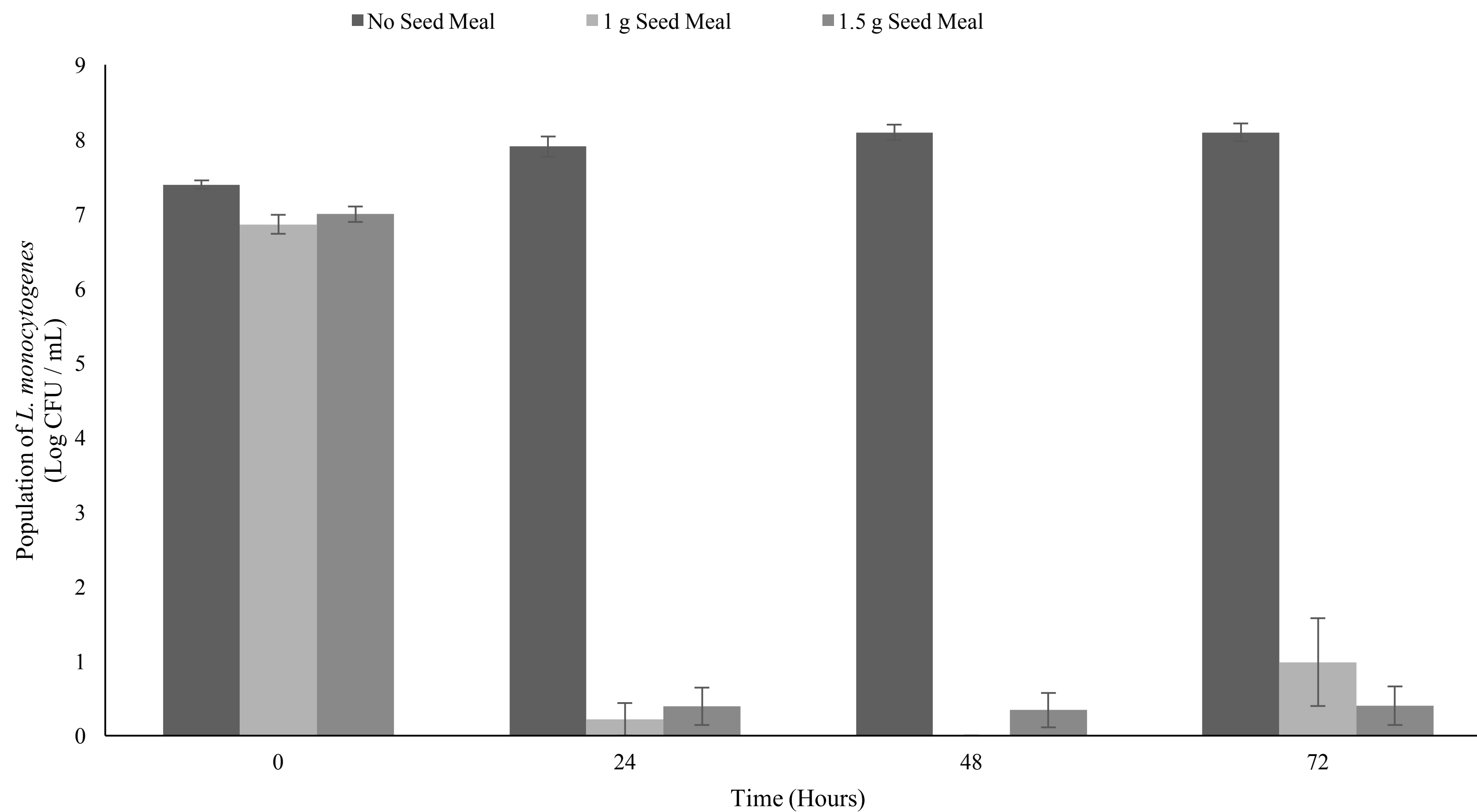

Figure 3 Mean *L. monocytogenes* values (log CFU/mL) and standard errors, of soil samples treated with mustard seed meal incubated at 20°C from 4 sample times (0, 24, 48, and 72 h) (n=6).

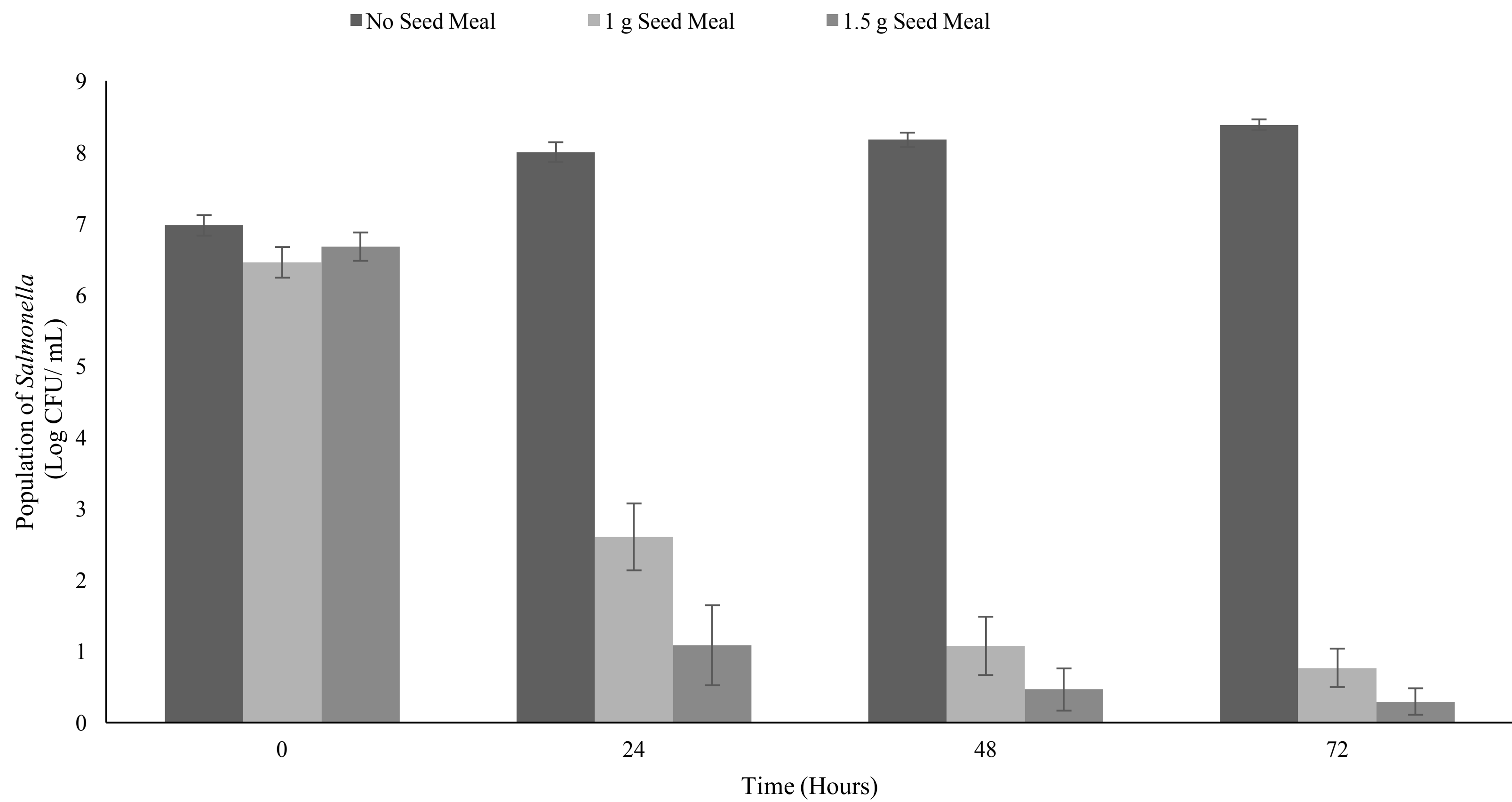

Figure 4 Mean *Salmonella* values (log CFU/mL) and standard errors, of soil samples treated with mustard seed meal incubated at 20°C from 4 sample times (0, 24, 48, and 72 h) (n=6).

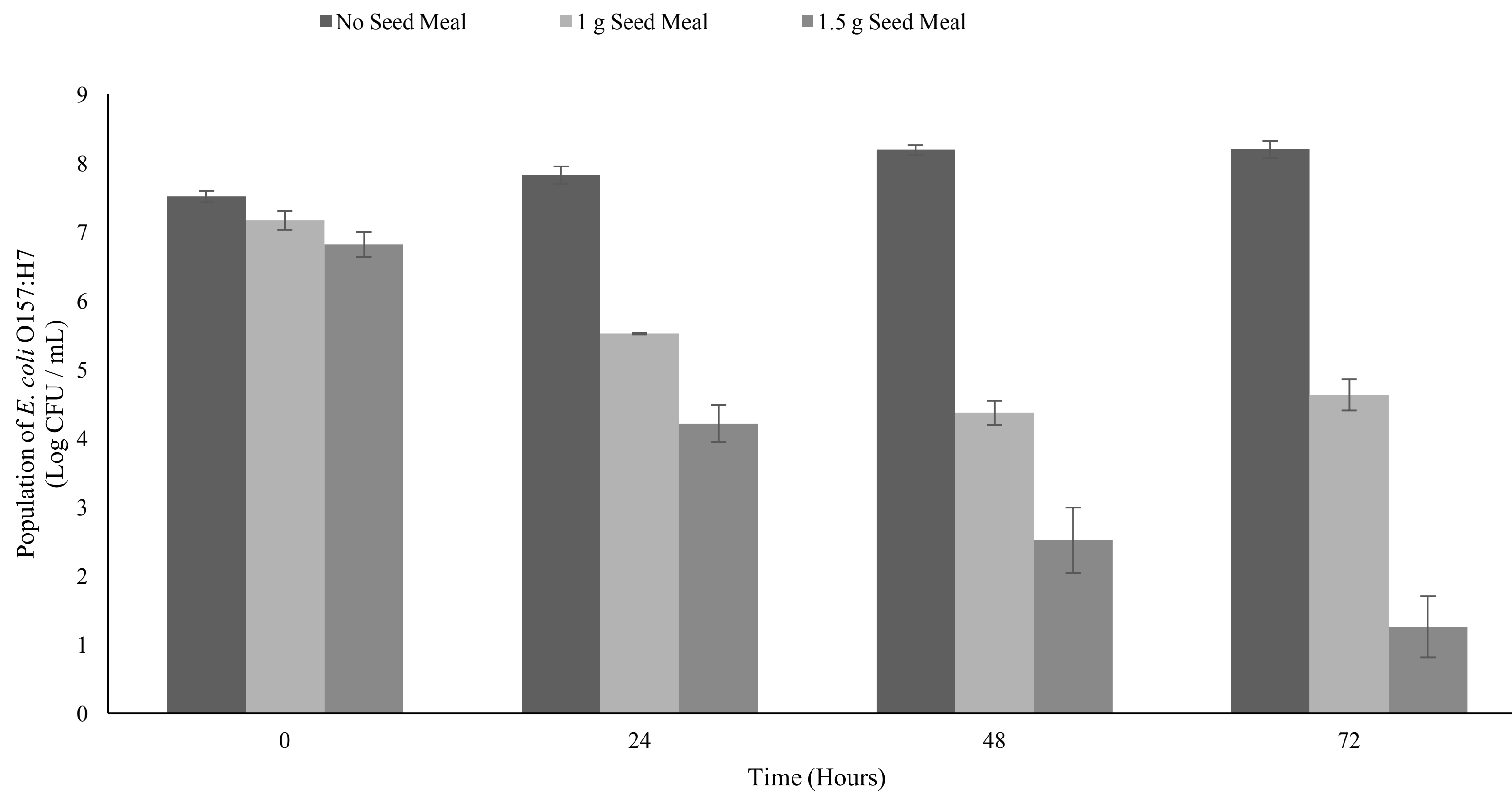

Figure 5 Mean *E. coli* O157:H7 values (log CFU/mL) and standard errors, of soil samples treated with mustard seed meal incubated at 20°C from 4 sample times (0, 24, 48, and 72 h) (n=6).

While the literature suggests AITC may be more effective against Gram-negative as opposed to Gram-positive, the alternative was observed in this study. However, it is important to consider that the effectiveness of AITC against bacteria can be altered by a variety of factors, including temperature and pH (Olaimat & Holley, 2013). Studies suggest that AITC is more effective against L. monocytogenes, a Gram-positive bacterium, at a neutral pH of 7.0, and more effective against *E. coli* and *Salmonella* at acidic pH of around 5.0 (Olaimat & Holley, 2013; Luciano & Holley, 2009). This may be due to the instability of AITC at neutral pH; Oliamat & Holley (2013) concluded that at a neutral pH AITC was unstable and the new compounds that had formed possessed bactericidal activity against *L. monocytogenes* but not *Salmonella*. In our experiment we mimic typical agricultural soil field conditions, pH 7.5 and temperature of 20 °C; conditions that effect the antimicrobial activity of AITC on Gram-positive and negative bacteria. In Brain Heart Infusion (BHI) broth (pH 7.4) at 21 °C the minimum inhibitory concentrations (MIC) for AITC against *L. monocytogene*s and *Salmonella* were as low as 20 ppm and 10 ppm; and the MIC for *E. coli* in Luria-Bertani broth (adjusted to pH 7.5) was 250 ppm (Oliamat & Holley, 2013; Luciano & Holley, 2009). These two studies show, while overall Gram-negative bacterium may be more sensitive to AITC, at an increased pH the instability of AITC may alter its effectiveness against Gram-negative and positive bacteria. The difference in pathogen resistance and sensitivity to AITC may be due in part to the interactive effects of temperature and pH, but it is also important to consider glucosinolate hydrolysis generates many byproducts. Aside from isothiocyanates, glucosinolate hydrolysis generates thiocyanates and nitriles that have also shown to have some antimicrobial activity (Matthiessen and Kirkegaard, 2006).

The literature clearly supports the antimicrobial potential of glucosinolate containing *Brassica* species, and highlights the importance of glucosinolate concentrations in effectiveness as biofumigants. It is evident that processed Brassicaceous seed meals contain higher levels of allyl glucosinolates, and thus may be more beneficial than the use of biofumigant cover crops (Table 14). Brassicaseous seed meals have successfully demonstrated suppressive activity towards a variety of insects, nematodes, fungi, and weeds (Reddy, 2013). There are also many advantages to the use of seed meals in comparison to cover crops, including quicker and more

flexible growing times, less water use, and no fertilizers needed (Rudolph, 2016). However, Brassicaceous seed meals are of limited supply, can be costly (~$1,600/ton), and are likely to be regarded as pesticides by regulatory authorities (Rudolph, 2016; Matthiessen and Kirkegaard, 2006). So, while the incorporation of Brassicaseous seed meals may be more effective as a biofumigant, there may be hurdles in implementation for farmers. Therefore, the potential of Brassicaseous plant matter as a biofumigant against foodborne pathogens in soil should also be investigated.

Table 14 Fresh weight and defatted meal allyl glucosinolate concentrations (mmol/100g) of glucosinolate vegetables

	Allyl glucosinolate content (mmol/100g)	
Cultivar	Fresh Weight	Defatted Seed Meal
Broccoli	0.0005	0.15
Brussels sprouts	0.0107	1.22
Cauliflower	0.0100	4.55
Collards	0.0970	4.13
Kale	0.0166	2.77
Mustard greens	0.7367	12.25
Kohlrabi	0.0000	0.05

Source: Carlson et al., 1987

3.3.2 *Brassica* Plant Matter

3.3.2.1 Antimicrobial activity in soil

Initial populations for *E. coli* O157:H7 in soil were 7.31, and 7.29 log CFU/g when treated with 10% and 15% Brussels sprouts, respectively. In absence of brussels sprout plant matter, concentrations of *E. coli* O157:H7 significantly increased in soil by 0.70 log CFU/g over the 72 h incubation period ($p < 0.05$). The addition of macerated Brussels sprouts, at both 10% and 15%, also resulted in a significant increase of *E. coli* O157:H7 populations in soil by 1.49 and 1.52 log CFU/g, respectively ($p < 0.05$) (Figure 4). There were no significant differences in *E. coli* O157:H7

concentrations between samples with 10% and 15% Brussels sprouts. For all samples there was a significant increase in *E. coli* concentrations after the first 24 h ($p < 0.05$); however, populations did not significantly increase after 24 h. Overall, the addition of Brussels sprout plant matter, regardless of concentration, resulted in increased *E. coli* O157:H7 populations in soil.

While there is evidence that the use of Brassicaseous cover crops has a suppressive effect on soilborne pests; some studies observed no suppression (Johnson et al., 1992), or even pathogen stimulation (Stephens et al., 1999; Cohen et al. 2005). The effectiveness of *Brassica* cover crops as biofumigants depends on a variety of factors. First, it is important to choose the right *Brassica* spp.; one with high concentrations of appropriate glucosinolates and high biomass potential, as glucosinolate concentrations are widely variable among cruciferous vegetables (Kirkegaard and Sarwar, 1998; Matthiessen and Kirkegaard, 2006). It was expected that the glucosinolate hydrolysis products of seed meal would have a greater effect on human pathogens in soil than those of *Brassica* plant matter, solely based on the major differences in glucosinolate concentrations and thus isothiocyanate production (Table 1). *Brassica* biomass and glucosinolate concentrations are directly proportionate, thus the amounts of *Brassica* plant matter applied during biofumigation is just as important as glucosinolate concentration. In both seed meal and plant matter experiments we used 10% and 15% of the respective *Brassica* products, meanwhile average glucosinolate concentrations in mustard seed meal (12.25 mmol/100 g) are greater than 1,000 times that of brussels sprout plant matter (0.0107 mmol/100 g). In addition, the Brussels sprouts used in this experiment were storebought, therefore time from harvest may also result in a decrease in glucosinolate levels present and therefore the theoretical AITC present.

The literature describes the types, concentrations and distribution of glucosinolates in different species; allowing one to calculate maximum potential isothiocyanate release upon tissue disruption and aids in crop selection. However, the release of isothiocyanates from incorporated *Brassica* tissues in soil is only approximately 1-5% of maximum potential (Gardiner et al., 1999; Morra & Kirkegaard, 2002). Not only is the isothiocyanate release in soil considerably less than what is calculated according to glucosinolate concentrations, but isothiocyanates are volatile and rapidly dissipate in soils. AITC was reported to have a half-life of 20-60 h, and the reactivity of

AITC with soil organic matter resulted in a negative effect on its short half-life (Borek et al., 1995). Studies have also shown that glucosinolate hydrolysis byproducts may also interact with soils natural microflora, sometimes even resulting in an increase of some bacteria, therefore these interactions which were not observed in autoclaved soils may significantly alter results (Ntalli et al., 2018; Hu et al., 2015; Omirou et al., 2011).

The timing for maximum isothiocyanate release following incorporation into soils varies (Gardiner et al., 1999; Bending and Lincoln, 1999; Morra and Kirkegaard, 2002). While all studies indicated most of the isothiocyanates would be released in the first four days, maximum isothiocyanate release was measured as early as 2 hours (Morra and Kirkegaard, 2002). The sample times used in this experiment were immediately after inoculation, 24, 48, and 72 hours. Since studies have shown that the isothiocyanate release from *Brassica* plant matter in soils can be highest as early as 2 hours after incorporation into soil, if there was a significant reduction in bacterial populations it may have not been observed due to a lack of analysis between time of inoculation and 24 hours.

3.3.2.2 Antimicrobial activity in Absence of Soil

In this experiment, the effect of glucosinolate hydrolysis products from *Brassica* spp. brussels sprouts and mustard green plant matter on *E. coli* O157:H7 populations was observed. In order to effectively assess the antimicrobial potential of Brussels sprouts and mustard green plant matter, modifications were made to experimental design to maximize glucosinolate and AITC concentrations. Modifications included observation in absence of soil to limit reactivity of AITC, an increase in plant biomass, and addition of pull times between incorporation and 24 hours. When both Brussels sprout and mustard green plant matter were used there was a significant increase in *E. coli* O157:H7 populations at 24 hours ($p < 0.05$) (Figure 5). There was no significant change in bacterial populations for the control over the 48-hour incubation period, while concentrations increased from 7.75 to 8.53 log CFU/ml with the addition of brussels sprouts and 7.68 to 8.25 log CFU/ml with the addition of mustard greens. For both brussels sprouts and mustard greens there was a slight reduction of 0.26 and 0.12 log, respectively, from time of incorporation to 2 hours. While this reduction was not statistically significant it may indicate the

time of maximum isothiocyanate release, and thus only observed decrease in *E. coli* concentrations (Morra and Kirkegaard, 2002).

While the biofumigation concept began with the use of *Brassica* spp. as cover crops, recent studies have shown that biofumigation potential can be enhanced with processed plant products, like seed meals, with much higher glucosinolate concentrations. Another, and perhaps more efficient, way to utilize *Brassica* spp. is to directly extract AITC from the plant and use as a pesticide (Wu et al., 2009). In 2014 and 2016, petitions were filed to the National Organic Program (NOP) by Isagro USA, Inc for the use of AITC, produced through chemical synthesis, as a pre plant fumigant for organic crop production (USDA, 2018). While it seems the petitions to the USDA for addition of AITC to the NOP list as an organic pre plant fumigant are at a standstill, Isagro USA, Inc received approval in 2013 from the U.S. EPA for their product DOMINUS, with active ingredient AITC, a broad-spectrum product that controls soil-borne fungi and pests (CropLife, 2013). For years AITC has been used as a biofumigant in organic and conventional farming, and the literature supports the fumigant effects of AITC (Table 15). This along with the studies assessing AITCs antimicrobial effects on food products (Table 16) and allyl isothiocyanates GRAS status may support its potential as a method to treat agricultural fields contaminated with human pathogens.

Biofumigation, using Brassicaseous plant matter or other processed plant products, like seed meals or oils, is a complicated process that depends on multiple factors. Studies show potential for inactivation of foodborne pathogens by glucosinolate hydrolysis product AITC. However, additional research is needed to determine if AITC will possess the same antimicrobial activity against foodborne pathogens in agricultural soils. This includes determining the minimum AITC concentrations needed to completely eliminate foodborne pathogen populations in soils ranging in pH and temperature, and assessing the economic feasibility of large-scale application.

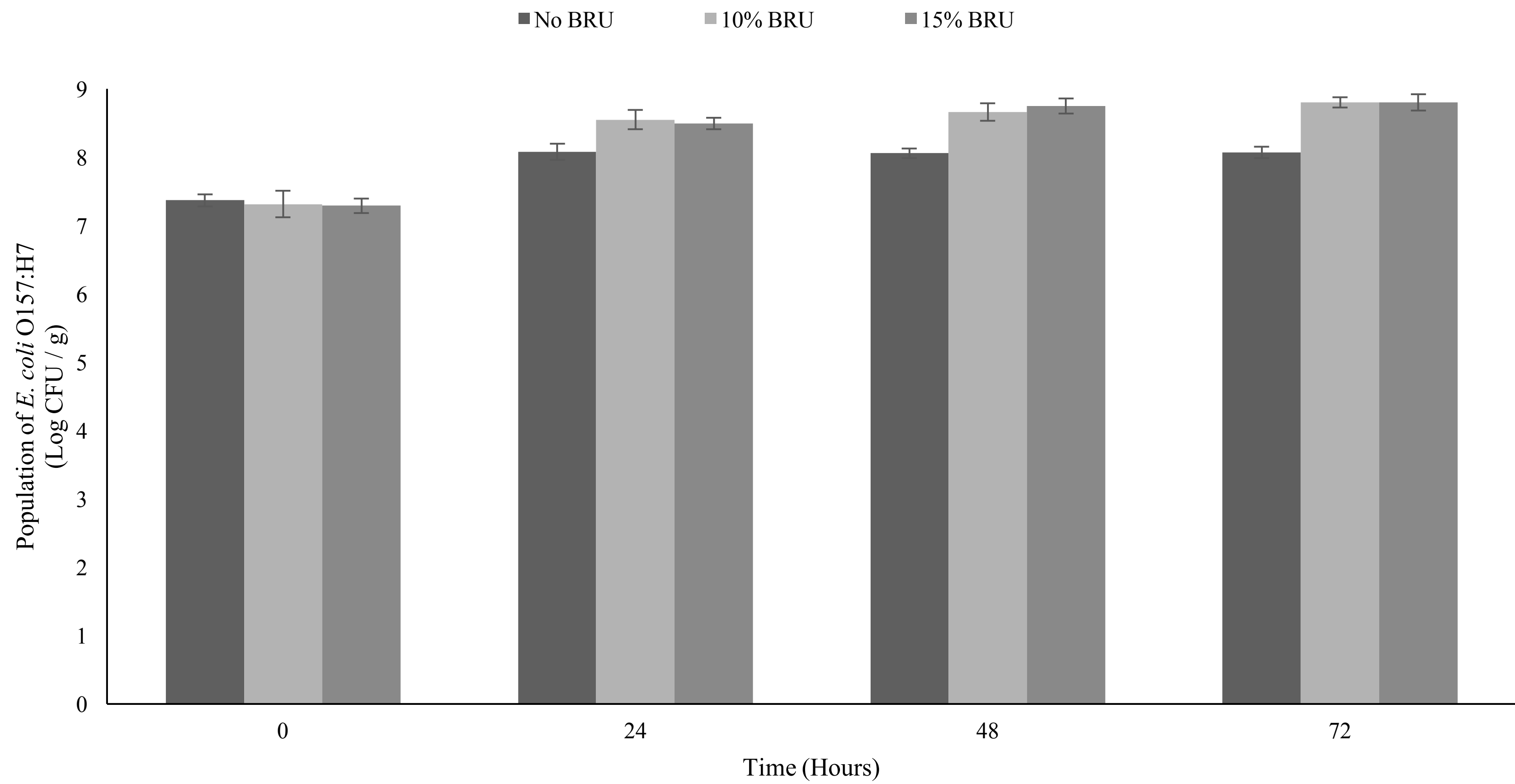

Figure 6 Mean *E. coli* O157:H7 values (log CFU/g) and standard errors, of soil samples treated with brussels sprout plant matter incubated at 20°C from 4 sample times (0, 24, 48, and 72 h) (n=4).

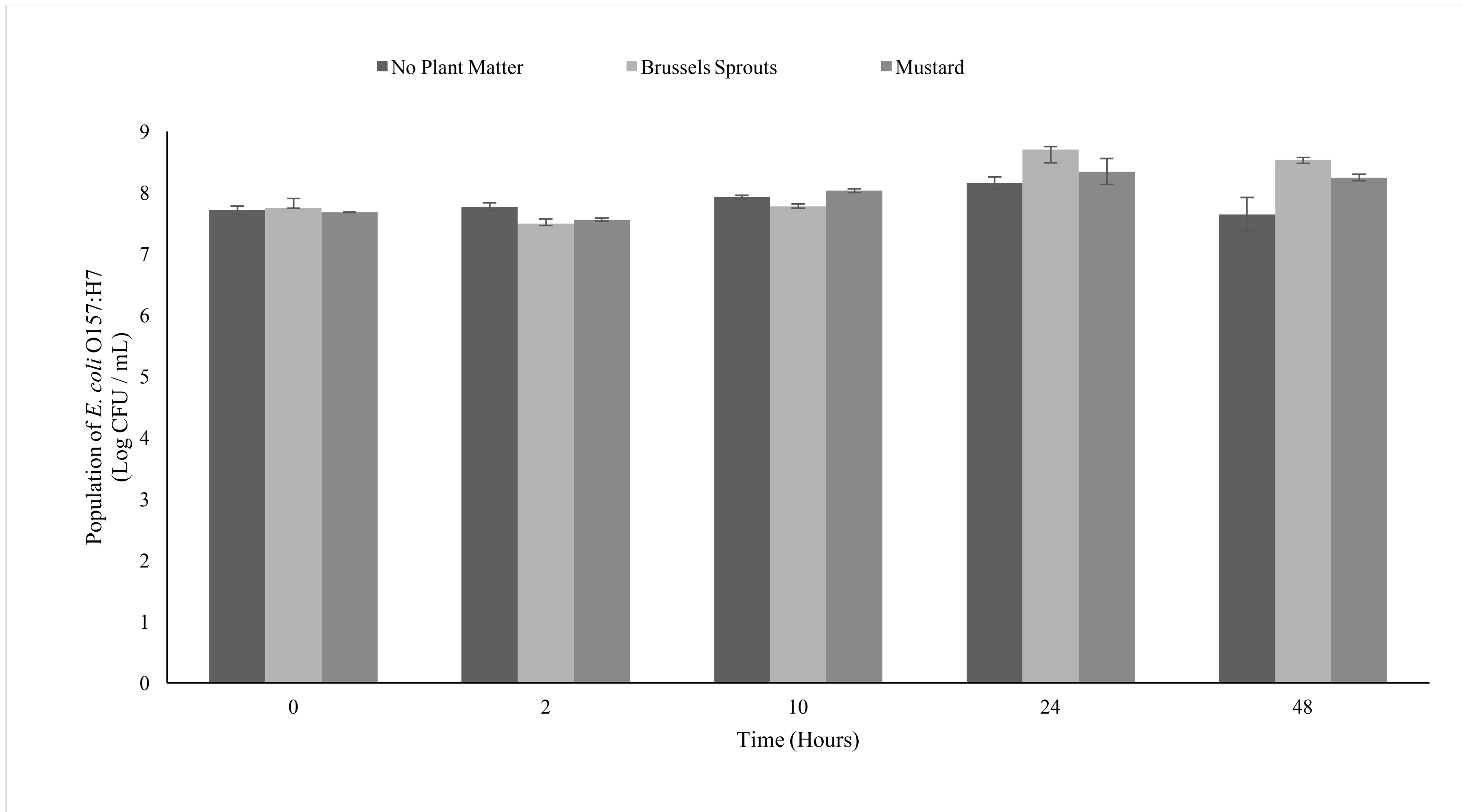

Figure 7 Mean *E. coli* O157:H7 values (log CFU/mL) and standard errors, of brussels sprout and mustard plant matter in inoculum incubated at 20°C from 4 sample times (0, 2, 10, 24, and 48 h) (n=2).

Table 15 Fumigant potential of AITC against a variety of plant pests and disease

Category	Species	Crop	Reference
Fungus	*Fusarium oxysporum, Verticillium dahliae*	Flowers and Strawberry	Hoffmann et al., 2020
	Aspergillus parasiticus	Nuts	Lopes et al., 2017
	Rhizoctonia solani	Snap bean and cabbage	Dhingra et al., 2003
	Basidiomycota, Glomeromycota, Zygomycota, Chytridiomycota	Tomato	Yao et al., 2020
	Fusarium oxysporum	Tomato	Yu et al., 2019
Insect	*Bradysia odoriphaga*	Chive seedlings	Shi et al., 2016
	Tribolium castaneum		Santos et al., 2011
	Solenopsis invicta		Du et al., 2020
Nematode	*Meloidogyne javanica*	Cucumber	Wu et al., 2011
	Criconemella, Hoplolaimus	Tomato	Yu et al., 2019
Pathogen	*Pythium ultimum,*	Flowers and Strawberry	Hoffmann et al., 2020
	Macrophomina phaseolina	Strawberry	Baggio et al., 2018
Weed	*Cyperus esculentus, Palmer amaranth, Digitaria sanguinalis*	Bell pepper	Bangarwa et al., 2011
	C. rotundus	Tomato	Yu et al., 2019
	Cyperus esculentus, Palmer amaranth, Digitaria sanguinalis	Tomato	Devkota and Norsworthy, 2014

Table 16 Antimicrobial potential of AITC against *E. coli*, *Salmonella*, and *L. monocytogenes* on food products

Food Product	Bacteria	Application	Reference
Apples, tomatoes, iceberg lettuce	*Salmonella*, *E. coli* O157:H7, *L. monocytogenes*	Direct application of AITC (98% purity)	Lin et al., 2000
Chicken breast	*L. monocytogenes, S. typhimurium*	AITC (purity > 95%) in controlled release vials, AITC-MAP system	Shin et al., 2010
Cooked Roast Beef	*E. coli* O157:H7, *L. monocytogenes*	Horseradish distillates in commercial grade canola oil	Ward et al., 1998
Dry fermented sausage	*E. coli* O157:H7	AITC (94% purity) in microcapsules	Chacon et al., 2006
Fresh beef, cured pork, sliced raw tuna, cheese, egg sandwich, noodles, pasta	*E. coli, S. typhimurium, S. enterditis*	AITC vapor	Isshiki et al., 1992
Fresh cantaloupe	*Salmonella*	AITC (95% purity), chitosan, and nisin coatings	Chen et al., 2012
Fresh cut onions	*E. coli* O157:H7, *L. monocytogenes*	Cyclodextrin entrapped AITC (94% purity)	Piercey et al., 2012
Fresh ground beef	*E. coli* O157:H7	Mustard flour and nitrogen flushed packaging	Nadarajah et al., 2005
Grape Tomatoes	*Salmonella*	Organic acid wash and chitosan-AITC coating	Mukhopadhyay et al., 2018
Ground Chicken	*E. coli* O157:H7	AITC essential oil and high-pressure processing	Huang et al., 2018
Hummus	*L. monocytogenes, S. enterica*	Direct application of AITC	Olaimat et al., 2018
Pork	*Salmonella, E. coli, L. monocytogenes*	AITC microencapsulation	Jin et al., 2020
Tomatoes	*E. coli*	Polylactic acid (PLA) films with AITC (AIT) vapor	Gao et al., 2018

3.4 Conclusions

Populations of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* significantly decreased in soil when treated with 10 or 15% of mustard seed meal at 21 °C ($p < 0.05$). *Brassica* spp. plant matter was not as effective against human pathogens. Populations of *E. coli* O157:H7 increased significantly in soil and in absence of soil at 21 °C when *Brassica* spp. plant matter was incorporated at 10, 15, or 75% ($p < 0.05$). In conclusion, the use of *Brassica* spp. cover crops as a method to decontaminate soils may not be sufficient. However, the use of higher glucosinolate containing processed products like defatted seed meals or extracts may be an effective strategy in reducing human pathogen concentrations in contaminated agricultural soils.

Chapter 4 Future Research

Our research highlights the contribution of food safety issues to food waste in the United States. However, the data only permitted analysis of 17% of recalls during 2015-2018 meeting our criteria. In order to increase the accuracy of the volume and monetary values of food wasted in food safety recalls, more consistent quantities need to be provided by the FDA. While this paper analyzes a small percentage of food safety recalls, it still provides justification for inclusion in future food waste analysis. After the New Era of Smarter Food Safety Blueprint is completely carried out, future analysis should investigate the impact on the efficiency food safety recalls and if this minimizes food waste due to these recalls.

Strategies to mitigate contamination of the food supply with foodborne pathogens should continue to be explored. While this paper supports the potential use of Brassica spp. processed products to decontaminate agricultural soils, additional research is needed to assess economic feasibility and practicality of large-scale application. Research efforts should be focused on determining the most effective Brassica species and the minimum concentration for seed meal application to achieve effective log reductions of foodborne pathogens in contaminated agricultural soils. Followed by analysis determining overall cost to farmers, and the willingness of farmers to pay for this treatment.

Extensive research has gone into the use of pure AITC as a pesticide, the existing pesticides including AITC as a main component should also be examined for use against foodborne pathogens in soil. If application of pesticides containing AITC are not sufficient alone, then combinations with methods like soil steaming, soil solarization, etc. should also be investigated. Once the use of either seed meal or pure AITC containing pesticides, alone or in combination with other methods, have proven to significantly decrease foodborne pathogens in a lab setting, surrogates should be identified to ensure these products possess the same antimicrobial activity in agricultural fields.

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Appendix A. Antimicrobial activity of Brassica spp. defatted seed meal against foodborne pathogens in agricultural soil

Table 17 Population of *L. monocytogenes* (log CFU/mL) when treated with mustard seed meal in agricultural soil during three days of incubation at 21 °C

	L. monocytogenes (log CFU/ml)			
Seed Meal Concentration (%)	0 Hour	24 Hour	48 Hour	72 Hour
None	7.39 ± 0.06^{Aa}	7.90 ± 0.14^{Ab}	8.09 ± 0.10^{Ab}	8.09 ± 0.12^{Ab}
10	6.86 ± 0.12^{Ba}	0.22 ± 0.22^{Bb}	0.00 ± 0.00^{Bb}	0.98 ± 0.59^{Bb}
15	7.00 ± 0.10^{Ba}	0.39 ± 0.25^{Bb}	0.34 ± 0.23^{Bb}	0.40 ± 0.26^{Bb}

Reported values are means ± standard errors (n=6). Means followed by different uppercase letters within columns and lowercase letters within rows are significantly different according to Tukey's test (p-value < 0.05).

Table 18 Population of *Salmonella* (log CFU/mL) when treated with mustard seed meal in agricultural soil during three days of incubation at 21 °C

	Salmonella (log CFU/ml)			
Seed Meal Concentration (%)	0 Hour	24 Hour	48 Hour	72 Hour
None	6.97 ± 0.14^{Aa}	8.00 ± 0.14^{Ab}	8.17 ± 0.10^{Ab}	8.38 ± 0.08^{Ab}
10	6.46 ± 0.22^{Aa}	2.60 ± 0.47^{Bb}	1.08 ± 0.41^{Bc}	0.76 ± 0.27^{Bc}
15	6.67 ± 0.20^{Aa}	1.08 ± 0.56^{Cb}	0.46 ± 0.29^{Bb}	0.29 ± 0.19^{Bb}

Reported values are means ± standard errors (n=6). Means followed by different uppercase letters within columns and lowercase letters within rows are significantly different according to Tukey's test (p-value < 0.05).

Table 19 Population of *E. coli* O157:H7 (log CFU/mL) when treated with mustard seed meal in agricultural soil during three days of incubation at 21 °C

	E. coli O157:H7 (log CFU/ml)			
Seed Meal Concentration (%)	0 Hour	24 Hour	48 Hour	72 Hour
None	7.51 ± 0.08^{Aa}	7.82 ± 0.13^{Aab}	8.19 ± 0.07^{Abc}	8.20 ± 0.13^{Ac}
10	7.17 ± 0.14^{ABa}	5.52 ± 0.01^{Bb}	4.37 ± 0.18^{Bc}	4.63 ± 0.23^{Bc}
15	6.81 ± 0.18^{Ba}	4.21 ± 0.27^{Cb}	2.51 ± 0.47^{Cc}	1.26 ± 0.45^{Cd}

Reported values are means ± standard errors (n=6). Means followed by different uppercase letters within columns and lowercase letters within rows are significantly different according to Tukey's test (p-value < 0.05).

Appendix B. Evaluation of the efficacy of Brassica spp. plant matter incorporation on the reduction of E. coli O157:H7 in soil and in absence of soil

Table 20 Populations of E. coli O157:H7 when treated with macerated Brussels sprouts in agricultural soil over three days of incubation at 21 °C

	E. coli O157:H7(log CFU/g)			
Brussels sprout plant matter (%)	0 Hour	24 Hour	48 Hour	72 Hour
None	7.37 ± 0.09^{Aa}	8.07 ± 0.12^{Ab}	8.06 ± 0.07^{Ab}	8.07 ± 0.08^{Ab}
10	7.31 ± 0.20^{Aa}	8.55 ± 0.14^{Bb}	8.66 ± 0.13^{Bb}	8.80 ± 0.08^{Bb}
15	7.29 ± 0.11^{Aa}	8.49 ± 0.08^{Bc}	8.75 ± 0.11^{Bc}	8.80 ± 0.12^{Bc}

Reported values are means ± standard errors (n=4). Means followed by different uppercase letters within columns and lowercase letters within rows are significantly different according to Tukey's test (p-value < 0.05).

Table 21 Populations of E. coli O157:H7 when treated with either macerated Brussels sprouts or mustard greens over three days of incubation at 21 °C

	E. coli O157:H7 (log CFU/ml)				
Plant matter at 75%	0 Hour	2 Hour	10 Hour	24 Hour	48 Hour
No Plant Matter	7.71 ± 0.07^{Aa}	7.77 ± 0.07^{Aa}	7.92 ± 0.03^{Aa}	8.15 ± 0.10^{Aa}	7.64 ± 0.28^{Aa}
Brussels Sprouts	7.75 ± 0.16^{Aa}	7.49 ± 0.07^{Ba}	7.78 ± 0.04^{Ba}	8.70 ± 0.05^{Ab}	8.53 ± 0.05^{Bb}
Mustard	7.68 ± 0.00^{Aab}	7.56 ± 0.03^{ABa}	8.03 ± 0.03^{Abc}	8.34 ± 0.21^{Ac}	8.25 ± 0.05^{ABc}

Reported values are means ± standard errors (n=2). Means followed by different uppercase letters within columns and lowercase letters within rows are significantly different according to Tukey's test (p-value < 0.05).