A STUDY OF THE MICROBIAL CONTAMINATION IN IMPORTED AND DOMESTIC FRESH PRODUCE AT RETAIL LEVEL IN ONTARIO

by

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A study of the microbial contamination in imported and local fresh produce at retail level in Ontario

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Abstract

Globalization has enabled the year-round availability of imported fresh produce in Toronto, supplementing the variety of locally grown produce in Ontario. Increased consumption of produce has led to more foodborne outbreaks, with *E. coli* O157:H7 as the second most frequent cause of illnesses. In this study, the levels of heterotrophic bacteria, coliforms, and generic *E. coli* were compared between three types of imported and local produce. Significantly higher levels ($p<0.04$) of heterotrophic bacteria were found in imported basil. Local romaine ($p<0.01$) and local spinach ($p<0.001$) contained significantly higher levels of coliforms. Local spinach also had a significantly higher ($p<0.005$) number of samples with coliform levels above 100 CFU/g. Although no statistical significance was found between the presence of *E. coli* and origin of produce, the five imported samples positive for *E. coli* compared to zero local samples supports the hypothesis that imported produce is more susceptible to microbial contamination.

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Chapter 1

Introduction

1.1 The Growing Produce Problem

Canada has one of the highest consumption rates of fresh fruits and vegetables per capita in the world (Kozak et al., 2013). According to the 2009 Food Statistics published by Statistics Canada (2010a), fresh fruit consumption, including citrus, and vegetable consumption, excluding potatoes, reached a record of 39.3 kg per person and 40.7 kg per person, respectively. Moreover, globalization and improved efficiency in producing, transporting, and distributing fresh produce have enabled year-round availability of a variety of produce but have also contributed to an increasingly complex food system (Fan et al., 2009; Olaimat, and Holley, 2012). The effects of globalization can be especially challenging to control since there is a lack of information and direct control over manufacturing processes and products abroad (Canadian Food Inspection Agency [CFIA], 2010). As farms become larger, produce fields have become situated closer to livestock and other potential environmental and health hazards, posing serious concerns that can impact the quality and safety of fresh produce (Kozak et al., 2013).

Since produce is minimally processed and often consumed raw, consumers face increased health risks and are exposed to foodborne pathogens that are not eliminated through a cooking process (Kozak et al., 2013). According to Health Canada, approximately 11 to 13 million Canadians are affected by foodborne illnesses each year (Health Canada, 2012a). An incident of contaminated produce often leads to sporadic cases and sometimes widespread disease outbreaks, creating significant barriers in tracing and detecting the origin of the contamination (Lynch et al., 2009). In a publication from the U.S. Centers for Disease Control and Prevention (CDC), approximately

46 percent of foodborne illness cases from 1998 to 2008 are linked to produce (Painter et al., 2013). Moreover, the consumption of leafy vegetables is among the top five leading causes of hospitalizations and deaths (Painter et al., 2013).

1.2 Canadian Produce and Produce Imports

Ontario's farmland is located in the southwest part of the province where the climate and soils provide farmers with ideal conditions for growing a wide selection of fruits and field vegetables (Lister, 2008; Bernier et al., 2010; Statistics Canada, 2011). The farm area encompasses 7.9 percent of Ontario's total land space, representing 12.7 million acres in 2011, a 4.8 percent decrease since 2006 (Statistics Canada, 2011). However, the average area per farm increased from 233 acres in 2006 to 244 acres in 2011 (Statistics Canada, 2011). Despite having a lower average farm size compared to other provinces, Ontario has the highest number of farms in Canada at 51,950 (Statistics Canada, 2011). Of the total farm area in Ontario in 2011, 70.5 percent was cropland, consisting of field crops, field vegetables, fruit, hay, sod, and nursery, and accounted for 10.2 percent of the total cropland in Canada (Statistics Canada, 2011). Additionally, vegetables comprised 1.5 percent of Ontario's cropland, and greenhouse vegetables accounted for 54.2 percent of Canada's total greenhouse area (Statistics Canada, 2011b).

Canada produces a diverse variety of fresh fruits and vegetables, although the production season is limited from April to October (Lister, 2008; Allen et al., 2013). Consequently, Canada faces challenges in meeting consumer demands for produce during months of limited availability and must therefore rely on imports (Allen et al., 2013; Kozak et al., 2013). In fact, approximately 41 percent of vegetables consumed by Canadians are imported (Kozak et al., 2013). The proportion of fresh produce consumed in Toronto that is imported is approximately 60 percent, with one

third of the imported produce consumed during Ontario's growing season (Lister, 2008). Despite that the majority of the imported produce consumed by Torontonians during Ontario's growing season can also be grown in the province (Lister, 2008), the increased demand for year-round and exotic produce has resulted in an increase in imports from foreign countries (Olaimat and Holley, 2012). In 2010, Canada imported 7.1 million dollars of vegetables, an increase from 3 million in 2005 (Gauthier, 2011). United States and Mexico were among the top countries that exported vegetables to Canada, followed by China, Peru, and Spain (Statistics Canada, 2010). The produce available in supermarkets is often sourced globally and the import process from distant countries increases the distance that the produce travels, consuming fossil fuels and emitting greenhouse gases (Kissinger, 2012; Caputo et al., 2013). In 2012, the main method of transportation for vegetables imported into Canada was by truck, followed by sea, together contributing over 800,000 tonnes of CO_2 emissions (Kissinger, 2012).

Although Canadian producers are becoming increasingly compliant with fresh produce standards and agricultural practices, standards may vary widely in exporting countries where information on production standards or practices may be limited or do not exist (Kozak et al., 2013). Consequently, fresh produce has become an increasingly frequent cause of foodborne disease outbreaks worldwide (Sewell and Farber, 2001; Kozak et al., 2013; Painter et al., 2013). In fact, the increased foodborne outbreaks caused by fresh produce has also coincided with increased sales of imported produce (Johnston, 2005). The multiple distribution stages and subsequent handling associated with importing can introduce foodborne pathogens to, or increase the prevalence of foodborne pathogens in, the imported produce (Lynch et al., 2009).

1.3 Food Safety in Canada

There are three authorities responsible for food safety in Canada. Health Canada establishes policies, regulations, and standards related to the quality and safety of food sold in Canada (Health Canada, 2013). The Canadian Food Inspection Agency (CFIA) enforces food safety policies and standards established by Health Canada (Health Canada, 2013). The Public Health Agency of Canada manages foodborne outbreak surveillance and epidemiology, and collaborates with the CFIA and Health Canada to provide support to the public during an outbreak (Health Canada, 2013). Health Canada and the CFIA maintained separate responsibilities until October 2013 when the responsibilities of the CFIA became adopted by Health Canada, strengthening the coordination and communication between the federal authorities and increasing the benefit to Canadians (Health Canada, 2013). Although Canada's food system is generally regarded as safe relative to other food systems worldwide, there are still opportunities for improving the surveillance of hazards and management of risks (Holley, 2010; Nesbitt et al., 2014).

Food safety is defined as ensuring that food will not cause harm to human health after being prepared or consumed according to its anticipated use (World Health Organization [WHO], 2000; Sun, 2012) and does not expose biological, chemical, and physical hazards to consumers (Munro et al., 2012). However, most biological pathogens are indiscernible to human senses, making it difficult for consumers to determine the safety of the foods they consume (Munro et al., 2012). To ensure that fresh produce is free from microbial contamination, the produce must comply with microbiological guidelines that are set by health authorities. In Canada, a "satisfactory" microbiological quality of fresh fruits and vegetables is based on a generic *Escherichia coli* threshold level of 100 CFU/g and the absence of pathogens such as *E. coli* O157:H7, *Salmonella, Campylobacter jejuni, Shigella, and Listeria monocytogenes*, and for

fresh produce the microbiological quality is based on a total coliform threshold level of 100 CFU/g (CFIA, 2010; Allen et al., 2013). *E. coli* levels between 100 and 1000 CFU/g require further investigation, and levels greater than 1000 CFU/g are considered as unsatisfactory (CFIA, 2010).

In Canada, Kozak and colleagues (2013) found that eight of the 27 produce-related outbreaks between 2001 and 2009 were from imported produce. However, the incidence of foodborne disease outbreaks related to produce has not been clearly documented, with Sewell and Farber (2001) reporting a lack of an observable pattern. Although the amount of produce consumed and the number of produce-related foodborne outbreaks have increased over the years, increased mass production of produce, as well as improved detection, may have revealed more produce-related illnesses that would have previously gone unreported (Sewell and Farber, 2001). For every reported case there are approximately 350 cases that remain unreported (Sewell and Farber, 2001). Moreover, since the emergence of the *E. coli* O157 strain, the number of produce-related outbreaks caused by this pathogen has increased (Sewell and Farber, 2001; Matthews, 2009; Olaimat and Holley, 2012; Kozak et al., 2013).

1.4 *E. coli* and Leafy Herbs and Vegetables

Since the 1990s, *E. coli* has been one of three major foodborne bacterial agents in the food system that have garnered a significant amount of attention and awareness from government agencies and the food industry (Newell et al., 2010). Although most outbreaks of *E. coli*-related illnesses have been linked to meat products, produce has become increasingly recognized as a cause of *E. coli* outbreaks (Barker-Reid et al., 2009; Kozak, et al. 2013). In addition, Barker-Reid and colleagues (2009) found that *E. coli* contamination in fresh produce closely followed

the leading cause of contamination – *Salmonella*. Mukherjee and colleagues (2004) noted that among fresh produce, lettuce was the most vulnerable to bacterial contamination. Moreover, investigations of total coliforms in produce found a significant prevalence of bacterial species that are part of the fecal coliform group (Mukherjee et al., 2004; Diez-Gonzalez, 2011). Although fecal coliforms are not agents that can cause disease, they indicate the potential presence of other undesirable microorganisms such as *E. coli* and pathogenic *E. coli* that may pose public health concerns for consumers (Carrero-Colon et al., 2011).

Culinary herbs and leafy vegetables have been increasingly associated with produce-related foodborne disease outbreaks (Matthews, 2009; CFIA, 2010; Kozak et al., 2013). Among fresh fruits and vegetables, herbs and leafy vegetables are considered by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as the highest priority with regards to microbial hazards (CFIA, 2010). In addition to the increased consumption of leafy vegetables, especially in its raw state, these produce possess intrinsic factors that make them susceptible to pathogen contamination (Wachtel et al., 2002; Matthews, 2009; Solomon and Sharma, 2009). For example, leafy vegetables are grown closer to the ground and are more likely to come in contact with soil, making them more vulnerable to contamination from pathogens in the soil (Matthews, 2009). Another factor is that leafy vegetables have large, fragile, and rough surfaces that are ideal breeding sites for pathogens and are difficult to clean effectively (Solomon et al., 2002; Wachtel et al., 2002).

1.5 Purpose of the Research

There is limited existing research that compares the quality of imported and local produce in Canada, as attention is mostly focused on organically and conventionally grown produce. Of the

studies related to imported and local produce, only surveys on overall microbial contamination have been carried out to examine the overall microbial levels in imported and local produce (Sagoo et al., 2001; CFIA, 2009, 2010), or only provide comparisons between imported and local produce but for a very limited produce selection (Johnston et al., 2006). Moreover, the testing of both imported and local varieties for microbial contamination for each type of produce was not always included for comparison (Johannessen et al., 2002). The majority of previous studies are also not specific to Canada. Research specific to Ontario is also limited, as only one study focuses on local produce, organic and conventional, that is grown in Ontario (Arthur et al., 2007). Another study examined imported produce available in Canada but only compared organic and conventional produce types (Allen et al., 2013).

The objective of this feasibility study is to compare the prevalence of microbial contamination in imported and domestic produce, based on the hypothesis that imported fresh produce is more susceptible to microbial contamination. Since an increased prevalence of *E. coli* associated with fresh produce has been well documented (Sewell and Farber, 2001; Allen et al., 2013; Kozak et al., 2013), this study will seek to compare the microbial loads between imported and local produce, using coliforms and *E. coli* as indicators of fecal contamination. Pre-harvest and post-harvest factors that can influence the microbial loads in produce at the farm level are also examined. Toronto is an ideal study area for investigating the quality of fresh produce as it is one of Canada's largest urban population centers (C. Ong, personal communication, September 30, 2013; Statistics Canada, 2011a). With the population within the Greater Toronto Area is estimated to reach 8.6 million by 2031 (Lister, 2008), there is an increasing concern to provide fresh produce to Toronto that is free from pathogens and other harmful biological agents that may cause harm to human health after consuming (Lister, 2008; Allen et al., 2013).

1.6 Composition of the Thesis

Chapter 2 presents a review of the literature and existing knowledge surrounding the microbial loads in fresh produce and the common pathogens associated with fresh produce outbreaks. The chapter also examines the factors that can influence the microbial loads in produce from the pre-harvest stage to the distribution stage. Chapter 3 details the methods and procedures used for sample collection, laboratory analysis, and statistical data analysis. The data and results from the statistical analysis are provided in Chapter 4. Chapter 5 presents a discussion of the findings and how they relate to other studies previously conducted in the field. Finally, a conclusion of the findings of the study, the ways in which this study contributes to the research on food safety and fresh produce, and recommendations for future research are provided in Chapter 6.

Chapter 2

Literature Review

2.1 Introduction

This chapter examines the literature surrounding fresh produce and pathogens that can compromise the quality of produce. Possible routes of exposure in which produce can become contaminated at the farm from the pre-harvest, harvest, and post-harvest stages to the distribution stage are explored.

2.2 *E. coli* and Coliforms

The term "coliform" was coined to describe the group of gram-negative, facultative anaerobic, rod-shaped bacteria that ferment lactose and produce acid and gas at 35°C within 48 hours (Leclerc et al., 2001). Members of the total coliform group are generally from the *Enterobacteriaceae* family and were originally thought to be found in the intestines of humans and warm-blooded animals (Carrero-Colon et al., 2011). However, it was later discovered that their presence is not consistently associated with fecal sources and that they can also be found naturally occurring in the environment such as in water, soil, and sediments (Caplenas and Kanarek, 1984; LeChevallier, 1990; Camper et al., 1991; Carrero-Colon et al., 2011). Although coliforms could be detected easily, their inconsistent association with fecal contamination was concerning (Carrero-Colon et al., 2011). As a result, the class of "fecal coliforms" was introduced and replaced coliforms as an indicator of fecal contamination (Feng et al., 2002)

Fecal coliforms, which was first defined by Eijkman in 1904, is a sub-category of total coliforms and is also referred to as 'thermotolerant coliforms' (Rompre et al., 2002). Fecal coliforms

possess the enzymes β-galactosidase and β-glucuronidase and are defined based on their ability to grow and ferment lactose and mannitol to produce acid, gas, and indole at higher temperatures between 44 °C and 45°C (Payment et al., 2003). The primary members of the fecal coliform group include *E. coli, Klebsiella* spp.*, Enterobacter* spp.*,* and *Citrobacter* spp. (Carrero-Colon et al., 2011). However, the fecal coliform assay is based on the assumption that *E. coli* is the only species from the group that can grow at higher temperatures (Dockins and McFeters, 1978). Although the association of *Enterobacter* spp. and *Klebsiella* spp. with fecal sources is uncommon, they are part of the fecal coliform group, making the correlation between fecal coliforms and fecal contamination not always reliable (Dockins and McFeters, 1978; Johannessen et al., 2002). Moreover, prior to the realization of this limitation, fecal coliforms were commonly used as indicators of fecal contamination in food (Diez-Gonzalez, 2011).

Other instances have questioned the efficacy of using fecal coliforms as an indication of fecal contamination (Edberg et al., 2000). For example, the Canadian Food Inspection Agency (1999) issued two recalls of alfalfa sprouts after the detection of fecal coliforms, specifically the species *Klebsiella pneumonia*. Although *Klebsiella pneumonia* has rarely been associated with fecal contamination, the recalls were nonetheless issued due to their association with fecal coliforms (Edberg et al., 2000; Johannessen et al., 2002). This recall highlights the limitations of using fecal coliforms and reveals issues surrounding their suitability as indicators of fecal contamination. These concerns increased after the development of single-step methods to rapidly detect *E. coli* in the 1980s (Edberg et al., 2000; Diez-Gonzalez, 2011).

The term *E. coli* was introduced in 1885 by Theodor Escherich, a German paediatrician, after he isolated the bacteria from the feces of a patient (Diez-Gonzalez, 2011). *E. coli* is a gram-

negative, facultatively anaerobic, rod-shaped organism from the *Enterobacteriaceae* family (Fields, 1979). Although other species such as *Salmonella* and *Shigella* are also members of the *Enterobacteriaceae* family, *E. coli* is the only species that is detected in the intestines of healthy humans and warm-blooded animals, and is present in over 90 percent of human and animal feces (Borczyk et al., 1987; Edberg et al., 2000; McElhany and Pillai, 2011). *E. coli* has been shown to be more widely distributed in terms of habitat than *Salmonella,* and can range from strains that are commensal with little to no virulence, to strains that have evolved over many years to become very infectious and virulent (Ochman and Wilson, 1987; Ochman and Davalos, 2006; McElhany and Pillai, 2011). In the 1890s, *E. coli* was first proposed as a possible indicator for fecal contamination after researchers noticed that it was frequently present in the feces of humans and animals yet absent in other niches (Diez-Gonzalez, 2011).

Technologies to rapidly detect *E. coli* were developed based on the fact that the β-glucuronidase enzyme was present in 95 percent of *E. coli* strains (Rompre et al., 2002). Chromogenic and fluorescent substrates, such as the 4-methylumbelliferyl-beta-D-glucuronide, increased the ease of isolating *E. coli* in liquid and solid media and these substrates were incorporated in many different commercial media formulations (Edge and Bohem, 2011). For almost a century, researchers have attempted to seek other coliform bacteria or indicators that could replace or complement *E. coli* as a fecal indicator (Diez-Gonzalez, 2011). However, *E. coli* remains the only microorganism to date that meets the largest number of guidelines for the ideal fecal indicator bacteria and has been the preferred indicator bacteria in a variety of food and water related studies (Johannessen et al., 2002; Johnston et al., 2006; Bohaychuk et al., 2009; Oliveira et al., 2010; Allen et al., 2013).

2.2.1 *E. coli* O157:H7

Pathogenic *E. coli* can be differentiated from commensal, or non-pathogenic, *E. coli* by the presence of pathogenicity-associated islands, where additional genetic material is present in the chromosomes (Johnson, 2011). Pathogenic *E. coli* can be categorized according to the types of genetic material present or absent in the chromosomes, or pathotypes, and the types of diseases they can cause in the host (Hacker et al., 1997; Kaper et al., 2004). *E. coli* O157:H7, or enterohemorrhagic *E. coli*, is a serotype of *E. coli* that possesses gene-encoding toxins and can cause severe disease, especially in vulnerable populations (McElhany and Pillai, 2011). The most common illnesses caused by a pathogenic *E. coli* infection include diarrheal disease and urinary tract and systemic infections (Forsythe, 2000). Compared to *E. coli* and *Salmonella, E. coli* O157:H7 has a relatively narrowly distributed habitat (Borczyk et al., 1987). Nevertheless, since *E. coli* O157:H7 is a serovar of *E. coli,* it was assumed at one point that commensal *E. coli* could also be a reliable indicator for serotype O157:H7 strains (Diez-Gonzalez, 2011). However, this assumption has not been proven in studies involving animal waste and fresh produce and the use of commensal *E. coli* as an indicator has thus been dismissed (Diez-Gonzalez, 2011).

E. coli O157:H7 is a zoonotic organism commonly found in the intestinal tracts and feces of cattle and small ruminants (Maule, 2000; Fremaux et al., 2008; McElhany and Pillai, 2011). The pathogen has also been associated with monogastric mammals as well as with birds and insects, although their colonization is temporary and may not cause disease to the host (Cizek et al., 1999; Elder et al., 2000). Since the main transmission route of the pathogen is through manure, it is imperative to determine its prevalence and fate in the environment in order to control its dissemination (Maule, 2000; Fremaux et al., 2008). Although the affinity of *E. coli* O157:H7 for

the intestinal tracts of large and small ruminants has been well documented, the prevalence and method of its colonization has been sporadic and less understood (Diez-Gonzalez, 2011).

E. coli O157:H7 became a prominent foodborne pathogen in the 1980s and was commonly linked to contaminated ground beef (Diez-Gonzalez, 2011). However, the pathogen has been increasingly implicated in ready-to-eat fresh vegetables and leafy vegetables over the years (Nguyen-the and Carlin, 1994; Kozak et al., 2013). A review of produce-related outbreaks associated with *E. coli* O157:H7 from 1984 to 1993 in Canada shows one outbreak in 1995 linked to spoiled lettuce received at and served from a hospital kitchen that affected 8 patients, 10 staff, and 3 volunteers (Sewell and Farber, 2001). In 2002, 17 confirmed cases, 81 possible cases, and 11 probable cases were attributed to the consumption of fresh vegetables used in prepared salads (Kozak et al., 2013). Among those infected, four people were hospitalized and two people died (Kozak et al., 2013). In 2006, a large-scale outbreak linked to spinach occurred in both Canada and the United States, with 207 cases and three deaths, although only three of those cases were documented in Canada (Jay et al., 2007). The sources of contamination were attributed to wildlife having access to the spinach crops and a cattle ranch downstream from the spinach field (Jay et al., 2007). In 2008, shredded iceberg lettuce caused 38 illnesses and 21 hospitalizations illnesses in Ontario (Kozak et al., 2013). An investigation revealed that the shredded lettuce originated from California and was sent to another company for processing before bagging (Kozak et al., 2013). In the same year, romaine lettuce was the culprit of 38 probable and 29 confirmed cases in Ontario (Kozak et al., 2013). However, the sources of contamination were not identified in either of the two lettuce outbreaks (Kozak et al., 2013).

2.2.2 *Salmonella*

Salmonella serovars also naturally occur in the gastrointestinal tracts of animals and its transmission route is commonly through direct contact with the animals or through direct or indirect contact with fecal contamination (Callaway et al., 2008). The *Salmonella* genus consists of over 2,500 different serovars, which originate from the *S. enterica* subspecies (D'Aoust and Maurer, 2007; Johnson, 2011). While most *Salmonella* serovars do not particularly colonize in a specific animal, some serovars such as *Enteritidis* and *Montevideon* have been frequently linked to poultry and ground beef, respectively (Callaway et al., 2008). *Salmonella* can colonize in mammals and farm animals such as cattle, swine, poultry, horses, reptiles, and domestic animals (Callaway et al., 2008; Diez-Gonzalez, 2011). *Salmonella* is not only an intestinal bacterium but can also cause many infections in the host, a characteristic that differentiates it from *E. coli* (Diez-Gonzalez, 2011). As such, it is regarded as one of the most diverse pathogens due to its ability to colonize and cause disease in many types of animal species (Callaway et al., 2008).

Since *Salmonella* has such a wide distribution in nature and is commonly associated with poultry, it has been responsible for 26 percent of foodborne diseases in the United States (Doyle, 1990; Johnson, 2011). In Canada, outbreaks of *Salmonella* related to produce have been commonly associated with alfalfa sprouts (1995-7), mung bean sprouts (2001, 2005), cucumbers (2004), roma tomatoes (2004), and cantaloupe (1991, 1996-7, 2002, 2006, 2008), (Sewell and Farber, 2001; Kozak et al., 2013). The sources of contamination were not always identified, but in cases where identification was possible, the sources were attributed to poor worker hygiene, equipment maintenance and sanitation, and pest management practices (Sewell and Farber, 2001; Kozak et al., 2013). Other sources included temperature abuse and using contaminated water to irrigate and clean or rinse produce (Sewell and Farber, 2001; Kozak et al., 2013).

2.2.3 *Shigella*

The genetic makeup of *Shigella* makes it nearly identical to *Escherichia*, with the exception that *Shigella* has a reduced ability to colonize in animals (Warriner et al., 2009). In the United States, *Shigella* has been estimated to be the cause of 6.6 cases of foodborne illnesses per 100,000 people and is considered the third most frequent foodborne disease-causing bacterial culprit in the United States (Diez-Gonzalez, 2011). *Shigella* is normally isolated from the intestinal tracts of humans and can also be present asymptomatically in humans (Diez-Gonzalez, 2011). As a foodborne pathogen, *Shigella* is typically associated with poor food handling practices resulting in direct fecal contamination or exposure to sewage or wastewater (Johnson, 2011). In fact, international outbreaks of shigellosis associated with fresh produce have occurred as a result of wastewater contamination (Kapperud et al., 1995). One of the concerns surrounding *Shigella* is the low infective dose required to cause illness (Sewell and Farber, 2001).

In 1998, an outbreak of *Shigella sonnei* was traced back to parsley and affected 400 people in Ontario, Alberta, and three U.S. states that ate at the same kiosk or restaurant (CDC, 1999; Sewell and Farber, 2001). The source of the outbreak was identified to be farms in Mexico and California where unchlorinated water was used to clean the parsley in the packing shed (CDC, 1999). Inadequate sanitation facilities and limited knowledge of food hygienic practices among farm workers added to the list of risk factors (Sewell and Farber, 2001). In 2001, an outbreak of *S. sonnei* linked to spinach in British Columbia resulted in 31 cases of illness (Kozak et al., 2013). Investigations into this outbreak revealed that the contamination originated from water used to wash the spinach, which was obtained from a ditch adjacent to the field (Kozak et al., 2013). Although *Shigella* was not isolated in the ditch water, *E. coli* was detected, suggesting that the contamination was due to sewage water from a septic system (Kozak et al., 2013).

2.3 Farming Practices

Within the foodborne outbreaks of *E. coli* O157:H7, *Salmonella*, and *Shigella*, the source of contamination, if identified, was often linked to a combination of factors such as contaminated irrigation water, inadequate farming practices during harvest and processing, and improper worker hygiene (Sewell and Farber, 2001; Kozak et al., 2013). Additionally, many of the outbreaks were linked to produce imported from farms outside of Canada (Sewell and Farber, 2001; Kozak et al., 2013). As a result of the increased scale of farms, the processing of produce with other commodities such as meat has become more common (Olaimat and Holley, 2012). Moreover, the short shelf-life of produce makes it difficult to investigate produce-related outbreaks since the implicated batch of produce may no longer be available for testing by the time an investigation is initiated (Kozak et al., 2013).

Sources of contamination can originate from pre-harvest processes and post-harvest processes (Beuchat and Ryu, 1997; Suslow et al., 2003). Although post-harvest measures such as pruning old leaves and cleaning aim to preserve the microbiological quality of fresh produce throughout the processing and distribution stages, proper management of pre-harvest farming practices is instrumental to ensuring high external and internal quality in produce (Nicola and Fontana, 2007). Contamination from humans occurs in both processes (Mukherjee et al., 2007; Barker-Reid et al., 2009). Several studies (Ibekwe et al., 2004; Doyle et al., 2005; Islam et al., 2005; Mukherjee et al., 2007; Barker-Reid et al., 2009) have found that *E. coli* is capable of persisting in soil and water and can be transmitted to produce through farm management practices during pre-harvest and post-harvest processes.

2.3.1 Sources of Contamination: Pre-harvest

The microbial contamination of farm environments from enteric pathogens that in turn affect the microbial levels in pre-harvest fresh produce has been well documented in field studies. Pre-harvest sources of enteric pathogens are generally associated with manure-based fertilizers or soil amendments (Hutchison et al., 2004; Johannessen et al., 2004; Millner, 2009), improperly composted manure and implications on pathogens introduced to soil (Gagliardi et al., 2003; Islam et al., 2004; Ingham et al., 2005; Johannessen et al., 2005), runoff caused by rain or floods (Muirhead et al., 2006; Millner, 2009), contaminated irrigation water (Duffy et al., 2005; Steele et al., 2005; Stine et al., 2005; Hamilton et al., 2006), animal farming and the presence of wildlife or domestic animals (Rice et al., 1995; Wallace et al., 1997; Doane et al., 2007; Millner, 2009), the mishandling of produce by workers or unhygienic worker practices (McEvoy et al., 2009; Millner, 2009), and improper maintenance and sanitation of equipment and processing facilities during harvest (Espinoza-Medina et al., 2006).

Many farmers apply manure-based fertilizer due to its high nutritional value and its benefits in maintaining soil quality (Suslow et al., 2003; Millner, 2009). Despite these benefits, untreated manure-based fertilizers applied to produce fields have been shown to significantly increase the survival of *E. coli* and the risk of contamination in fresh produce, especially those grown on organic farms (Kudva et al., 1998; Lung et al., 2001; Hutchison et al., 2004; Mukherjee et al., 2007; Fremaux et al., 2008). Among the different types of manure-based fertilizers, which include cattle, chicken, swine, and horse manure, cattle-based fertilizers posed the highest risk for *E. coli* contamination (Millner, 2009). Various studies have demonstrated the ability of *E. coli* and other enteric pathogens to survive in soils for extended periods of time after being amended with manure or treated with manure-based fertilizers (Jiang et al., 2002; Topp et al.,

2003; Avery et al., 2004; Islam et al., 2004; Berry and Miller, 2005; Johannessen et al., 2005; Sinton et al., 2007; Fremaux et al., 2008). Factors such as the type of organism and presence of other indigenous organisms, as well as the soil moisture, texture, and nutrients can influence the survivability of these pathogens in the environment (Jiang et al., 2002; Topp et al., 2003; Sinton et al., 2007; Byappanahalli et al., 2011). In one study, cattle manure was applied to a pasture and *E. coli* survived approximately 48 days, which was 10 days longer than the survival time of *Salmonella* (Bolton et al., 1999; Sinton et al., 2007). Similarly, Sinton et al. (2007) observed varying growth levels of *E. coli* O157:H7 and *S. enterica* in cow pats on pastures, suggesting that cattle manure not only introduced enteric bacteria into the environment but also provided an environment and nutrients for these pathogens to flourish in the soil. *E. coli* O157:H7 has also been found to out-survive other enteric pathogens in manure (Kudva et al., 1998; Guan and Holley, 2003). However, Gagliardi and Karns (2002) did not observe that the addition of manure to soil affected the persistence of the pathogen. In addition to animal manure being a contributor to the growth of enteric bacteria in soils, chemical fertilizers have also been shown to encourage the growth of *E. coli* (Byappanahalli and Fujioka, 2004; Whitman et al., 2006).

In addition to surviving in soil for extended periods of time, *E. coli* can also attach to and even be internalized into produce (Solomon et al., 2002; Wachtel et al. 2002). *E. coli* was found on lettuce plants grown in both greenhouse and field conditions after direct contact with manure-based fertilizers or contaminated irrigation water (Solomon et al., 2002; Cooley et al., 2006; Franz et al., 2008). After compost inoculated with *E. coli* O157:H7 was applied to soil, the pathogen survived over 5 months and was also detected on crops that were planted in the soil (Islam et al., 2004). In another study, Solomon and colleagues (2002) sprayed water inoculated with 10^7 CFU per ml of *E. coli* O157:H7 onto greenhouse-grown lettuce and the strain was

detected in the lettuce samples after 20 days. In contrast, a study on the potential for *E. coli* O157:H7 contamination in leafy greens and herbs after the application of composted, manure-based fertilizer (Johannessen et al., 2004) showed that *E. coli* O157:H7 could not be detected in lettuce samples even though the pathogen was present in all the manure fertilizers applied to the soils. Johannessen and colleagues (2004) concluded that further research was required to investigate how the lettuce avoided contamination. Moreover, several studies (Sagoo et al., 2001; Johannessen et al., 2002; Mukherjee et al., 2004) have attempted to detect *E. coli* O157:H7 in fresh fruits and vegetables, though no substantial evidence of *E. coli* contamination during the pre-harvest stage has been found.

The implementation of proper Good Agricultural Practices is also extremely important to ensure that the potential for microbial contamination is minimized in pre-harvest produce (Delazari et al., 2006). These practices include applying properly composted manure, using irrigation water from a potable source, and applying pesticides made from potable water to minimize exposure of fruits and vegetables in the field to contaminants (Delazari et al., 2006). Other factors that should be managed include controlling domestic animals, wildlife, and insects, which are potential vectors for enteric pathogens, and providing regularly-maintained toilets, hand washing stations, and sanitation facilities to field workers (Suslow et al., 2003; Hajmeer and Crozier-Dodson, 2012). For example, Canadian good agricultural practices advise that uncomposted manure should be applied more than four months before harvesting (Martin, 2005; Blakely et al., 2008). Studies also found that ageing non-composted manure for more than 6 months significantly reduced the risk of microbial contamination among organic produce (Mukherjee et al., 2006; Millner, 2009). Hutchison and colleagues (2004) reported that applying aged manure on top of soil without mixing the manure into the soil significantly reduced pathogen levels.

2.3.2 Pre-harvest: Irrigation and Water Source Contamination

Agriculture is one of the largest uses for fresh water, as more than 70 percent of fresh water is used for irrigation purposes (Gerba and Choi, 2009). As a result of increased outbreaks of *E. coli* O157:H7 in recreational water, there have been increased concerns of the ability of *E. coli* to survive in freshwater environments (Leclerc et al., 2002; Muniesa et al., 2006). In river water, *E. coli* was able to survive at low temperatures, and for longer periods in circumstances with low levels of indigenous microorganisms (Flint, 1987; Bogosian et al., 1996; Byappanahalli and Ishii, 2011). Vital and colleagues (2008) reported that the growth of *E. coli* was enhanced in sterile river water, which had higher temperatures and a high availability of nutrients such as organic carbon. Moreover, water microcosm studies revealed that depending on the water source, *E. coli* O157:H7 could survive over 12 weeks at 8°C and up to 12 weeks at 25°C (Diez-Gonzalez, 2011).

Agricultural farmers often use nearby waterways such as rivers, streams, and ponds as sources of irrigation water (Ijabadeniyi et al., 2011). However, most of these water sources do not meet microbial standards required for irrigation (Ijabadeniyi et al., 2011). The irrigation of produce fields with contaminated water is an increasing international concern that poses significant risks to consuming fresh produce, especially for the variety of produce that is usually consumed raw (Beuchat, 2002). Produce with surfaces that are furrowed or that can retain water is also more vulnerable to contamination (Beuchat and Ryu, 1997). Since the portion of the produce grown near or on the surface of the soil is often the part that is consumed, there is a higher potential for contamination due to the increased contact with soil and water (Beuchat and Ryu, 1997). Contaminated irrigation water was among the factors that caused an outbreak of *E. coli* resulting in a large recall of spinach that affected many Americans and Canadians (Gagliardi et al., 2003).

The method of irrigation can also encourage the spreading of enteric bacteria, as sprinkle irrigation is more likely to disperse contaminated water onto the edible portion of the produce than furrow irrigation (Gerba and Choi, 2009). The quality of produce is not only affected by the water used for irrigation, but also by the methods used to disperse pesticides or treat foliage (Suslow et al., 2003; Hajmeer and Crozier-Dodson, 2012). The quality of water used to create and disperse pesticides can introduce pathogens to the soil and to the produce grown in the soil (Suslow et al., 2003; Hajmeer and Crozier-Dodson, 2012). Similarly, Gerber and Choi (2009) found that the use of untreated water in pesticide sprays was a highly suspected source of enteric bacteria in produce.

Rainfall and extreme rain events can increase the potential for contamination of irrigation water sources. Rainfall can spread pathogens through runoff, especially if manure has been applied upstream on agricultural land (Siller-Cepeda et al., 2009). In Quebec, Canada, a study found that 70 percent of samples retrieved from a river were contaminated with human and swine enteroviruses as a result of pig farming in the area (Payment, 1989). Flooding can also transport pathogens from locations where animals have grazed on or been confined, onto produce fields (Siller-Cepeda et al., 2009). Additionally, in areas where animal manure is applied to soils, immediate precipitation can disperse fecal indicator bacteria to nearby waterways (Meals and Braun, 2006; Mishra et al., 2008). In severe rain events, flooding can overwhelm or damage wastewater treatment plant systems, contaminating the water with human, municipal, as well as industrial wastes, and transport contaminants downstream to be deposited in produce fields (Siller-Cepeda et al., 2009). Pathogens such as *E. coli* O157:H7 have been found to survive more than 10 days in soil after being deposited from a flood (Tate, 1978).

Watersheds provide water for drinking and recreational purposes, for irrigating in agricultural areas, and for various livestock and wildlife (Byappanahalli and Ishii, 2011). However, watersheds are vulnerable to contamination from animal wastes, especially in agricultural areas (Byappanahalli and Ishii, 2011). Fecal indicator bacteria usually reside in the upper layer of soil and can be transported or dispersed by infiltration and surface runoff, potentially contaminating groundwater and adjacent waterways (Unc and Goss, 2004). For example, animals kept in pastures produce waste that often accumulate and can contribute to the contamination of groundwater and adjacent waterways (Edge et al., 2012). Thus, farm animals have been identified as one of the main sources of fecal indicator bacteria in agricultural watersheds and their presence has often resulted in increased levels of indicator bacteria in watershed runoff (Doran and Linn, 1979; Jawson et al., 1982; Okabe et al., 2007; Vogel et al., 2007; Kon et al., 2009). Improper waste management from farm animals can result in leaching or runoff and introduce fecal indicator bacteria present in these wastes into adjacent waterways (Byappanahalli and Ishii, 2011).

In developing countries, rapidly growing populations have increased the demand for food and water and correspondingly generated increasing amounts of domestic wastewater (Hanjra et al., 2012). The slow development of wastewater treatment facilities or, in some countries, a lack of wastewater treatment has resulted in the release of untreated wastewater to surface water bodies, affecting surface water quality (Lazarova and Bahri, 2005; Qadir et al., 2010). However, disinfecting wastewater effluents prior to discharging is not a common practice in developing countries and in other areas of the world such as Europe, although it is required in U.S. and Canada (Gerba and Choi, 2009). Consequently, farmers have been both voluntarily and involuntarily using contaminated water to irrigate crops, especially in areas with scarce

precipitation where contaminated water is the only reliable source of water (Keraita and Drechsel, 2004; Scott et al., 2004). Irrigating with contaminated surface water runoff has been documented as a potential source of pathogens, although there are many benefits of the practice including its ease of access in remote areas and its nutritional value (Hanjra et al., 2012; Steele et al., 2005). Despite the benefit of added nutrients to farmed produce irrigated with diluted and untreated wastewater, there are also health-related implications associated with consuming raw crops irrigated with untreated wastewater (Hanjra et al., 2012). These implications include negative impacts to human health, especially among children in developing countries where diarrheal diseases is the leading cause of death and government agencies are unaware of such health effects (Hanjra et al., 2012). The limited information on water use makes it difficult to estimate future water needs, as farming activities are often not included in official statistics in some developing countries (Qadir et al., 2010).

Vegetables in developing countries are often grown close to the markets where they are sold due to a lack of refrigerated transportation and storage facilities (International Water Management Institute, 2006). Clean water sources are often scarcer closer to city centers and there is no other source of irrigation water other than contaminated water (International Water Management Institute, 2006; Qadir et al., 2010). Examples of regions that experience water scarcity include Mexico, Pakistan, Africa, and Vietnam, and wastewater irrigation is a common practice that is used for a high percentage of crop production (International Water Management Institute, 2006; Cirelli et al., 2012). Wastewater irrigation is used for 25 percent all vegetables grown in Pakistan and for 60 to 100 percent of vegetables provided to most cities in Sub-Saharan Africa (International Water Management Institute, 2006). In Mexico, the agricultural land irrigated with wastewater supports over 450,000 people (International Water Management Institute,

2006). Between 10,000 and 30,000 hectares of land in Vietnam and Pakistan are irrigated with undiluted wastewater and this figure does not include areas irrigated with diluted wastewater (International Water Management Institute, 2006). Globally, the figures for areas irrigated with both undiluted and diluted wastewater are unclear; however, it is estimated that approximately 3.5 million hectares of land are irrigated with some form of wastewater (Jimenez and Asano, 2004; Qadir et al., 2010). In their studies, Ibekwe and colleagues (2004), Islam and colleagues (2005), and Barker-Reid and colleagues (2009) demonstrated that produce irrigated with contaminated water was a significant pre-harvest transmission route for produce contamination. In areas such as Africa and Vietnam, the water used to wash, sprinkle, or cool harvested produce sold in markets is often from the same source as the water used for irrigation, which can harbour pathogens if the irrigation water is already contaminated (Drechsel et al., 2007; Ogunsola and Adesiji, 2008; Qadir et al., 2010; Tram et al., 2008).

2.3.3. Farm Animals and Wildlife

Aside from the risks of applying manure-based fertilizer directly to agricultural soil, runoff from animal grazing areas or poorly segregated animal farming areas can also increase the risk of produce contamination (Muirhead et al., 2006; Millner, 2009). Higher animal densities have been attributed to increased efficiency of animal farming operations but have also increased the production of animal waste (Millner, 2009). Moreover, disposing of the increased animal waste has become a significant waste management issue (Suslow et al., 2003). Possible waste management strategies to prevent contamination of watersheds around agricultural farms include storing manure in secure locations prior to use on farmland to minimize the potential for contamination through runoff or infiltration, applying manure in the late fall or early spring

seasons, and growing vegetation to minimize erosion around the field (Natvig et al., 2002; Meals and Braun, 2006; Byappanahalli and Ishii, 2011).

Wildlife has been identified in various studies as a significant non-point source of fecal bacteria, although it is difficult to identify the specific wild animal species responsible for the contamination (Scott et al., 2002; Stoeckel et al., 2004; Field and Samadpour, 2007). There are often limitations related to specific geographical areas and sample sizes within studies that attempt to identify the species of animals positive for a specific pathogen (Rice, 2009). For example, deer, raccoons, as well as water birds consisting of geese, gulls, and waterfowl are all common sources of fecal contamination in waterways (Ishii et al., 2007; Vogel et al., 2007; Kon et al., 2009). To complicate matters, these animals are also known to carry the human pathogens that have been linked to produce outbreaks (Parish 1997; Jijon et al., 2007). For example, certain pathogenic *E. coli* strains have been isolated from deer, rabbits, and feral swine (Akasura et al., 1998; Fischer et al., 2001; Wahlstrom et al., 2003; Ishii et al., 2007; Jay et al., 2007; Sanchez et al., 2009), as well as from cattle, goat, sheep, and domestic animals (Byappanahalli and Ishii, 2011).

However, the potential for fecal contamination in agricultural areas or waterways by these wild animals is considered more localized than the types of fecal contamination caused by migratory birds traveling long distances that disperse pathogens across many areas (Gabrey, 1996). Moreover, there is limited data that identify wild animals as the source of fecal contamination in produce-related outbreaks and most studies are incapable of providing accurate prevalence estimates in specific populations of animals (Rice, 2009). Although greenhouses and physical barriers are effective measures to prevent access by wild animals, the protection of crops is limited and not always feasible for outdoor fields (Byappanahalli and Ishii, 2011). For example,

if constructing physical barriers in outdoor fields is not feasible, the Leafy Green Marketing Agreement in California advises growers against harvesting lettuce and leafy greens if deer or feral swine have had heavy contact with the field (Byappanahalli and Ishii, 2011).

2.3.4 Sources of Contamination: Harvest

Field practices during the harvesting of produce may also introduce or encourage the growth of pathogens (Hajmeer and Crozier-Dodson, 2012). During harvest in the field, collection bins are often placed directly on the soil, which can transfer pathogens from the soil to the bottom of the bins (Matthews, 2009). The pathogens can then be transferred to the produce if the bins are stacked on top of one another when being transported to packing sheds or processing facilities (Matthews, 2009). The maturity of the produce harvested also influences its susceptibility to contamination (Brandl, 2008). For example, Brandl (2008) observed higher *E. coli* levels on younger leaves compared to older-aged leaves, demonstrating the affinity of *E. coli* for produce that is harvested earlier in the growing cycle.

During the harvesting of lettuce, coring and trimming the outer leaves is a common practice in the field to reduce shipping weight (McEvoy et al., 2009). However, this practice creates openings that can serve as breeding sites for pathogens as they attach better to cut surfaces rather than intact or whole leaf surfaces (Takeuchi and Frank, 2000). The affinity of pathogens for cut stems is due to the production of latex after a stem is cut, which provides nutrients that encourage the proliferation of potential pathogens (Takeuchi and Frank, 2000). For example, *E. coli* O157:H7 that was inoculated onto cut lettuce stems grew more than tenfold after being incubated for four hours at 28°C (Brandl, 2008). *Salmonella* and *Shigella* also grew more rapidly and to higher levels on chopped leaves of cilantro and parsley compared to whole leaves

(Wu et al., 2000; Campbell et al., 2001). In Brandl's (2008) study, the levels of *E. coli* O157:H7 on mechanically and disease-induced damaged romaine lettuce grew by four and 11 fold, respectively, within four hours.

The aforementioned studies underscore the potential for pathogens to grow rapidly on produce leaves as a result of intentional cutting and trimming, or accidental bruising, to levels that can pose significant public health concerns (Matthews, 2009). Moreover, the handling from field workers can also introduce pathogens to the produce (Beuchat and Ryu, 1997; Suslow et al., 2003.). As such, proper worker hygiene must be exercised, and regularly maintained toilet and sanitation facilities must be provided to all field workers to prevent contamination from handling (Beuchat and Ryu, 1997; Kitinoja and Kader, 2003; Suslow et al., 2003). Although the handling of produce in the field during harvest may increase the susceptibility for contamination, the problem can be further magnified through post-harvest processing, transporting, and storing if hygienic and handling practices are not followed (McEvoy et al., 2009).

2.3.5 Sources of Contamination: Post-harvest

Post-harvest activities that can affect the microbial presence in produce include transporting to a processing or packing facility, washing or rinsing, and storing prior to distribution (Beuchat, 2002; Drechsel et al., 2007; Qadir et al., 2010). During the processing stage, the produce often comes in direct contact with humans and equipment, is rinsed or cleaned in water, and is further cut or pruned (Beuchat and Ryu, 1997; Brackett, 1999). Improper handling and poor worker hygiene are major factors that can influence the quality of produce during post-harvest activities (Suslow et al., 2003; Matthews, 2009). Pathogens present on the hands of processing workers can be transferred from the infected worker to the produce (Suslow et al., 2003). The handling of

harvested produce throughout the distribution chain, from the packing shed or processing facility to the retail market or food service establishment, can also affect the level of microbial presence in the produce (Johnston et al., 2006).

The cooling process is another factor that can significantly affect the microbial safety of leafy greens (Matthews, 2009). Leafy vegetables are generally refrigerated under forced air or in vacuums using passive refrigeration, with the latter being the most common method (Matthews, 2009). Cooling produce to 4°C can slow the growth of pathogens; however, Hsu and colleagues (2006) have shown that bacteria such as *E. coli* can persist or even grow under standard storage conditions. For example, Li and colleagues (2008) also found that the infiltration of *E. coli* was actually promoted by the cooling process, whereas Koseki and Isobe (2005) demonstrated that levels of *E. coli* O157:H7 and *Salmonella* on iceberg lettuce remained unchanged at standard refrigeration temperatures. Similarly, *E. coli* O157:H7 levels either remained the same or decreased minimally in bagged lettuce stored at or below 4°C (Delaquis et al., 2007). Delaquis and colleagues (2007) observed that the levels of *E. coli* O157:H7 and *Salmonella* on various culinary herbs did not decline significantly after 19 days in a refrigerator set at 4°C. Despite the inconsistent findings regarding temperature controls and pathogen proliferation, maintaining proper temperatures after processing and during transporting is recommended to prevent the growth of foodborne pathogens (Matthews, 2009; Allen et al., 2013).

Proper maintenance and sanitization of packing equipment such as scrubbers, spray nozzles, and conveyor belts are other important practices that can prevent the introduction or dissemination of pathogens to produce (Keller et al., 2002; Duffy et al., 2005). In typical post-harvest processes, produce can be exposed to as many as 12 different containers or pieces of processing equipment (Suslow et al., 2003). However, most of the equipment and containers that come in contact with

the produce during these processes are difficult to clean (Suslow et al., 2003). For example, conveyor belts commonly found in packing sheds are often constructed from a rough material and contain bristle-like textures that make them difficult to clean thoroughly (Duffy et al., 2005).

In packing sheds, soil and other debris are rinsed or washed from harvested produce to reduce the microbial presence and prolong shelf life (Herdt and Feng, 2009; Solomon and Sharma, 2009). Sanitizers are generally used to maintain the microbial quality of the wash water rather than of the produce being washed (Brackett, 1999). Chlorine is a common additive to wash water that is used to rinse produce and has been shown to reduce microbial loads by a maximum of two $\log_{10}$ units (Beuchat, 1998). Johnston and colleagues (2006) found that although chlorine is a widely adopted chemical used to effectively disinfect common surfaces, drinking water, and recreational water, its disinfectant properties on produce are less effective.

Beuchat and Brackett (1990) found that chlorine-based sanitizers were initially successful in reducing microbial loads in lettuce; however, the microbial presence increased significantly after prolonged storage. In separate studies, Senter and colleagues (1985) and Johnston and colleagues (2006) reported that the use of chlorinated sanitizers did not significantly lower the microbial load in tomatoes, herbs, and mustard greens. However, similar to Beuchat and Brackett (1990), Johnston and colleagues (2006) observed increased microbial loads in cilantro and parsley that had been washed. Han and colleagues (2001) reported the difficulty of completely removing pathogens from leaf surfaces, especially in protected areas or on open areas caused by injuries. In addition, Beuchat and Brackett (1990) observed no difference between chlorinated and non-chlorinated water in their effectiveness in removing pathogens on lettuce. Similarly, Li and colleagues (2001) found that the use of 20 ppm of chlorine at different temperatures did not significantly reduce *E. coli* O157:H7 populations compared to the use of

non-chlorinated water. Sanitizers with higher concentrations of chlorine (200-ppm) were also unable to completely inactivate the *E. coli* O157:H7 (Solomon et al., 2002).

However, packing sheds are considered to be relatively controlled environments in which the exposure for post-harvest sources of contamination such as improperly maintained equipment and wash water can be monitored and minimized (Beuchat, 1996). Although current sanitization methods may remove soil and debris, Nicola and Fontana (2007) found that post-harvest measures only delay the spoilage of produce and do not improve its overall microbial quality. The inability to remove pathogens in produce is an increasing concern especially in cases where pathogens have become internalized into leaf tissues (Wachtel et al., 2002; Solomon and Sharma, 2009). As such, pre-harvest contamination control is arguably of higher priority than post-harvest sanitation measures (Barker-Reid et al., 2009).

2.3.6 Post-harvest: Water Use

Water is not only crucial to the growth of produce prior to and during harvest but also essential in processing and transporting the produce after harvest (Gerba and Choi, 2009). Water has consistently been identified as a vehicle for pathogen transmission and maintaining its microbial quality is one major way to prevent contamination of harvested produce (Beuchat and Ryu, 1997). For example, processing equipment can become compromised after being exposed to contaminated water, which can transfer contaminants to the produce (Mena, 2006). Additionally, refrigeration is required during transporting to maintain proper temperatures, and in some cases, the ice used for refrigeration can be another potential source of contamination (Kitinoja and Kader, 2003). Ice can introduce pathogens to the produce if the water used to make the ice is contaminated (Beuchat and Ryu, 1997; Mena, 2006). As such, using water that is

clean and potable can prevent the dissemination of pathogens (Kitinoja and Kader, 2003, Herdt and Feng, 2009).

Currently, the use of wash water containing between 50 and 200 ppm of chlorine is a common commercial antimicrobial measure to sanitize fresh produce during processing (Stopforth et al., 2008). Pathogens introduced to the wash water may grow when the water is not maintained properly or replenished sufficiently (Keller et al., 2002; Duffy et al., 2005). Reusing wash water can also encourage the dissemination of pathogens, which can occur when the pathogens present in the wash water are transferred to other uncontaminated batches of produce in subsequent washings (Herdt and Feng, 2009). As such, maintaining clean wash water during processing is essential to prevent pathogens from spreading (Johnston et al., 2006).

2.3.7 Other Sources of Contamination: Distribution and Handling

The distribution stage includes all the points between the farm, packing shed, or processing facility and the food service establishments, retail markets, or consumers (CDC, 2013). A major challenge of ensuring the microbial quality of produce throughout the distribution stage is maintaining proper temperatures during transit and storage between the two points (CDC, 2013). The process becomes more complex with the extended transport time required for imported produce (Allen et al., 2013). McKellar and colleagues (2012) observed temperature variations during the distribution stages and at the retail market. They also found that the growth of *E. coli* and other pathogens can be slowed or prevented if proper temperatures are maintained throughout the distribution chain (McKellar et al., 2012). However, maintaining temperatures that are too low may result in chilling injuries that can cause premature decay and discolouration, resulting in produce that is less marketable to consumers (Kitinoja and Kader, 2003).

Factors affecting the microbial safety of produce extend beyond the farm level to include worker health and hygiene when handling produce, processing facilities, retail establishments, and hygienic practices exercised by consumers during food preparation (Hajmeer and Crozier-Dodson, 2012). Proper worker hygiene during preparation at the retail and food service level is also important even if pre-harvest and post-harvest practices do not introduce pathogens to the produce (Qadir et al., 2010). Individuals throughout the production and distribution chain who handle produce must exercise proper hygienic practices to prevent or minimize the risk of transmitting foodborne pathogens (Diez-Gonzalez, 2011). Although there is no exact definition of personal hygiene, it includes the efforts taken and practices exercised to reduce the risk of disease transmission by workers handling the produce (Michaels and Todd, 2006).

Many studies conducted on produce outbreaks have found incidences occurring at the restaurant level as a result of improper hygienic practices (Kozak et al., 2013; Sewell and Farber, 2001). Infected workers can unintentionally contaminate the produce itself, the water used to clean or rinse the produce, and fellow coworkers (Michaels and Todd, 2006). Moreover, unsanitary working conditions such as a lack of washing facilities may prevent workers from practicing proper hygiene (Kitinoja and Kader, 2003). Keratita and Dreschsel (2004) and Ogunsola and Adesiji (2008) also noted that in areas with limited water resources, hand wash water can often be shared by many individuals, causing the hands of many workers to become contaminated. Therefore, proper education and training on proper handling, personal hygiene practices, and standard sanitation procedures must be provided to all produce handlers not only at the farm (e.g. growers, packers, processers), but also at the retail or food service establishments (e.g. supermarket employees, kitchen staff) (Kitinoja and Kader, 2003; Michaels and Todd, 2006).

Overall, based on self-reported behaviour, consumers believe they engage in safe food handling practices in the kitchen; however, studies have shown that members from every demographic group overstate their adherence to safe food handling guidelines and mishandle produce in ways that can increase the potential for contamination (Bruhn, 2006). In general, proper handling practices that consumers should exercise include washing produce thoroughly prior to consuming and selecting produce that is absent of bruises, decay, excessive soil on the edible portions, and cuts, as they expose the produce to potential contamination (Hajmeer and Crozier-Dodson, 2012). In addition, other measures to minimize cross-contamination include separating raw meat, poultry, and fish products, sanitizing all surfaces and utensils, especially ones that have been exposed to raw meat, poultry and fish, and following labels when storing produce (Hajmeer and Crozier-Dodson, 2012). Storing produce at the proper temperature is a practice that consumers often neglect (Bruhn, 2006), and hand washing prior to and during food preparation is a practice that 20 percent of consumers do not exercise (Anderson et al., 2004).

2.3.8 Fecal-Oral Route

Foodborne pathogens, especially those associated with fresh produce outbreaks, are generally transmitted to human populations via the fecal-oral route (De Roever, 1999). In this transmission route, pathogenic bacteria isolated from feces is indirectly transmitted from an infected worker or animal, through food, water, soil, or equipment, to a person who eventually becomes ill after consuming the food (Diez-Gonzalez, 2011). The transmission can stem from poor hygienic conditions, close contact between workers who handle the produce from the field and processing facilities, food preparation methods in retail market and food service establishments, and improper preparation within consumer households (Beuchat and Ryu, 1997; Karch et al., 1999; Solomon and Sharma, 2009). Modern food production and distribution

processes have extended the fecal-oral route between the point where animal waste is produced to the point where the produce is consumed, making it difficult to control the factors in between that can compromise the quality of fresh produce (Diez-Gonzalez, 2011).

An example of a pathogen that had been transmitted via the fecal-oral route and caused widespread illness is the *E. coli* O157:H7 outbreak in 2006 that resulted in a large recall of spinach in the U.S. and Canada (Kozak et al., 2013). This outbreak affected 204 people, with one case reported in Canada, and caused three deaths in the U.S (CDC, 2006; Kozak et al., 2013). The investigation attributed the outbreak to fecal contamination of prepackaged spinach, which occurred when the pathogen spread from cattle on a farm adjacent to the spinach field (Kozak et al., 2013). Other potential factors that likely contributed to the transmission of the pathogen include runoff of contaminated water from a nearby ditch to the spinach field and feral pigs that had contact with the field (Jay et al., 2007). After harvesting, the implicated spinach was transported to a processing plant where it was washed and packaged, further spreading the pathogen to other spinach leaves (CDC; 2006; Jay et al., 2007). The bagged spinach was then distributed to retail markets, thus exposing consumers to the pathogen and completing the fecal-oral route (Jay et al., 2007).

2.4 Studies Comparing the Microbial Contamination in Produce

There have been five studies that have examined the overall quality of imported and local produce. Among the studies, only Johnston and colleagues (2006) investigated differences in the microbial contamination between imported and local produce, while others surveyed the overall microbial loads in imported and local produce (Sagoo et al., 2001; Johannessen et al., 2002; CFIA, 2009, 2010). The remaining studies surrounding the topic compared organically and conventionally grown produce that were solely of imported origin (McMahon and Wilson, 2001; U.S. FDA, 2001; Allen et al., 2013) or solely of local origin (U.S. FDA, 2003; Loncarevic et al., 2005; Arthur et al., 2007; Abadias et al., 2008; Bohaychuk et al., 2009; Oliveira et al., 2010).

There have been three studies conducted in North America that have analyzed imported and local produce (Johnston et al., 2006; CFIA 2009, 2010). Johnston and colleagues (2006) compared 466 local (southern U.S.) and imported (Mexico) leafy vegetables throughout the various processing stages in a packing shed. The produce types tested included leafy greens, herbs, melons, and broccoli (Johnston et al., 2006). The study determined that the general microbial loads of aerobic plate counts, coliforms, and generic *E. coli* were equivalent, if not higher, in local produce than in imported produce, especially in herbs (Johnston et al. 2006). In an effort to analyze the overall quality of produce in Canada, the CFIA conducted two studies, one of leafy green vegetables between 2008 and 2009, and the other of leafy herbs between 2009 and 2010. In the 2008 to 2009 study, 433 imported and 168 domestic leafy green vegetables were sampled and the results indicated that none of the samples tested exceeded unsatisfactory thresholds (CFIA, 2009). However, the types of leafy green vegetables that were tested in the study were not specified. In the 2009 to 2010 study, 816 imported and 408 domestic fresh leafy herbs were tested and the analysis showed elevated or high levels of *Salmonella* and *E. coli* in 1.6 percent of

all samples (CFIA, 2010). Elevated levels of *E. coli* (between 100 and 1000 CFU/g) were found in 0.8 percent of the samples, with nine being imported and one being domestic (CFIA, 2010). High or unsatisfactory levels of *E. coli* (>1000 CFU/g) were found in 0.7 percent of the samples, with eight being imported and one being domestic (CFIA, 2010). Both studies conducted by the CFIA (2009, 2010) only surveyed the overall microbial loads in imported and local produce. Pathogenic bacteria such as *E. coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* was not detected in any of the samples (Johnston et al., 2006; CFIA, 2009, 2010).

Outside of North America, there have been two major studies in recent years that tested imported and local produce, one in Norway (Johannessen et al., 2002) and the other in the United Kingdom (Sagoo et al., 2001). Johannessen and colleagues (2002) tested imported and local produce including pre-cut salads, culinary herbs, mushrooms, and strawberries (Johannessen et al., 2002). However, both imported and domestic varieties were not available for all of the produce types tested (Johannessen et al., 2002). Additionally, Sagoo and colleagues (2001) surveyed 3200 ready-to-eat organic vegetables available for purchase in retail establishments across the United Kingdom. In their study, generic *E. coli* and *Listeria* spp. were detected in only 1.5 percent (48 samples) and 0.2 percent (six samples) of the produce tested, respectively (Sagoo et al., 2001). Although the study tested both imported and local produce, no comparisons were made between the two types (Sagoo et al., 2001). Pathogenic bacteria such as *E. coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* was not detected in any of these studies (Sagoo et al., 2001; Johannessen et al., 2002).

Among studies that surveyed imported produce, most compared organic against conventional produce varieties. Allen and colleagues (2013) analyzed 106 imported samples consisting of

herbs, leafy vegetables, and spinach, both organically and conventionally farmed, that were available in Canada during a period of limited local produce availability. Although a statistical difference was found in the levels of coliforms between organic and conventional produce, no statistical difference was found in the levels of *E. coli* between the imported and local samples, nor were pathogenic *E. coli* or *Salmonella* detected in any of the samples (Allen et al., 2013). Their findings differed from the study conducted by the U.S. FDA (2001) on imported produce from 21 countries. In the U.S. FDA study, 1003 produce samples consisting of broccoli, lettuce, tomatoes, strawberry, cantaloupe, celery, cilantro, culantro, parsley, and scallions were collected and tested (U.S. FDA, 2001). The results found that pathogenic *Salmonella* and *Shigella* were present in 4.4 percent of the samples tested, including 16 cilantro, 6 culantro, 2 parsley, 2 lettuce, and 3 scallion samples (U.S. FDA, 2001). In another study, McMahon and Wilson (2001) tested 86 organic vegetables from supermarket chains and did not detect *E. coli* or pathogenic bacteria such as *E. coli* O157, *Listeria, Salmonella,* and *Campylobacter* in any of the samples. Allen and colleagues (2013) have noted the limited data on studies of imported produce in Canada.

Studies conducted in Canada on domestic produce have also mainly involved comparisons between organic and conventional produce (Arthur et al., 2007; Bohaychuk et al., 2009). In 2007, Arthur and colleagues (2007) surveyed organic and conventionally grown produce from various retail distribution centers, farmers' markets, organic wholesale locations and organic farms in Ontario. The produce types tested included leaf lettuce, head lettuce, parsley, cilantro, tomatoes, green onions, and muskmelon. Of the 1,183 samples, *Salmonella* was detected in two samples (roma tomato and organic leaf lettuce), while *E. coli* O157:H7 and *Shigella* were not detected in any of the samples (Arthur et al., 2007). Significant differences were observed between the *E. coli* levels of the produce types, with parsley and organic leaf lettuce having the

highest levels, followed by cilantro (Arthur et al., 2007). The lowest prevalence of *E. coli* was found in tomatoes, muskmelon, and head lettuce (Arthur et al., 2007). Bohaychuk and colleagues (2009) compared organically and conventionally-grown produce in Alberta and detected *E. coli in* 8.2 percent of all samples, although no statistical relationship was found between the levels of *E. coli* and the type of produce. *E. coli* O157:H7, *Salmonella,* and *Campylobacter* were not detected in any of the samples (Bohaychuk et al., 2009).

Other studies outside of Canada have also examined the microbial contamination in domestic produce. In 2000, the U.S. FDA surveyed 1028 domestic produce samples to compare against their study on imported produce conducted in 1999. The results revealed that *Salmonella* and *Shigella* were present in 1.1 percent of all samples, including three scallion samples and one sample of each celery, parsley, and lettuce (U.S. FDA, 2003). In Spain, Abadias and colleagues (2008) examined 300 local produce samples purchased from retail markets and detected generic *E. coli* in 7.1 percent of whole vegetables. In another study conducted in Spain, Oliveira and colleagues (2010) compared 72 organically and conventionally grown local lettuce samples and found *E. coli* in 12.5 percent of conventional lettuce samples and 22.2 percent of organic lettuce samples. In Norway, Loncarevic and colleagues (2005) examined 179 samples of organically grown lettuce and detected *E. coli* in 8.9 percent of samples but in low concentrations of less than 100 CFU/g. In all three studies, *E. coli* O157:H7 and *Salmonella* were not detected in any of the samples (Loncarevic et al., 2005; Abadias et al., 2008; Oliveira et al., 2010).

Chapter 3

Methods

3.1 Introduction

This chapter outlines the experimental approach of the study. The methods used to determine the components of the experimental portion such as the sample size and sample collection are discussed. Moreover, this chapter details the methods used to determine the microbial loads of local and imported produce as well as the types of data analyses used to interpret the results.

3.2 Sample Size

The sample size was calculated based on preliminary results obtained between October 2013 and December 2013. The preliminary results were based on 14 samples of local and imported basil and romaine. Data from a preliminary study was used to determine the inputs into Russ Lenth's power calculator (University of Iowa, Iowa, USA) to calculate the sample size required for this study. To obtain a statistical power of 0.8 and a type I error of 0.05, a total of 60 samples were required, with 30 of each local and imported samples. In anticipation of a lack of local produce in the early 2014 season and to increase specimen variety, the produce types used for the study were expanded to include spinach in addition to basil and romaine.

3.3 Sample Collection

Major supermarket locations carrying the required produce within the downtown Toronto core were visited in a random order on a weekly basis between January 2014 and April 2014.

Samples of basil, romaine, and spinach of both local and imported origin were collected. Only produce labelled with precautions to wash or rinse before consuming was purchased.

A list of farmers markets available in 2014 was obtained from the Toronto Farmers Markets Network website. Farmers markets with basil, romaine, and spinach available for purchase were visited in a random order on a weekly basis between late April 2014 and June 2014. With the limited production season for produce in Ontario, the majority of the produce purchased at farmers markets was greenhouse grown. Prior to purchasing, vendors were asked questions regarding the produce. These questions included its origin, to ensure that the produce was grown within Ontario, and whether the produce required rinsing or washing prior to consuming, to ensure that it was as similar to the imported produce purchased so that a fair comparison could be made.

Both imported and local produce were purposefully sampled from major supermarkets and farmers markets, respectively. All produce was purchased based on requirements that resembled consumer purchasing preferences and habits (U.S. FDA, 2001, 2003). For example, produce that was visibly browning or wilting was not purchased (U.S. FDA, 2001, 2003). Samples were stored in separate bags to prevent cross contamination and refrigerated within 30 minutes of purchasing. Produce samples were stored in a refrigerator for a maximum of two days after purchase prior to testing.

3.4 Laboratory Analysis

All specimens were tested using both the U.S. Food and Drug Administration's Sub-Sample Rinse Method and the solid medium method for coliforms (U.S. FDA, 2002), as well as Health

Canada's 2012 update of the MFHPB-27 direct plating method for enumerating *Escherichia coli* in foods, with slight modifications (Health Canada, 2012). The process for testing each specimen was the same for both imported and local produce. Produce samples were prepared in a way that resembled minimal consumer preparations such as removing visible browning, severely wilted outer leaves, and dirt. Prior to testing, the physical quality of the specimens was recorded along with information such as the country of origin, pack dates, best before dates, lot numbers, brand or producer, and the vendor e.g. grocery store or farmers market.

Sub-Sample Rinse and Solid Medium Method

The procedure used for specimen testing using the rinse method began with 10 grams of produce weighed and placed aseptically into sterile 4 oz. Whirlpak bags (Spectrum-Nasco, Ontario, Canada) along with 100 ml of sterile, deionized water. The specimens were washed by agitation in an orbital shaker for 30 minutes at 7.5 rpm, and flipped once half way through the wash. After the first wash, the water was discarded and another 100 ml of sterilized deionized water was poured into the Whirlpak bag for the produce to be washed a second time. The resulting wash water was then diluted by a factor of 1:100 (some of the early samples were diluted by a factor of 1:10) using sterile, deionized water for inoculation on culture media.

Direct Plating Method

The direct plating method was used to compare against results from the rinse method. For this method the wash water that was used in the rinse method was discarded and the remaining 10 grams of specimen left in the 4 oz. Whirlpak bag was placed into a sterilized blender with 99 ml of sterile, deionized water. The blender was run on the highest speed for 10 seconds. Prior to

inoculation onto culture media, the homogenized sample was diluted by factor of 1:100 in another 4 oz. sterile Whirlpak bag.

Aerobic Plate Counts

Aerobic plate counts of heterotrophic flora were determined using nutrient agar. Since heterotrophic bacteria utilize carbohydrates, proteins, and fats from other organisms, this type of bacteria was used to determine overall microbial loads in produce samples. Using a 1 ml pipette, 0.33 ml of the diluted wash water was inoculated onto nutrient agar. The media were incubated at 37°C for 24 hours. Colony forming units (CFUs) counts were performed to enumerate the resulting number of microorganisms on the media. The number and description of CFUs were observed, counted manually, and recorded.

Gram-Negative Bacteria

Gram-negative bacteria were isolated using selective culture media. Using a 1 ml pipette, 0.33 ml of the diluted wash water was inoculated onto MacConkey agar and incubated at 37°C for 24 hours. The number and description of CFUs were observed, counted manually, and recorded. Morphological characteristics were used to differentiate between groups of bacteria, such as lactose-fermenting bacteria and non-lactose fermenting bacteria. Lactose-fermenting bacteria were characterized by deep pink/burgundy coloured colonies, which typically indicate the presence of coliforms. Non-lactose fermenting bacteria were characterized by colourless and beige or cream coloured colonies and served as indicators for the potential presence of pathogens such as *Salmonella* and *Shigella*.

Coliforms and *E. coli*

Enumeration of coliforms and *E. coli* was conducted using membrane filtration. Colilert tests were initially used as a qualitative method to determine the presence or absence of coliforms and *E. coli*, and membrane filtrations were used to quantify the microbial load. A presence of coliforms indicates a potential presence of *E. coli,* which are both indicators of fecal contamination.

Membrane Filtration

In order to quantify the levels of coliforms and *E. coli* present in each sample, 10 ml of undiluted wash water from each sample was filtered through a sterile 0.45 μm cellulose nitrate membrane filter (Whatman Limited, Maidstone, England). The membrane filter was then plated on differential coliform agar (DC Medium, Oxoid, Napean, Ontario) and incubated at 37°C for 24 hours. Following incubation, the number and description of colonies was observed, counted manually, and recorded. Morphological characteristics were used to differentiate between various groups of bacteria such as *E. coli*, which is characterized by dark blue/purplish coloured colonies, and coliforms, which are characterized by pink coloured colonies.

Colilert Tests

The Colilert test (IDEXX Laboratories, Inc., Maine, USA) was used to determine the presence or absence of *E. coli* and coliforms. To account for the increased wash water required for this test, the same 1:10 ratio of produce to sterile, deionized water was applied. For this test, 15 g of specimen, instead of 10 g, were weighed and 150 ml of sterile, deionized water was poured into the 4 oz. Whirlpak bag. The specimens underwent the same washing procedure used previously

for the 10 g samples of weighed produce. After two washes, 100 ml of undiluted wash water was poured into a Colilert bottle with the reagent already placed into the 120 ml bottle. The bottles were incubated at 37°C for 24 hours. After incubation, the sample was observed under natural and UV light and the presence or absence of coliforms and *E. coli* was recorded. A dark yellow colour observed under natural lighting indicated a presence of coliforms, and blue fluorescence observed under UV lighting indicated a presence of coliforms and *E. coli.*

Controlling for Baseline Contamination

Negative controls were conducted for each batch of samples to prevent baseline contamination. For the negative controls, the same batch and volume of sterile, deionized water used to inoculate culture media was used in the rinse method, the direct plating method, membrane filtration, and the Colilert tests. Culture media used to conduct negative controls included nutrient agar, MacConkey agar, and DC agar. All negative controls were incubated along with inoculated culture media for 24 hours at 37°C. The results of a batch were excluded from the analysis if the negative control showed microbial growth.

3.5 Statistical Analysis

The imported and local data was tabulated into contingency tables according to the type of testing conducted, including levels of heterotrophic bacteria, lactose-fermenting bacteria, coliforms, coliforms greater than 100 CFU/g, and *E. coli*. Prior to comparing individual imported and local produce types, all produce were compared together. All imported and local comparisons, except coliform levels greater than 100 CFU/g and *E. coli,* were analyzed together in 2x3 contingency tables. Comparisons between imported and local produce in terms of

coliforms greater than 100 CFU/g and *E. coli* were conducted using 2x2 contingency tables. All colony counts enumerated were organized into qualitative categories of low, medium, and high. The thresholds for determining the categories were calculated separately based on the type of bacteria tested and the ranges in the data sets. The thresholds were determined by sorting the combined results from both imported and local produce and separating the levels into three groups such that each group generally contained an equal number of samples. Microsoft Excel and the XLSTAT statistical package (Addinsoft, New York, U.S.A.) were used to compile the contingency tables and perform the Fisher's exact and chi-squared tests.

The imported and local data sets were first compared using a chi-squared or Fisher's exact test with data from a summary table of all three produce types. The Freeman-Halton extension of the Fisher's exact test was used for all 2x3 contingency tables where the use of the chi-squared test was not suitable (Freeman & Halton, 1951; Lowry, 2014). A description of this extension is provided in Appendix A. From the summary table, further analysis was performed using chi-squared or Fisher's exact test by extracting data for each produce type. Separate analyses were conducted for each produce type in order to determine significances in the data that could have been masked by confounding factors in the comparisons of overall imported and local produce. Also, chi-squared tests or Fisher's exact tests were used to determine whether relationships existed between the presence of *E. coli* and the origins of the produce. When the expected values did not enable the use of chi-squared tests, Fishers exact test was used instead. Significant differences between local and imported samples were determined using a statistical significance threshold, or α, of 0.05. Visual representations of the contingency tables were also observed for trends and relationships in the data sets.

Chapter 4

Results

4.1 Introduction

The results from the local and imported analysis are presented in this chapter. The data from the testing was tabulated into contingency tables and a combination of the chi-squared test and Fisher's exact test was utilized to interpret and analyze the results.

4.2 Sample Collection and Statistical Analyses

The availability of local produce was limited due to unusually adverse weather conditions in the winter of 2013-2014. In total, 31 local samples were collected, including 13 basil, 7 romaine lettuce, and 11 spinach, from 3 farmers' markets. For imported produce, 47 samples consisting of 14 basil, 18 romaine lettuce, and 15 spinach, were collected from six supermarkets. The chi-squared test and Fisher's exact test were used to analyze the data. The statistical significance threshold, or alpha, was set at 0.05. Table 1 and Table 2 show the breakdown of imported produce samples and the origins of the imported produce collected, respectively. Table 3 shows the breakdown of the local produce sampled and Table 4 shows the overall microbial loads in imported and local produce. It is important to note that only three farmers markets were visited and the majority of samples were collected from one farmers market during the sample collection period due to limited availability of local produce. The imported and local produce test results are provided in Appendix B and Appendix C, respectively.

Table 1. Imported Produce Sampling Results

Supermarket	Total Basil	Total Romaine	Total Spinach	Total
Loblaw's	3	3	2	8
Longos	3	4	4	11
Metro	2	2	4	8
No Frills	2	3	3	8
Sobey's	3	3	2	8
T&T	1	3	0	4
Total	14	18	15	47

Table 2. Summary of Imported Produce Origin

	U.S.A.	Mexico	Dominican Republic	Colombia	Vietnam	Costa Rica	Total
Basil	0	6	2	4	1	1	14
Romaine	18	0	0	0	0	0	18
Spinach	8	7	0	0	0	0	15
Total	26	13	2	4	1	1	47

Table 3. Local Produce Sampling Results

	Basil	Romaine	Spinach	Total
St. Lawrence Farmers Market	13	7	9	29
Leslieville Farmers Market	0	0	1	1
The Stop's Wychwood Barns Farmers Market	0	0	1	1
Total	13	7	11	31

Table 4. Microbial Loads in Imported and Local Produce

Produce Type	Heterotrophic No. Positive	Heterotrophic Geometric Mean** (CFU/g)	Heterotrophic Range (CFU/g)	Lactose-fermenting No. Positive	Lactose-fermenting Geometric Mean** (CFU/g)	Lactose-fermenting Range (CFU/g)	No. Samples with Coliforms	No. Samples with Generic *E. coli*
Imported								
Basil (n=14)	14	1.6×10^5	$2.4 \times 10^4 – 6.6 \times 10^5$	13	4.7×10^4	$0 – 4.5 \times 10^5$	10 (71.4%)	3 (21.4%)
Romaine (n=18)	18	2.1×10^5	$3.0 \times 10^4 – 3.4 \times 10^6$	15	9.1×10^4	$0 – 1.8 \times 10^6$	8 (44.4%)	1 (5.6%)
Spinach (n=15)	15	3.3×10^5	$1.7 \times 10^4 – 1.1 \times 10^6$	15	7.5×10^4	$1.8 \times 10^4 – 8.0 \times 10^5$	6 (40.0%)	1 (6.7%)
Local								
Basil (n=13)	13	9.4×10^4	$2.7 \times 10^4 – 4.8 \times 10^5$	12	4.3×10^4	$0 – 3.2 \times 10^5$	13 (100.0%)	UD*
Romaine (n=7)	7	8.8×10^4	$1.5 \times 10^4 – 3.8 \times 10^6$	6	4.5×10^4	$6.0 \times 10^3 – 1.2 \times 10^5$	7 (100.0%)	UD*
Spinach (n=11)	11	4.4×10^5	$7.9 \times 10^4 – 1.3 \times 10^6$	10	2.0×10^5	$0 – 1.2 \times 10^6$	11 (100.0%)	UD*

*UD – Undetected

**Geometric mean used instead of arithmetic mean as the data was not normally distributed due to the exponential growth nature of bacteria

4.3 Heterotrophic Plate Counts

Table 5 is a summary of the heterotrophic bacteria levels in both imported and local produce, based on the data from Table 4. The levels were categorized as either low (0 to 1.2 x 10^5 CFU/g), medium (1.2 x 10^5 to 5.0 x 10^5 CFU/g), or high (5.0 x 10^5 to 1.0 x 10^8 CFU/g). The analysis consisted of comparing all three produce types together, followed by basil, romaine lettuce, and spinach separately. Among the imported produce samples, basil and romaine had relatively an evenly balanced number of samples across the low to high categories. However, the samples of imported spinach had varying levels of heterotrophic bacteria and generally fell within the medium to high categories. On the other hand, over half of the local produce samples fell within the low category. Imported produce generally had a higher number of samples within the medium and high categories compared to local produce, and the total percentage of imported samples in these categories was approximately two times higher than the total percentage of local produce in the categories. Among the local produce, basil and romaine had more samples with heterotrophic bacteria levels in the low category, whereas local spinach had more samples in the high category. Moreover, the majority of both imported and local spinach samples fell within the high category. On the other hand, local produce had more samples with heterotrophic bacteria levels in the lower range. In fact, 52 percent (16/31) of the local samples were in the low category. The findings suggest that overall heterotrophic bacteria levels differ between imported and local produce, an observation that is also statistically significant ($p=0.035$). The expected frequencies for all imported and local produce comparisons can be found in Appendix E.

Table 5. Summary of Imported and Local Samples Count Frequencies for Heterotrophic Bacteria

	Low	Medium	High	Total
Imported	11	17	19	**47**
Row %	23.40%	36.17%	40.43%	100.00%
Basil	4	5	5	14
Romaine	6	6	6	18
Spinach	1	6	8	15
Local	16	8	7	**31**
Row %	51.61%	25.81%	22.58%	100.00%
Basil	9	4	0	13
Romaine	5	1	1	7
Spinach	2	3	6	11
Total	**27**	**25**	**26**	**78**
Row %	34.62%	32.05%	33.33%	100.00%

Figure 1 is a visual representation of Table 5. The distribution of the levels of heterotrophic bacteria in imported and local produce appear inverted with imported produce increasing in frequency from the low to high categories and local produce decreasing in frequency across the categories. The analysis to follow will separate the produce types to analyze basil, romaine lettuce, and spinach separately.

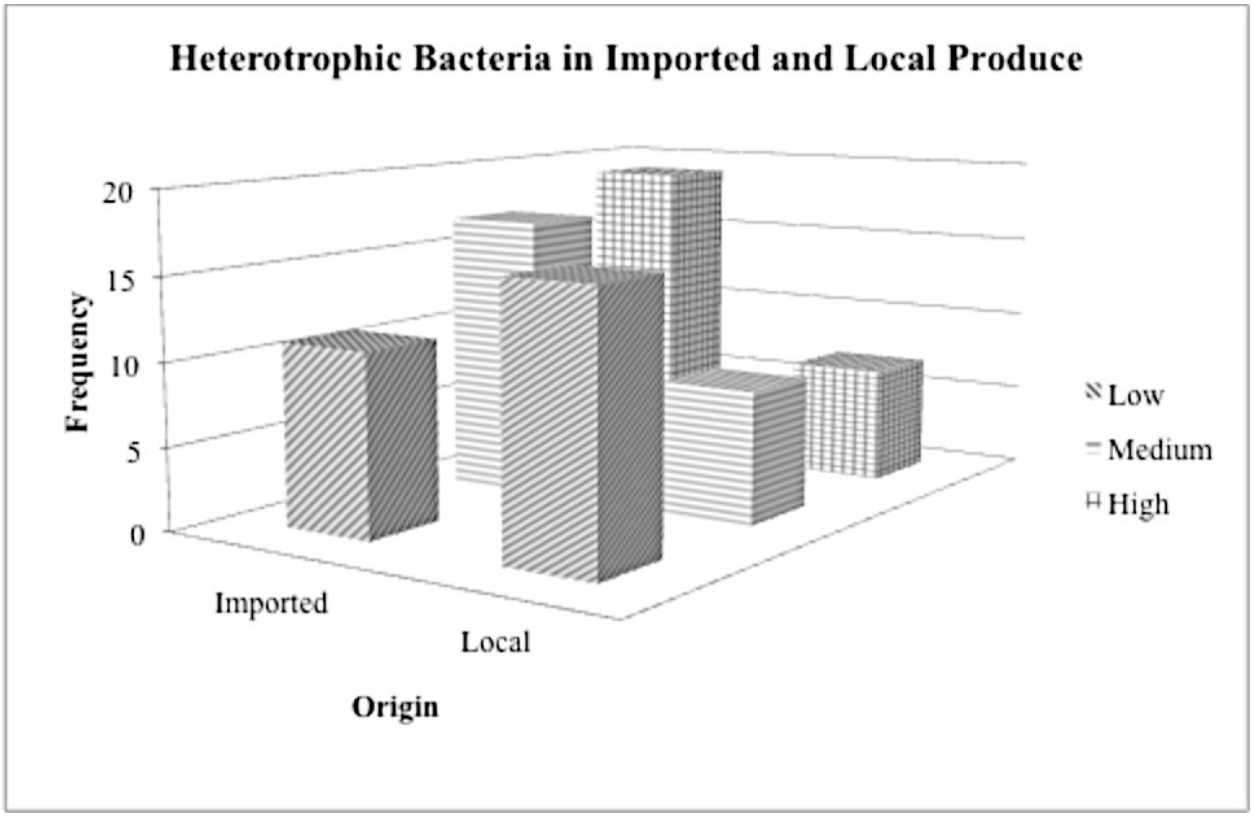

Figure 1. Heterotrophic Bacteria in Imported and Local Produce

Table 6 is a comparison of imported and local basil. Again, the levels of heterotrophic bacteria varied more in local basil than in imported basil with the majority of local samples concentrated in the low category. In addition, the percentage of local basil samples in the low category was more than double the percentage in the medium category, and there were no local basil samples in the high category. For imported basil, the levels of heterotrophic bacteria were more evenly distributed across the categories. It is worthy to note that there were five imported basil samples in the high category compared to zero local samples. Local basil had a higher percentage of samples within the low category compared to imported basil, but imported basil had a higher percentage of samples in the medium and high categories compared to local basil. While the findings are based on a small number of samples, it appears that overall, imported basil had higher heterotrophic bacteria levels than local basil, an observation supported by a statistically significant result ($p=0.035$[1]) from Fisher's exact test.

Table 6. Heterotrophic Bacteria in Imported and Local Basil

	Low	Medium	High	Total
Imported	4	5	5	14
Row %	28.57%	35.71%	35.71%	100.00%
Local	9	4	0	13
Row %	69.23%	30.77%	0.00%	100.00%
Total	13	9	5	27
Row %	48.15%	33.33%	18.52%	100.00%

[1] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 2, a visual representation of Table 6, highlights the large proportion of local basil samples with low levels of heterotrophic bacteria. Again, the distribution of the samples from the low to high categories was inverted between imported and local basil, with frequencies in imported basil increasing and frequencies in local basil descending. There were five samples in the high category in imported basil, compared to zero in the local basil samples.

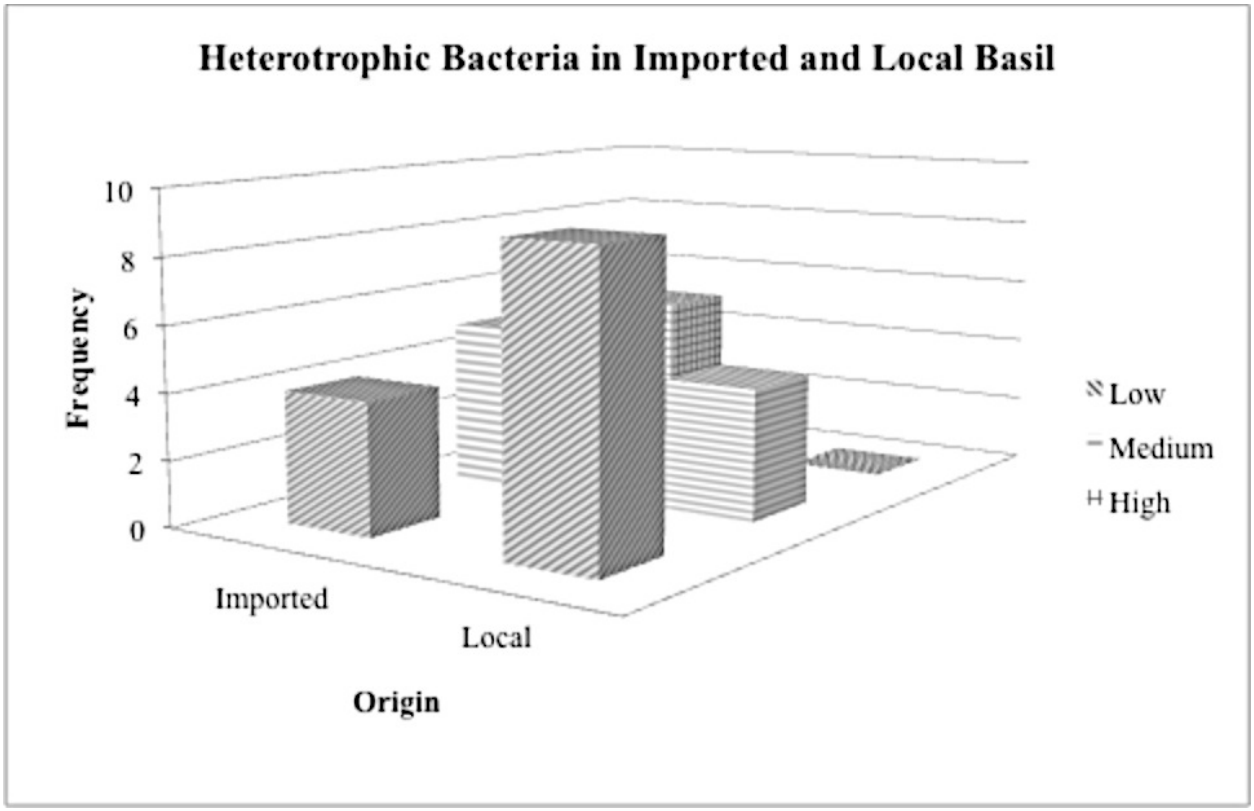

Figure 2. Heterotrophic Bacteria in Imported and Local Basil

In imported romaine, the levels of heterotrophic bacteria were evenly distributed across the categories, as shown in Table 7. On the other hand, the levels of local romaine were heavily concentrated in the low category. Also, the percentage of imported romaine samples in both the medium and high categories was more than twice as large as the percentage of local samples in those categories. While the relationship was not statistically significant ($p=0.31$[2]), the concentration of local romaine samples in the low end of the range is worth noting. Future studies should track this finding and see if the relationship continues to exist as the sample size increases.

Table 7. Heterotrophic Bacteria in Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	6	6	6	18
Row %	33.33%	33.33%	33.33%	100.00%
Local	5	1	1	7
Row %	71.43%	14.29%	14.29%	100.00%
Total	11	7	7	25
Row %	44.00%	28.00%	28.00%	100.00%

[2] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 3 also highlights the larger proportion of local romaine lettuce samples concentrated in the low category, while the levels in imported romaine lettuce were evenly distributed across the categories.

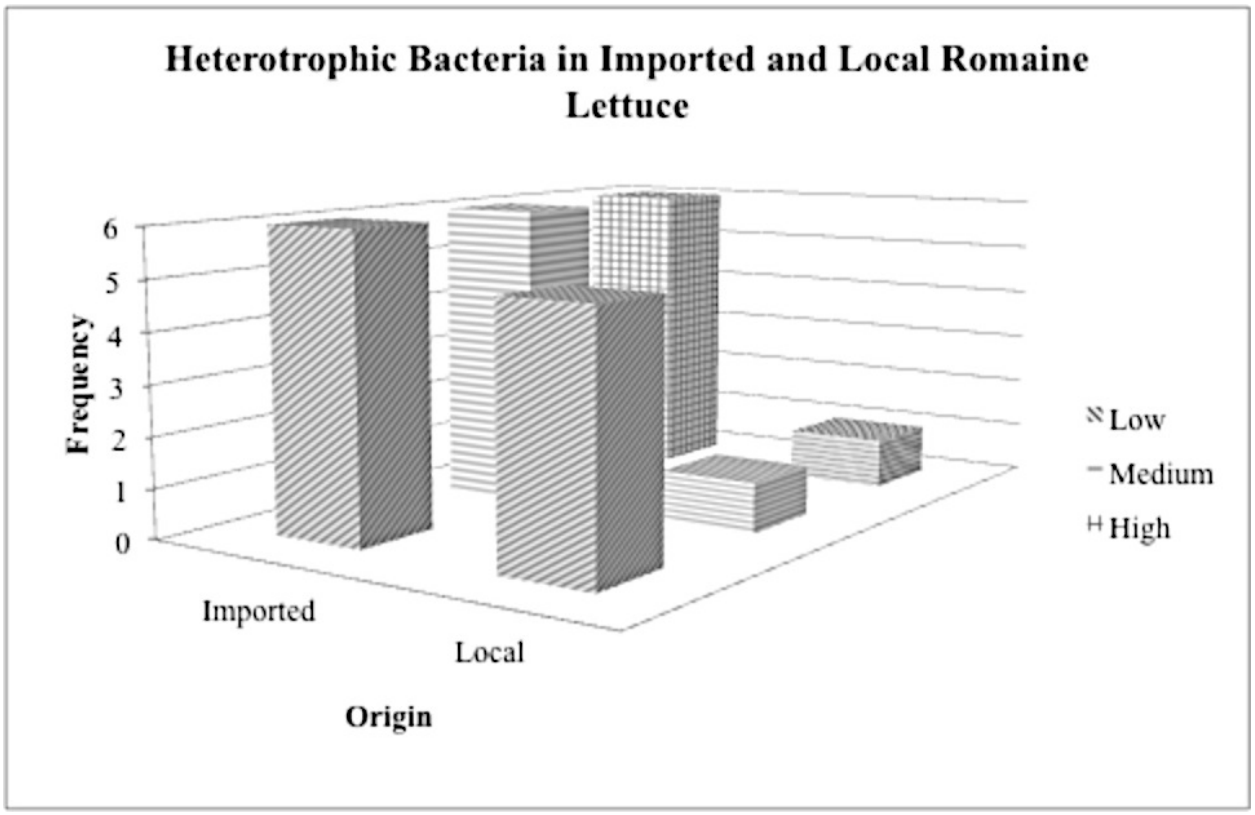

Figure 3. Heterotrophic Bacteria in Imported and Local Romaine Lettuce

Table 8 compares imported and local spinach in terms of heterotrophic bacteria levels. The percentage of samples with heterotrophic bacteria levels in the high category was the greatest for both imported and local spinach. Fourteen of 15 (93 percent) imported samples and nine of 11 (82 percent) local samples were within the medium and high categories. The low category had the lowest percentage of samples for both the imported and local spinach. Both imported and local samples had similar distributions across the categories and there appeared to be no differences in overall heterotrophic bacteria levels between imported and local spinach. The results also failed to achieve any statistical significance ($p=0.74$[3]).

Table 8. Heterotrophic Bacteria in Imported and Local Spinach

	Low	Medium	High	Total
Imported	1	6	8	15
Row %	6.67%	40.00%	53.33%	100.00%
Local	2	3	6	11
Row %	18.18%	27.27%	54.55%	100.00%
Total	3	9	14	26
Row %	11.54%	34.62%	53.85%	100.00%

[3] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 4 shows the concentration of both imported and local samples within the medium and high categories, as well as the low frequencies in the low category.

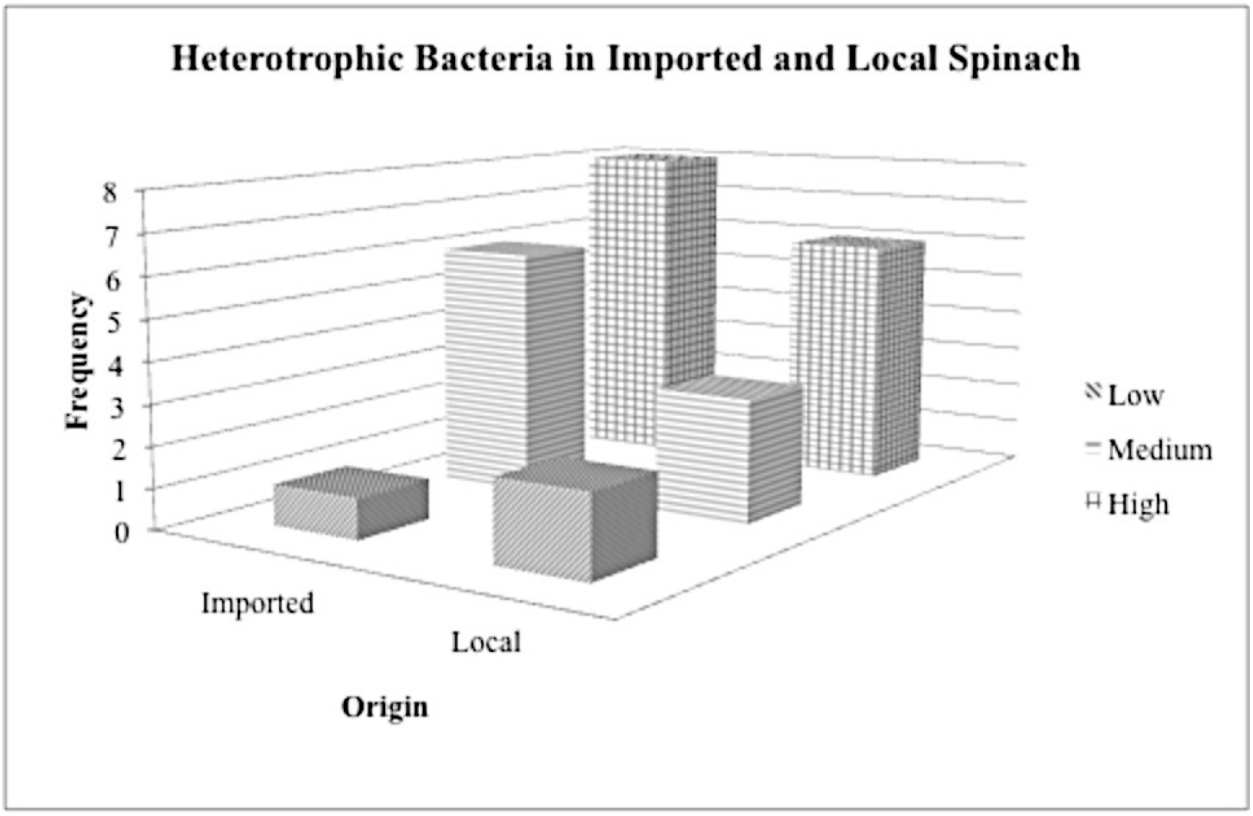

Figure 4. Heterotrophic Bacteria in Imported and Local Spinach

4.4 Lactose-Fermenting Plate Counts

The presence of lactose-fermenting bacteria indicates a potential presence of coliforms. Table 9 below shows the levels of lactose-fermenting bacteria in both imported and local produce, based on data from Table 4. The bacteria levels were categorized as either low (0 to 3.0 x 10^4 CFU/g), medium (3.0 x 10^4 to 1.5 x 10^5 CFU/g), or high (1.5 x 10^5 to 1.0 x 10^8 CFU/g). Among the imported produce samples, the levels of lactose-fermenting bacteria appeared fairly evenly distributed. On the other hand, the lactose-fermenting bacteria levels for local produce showed more distinct differences between the produce types. The majority of the local basil and local romaine samples fell within the low and medium categories, while the lactose-fermenting bacteria levels in local spinach were concentrated in the medium to high categories. Further analysis will perform comparisons between imported and local produce using all three produce types, as well as basil, romaine lettuce, and spinach separately. There did not appear to be a relationship in overall lactose-fermenting bacteria levels between imported and local produce, and the results were not statistically significant ($p=0.4$).

Table 9. Summary of Imported and Local Produce Count Frequencies for Lactose-Fermenting Bacteria

	Low	Medium	High	Total
Imported	18	13	16	**47**
Row %	38.30%	27.66%	34.04%	100.00%
Basil	7	2	5	14
Romaine	7	5	6	18
Spinach	4	6	5	15
Local	9	13	9	**31**
Row %	29.03%	41.94%	29.03%	100.00%
Basil	6	4	3	13
Romaine	2	5	0	7
Spinach	1	4	6	11
Total	**27**	**26**	**25**	**78**
Row %	34.62%	33.33%	32.05%	100.00%

A visual representation of Table 9 is shown in Figure 5, displaying the generally balanced levels of lactose-fermenting bacteria in both imported and local produce across the three categories.

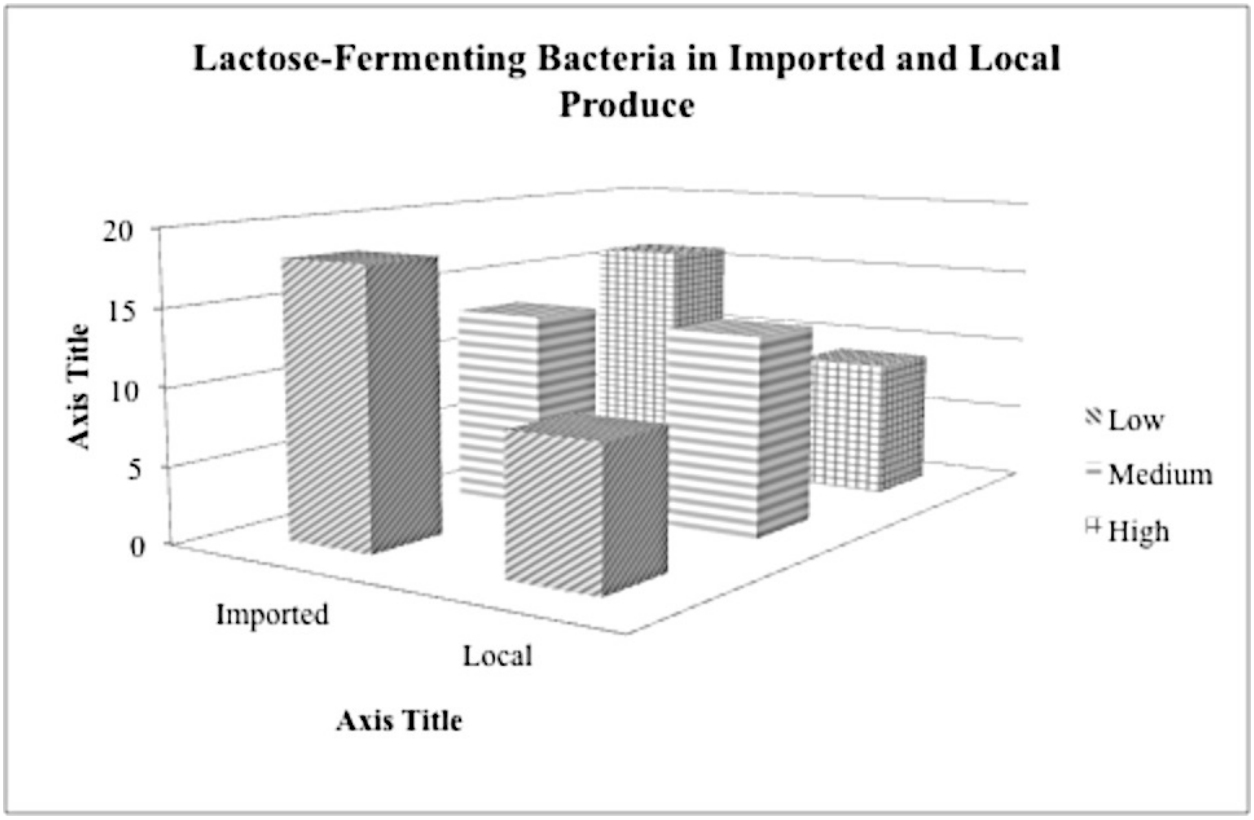

Figure 5. Lactose-Fermenting Bacteria in Imported and Local Produce

In examining the produce types separately, Table 10 shows the contingency table for the levels of lactose-fermenting bacteria in imported and local basil. The majority of imported basil samples fell within the low and high categories, while the local basil samples were balanced across the categories but concentrated slightly in the low and medium ranges. Overall, there were no noticeable patterns detected. This lack of a relationship was further supported by Fisher's exact test, which revealed a p-value of 0.7.

Table 10. Lactose-Fermenting Bacteria in Imported and Local Basil

	Low	Medium	High	Total
Imported	7	2	5	14
Row %	50.00%	14.29%	35.71%	100.00%
Local	6	4	3	13
Row %	46.15%	30.77%	23.08%	100.00%
Total	13	6	8	27
Row %	48.15%	22.22%	29.63%	100.00%

Figure 6 visually depicts the observation that the distributions of both the imported and local basil samples were balanced across the categories, with some slight concentration of samples in the low category.

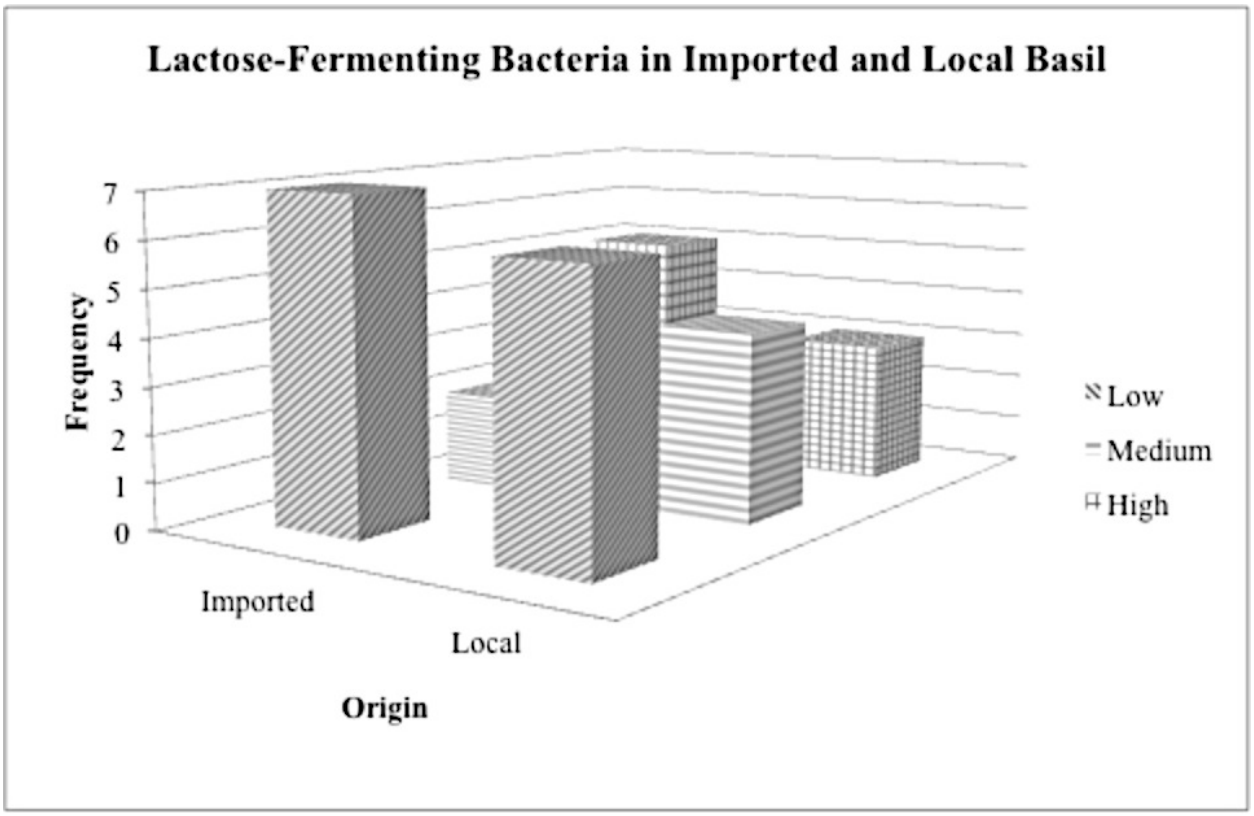

Figure 6. Lactose-Fermenting Bacteria in Imported and Local Basil

Table 11 compares lactose-fermenting bacteria levels in imported and local romaine lettuce. The imported romaine samples were noticeably evenly spread out across the categories, with no single category containing more than 40 percent of the samples. All of the local romaine samples fell within the low and medium categories, with no samples in the high category. Overall, these patterns were similar to the results found in the comparison using basil, although the concentration of the local produce in the low category was more evident. While the small samples failed to achieve statistical significance ($p=0.11$[4]), further studies with more samples should explore the overall lower lactose-fermenting bacteria levels in local produce.

Table 11. Lactose-Fermenting Bacteria in Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	7	5	6	18
Row %	38.89%	27.78%	33.33%	100.00%
Local	2	5	0	7
Row %	28.57%	71.43%	0.00%	100.00%
Total	9	10	6	25
Row %	36.00%	40.00%	24.00%	100.00%

[4] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 7 shows the generally evenly distributed imported romaine samples, as well as the heavy concentration of local romaine samples within the low and medium categories. The most noticeable difference was that one third (6/18) of the imported romaine samples were in the high category, compared to zero of the local romaine samples.

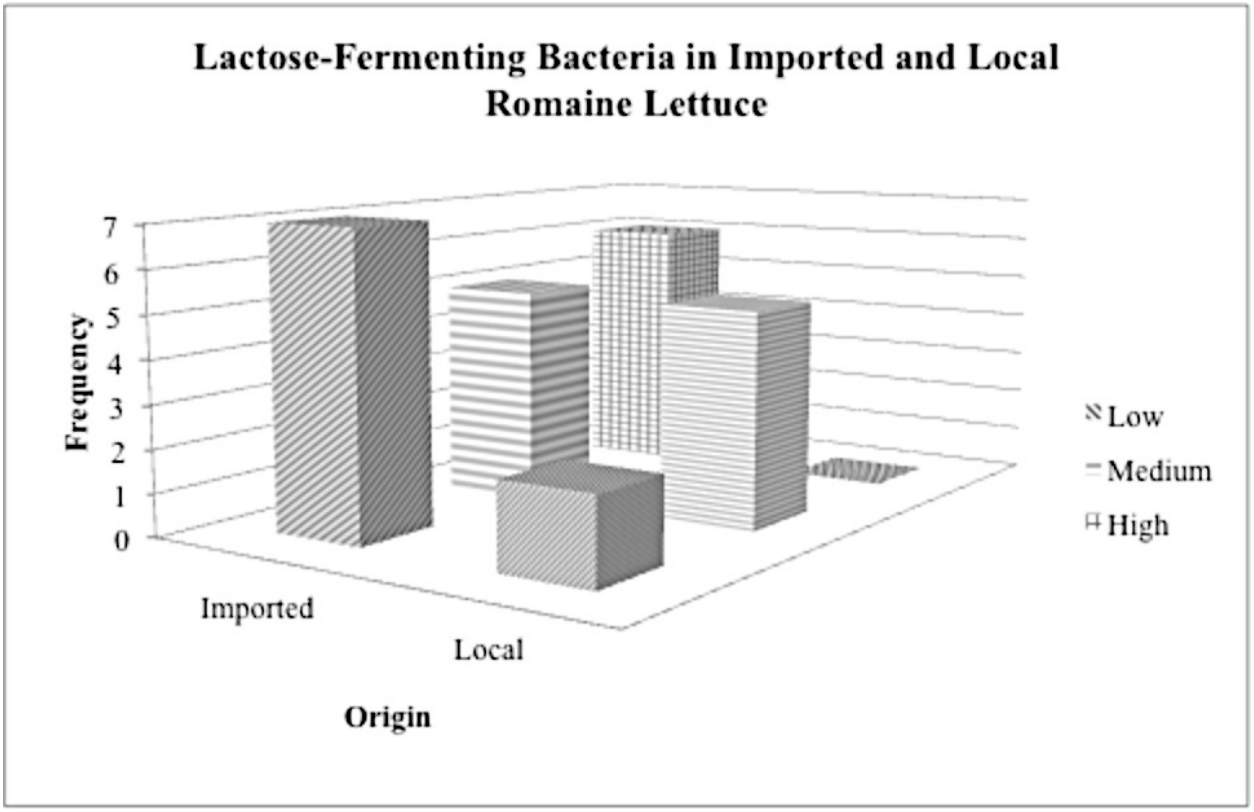

Figure 7. Lactose-Fermenting Bacteria in Imported and Local Romaine Lettuce

Table 12 shows the levels of lactose-fermenting bacteria in imported spinach to be relatively balanced across the three categories, which was similar to the distribution of imported romaine samples. However, local spinach samples were largely concentrated in the upper range as 10 of 11 (91 percent) samples were within the medium and high categories. While this potential relationship was not statistically significant ($p=0.46$[5]), further studies should examine the higher proportion of local samples with high levels of lactose-fermenting bacteria.

Table 12. Lactose-Fermenting Bacteria in Imported and Local Spinach

	Low	Medium	High	Total
Imported	4	6	5	15
Row %	26.67%	40.00%	33.33%	100.00%
Local	1	4	6	11
Row %	9.09%	36.36%	54.55%	100.00%
Total	5	10	11	26
Row %	19.23%	38.46%	42.31%	100.00%

[5] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 8 shows the generally balanced distribution of the imported spinach samples, while highlighting the concentration of local samples in the medium and high categories.

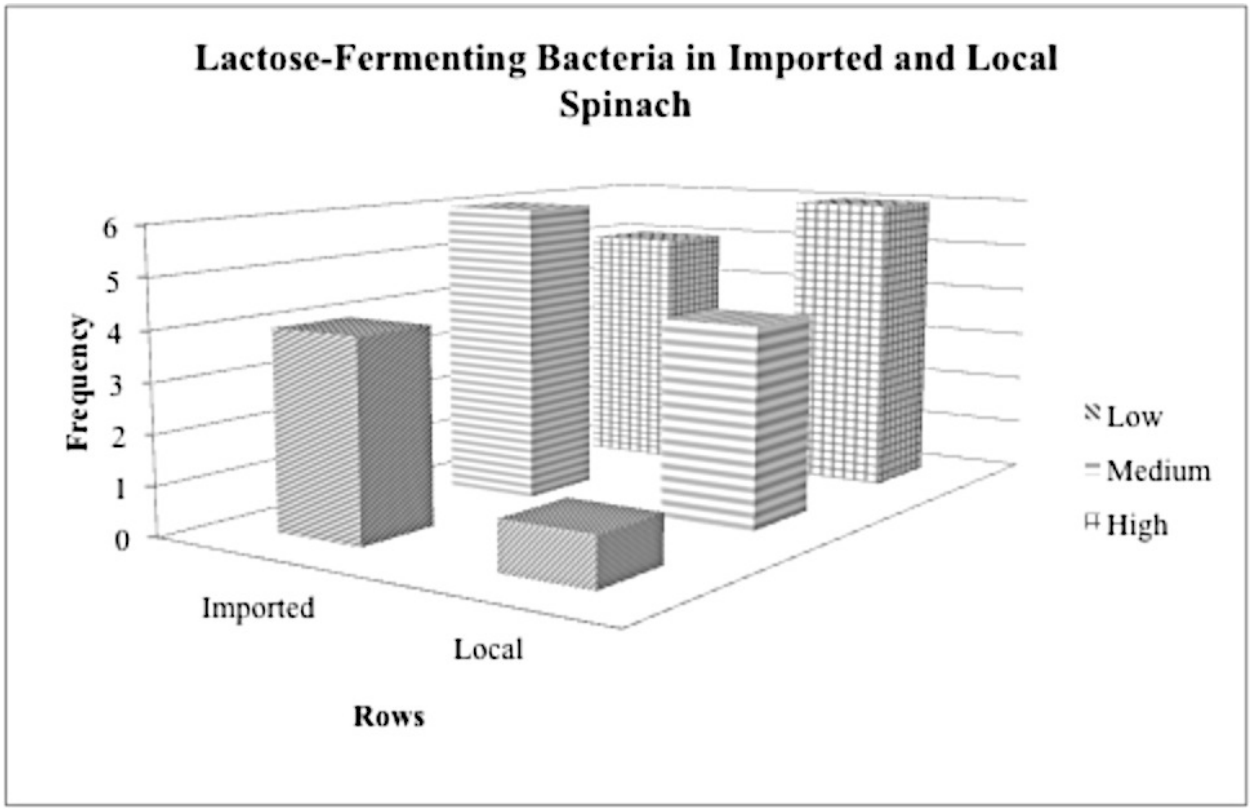

Figure 8. Lactose-Fermenting Bacteria in Imported and Local Spinach

4.5 Coliform Counts

Usually when coliforms are detected, there is also a potential presence of *E. coli,* both of which are indicators of fecal contamination. Coliforms were enumerated by differentiating pink coloured colonies on the membrane filter from the DC agar growth media. The coliform levels for both imported and local produce, shown in Table 13, are based on Table 4. The levels were categorized as either low (0 to 5 CFU/g), medium (5 to 300 CFU/g), or high (300 to 1.0 x 10^5 CFU/g). Based on Table 15, there was a significant concentration of imported samples in the low category, as the number of samples in this category was greater than the number of samples in both the medium and high categories combined. Within the imported produce, romaine and spinach both had the greatest proportion of samples in the low category. On the other hand, local produce samples were heavily distributed in the medium and higher categories. This pattern was consistent within individual produce types as well, as both local basil and local romaine had no samples in the low category at all. There was only one sample within local basil that fell within the low category. There appeared to be a distinct difference between the coliform levels of imported and local samples as 30 of 31 (97 percent) local samples were in the medium and high categories, compared to only 21 of 47 (45 percent) imported samples. This apparent relationship was also statistically significant ($p<0.001$).

Table 13. Summary of Imported and Local Produce Count Frequencies for Coliforms

	Low	Medium	High	Total
Imported	26	11	10	**47**
Row %	55.32%	23.40%	21.28%	100.00%
Basil	5	3	6	14
Romaine	11	3	4	18
Spinach	10	5	0	15
Local	1	13	17	**31**
Row %	3.23%	41.94%	54.84%	100.00%
Basil	1	4	8	13
Romaine	0	3	4	7
Spinach	0	6	5	11
Total	**27**	**24**	**27**	**78**
Row %	34.62%	30.77%	34.62%	100.00%

Figure 9 shows the large difference in the levels of coliforms detected in imported produce compared to local produce in the low category. The concentration of imported samples on the low range, and the concentration of local samples on the upper ranges, can be clearly seen.

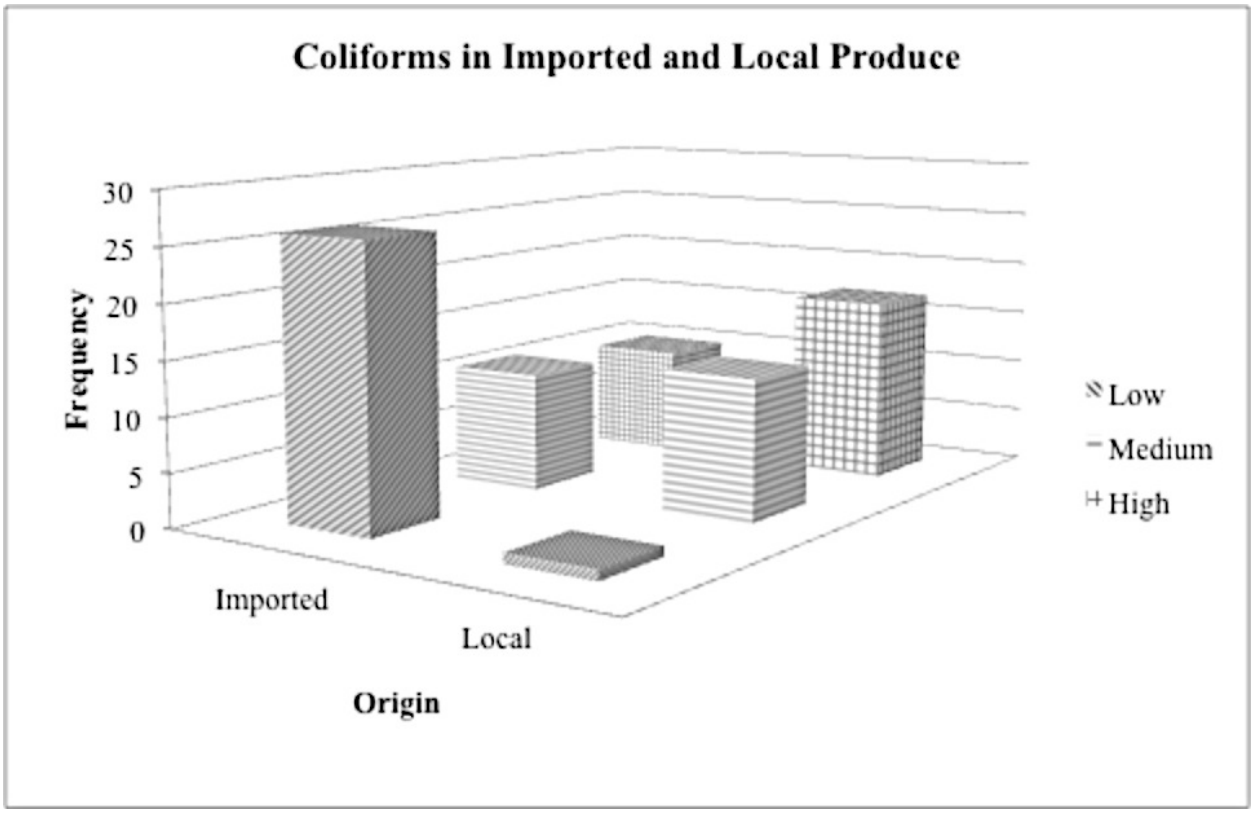

Figure 9. Coliforms in Imported and Local Produce

The coliform levels within imported and local basil are shown in Table 14. The distribution of the imported basil samples was generally balanced across the categories. However, local basil samples were concentrated in the high category, which contained eight out of 13 (62 percent) of the local samples. Together, the medium and high categories contained 12 out of 13 (92 percent) of the local basil samples. While imported basil was spread out and contained a high number of samples in the high category as well, the higher overall coliform levels in local produce are worth further examining, despite failing to achieve statistical significance ($p=0.27$[6]).

Table 14. Coliforms in Imported and Local Basil

	Low	Medium	High	Total
Imported	5	3	6	14
Row %	35.71%	21.43%	42.86%	100.00%
Local	1	4	8	13
Row %	7.69%	30.77%	61.54%	100.00%
Total	6	7	14	27
Row %	22.22%	25.93%	51.85%	100.00%

[6] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

The distribution of the coliform levels in imported and local basil is shown in Figure 10. The majority of local basil samples were within the high category, while imported basil samples were more dispersed across the categories.

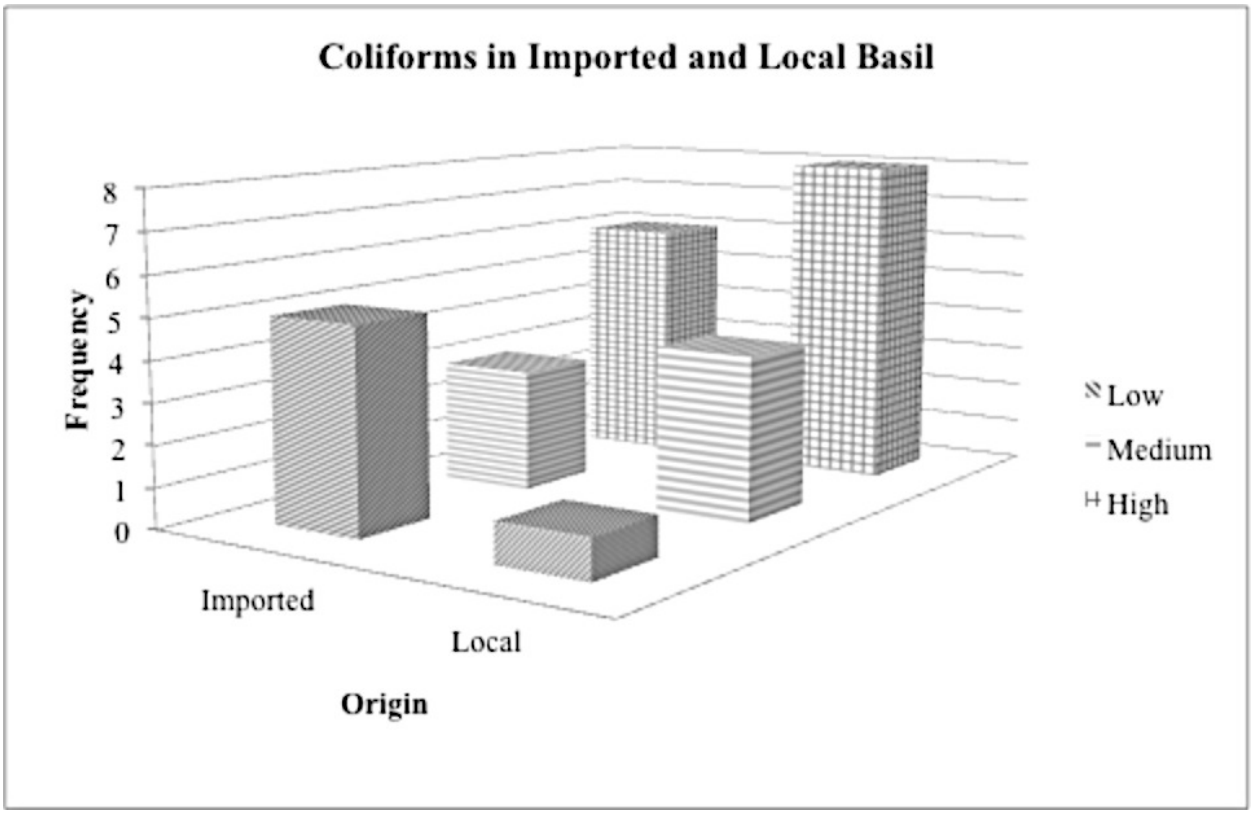

Figure 10. Coliforms in Imported and Local Basil

Table 15 shows the coliform levels within imported and local romaine lettuce. Unlike the imported basil samples, the imported romaine samples were more evidently concentrated in the low category, as 11 of 18 (61 percent) imported samples were in the low range. On the other hand, local romaine samples were again heavily concentrated in the medium and high categories, with zero samples in the low category. It appeared that local romaine contained higher levels of coliforms, while imported romaine contained lower levels, a relationship that was also statistically significant ($p=0.01$[7]). However, the number of local samples tested was low. Further studies with larger sample sizes should examine whether local romaine continues to exhibit higher coliform levels.

Table 15. Coliforms in Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	11	3	4	18
Row %	61.11%	16.67%	22.22%	100.00%
Local	0	3	4	7
Row %	0.00%	42.86%	57.14%	100.00%
Total	11	6	8	25
Row %	44.00%	24.00%	32.00%	100.00%

[7] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 11 shows the heavy concentration of imported romaine in the low category and the concentration of local romaine in the upper ranges.

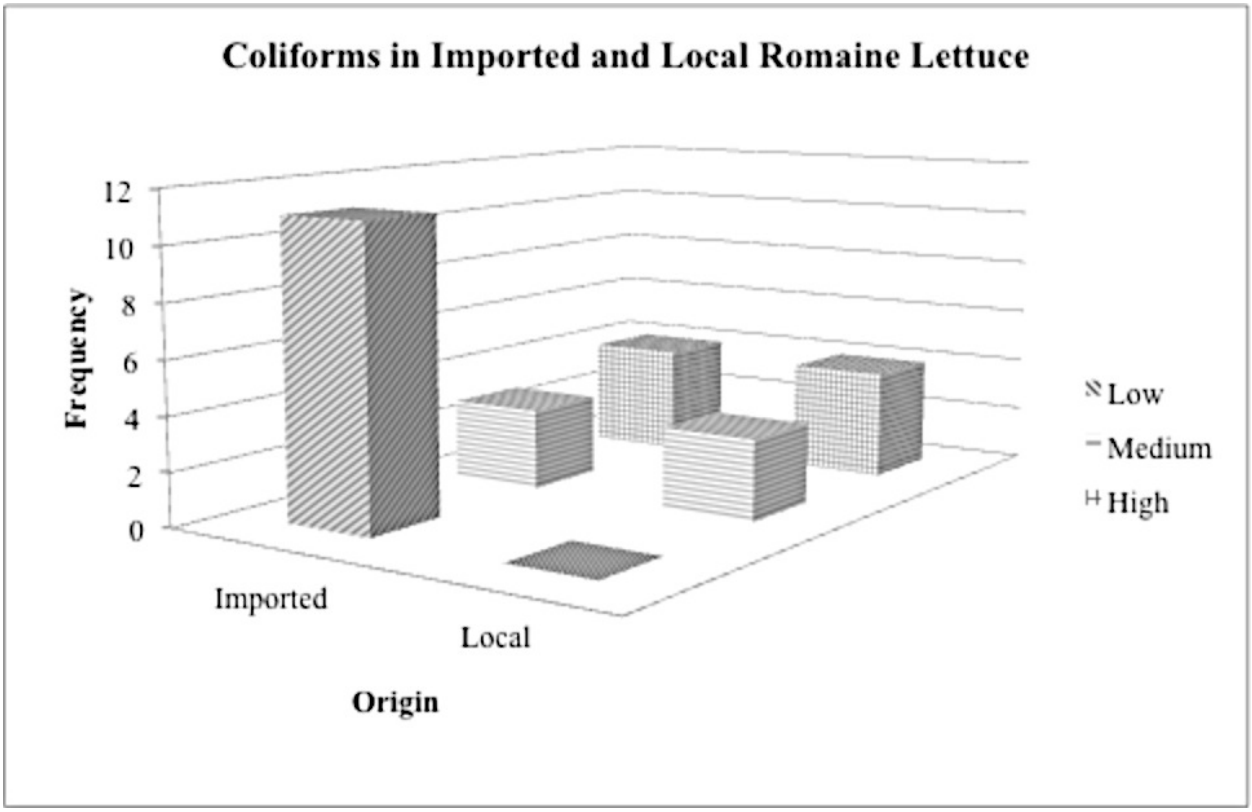

Figure 11. Coliforms in Imported and Local Romaine Lettuce

The levels of coliforms for imported and local spinach samples are shown in Table 16. Similar to the results for romaine lettuce, imported spinach samples were concentrated in the low category, which contained two thirds (10/15) of the imported samples. In fact, there were zero samples of imported spinach with coliforms in the high category. Also, local spinach samples were once again concentrated in the medium and high categories, with zero samples in the low category. There appeared to be a strong relationship that local spinach contained higher coliform levels than imported spinach, a result that was also statistically significant ($p< 0.0001$[8]). Attempts should be made to verify that this relationship continues to exist in larger studies.

Table 16. Coliforms in Imported and Local Spinach

	Low	Medium	High	Total
Imported	10	5	0	15
Row %	66.67%	33.33%	0.00%	100.00%
Local	0	6	5	11
Row %	0.00%	54.55%	45.45%	100.00%
Total	10	11	5	26
Row %	38.46%	42.31%	19.23%	100.00%

[8] Calculated using the Freeman-Halton extension of Fisher's exact test. The p-value shown is the larger of P_A and P_B.

Figure 12 shows the concentration of imported spinach samples in the low range and the heavy concentration of local spinach samples in the medium and high categories.

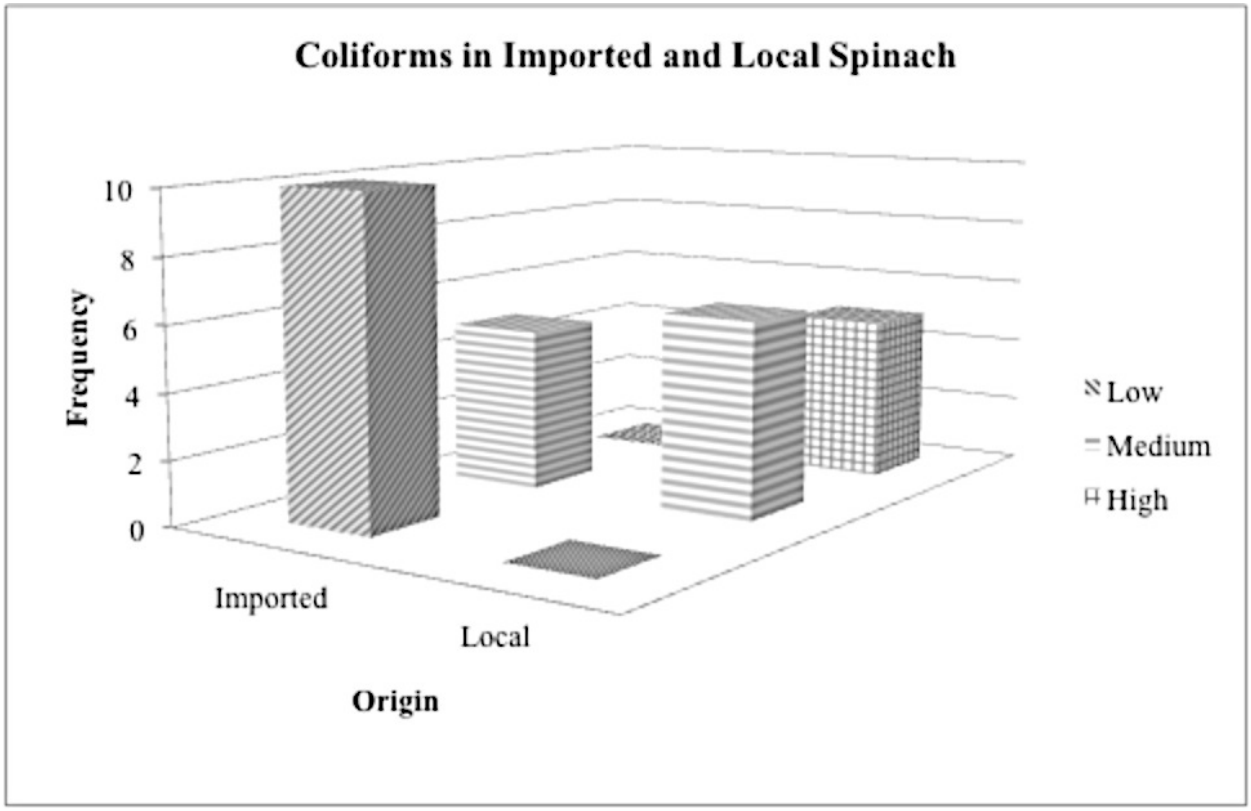

Figure 12. Coliforms in Imported and Local Spinach

The imported and local samples were also analyzed in terms of whether or not their coliform levels exceeded 100 CFU/g, a provincial and federal threshold. The results are shown in Table 17. There was a distinct relationship of imported samples having fewer samples with coliform levels above 100 CFU/g, while local samples had more samples with coliform levels above 100 CFU/g. Among imported produce, it appeared that this pattern was most evident in romaine and spinach samples. This was consistent with the findings from the total coliform analysis, which found a relationship between the produce origin and the coliform levels in both romaine and spinach. Among local produce, basil and spinach appeared to have higher proportions of samples with coliform levels exceeding100 CFU/g. Fifteen of 47 (32 percent) imported samples contained coliforms exceeding 100 CFU/g, compared to 21 of 31 (68 percent) local samples. This finding, which was also statistically significant ($p=0.002$), suggests that a greater proportion of local produce contained coliform levels that were above the threshold and should be further explored in greater detail.

Table 17. Summary of Coliforms >100 CFU/g Count Frequencies in Imported and Local Produce

	Yes	No	Total
Imported	15	32	**47**
Row %	31.91%	68.09%	100.00%
Basil	7	7	14
Romaine	6	12	18
Spinach	2	13	15
Local	21	10	**31**
Row %	67.74%	32.26%	100.00%
Basil	9	4	13
Romaine	4	3	7
Spinach	8	3	11
Total	**36**	**42**	**78**
Row %	46.15%	53.85%	100.00%

Figure 13 shows the greater proportion of imported samples under the threshold and the greater proportion of local samples above the threshold.

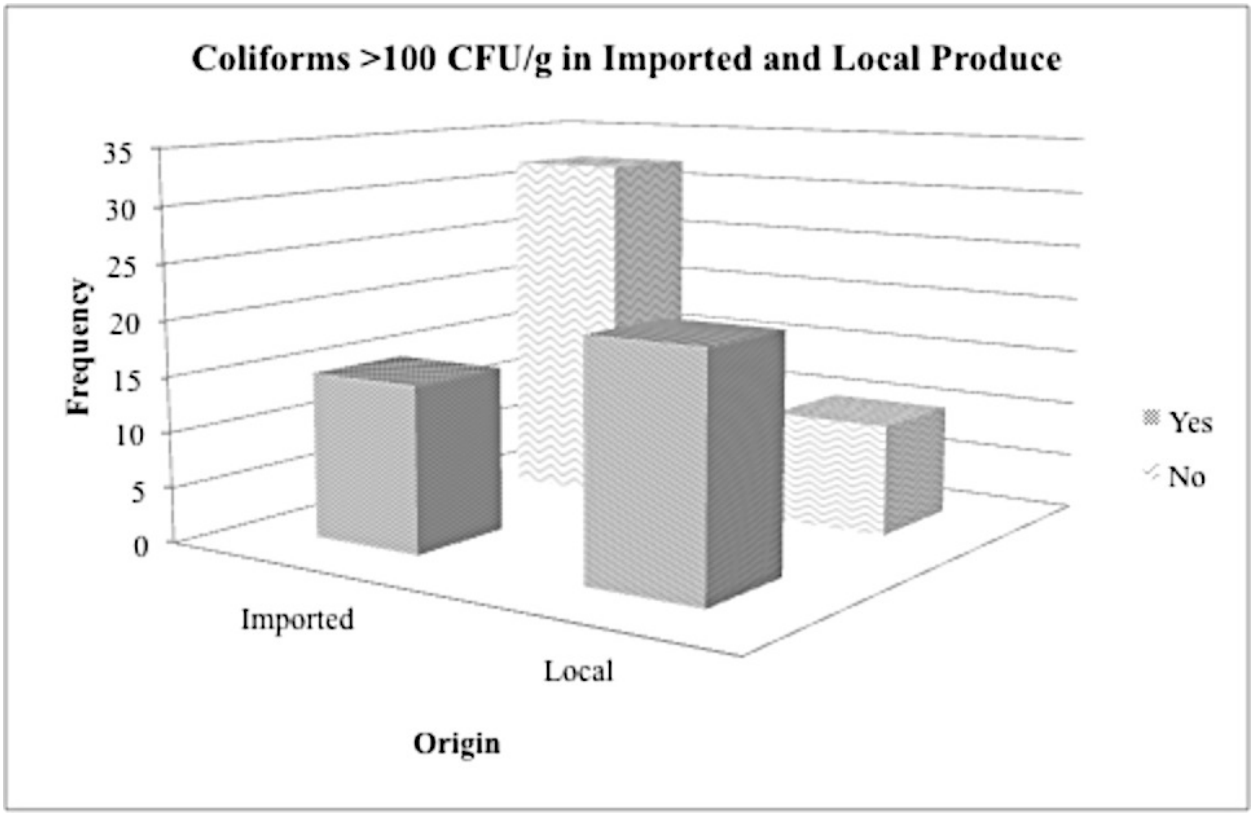

Figure 13. Coliforms >100 CFU/g in Imported and Local Produce

The number of imported and local basil samples with coliform levels exceeding 100 CFU/g is shown in Table 18. Imported basil samples were split evenly, with half exceeding the coliform threshold and half under the threshold, while nine of 13 (69 percent) of local basil exceeded the threshold. The results did not show any distinct relationships, and the small numbers were unable to achieve statistical significance (p=0.38). However, it suggested that a greater proportion of local basil failed to meet the threshold.

Table 18. Coliforms >100 CFU/g in Imported and Local Basil

	Yes	No	Total
Imported	7	7	14
Row %	50.00%	50.00%	100.00%
Local	9	4	13
Row %	69.23%	30.77%	100.00%
Total	16	11	27
Row %	59.26%	40.74%	100.00%

Figure 14 also shows no distinct visual relationships between imported and local basil in terms of samples exceeding the 100 CFU/g threshold.

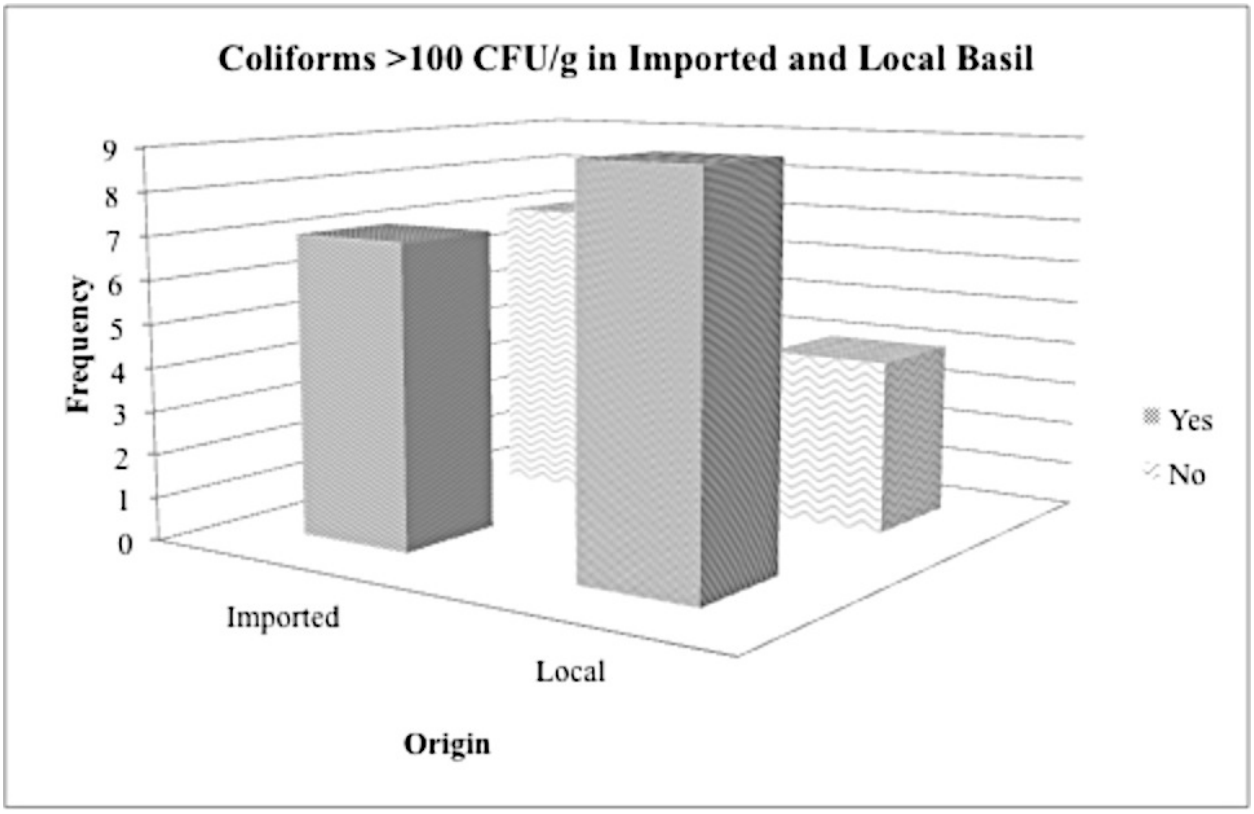

Figure 14. Coliforms >100 CFU/g in Imported and Local Basil

The comparison between imported and local romaine lettuce in terms of samples exceeding the 100 CFU/g coliform threshold is shown in Table 19. Out of the local romaine samples, 57 percent (4/7) of the samples contained coliform levels above 100 CFU/g, compared to only one third (6/18) of the imported samples. It appears that local produce contains a higher proportion of samples containing levels of coliforms above the threshold. While the results were not statistically significant (p=0.38), they are consistent with the findings thus far that suggest local produce contains higher coliform levels, and should be explored in more detail.

Table 19. Coliforms >100 CFU/g in Imported and Local Romaine Lettuce

	Yes	No	Total
Imported	6	12	18
Row %	33.33%	66.67%	100.00%
Local	4	3	7
Row %	57.14%	42.86%	100.00%
Total	10	15	25
Row %	40.00%	60.00%	100.00%

Figure 15 shows the concentration of imported samples that exceeded the 100 CFU/g coliform threshold, as well as the evenly split nature of the local samples. Overall, there appeared to be no visual relationship between the origin of the romaine and the number of samples exceeding the threshold.

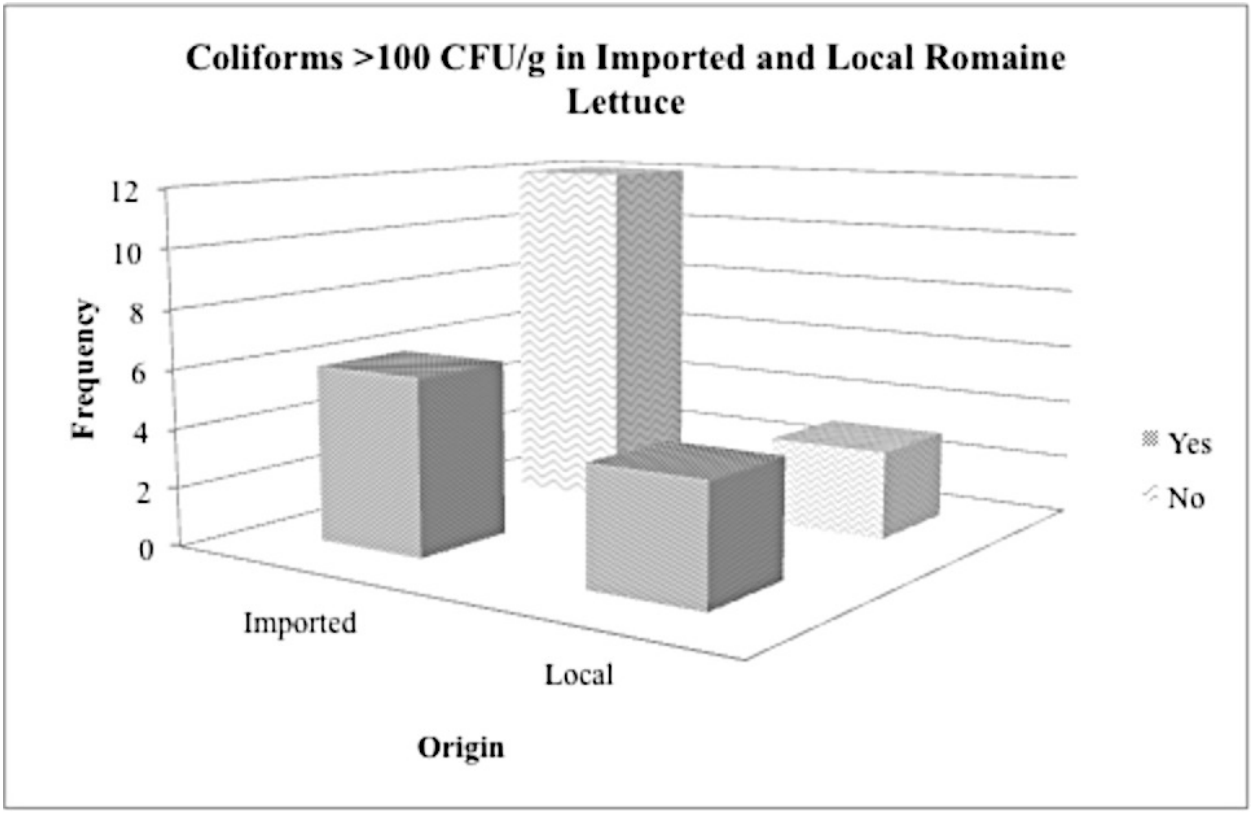

Figure 15. Coliforms >100 CFU/g in Imported and Local Romaine Lettuce

The number of imported and local spinach samples with coliform levels exceeding the 100 CFU/g coliform threshold is shown in Table 20. A more distinct relationship could be seen among the spinach samples, in contrast to the analyses with basil and with romaine. Eight out of 11 (87 percent) local spinach samples had coliform levels above the threshold, compared to only two out of 15 (13 percent) imported samples . The results show that a much larger proportion of local spinach contained coliform levels above the threshold, a result that was also statistically significant (p=0.004) and consistent with the findings from the analyses with the other produce types.

Table 20. Coliforms >100 CFU/g in Imported and Local Spinach

	Yes	No	Total
Imported	2	13	15
Row %	13.33%	86.67%	100.00%
Local	8	3	11
Row %	72.73%	27.27%	100.00%
Total	10	16	26
Row %	38.46%	61.54%	100.00%

Figure 16 presents a clear visual interpretation of the inverse relationship. Imported spinach samples largely had coliform levels below the threshold, while local samples mostly had coliform levels above the threshold.

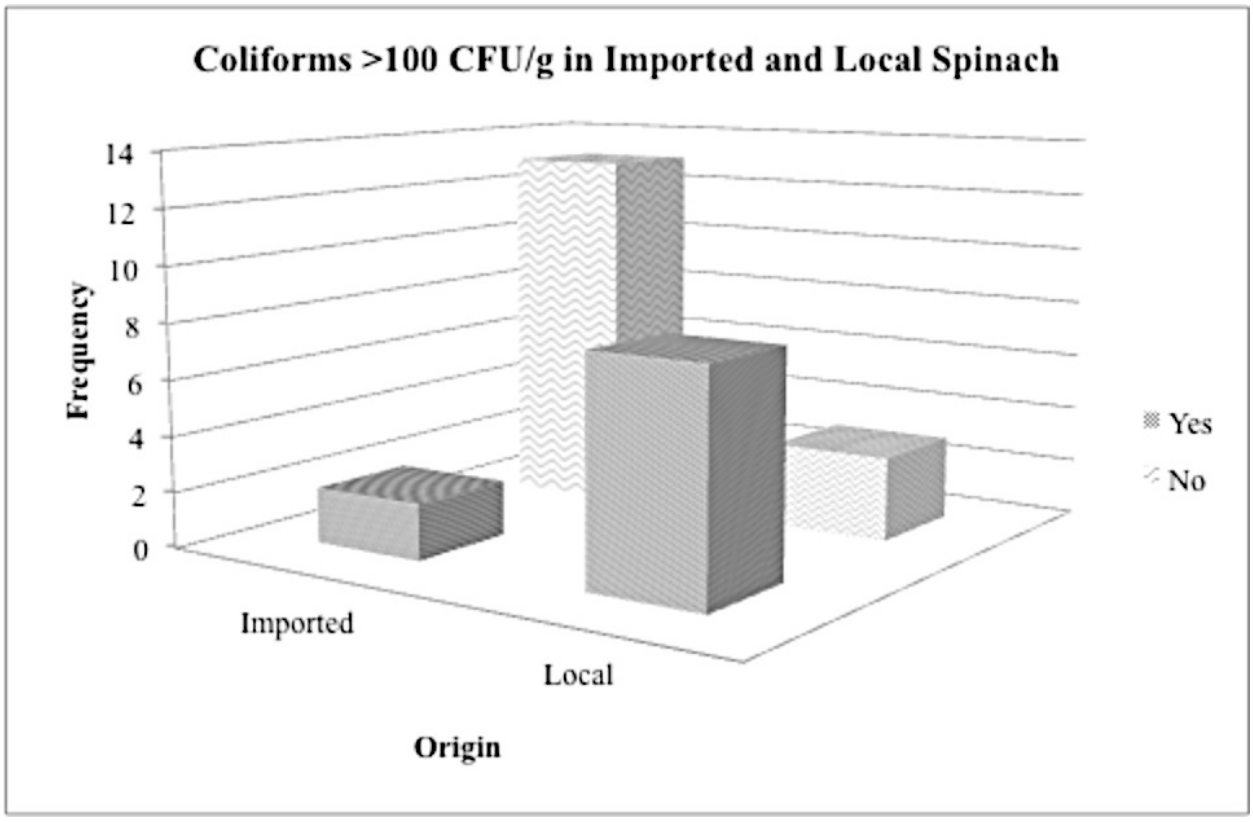

Figure 16. Coliforms >100 CFU/g in Imported and Local Spinach

4.6 *E. coli*

The presence of *E. coli,* represented by dark blue colonies on the membrane filter, indicates fecal contamination. The acceptable level for generic *E. coli* used by the CFIA is 100 CFU/g. Table 21 summarizes the levels of *E. coli* detected within imported and local produce samples. No *E. coli* was detected in any of the local produce samples; however, *E. coli* was detected in five, or 10.6 percent, of the imported produce samples. Of the imported produce, basil contained the highest proportion of samples (21.4 percent) that tested positive for *E. coli*, followed by spinach (6.7 percent), and romaine (5.6 percent). In total, the prevalence of *E. coli* among both imported and local produce samples, across all produce types, was 6.4 percent. However, the finding that only imported samples tested positive for *E. coli* should be examined further in greater detail, and future studies should attempt to verify the lack of *E. coli* in local produce.

Table 21. Summary of *E. coli* Count Frequencies in Imported and Local Produce

	Yes	No	Total
Imported	5	42	**47**
Row %	10.64%	89.36%	100.00%
Basil	3	11	14
Romaine	1	17	18
Spinach	1	14	15
Local	0	31	**31**
Row %	0.00%	100.00%	100.00%
Basil	0	13	13
Romaine	0	7	7
Spinach	0	11	11
Total	**5**	**73**	**78**
Row %	6.41%	93.59%	100.00%

Table 22 shows the origins and *E. coli* levels of the five imported produce samples that tested positive. In each sample, the overall levels of generic *E. coli* detected were well below the 100 CFU/g threshold used by the CFIA.

Table 22. Levels of *E. coli* in Imported Samples

Produce Type	Country of Origin	*E. coli* CFU/g
Basil	Mexico	2
Basil	Vietnam	20
Basil	Mexico	16
Romaine	U.S.A.	1
Spinach	U.S.A.	7

Figure 17 shows that the majority of imported produce samples were not positive for generic *E. coli*. Also, none of the local produce samples tested positive for generic *E. coli*.

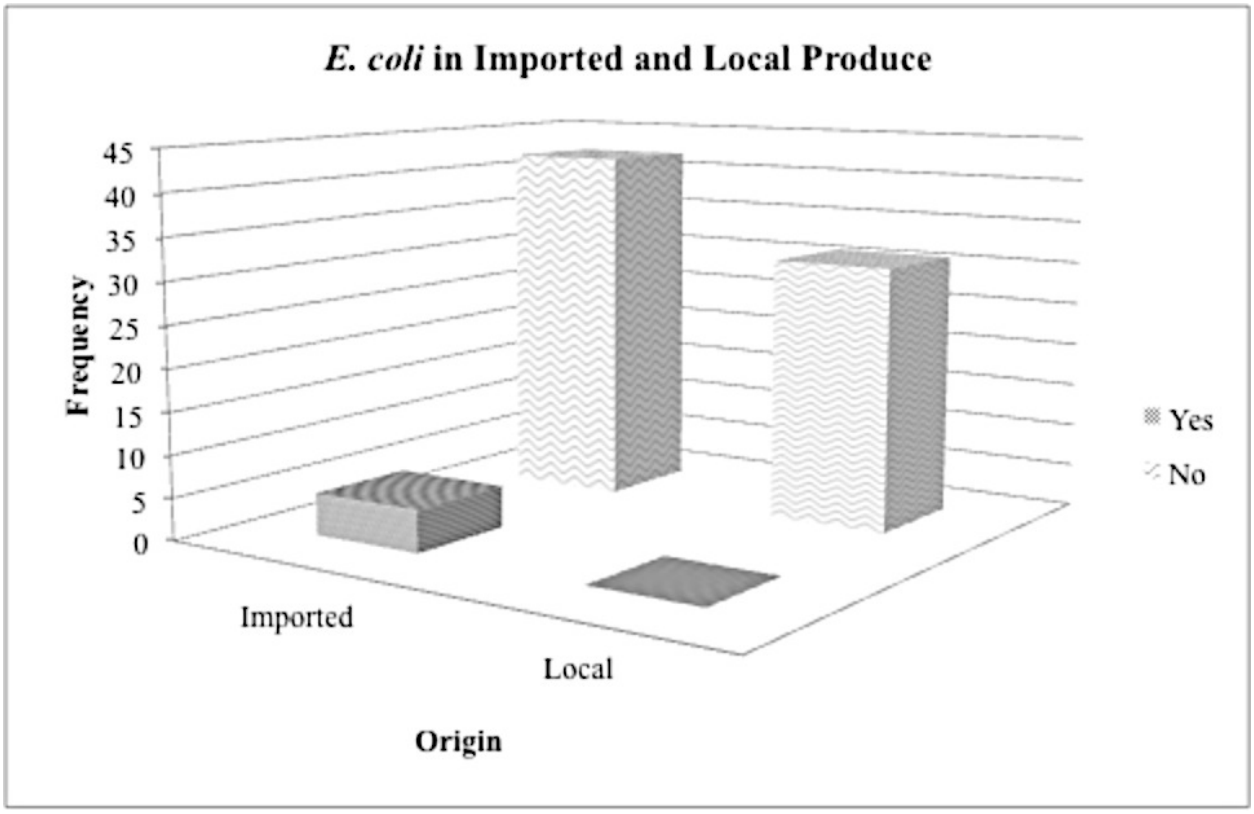

Figure 17. *E. coli* in Imported and Local Produce

Tables 23, 24, and 25 summarize the presence of *E. coli* in imported and local samples of basil, romaine lettuce, and spinach, respectively. In summary, *E. coli* was detected in five of 47 imported samples, consisting of three of 14 imported basil samples, one of 18 imported romaine samples, and one of 15 imported basil samples. In contract, none of the local produce samples tested positive for *E. coli*. Again, although the results were not statistically significant, the finding that only local produce contained *E. coli* is one worth studying in greater detail, and more samples should be tested to attempt to verify this distinction.

Table 23. *E. coli* in Imported and Local Basil

	Yes	No	Total
Imported	3	11	14
Row %	21.43%	78.57%	100.00%
Local	0	13	14
Row %	0.00%	92.86%	100.00%
Total	3	24	28
Row %	10.71%	85.71%	100.00%

Table 24. *E. coli* in Imported and Local Romaine Lettuce

	Yes	No	Total
Imported	1	17	18
Row %	5.56%	94.44%	100.00%
Local	0	7	7
Row %	0.00%	100.00%	100.00%
Total	1	24	25
Row %	4.00%	96.00%	100.00%

Table 25. *E. coli* in Imported and Local Spinach

	Yes	No	Total
Imported	1	14	15
Row %	6.67%	93.33%	100.00%
Local	0	11	11
Row %	0.00%	100.00%	100.00%
Total	1	25	26
Row %	3.85%	96.15%	100.00%

Chapter 5

Discussion

5.1 Introduction

This chapter presents an interpretation of the results from the analysis of local and imported produce. Major trends and relationships are identified along with rationales for likely causes. These observations are compared with results from other studies in the field and are linked back to the research question. Furthermore, limitations of the research methodology are explored.

5.2 Sample Collection and Analysis

In this study, a total of 78 samples of imported and local basil, romaine lettuce, and spinach were analyzed for overall microbial loads and fecal contamination. The objective of this study was to compare the prevalence of microbial contamination in imported and domestic produce, which is based on the hypothesis that imported produce is more susceptible to microbial contamination. In total, 47 imported samples and 31 domestic samples were tested, which exceeded the original target of 30 imported and 30 local samples. However, local basil, spinach, and especially romaine lettuce were not available as early as initially expected due to adverse weather conditions in Ontario in the early 2014 production season. As a result, the number of local samples collected and tested was lower compared to imported produce. Moreover, the lack of local produce was reflected by the fact that visits were made to only three farmers' markets within the GTA, with the majority of local samples originating from one market.

5.3 Heterotrophic Bacteria

Overall, imported produce samples generally had higher levels of heterotrophic bacteria than the local samples. Within imported produce, 77 percent (36/47) of the samples contained medium or high levels of heterotrophic bacteria, compared to only 48 percent (15/31) of the local samples. In terms of produce types, heterotrophic bacteria levels in local basil and local romaine samples were heavily concentrated in the low category compared to imported basil and romaine, while the relationship was less apparent in spinach. The observation that imported produce contained higher levels of heterotrophic bacteria differed from the findings of Johnston and colleagues (2006), who performed a study in the U.S. which showed that the levels of heterotrophic bacteria were equivalent, if not higher, in local produce than in imported produce. The levels of heterotrophic bacteria in imported basil in the present study were also significantly higher, with a geometric mean of 1.6×10^5 CFU/g, than in local basil, which had a geometric mean of 9.4×10^4 CFU/g. However, the geometric mean levels of heterotrophic bacteria were higher in local spinach (4.4×10^5 CFU/g) than imported spinach (3.3×10^5 CFU/g), which is consistent with Johnston and colleagues' (2006) findings regarding local and imported produce.

The mean level of heterotrophic bacteria in imported spinach was approximately two times higher than both the mean level in imported basil and the mean level in imported romaine lettuce (Table 4). The gap was more evident in locally grown spinach, which had significantly higher levels of heterotrophic bacteria i.e. approximately five and ten times higher than the levels in local basil and romaine lettuce, respectively. Abadias and colleagues (2008) also reported higher levels of heterotrophic bacteria in spinach and lower levels in lettuce. The higher levels found in spinach could be attributed to the fact that they are grown in topsoil and have open leaves that could be exposed to or come in contact with soil and irrigation water (Abadias et al., 2008).

These factors can differ between countries and affect the susceptibility of produce to microbial contamination at the farm level, underscoring the influence of farming practices and the importance of subsequent processing stages as potential sources of contamination (Abadias et al., 2008).

An extensive literature review identified only two existing studies that compared the quality of imported and local produce, with one conducted in Norway (Johannessen et al., 2002), and the other conducted in the U.S. (Johnston et al., 2006). Most of the existing studies compare organically and conventionally grown produce (Bohaychuk et al., 2008; CFIA, 2009, 2010; Oliveira et al., 2010; Allen et al., 2013) with focus placed either on imported produce (U.S. FDA, 2001; Allen et al., 2013;), or domestic produce (U.S. FDA, 2003; Arthur et al., 2007; Abadias et al., 2008). Therefore, the imported and local results from this study will be separately compared to the imported or local results from other studies.

The levels of heterotrophic bacteria in imported produce were similar to those found by Allen and colleagues (2013). The levels of heterotrophic bacteria in imported basil (from Colombia, Mexico, and Dominican Republic) ranged from 2.5 $x10^4$ to 5.1 x 10^8 CFU/g in Allen and colleagues' (2013) study. The levels of heterotrophic bacteria in the present study for imported basil ranged from 2.4 x 10^4 to 6.6 x 10^5 CFU/g, which fell within the range reported by Allen and colleagues (2013). For spinach, Allen and colleagues (2013) found heterotrophic bacteria levels ranging from 4.0 x 10^5 to 1 x 10^8 CFU/g, whereas levels in the present study were from 1.7 x 10^4 to 1.3 x 10^6 CFU/g. This placed imported spinach from this study on the lower end of the range reported by Allen and colleagues (2013). The higher levels observed in Allen and colleagues' study could be attributed to the inclusion of organic produce. Also, Allen and colleagues (2013)

did not report separate heterotrophic counts for organic and conventional produce, whereas only produce grown conventionally was tested in the present study. Moreover, they did not clarify the reason for an overall lack of a significant difference between organic and conventional produce but determined that it was likely influenced by the small sample size (Allen et al., 2013). Since the U.S. FDA (2001) did not conduct aerobic plate counts in their survey of imported produce, the findings from their study could not be compared.

While the focus of Allen and colleagues' (2013) study was not to compare organically grown and conventionally grown produce, the majority of other studies examined the level of microbial contamination based on this topic in regions including Ontario (Arthur et al, 2007), Alberta (Bohaychuk et al., 2009), and Spain (Abadias et al., 2008; Oliveira et al., 2010). Thus, only the levels of conventionally grown produce found in these studies can be compared to the results from the present study. Oliveira and colleagues (2010) found a mean level of heterotrophic bacteria for conventionally grown lettuce in Spain of 4.7×10^5 CFU/g. Abadias et al., (2008) found that romaine lettuce had a mean aerobic bacteria level of 1.0×10^6 CFU/g, and that spinach had a mean aerobic bacteria level of 2.5×10^7 CFU/g. The levels of heterotrophic bacteria found by Oliveira and colleagues (2010) were higher than the level of heterotrophic bacteria for romaine lettuce (4.7×10^4 CFU/g) found in this study. Similarly, the levels reported by Abadias and colleagues (2008) for local romaine lettuce and local spinach were also higher than the values found in the present study, which were 4.7×10^4 CFU/g for romaine lettuce and 4.4×10^5 CFU/g for spinach. The reasons for the varying results are not well understood but can likely be attributed to a combination of factors such as the geographical location, differences in farming practices, source of produce (for example the farm, retail markets, farmers' markets, and processing facilities), and types of produce tested (Bohaychuk et al., 2009). Again, these factors

can differ between regions, introducing sources of contamination as early as at the farm level. Although aerobic plate counts do not indicate hazards that compromise the safety of a food product for consumption, it is, however, an indication of the overall quality and shelf life (Pianetti et al., 2008; Oliveira et al., 2010). As such, lower values of aerobic bacteria in this study indicate that the produce tested is not necessarily superior to the produce tested in other studies but will likely have a longer shelf-life (Abadias et al., 2008; Oliveira et al., 2010).

Studies surveying microbial contamination in locally grown produce in Canada include one study that tested Ontario-grown produce (Arthur et al., 2007) and another that tested Alberta-grown produce (Bohaychuk et al., 2009). Heterotrophic bacteria were not enumerated in either study. Arthur and colleagues (2007) examined the presence of pathogenic bacteria such as *E. coli, Shigella,* and *Salmonella,* and Bohaychuk and colleagues (2009) compared organically grown and conventionally grown produce in Alberta and evaluated the presence of pathogenic bacteria such as *E. coli, Salmonella,* and *Campylobacter.*

5.4 Coliforms and *E. coli*

Overall, local produce had higher levels of coliforms as 97 percent (30/31) of local produce samples contained coliform levels in the medium and high category, compared to only 47 percent (21/47) of imported produce samples. The relationship was also discovered in the analyses under the individual produce types, especially in spinach, which showed a strong distinction in the coliform levels between the imported and local samples. In comparison with other studies, coliforms were found in 40 percent of imported spinach in the present study, which is consistent with the findings in Allen and colleagues' (2013) study where the figure ranged from 46.7 to 60.0 percent. In line with the observations on total coliforms, further analysis

suggested that local produce contained a higher proportion of samples with coliform levels exceeding the 100 CFU/g threshold. Interestingly, these observations contrast the results from the analysis using heterotrophic bacteria where imported produce, specifically basil, had higher levels than local produce. Although the reasons for this contrast are beyond the scope of this study, it would be a topic worth investigating further in depth.

Coliforms were found in 71 percent of imported basil and 100 percent of the local basil samples. The prevalence of coliforms within the various produce types is consistent with the findings in Johnston and colleagues' (2006) study, where the mean levels of coliforms in domestic herbs were higher than in imported herbs. However, other studies that surveyed fresh produce focused more on evaluating the presence or absence of *E. coli* and other pathogenic bacteria.

In the present study, six percent (5/79) of samples overall tested positive for *E. coli*, all of which were imported. Although the small numbers did not achieve statistical significance, the fact that *E. coli* was isolated only in imported produce is a distinction worth investigating further in larger studies involving more samples. However, it is also worth noting that the levels of *E. coli* detected in the positive samples were low and did not exceed the investigative assessment threshold for *E. coli* of 100 CFU/gram (CFIA, 2010). Interestingly, the absence of *E. coli* in local produce contrasts with the finding that local produce contained higher levels of coliforms and more samples exceeding the 100 CFU/g coliform threshold. This suggests that while local produce contained higher coliform levels, it had less *E. coli* contamination. Imported produce had lower coliform levels and fewer samples above the threshold, but *E. coli* was detected in five samples with at least one sample from each of the three produce types testing positive for *E. coli*. The findings of this study are generally consistent with those reported in other relevant studies

(Johannessen et al., 2002; Johnston et al., 2006; Abadias et al., 2008; Bohaychuk et al., 2009; CFIA, 2009, 2010; Oliveira et al., 2010; Allen et al., 2013). However, the reasons for these differences between imported and local produce are not fully understood at this time.

The higher prevalence of *E. coli* in imported produce was similar to the results from the CFIA (2010) study on leafy herbs, although statistical analysis was not performed in that study. Of the samples that tested positive for *E. coli,* two were from basil, two were from romaine lettuce, and one was from spinach. The overall proportion of imported samples positive for *E. coli* of 10.6 percent fell within the range of the proportions of *E. coli* found in other studies where the figure was higher (Johannessen et al., 2002; Bohaychuk 2009; Oliveira et al., 2010), or lower (Arthur et al., 2007; Johnston et al., 2006), or varied (Abadias et al., 2008). For example, Johannessen and colleagues (2002) isolated *E. coli* in only one lettuce sample (<1 percent) and three herb samples (3 percent), which did not include basil. This finding is lower than the proportion of lettuce samples that were positive for *E. coli* in the present study. However, the presence of *E. coli* in the samples in Johannessen and colleagues' (2002) study was attributed to possible exposure to water or soil that was fecally contaminated or from contamination during handling. Moreover, coliform counts were not conducted in the U.S. FDA's (2001, 2003) survey of imported produce in 1999 or their survey of domestic produce in 2000.

Varying results between coliforms and *E. coli* were also found in the present study. Imported produce exhibited lower mean levels of coliforms compared to local produce, yet imported produce exhibited higher occurrences of *E. coli.* These mixed findings were similar to those reported by Johnston and colleagues (2006) where domestic herbs (347 CFU/g) had higher levels of coliforms than Mexican herbs (56 CFU/g). Contrastingly, levels of *E. coli* were higher in

domestic herbs (19 CFU/g) than in Mexican herbs (7 CFU/g). Although the present study did not test for pathogenic *E. coli*, it is interesting to note that these bacteria were not detected in any of the herb samples in the study by Johnston and colleagues (2006). The geometric mean level of coliforms in imported produce (34 CFU/g) in the present study was higher than the mean level in the imported (Mexican) produce found in Johnston and colleagues' (2006) study. However, the geometric mean level of coliforms in local produce (61 CFU/g) was lower than the levels found in Johnston and colleagues' (2006) study. The overall low prevalence of *E. coli* found in the present study is consistent with other studies that compared imported and local produce (Johannessen et al., 2002; Johnston et al., 2006).

For studies conducted on domestic produce, Arthur and colleagues (2007) surveyed organically grown Ontario-grown produce and found that the levels of generic *E. coli* were higher in leaf lettuce and cilantro. In their results, *E. coli* was found in 6.5 percent of lettuce, 4.9 percent of cilantro, and 13.4 percent of parsley (Arthur et al., 2007). In another study, Bohaychuk and colleagues (2009) compared organically grown and conventionally grown produce in Alberta and found that 3.5 percent of conventional lettuce and 2.1 percent of spinach tested positive for *E. coli.* *E. coli* O157:H7 was not isolated in any of the produce samples. Abadias and colleagues (2008) did not detect any *E. coli* in local romaine lettuce, whereas *E. coli* was detected in 20 percent of local spinach. These findings differ from those of the present study as no local samples tested positive for *E. coli.* The higher proportion of *E. coli*-positive samples in Arthur and colleagues' (2007) study is likely due to the testing of organic produce and a larger sample size. However, some similarities of the Arthur and colleagues' (2007) study support the challenges of determining the microbial loads in local produce in Ontario. The short production season in Ontario poses challenges to collecting the necessary amount of samples required to

determine the prevalence of pathogens at a statistically significant level (Arthur et al., 2007). In addition, lettuce, parsley, and cilantro possess large surface leaf areas and are grown close to the ground, increasing the potential for contact with irrigation water and soil (Arthur et al., 2007; Abadias et al., 2008). Since these types of produce have short growing periods, their exposure to environmental stresses is reduced and thus the potential for pathogens to survive is increased (Arthur et al., 2007). Moreover, these types of produce are also handled more frequently by employees (Arthur et al., 2007).

Considering that coliforms are microorganisms that occur naturally on produce and are not necessarily of fecal origin, their use as fecal indicators is limited (Allen et al., 2013). Johannessen and colleagues (2002) screened produce for thermotolerant coliform bacteria; however, they found that the presence of these bacteria in the tested produce originated from non-fecal sources such as *Enterobacter* spp. *or Klebsiella* spp. These types of bacteria are similar to *E. coli* in that they can also grow at higher temperatures, further contributing to the limitations of using coliforms as fecal indicators (Johannessen et al., 2002; Allen et al., 2013). As such, many studies consider *E. coli* to be the best indicator of fecal contamination (Johannessen et al., 2002; Abadias et al., Abadias et al., 2008; Bohaychuk et al., 2009; Oliveira et al., 2010; Allen et al., 2013). However, recent studies have also raised concerns regarding the validity of using *E. coli* as a fecal indicator. For example, Luo and colleagues (2011) found that *E. coli* possesses a specific genomic content that has been shown to favour survival in the environment more than in the intestines of mammals. Although these strains may not be of significant importance in public health, *E. coli* continues to be the preferred indicator for microbial contamination in fresh produce (Allen et al., 2013).

The sources of contamination examined in the literature review provide some insight into the possible explanations for the differences in microbial loads between imported and local produce found in this study. However, without investigating specific factors at the farm level or throughout the transportation or distribution stages, the reasons for the relationships found in this study between microbial loads and the origin of the produce cannot be pinpointed. The review also revealed some of the major differences in farming practices used in Canada, U.S., and other countries that export fresh produce to Canada. For example, the quality of water used for irrigation can be a significant source of pathogenic contamination and plays an instrumental role in all stages from pre-harvest, to harvest, to post-harvest, to being sold in retail markets or served in food service establishments (Beuchat, 2002; Suslow et al., 2003; Gerba and Choi, 2009; Ijabadeniyi et al., 2011; Cirelli et al., 2012; Hanjra et al., 2012;). Since produce contamination most commonly occurs by way of the fecal-oral route (Beuchat and Ryu, 1997), the increased consumption of produce, coupled with the increased prevalence of foodborne outbreaks associated with produce, demonstrates the importance of improving measure to prevent microbial contamination in fresh produce (Allen et al., 2013). However, although an investigation of the extent to which these factors influence the microbial loads in produce sampled is beyond the scope of this study, the fact that *E. coli* was present in five imported samples compared to zero local samples and the differences in microbial loads between the two types of produce make examining the effects of these farming practices worthwhile.

5.5 *E. coli* O157:H7

This study did not screen produce samples for the presence of pathogenic *E. coli* such as *E. coli* O157:H7. However, within all the studies that surveyed microbial loads in produce, *E. coli* O157:H7 was not detected in any the samples despite the occurrence of other foodborne pathogens such as *Salmonella* (Arthur et al., 2007). Canadian studies that did not report any samples positive for this strain include a study that tested 106 imported fresh produce samples across Canada (Allen et al., 2013), two studies that tested 601 imported and domestic leafy vegetables and 1224 imported and domestic leafy herbs (CFIA 2009, 2010), a study comparing 673 organic and conventional produce samples grown in Alberta (Bohaychuk et al., 2009), and a study surveying 1,183 samples of Ontario grown organic and conventional produce (Arthur et al., 2007). International studies include a study comparing 466 imported and domestic produce samples from the United States and Mexico (Johnston et al., 2006), separate larger-scale studies surveying 1003 imported and 1028 domestic produce samples in the United States (U.S. FDA, 2001, 2003), a study comparing 890 imported and local produce samples tested in Norway (Johannessen et al., 2002), a study comparing144 organic and conventional lettuce samples in Spain (Oliveira et al., 2010), and a study examining 300 samples of fresh produce from retail markets in Spain (Abadias et al., 2008). Although tests for *E. coli* O157:H7 were not performed in the present study, the presence of *E. coli* in imported samples suggests a potential for fecal contamination and therefore demonstrates the need to minimize the exposures of fecal contamination to produce by improving the food production chain.

5.7 Research Limitations

Since the differences in handling by employees and consumers cannot be determined or controlled, an examination of employee or consumer handling at retail markets or throughout the distribution stages that may introduce contamination to post-harvest produce was beyond the scope of this study. Considering that the production and distribution system is already complex for local produce, the added obstacle of crossing international borders further increases the complexities of the production and distribution system such as maintaining proper temperatures during transportation (Olaimat and Holley, 2012; Allen et al., 2013). For example, the methods in which the produce is harvested, washed, stored, or transported to the retail markets could not be determined for each sample and thus it could not be guaranteed that all samples were equally clean at the point of collection. Also, since the distribution chain for imported produce is much longer than for local produce, it is possible that the prolonged transportation and distribution associated with imported produce increases the chances for pathogens to grow (Allen et al., 2013). However, it was not possible to determine the extent of these effects on the imported or local samples tested. To minimize consumer or employee handling at the supermarket, this study utilized bagged produce varieties. As an attempt to ensure similar levels of cleanliness within the samples at the farmers' markets, similar bagged produce that were confirmed by the local vendors as requiring rinsing or washing prior to consumption was selected.

The sample size was calculated based on preliminary test results, which was comprised of a small sample size (n=14). The length of the preliminary study was shortened due to the limited seasonal availability of produce during the study period, which was reflected in the small sample size. Arthur and colleagues (2007) also experienced this challenge in their study of Ontario

grown produce. In the preliminary study, local produce availability was also a major constraint, which resulted in fewer local samples collected compared to imported samples. Since the availability of local produce in Ontario was also very limited in the early 2014 season due to weather conditions (an usually long and colder than average winter in 2013), the number of farmers' markets visited and produce purchased was much more limited compared to imported produce.

The use of sample size calculators have inherent risks such as sensitivity to errors since a slight difference in the input assumptions can result in large differences in the calculated sample size (Noordzij, et al., 2010). In addition, the required sample size can be very sensitive to the assumptions made or the parameters chosen and therefore result in a lack of precision (Noordzij, et al., 2010). Since the preliminary study was based on a relatively small sample size, the required sample size calculated for the present study may not be representative of all of the produce types. Moreover, the introduction of other produce types such as spinach also reduced the confidence level of the study. However, since this study is intended for feasibility purposes, it is recommended for future studies to base sample size calculations on a comparable study with a larger sample size and using a single produce type.

Sampling bias was introduced in this study in two ways: purposeful sampling and the collecting the majority of local samples from one farmers' market. Since both imported and local produce were purposefully sampled, this type of sampling can introduce bias and other confounding variables to the study (Suri, 2011). The handling of samples collected was similar to that of the U.S. FDA's (2001, 2003) survey on imported and domestic produce, and aimed to mimic handling similar to typical consumer habits such as selecting produce that is free from extraneous

dirt, browning, bruising, or wilting leaves. Future studies can employ random sampling as this will improve the credibility and generalizability of the results, minimize potential bias in sample selection, and control for potential confounding variables (Suri, 2011; Palinkas, et al., 2013). In addition, the limited produce availability and adverse weather conditions were reflected in the study by the fact that the majority of local samples was collected from one farmers' market. Collecting the majority of samples from one market can introduce confounding variables such as potential sources of contamination specific to the location or facility holding the farmers' market. Despite the constraints preventing sampling from more or other farmers' markets, future studies should randomize sampling, ensure that sampling locations are representative of all markets available, and not heavily skewed to one specific location.

Another limitation relates to the method used to test the produce samples. Most studies homogenize produce samples in a stomacher (Johnston et al., 2006; Abadias et al., 2008; Bohaychuk et al., 2009; Oliveira et al., 2010; Allen et al., 2013); however, one of the main reasons why homogenization of samples was not used consistently throughout this study was the limited supply of blenders available for use in homogenization. There was also a significantly higher chance of cross-contamination since certain plastic parts of the blender could not be sterilized. In this study, non-homogenized samples of basil, romaine, and spinach were compared to nine homogenized samples. The comparisons did not differentiate between produce types, as the main objective was to determine whether there was a difference between the two methods of testing. A two-tailed u-test was performed and revealed no statistically significant difference between heterotrophic bacteria levels or coliform levels under the two methods for either imported or local produce. For imported product, the p-values for the comparisons in heterotrophic bacteria levels and coliform levels between the two methods were 0.7622 and

0.6293, respectively. For local product, the p-values for the comparisons in heterotrophic bacteria levels and coliform levels between the two methods were 0.4970 and 0.1613, respectively. Based on the lack of statistical differences between the results from the two methods, the disadvantages of possible cross-contamination and potentially confounding results outweighed the benefits of using homogenized samples, and the use of the method was discontinued.

Since many produce-related recalls have been associated with *E. coli* O157:H7 (Warriner et al., 2009; Olaimat and Holley, 2012; Allen et al., 2013; Kozak et al., 2013), the ability to identify specific strains or pathogenicity would have been beneficial in this study. Moreover, another limitation within this study, as well as within other studies that examine the microbial loads in produce, was the exclusion of foodborne viral agents (Allen et al., 2013). Although the use of MacConkey and DC agar was a cost-effective means to identify the presence and levels of coliforms and generic *E. coli*, identifying specific strains or determining pathogenicity would not have been possible without further verification. These verification methods, although effective, were not carried out due to limitations in time and resources. These limitations also prevented serial dilutions in the laboratory analysis. Instead, 1:100 dilutions were conducted for the samples as this level of dilution was determined to be able to yield countable results.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

The results of this feasibility study indicate that overall there were five imported samples that were positive for *E. coli* compared to zero local samples. There were other relationships between imported and local produce, but the significances were inconsistent across the analyses on heterotrophic bacteria, coliforms, and *E. coli.* Total coliform levels were higher in local samples, and there were more local produce samples with coliform levels greater than 100 CFU/g, especially within spinach. However, in terms of heterotrophic bacteria levels, it was imported produce that had higher levels, especially within basil. The mixed relationships identified in this study show similarities with the findings from other studies characterizing the overall microbial loads in imported and local produce (Johnston et al., 2006; CFIA, 2009, 2010). While these findings highlight relationships between certain produce types under different types of bacteria indicators, there was no consistent overall difference between bacteria levels in imported and domestic produce.

Imported produce generally had higher levels of heterotrophic bacteria, while local produce generally had higher coliform levels and more samples with coliforms levels exceeding 100 CFU/g. Another significant finding in this study was the presence of *E. coli* in five imported samples, with at least one sample from each of the three produce types, compared to zero local samples. Although no relationship was found between the presence of *E. coli* and the origin of produce and the levels of *E. coli* detected in the five imported samples were low, this finding nonetheless supports the hypothesis that imported produce is more susceptible to microbial

contamination. Further research should be directed towards investigating potential reasons for the increased susceptibility in imported produce.

While the detection of pathogenic bacteria was not included in the study, generic *E. coli* was detected in five imported samples, although none of the *E. coli* levels exceeded CFIA's satisfactory threshold. Nevertheless, the presence of coliforms and *E. coli* indicate the potential presence of undesirable and pathogenic microorganisms that can pose serious public health concerns for consumers. The extent to which farming practices could have affected produce quality throughout the stages, from pre-harvest to distribution at the retail market or food service establishment, was reviewed extensively but not examined in this study. However, the fact that five imported samples compared to zero local samples tested positive for *E. coli*, along with the fact that Ontario imports the majority of the produce in its retail markets, demonstrates the necessity of ensuring low microbial contamination of produce imported into Canada.

6.2 Contributions and Future Research

The findings of this study serve as a feasibility study for imported and local produce in Ontario. The results of this study were not intended to draw specific conclusions between imported and local produce, but rather to solicit further research focused on this topic. Although the CFIA conducted surveys on the overall microbial contamination in imported and local produce in Canada, a study comparing the microbial contamination in imported and local produce has not been conducted in Toronto or Ontario. Moreover, Bohaychuk and colleagues (2009) noted that varying microbial loads found in other studies could be attributed to many factors including differences in geographical location, farming practices, produce source (e.g., farm, retail markets, farmers' markets, and processing facilities), and produce types tested. As such, the

findings from one study may not necessarily be generalized to other areas, and localized studies comparing imported and local produce within specific regions, especially in Toronto and Ontario, are encouraged.

Moreover, although the findings of this study indicated a lack of a statistical difference between heterotrophic bacteria levels and coliform levels in imported and local produce, there were more countable occurrences of *E. coli* in imported produce than local. While *E. coli* occurrences do not necessarily indicate the presence of pathogens, the study did not include advanced pathogen detection methods and thus the presence of *E. coli* in the five imported produce samples could not be investigated further. Nonetheless, the increased occurrence of *E. coli* in imported produce is an indicator of fecal contamination. These findings highlight the fact that improvements can be made in pre-harvest and post-harvest farming practices to prevent the introduction of microbial contamination into imported produce throughout the processing and distribution stages.

Future research can be directed in three main ways: conducting case studies that compare farms or processing facilities in Ontario with those in foreign regions or countries, testing imported and local produce from the same time periods during the year, and focusing on a large quantity of a single type of produce. Studies that focus on comparing farms or processing facilities in settings where both imported and local produce are grown would be beneficial in providing information to the local and foreign farming communities. Such studies would also allow for more in-depth analyses of how farming practices influence the quality of produce grown in the regions. Investigations of farms or processing facilities abroad would provide insight into measures that could be adopted in other countries to prevent microbial contamination in produce.

Similar to the study by Johnston and colleagues' (2006), localized case studies would also present a better understanding of the local production and distribution chain for produce from the farms to the retail markets or food service establishments. For example, in Ontario, such an investigation would uncover areas of potential improvement to prevent microbial contamination in locally grown produce, especially spinach since it was found in the present study to have higher microbial loads.

Another topic of study that would benefit from further research is the surveying of imported and local produce available during the same time period in Toronto. The present study did not test imported and local produce from the same weeks, or even months, of the year due to resource constraints and overall limitations in produce availability. However, the findings from the testing of both types of produce from similar time periods can be used to help determine whether Toronto's reliance on imported produce can be reduced, especially during Ontario's most productive growing period of the year. The importing process also produces significant amounts of carbon emissions and consumes fossil fuels (Kissinger, 2012; Caputo et al., 2013). As such, promoting local consumption of produce can help decrease global carbon emissions and increase support for the local economy.

Finally, the third potential approach in future studies is to test a large quantity of a single type of produce. The present study included three types of produce, albeit having relatively small sample sizes compared to other studies. Given the resources required to obtain a sufficient sample size capable of producing statistically significant comparisons, along with the complications of grouping several different produce types when performing statistical analysis, future studies can focus on a larger quantity of a single type of produce. This would reduce the

effects of possible confounding factors and interactions associated with multiple produce types. The results may allow for comparisons that are more straightforward and provide a better representation of the overall produce quality.

Appendix A. Freeman-Halton Extension of Fisher's Exact Test

Fisher Exact Probability Test: 2x3

This unit will perform the Freeman-Halton extension of the Fisher exact probability test for a two-rows by three-columns contingency table, providing that the total size of the data set is no greater than N=300. The test will yield two probability values, P_A and P_B, defined as follows:

P_A = the probability of the observed array of cell frequencies plus the sum of the probabilities of all other cell-frequency arrays (such as would be consistent with the observed marginal totals) that are *equal to or smaller* than the probability of the observed array.

P_B = the probability of the observed array of cell frequencies plus the sum of the probabilities of all other cell-frequency arrays (such as would be consistent with the observed marginal totals) that are *smaller* than the probability of the observed array.

Note that P_A and P_B are both non-directional (two-tailed) probabilities.

A chi-square test will also be performed on the data set, providing that at least 80% of the cells have an expected frequency of 5 or greater, and that no cell has an expected frequency smaller than 1.0.

Data Entry

	C_1	C_2	C_3	Totals
R_1				---
R_2				---
Totals	---	---	---	---

Clear Calculate

Fisher Exact Probability Test

P_A =

P_B =

No. of tables evaluated =

The Fisher test is performed only if N≤300.

Note that P_A and P_B are both non-directional (two-tailed).

Chi-Square Test (df=2)

Chi-square =

P =

The chi-square test is performed only if at least 80% of the cells have an expected frequency of 5 or greater, and no cell has an expected frequency smaller than 1.0.

Home	Click this link **only** if you did not arrive here via the VassarStats main page.

(Freeman-Halton, 1951; Lowry, 2014)

Appendix B. Imported Produce Test Results

Produce						Results							
						Nutrient Agar		MacConkey Agar		DC Agar (Coliforms)		DC Agar (*E. coli*)	
Test Date	Item	Source	Country	Weight (grams)	Wash Water (ml)	CFU/g	Category	CFU/g	Category	CFU/g	Category	*E. coli* (Y/N)	*E. coli* (CFU/g)
Jan-30-2014	Spinach	Metro	Mexico	15.01	150.00	17,261	Low	1,817	Low	2.00	Low	N	0
Jan-30-2014	Romaine	T&T	U.S.A.	15.02	150.00	73,841	Low	62,644	Low	0.00	Low	N	0
Feb-03-2014	Basil	Loblaws	Basil-1 Mexico	15.00	150.00	54,545	Low	4,545	Low	TNTC	High	N	0
Feb-03-2014	Basil	Loblaws	Basil-2 Colombia	14.50	145.00	28,182	Low	7,273	Low	0.00	Low	N	0
Feb-03-2014	Spinach	Longos	Mexico	15.01	150.00	TNTC	High	55,115	Low	0.00	Low	N	0
Feb-03-2014	Basil	Longos	Colombia	15.01	150.00	TNTC	High	39,065	Low	2.66	Low	N	0
Feb-06-2014	Basil	No Frills	Mexico	15.02	150.00	387,362	Low	269,338	Low	17.98	Low	Y	2
Feb-06-2014	Spinach	No Frills	U.S.A.	15.02	150.00	223,944	Low	3,026	Low	0.00	Low	N	0
Feb-06-2014	Romaine	No Frills	U.S.A.	15.03	150.00	133,067	Low	60,485	Low	0.00	Low	N	0
Feb-06-2014	Basil	Sobeys	Costa Rica	15.00	150.00	133,333	Low	63,636	Low	0.00	Low	N	0
Feb-06-2014	Romaine	Metro	U.S.A.	15.02	150.00	587,096	Medium	348,021	Low	0.00	Low	N	0
Feb-06-2014	Basil	Metro	Dominican Republic	15.01	150.00	302,828	Low	324,026	Low	TNTC	High	N	0
Feb-06-2014	Spinach	Metro	Mexico	15.01	150.00	TNTC	High	381,564	Low	0.00	Low	N	0
Feb-13-2014	Spinach	Metro	U.S.A.	15.01	150.00	136,273	Low	21,198	Low	0.00	Low	Y	7
Feb-13-2014	Romaine	Sobeys	U.S.A.	15.01	150.00	45,424	Low	21,198	Low	0.00	Low	N	0
Feb-13-2014	Spinach	No Frills	U.S.A.	15.01	150.00	766,156	Medium	187,754	Low	0.00	Low	N	0

Produce						Results							
						Nutrient Agar		MacConkey Agar		DC Agar (Coliforms)		DC Agar (*E. coli*)	
Test Date	Item	Source	Country	Weight (grams)	Wash Water (ml)	CFU/g	Category	CFU/g	Category	CFU/g	Category	*E. coli* (Y/N)	*E. coli* (CFU/g)
Feb-17-2014	Romaine	Loblaws	U.S.A.	15.01	150.00	1,280,964	High	581,431	Medium	0.00	Low	N	0
Feb-17-2014	Romaine	Loblaws	U.S.A.	15.01	150.00	1,280,964	High	581,431	Medium	0.00	Low	N	0
Feb-17-2014	Basil	Loblaws	Mexico	15.01	150.00	218,036	Low	448,186	Low	0.00	Low	N	0
Feb-17-2014	Romaine	T&T	U.S.A.	15.01	150.00	320,998	Low	354,309	Low	0.00	Low	N	0
Feb-24-2014	Spinach	Sobeys	Mexico	12.01	120.00	439,028	Low	99,917	Low	0.00	Low	N	0
Feb-24-2014	Romaine	No Frills	U.S.A.	12.01	120.00	139,278	Low	3,028	Low	0.00	Low	N	0
Feb-24-2014	Spinach	Metro	Mexico	12.02	120.00	919,679	Medium	114,960	Low	0.00	Low	N	0
Mar-14-2014	Spinach	Loblaws	U.S.A.	12.01	120.00	947,695	Medium	157,445	Low	0.00	Low	N	0
Mar-14-2014	Romaine	Sobeys	U.S.A.	12.01	120.00	TNTC	High	72,667	Low	104.91	High	N	0
Mar-14-2014	Basil	Sobeys	Mexico	12.01	120.00	TNTC	High	9,083	Low	TNTC	High	Y	16
Mar-14-2014	Basil	Longos	Colombia	12.01	120.00	175,611	Low	0	Low	TNTC	High	N	0
Mar-14-2014	Romaine	Longos	U.S.A.	12.01	120.00	314,889	Low	0	Low	4.16	Low	N	0
Mar-14-2014	Spinach	Longos	Mexico	12.01	120.00	753,917	Medium	18,167	Low	153.21	High	N	0
Mar-20-2014	Romaine	Metro	U.S.A.	10.00	100.00	30,303	Low	39,394	Low	262.00	High	Y	1
Mar-20-2014	Basil	Sobeys	Mexico	10.00	100.00	663,636	Medium	12,121	Low	140.00	High	N	0
Mar-20-2014	Spinach	Sobeys	U.S.A.	10.00	100.00	139,394	Low	90,909	Low	64.00	Medium	N	0
Mar-20-2014	Spinach	No Frills	U.S.A.	10.00	100.00	160,606	Low	127,273	Low	56.00	Medium	N	0
Mar-20-2014	Spinach	Longos	U.S.A.	10.00	100.00	806,061	Medium	803,030	Medium	212.00	High	N	0
Apr-01-2014	Basil	Longos	Colombia	10.00	100.00	24,242	Low	27,273	Low	TNTC	High	N	0

Produce						Results							
						Nutrient Agar		MacConkey Agar		DC Agar (Coliforms)		DC Agar (*E. coli*)	
Test Date	**Item**	**Source**	**Country**	**Weight (grams)**	**Wash Water (ml)**	**CFU/g**	**Category**	**CFU/g**	**Category**	**CFU/g**	**Category**	***E. coli* (Y/N)**	***E. coli* (CFU/g)**
Apr-01-2014	Romaine	Longos	U.S.A.	10.00	100.00	1,009,091	High	621,212	Medium	TNTC	High	N	0
Apr-01-2014	Romaine	Loblaws	U.S.A.	10.00	100.00	290,909	Low	12,121	Low	TNTC	High	N	0
Apr-08-2014	Spinach	Loblaws	U.S.A.	10.00	100.00	1,057,576	High	775,758	Medium	0.00	Low	N	0
Apr-08-2014	Romaine	Loblaws	Vietnam	10.00	100.00	248,485	Low	115,152	Low	0.00	Low	N	0
Apr-08-2014	Romaine	T&T	U.S.A.	10.00	100.00	796,970	Medium	863,636	Medium	TNTC	High	N	0
Apr-08-2014	Basil	No Frills	Mexico	10.00	100.00	503,030	Medium	321,212	Low	TNTC	High	N	0
Apr-15-2014	Romaine	Longos	U.S.A.	10.00	100.00	3,387,879	High	1,757,576	High	11.00	Low	N	0
Apr-15-2014	Romaine	Sobey's	U.S.A.	10.00	100.00	30,303	Low	0	Low	0.00	Low	N	0
Apr-15-2014	Basil	Metro	Dominican	10.00	100.00	45,455	Low	6,061	Low	0.00	Low	N	0
May-27-2014	Romaine	Longos	U.S.A.	10.00	100.00	69,697	Low	12,121	Low	TNTC	High	N	0
May-27-2014	Spinach	Longos	Mexico	10.00	100.00	266,667	Low	121,212	Low	31.00	Low	N	0
Jun-04-2014	Basil	T&T	Vietnam	10.00	100.00	600,000	Medium	269,697	Low	42.00	Low	Y	20

Appendix C. Local Produce Test Results

Produce					Results							
					Nutrient Agar		MacConkey		DC Agar (Coliforms)		DC Agar (E. coli)	
Test Date	Item	Source	Weight (grams)	Wash Water (ml)	CFU/g	Category	CFU/g	Category	CFU/g	Category	*E. coli* (Y/N)	*E. coli* (CFU/g)
Apr-21-2014	Basil	St. Lawrence A	10.00	100.00	42,424	Low	48,485	Low	TNTC	High	N	0
Apr-21-2014	Spinach	St. Lawrence A	10.00	100.00	118,182	Low	30,303	Low	TNTC	High	N	0
Apr-21-2014	Basil	St. Lawrence B	10.00	100.00	72,727	Low	6,061	Low	TNTC	High	N	0
Apr-21-2014	Romaine	St. Lawrence B	10.00	100.00	69,697	Low	27,273	Low	TNTC	High	N	0
Apr-30-2014	Romaine	St. Lawrence A	10.00	100.00	136,364	Low	121,212	Low	TNTC	High	N	0
Apr-30-2014	Basil	St. Lawrence B	10.00	100.00	127,273	Low	24,242	Low	TNTC	High	N	0
Apr-30-2014	Spinach	St. Lawrence B	10.00	100.00	1,175,758	High	396,970	Low	TNTC	High	N	0
May-13-2014	Basil	St. Lawrence A	10.00	100.00	103,030	Low	6,061	Low	TNTC	High	N	0
May-13-2014	Spinach	St. Lawrence A	10.00	100.00	1,021,212	High	215,152	Low	TNTC	High	N	0
May-13-2014	Basil	St. Lawrence B	10.00	100.00	333,333	Low	321,212	Low	TNTC	High	N	0
May-13-2014	Romaine	St. Lawrence B	10.00	100.00	27,273	Low	75,758	Low	TNTC	High	N	0
May-20-2014	Basil	St. Lawrence A	10.00	100.00	115,152	Low	6,061	Low	256	High	N	0
May-20-2014	Spinach	St. Lawrence A	10.00	100.00	260,606	Low	296,970	Low	185	High	N	0
May-20-2014	Basil	St. Lawrence B	10.00	100.00	54,545	Low	69,697	Low	54	Medium	N	0
May-20-2014	Romaine	St. Lawrence B	10.00	100.00	3,748,485	High	136,364	Low	TNTC	High	N	0
May-21-2014	Spinach	The Stop's	10.00	100.00	1,306,061	High	1,218,182	High	148	High	N	0
May-26-2014	Basil	St. Lawrence A	10.00	100.00	124,242	Low	227,273	Low	336	High	N	0
May-26-2014	Romaine	St. Lawrence A	10.00	100.00	15,152	Low	54,545	Low	85	Medium	N	0
May-26-2014	Basil	St. Lawrence B	10.00	100.00	112,121	Low	242,424	Low	5	Low	N	0
May-26-2014	Spinach	St. Lawrence B	10.00	100.00	1,203,030	High	875,758	Medium	16	Low	N	0

Produce					Results							
					Nutrient Agar		MacConkey Agar		DC Agar (Coliforms)		DC Agar (*E. coli*)	
Test Date	Item	Source	Weight (grams)	Wash Water (ml)	CFU/g	Category	CFU/g	Category	CFU/g	Category	*E. coli* (Y/N)	*E. coli* (CFU/g)
May-27-2014	Spinach	St. Lawrence C	10.00	100.00	509,091	Medium	139,394	Low	143	High	N	0
May-27-2014	Spinach	Leslieville Farmers Market	10.00	100.00	78,788	Low	0	Low	33	Low	N	0
Jun-01-2014	Basil	St. Lawrence A	10.00	100.00	484,848	Low	51,515	Low	TNTC	High	N	0
Jun-01-2014	Spinach	St. Lawrence A	10.00	100.00	487,879	Low	63,636	Low	TNTC	High	N	0
Jun-01-2014	Romaine	St. Lawrence B	10.00	100.00	27,273	Low	106,061	Low	99	Medium	N	0
Jun-01-2014	Basil	St. Lawrence B	10.00	100.00	27,273	Low	18,182	Low	11	Low	N	0
Jun-01-2014	Spinach	St. Lawrence C	10.00	100.00	121,212	Low	33,333	Low	72	Medium	N	0
Jun-07-2014	Basil	St. Lawrence A	10.00	100.00	36,364	Low	142,424	Low	TNTC	High	N	0
Jun-07-2014	Spinach	St. Lawrence A	10.00	100.00	818,182	Medium	493,939	Low	TNTC	High	N	0
Jun-07-2014	Basil	St. Lawrence B	10.00	100.00	81,818	Low	0	Low	23	Low	N	0
Jun-07-2014	Romaine	St. Lawrence B	10.00	100.00	103,030	Low	6,061	Low	75	Medium	N	0

Appendix D. Expected Frequencies for Heterotrophic bacteria, Lactose-fermenting bacteria, Coliforms, and *E. coli*

Heterotrophic Bacteria

Heterotrophic Bacteria: Imported and Local Produce

	Low	Medium	High	Total
Imported	16.269	15.064	15.667	47.000
Local	10.731	9.936	10.333	31.000
Total	27	25	26	78

Heterotrophic Bacteria: Imported and Local Basil

	Low	Medium	High	Total
Imported	6.741	4.667	2.593	14.000
x	6.259	4.333	2.407	13.000
Total	13	9	5	27

Heterotrophic Bacteria: Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	7.920	5.040	5.040	18.000
Local	3.080	1.960	1.960	7.000
Total	11	7	7	25

Heterotrophic Bacteria: Imported and Local Spinach

	Low	Medium	High	Total
Imported	1.731	5.192	8.077	15.000
Local	1.269	3.808	5.923	11.000
Total	3	9	14	26

Lactose-Fermenting Bacteria

Lactose-Fermenting Bacteria: Imported and Local Produce

	Low	Medium	High	Total
Imported	16.269	15.667	15.064	47.000
Local	10.731	10.333	9.936	31.000
Total	27	26	25	78

Lactose-Fermenting Bacteria: Imported and Local Basil

	Low	Medium	High	Total
Imported	6.741	3.111	4.148	14.000
Local	6.259	2.889	3.852	13.000
Total	13	6	8	27

Lactose-Fermenting Bacteria: Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	6.480	7.200	4.320	18.000
Local	2.520	2.800	1.680	7.000
Total	9	10	6	25

Lactose-Fermenting Bacteria: Imported and Local Spinach

	Low	Medium	High	Total
Imported	2.885	5.769	6.346	15.000
Local	2.115	4.231	4.654	11.000
Total	5	10	11	26

Coliforms

Coliforms: Imported and Local Produce

	Low	Medium	High	Total
Imported	16.269	14.462	16.269	47.000
Local	10.731	9.538	10.731	31.000
Total	27	24	27	78

Coliforms: Imported and Local Basil

	Low	Medium	High	Total
Imported	3.111	3.630	7.259	14.000
Local	2.889	3.370	6.741	13.000
Total	6	7	14	27

Coliforms: Imported and Local Romaine Lettuce

	Low	Medium	High	Total
Imported	7.920	4.320	5.760	18.000
Local	3.080	1.680	2.240	7.000
Total	11	6	8	25

Coliforms: Imported and Local Spinach

	Low	Medium	High	Total
Imported	5.769	6.346	2.885	15.000
Local	4.231	4.654	2.115	11.000
Total	10	11	5	26

Coliforms >100 CFU/g

Coliforms >100 CFU/g: Imported and Local Produce

	Yes	No	Total
Imported	21.692	25.308	47.000
Local	14.308	16.692	31.000
Total	36	42	78

Coliforms >100 CFU/g: Imported and Local Basil

	Yes	No	Total
Imported	8.296	5.704	14.000
Local	7.704	5.296	13.000
Total	16	11	27

Coliforms >100 CFU/g: Imported and Local Romaine Lettuce

	Yes	No	Total
Imported	7.200	10.800	18.000
Local	2.800	4.200	7.000
Total	10	15	25

Coliforms >100 CFU/g: Imported and Local Spinach

	Yes	No	Total
Imported	5.769	9.231	15.000
Local	4.231	6.769	11.000
Total	10	16	26

E. coli

E. coli: Imported and Local Produce

	Yes	No	Total
Imported	3.013	43.987	47.000
Local	1.987	29.013	31.000
Total	5	73	78

E. coli: Imported and Local Basil

	Yes	No	Total
Imported	1.556	12.444	14.000
Local	1.444	11.556	13.000
Total	3	24	27

E. coli: Imported and Local Romaine Lettuce

	Yes	No	Total
Imported	0.720	17.280	18.000
Local	0.280	6.720	7.000
Total	1	24	25

E. coli: Imported and Local Spinach

	Yes	No	Total
Imported	0.577	14.423	15.000
Local	0.423	10.577	11.000
Total	1	25	26

Appendix E. Figures for imported and local basil, romaine, and spinach compared with the presence of *E. coli*

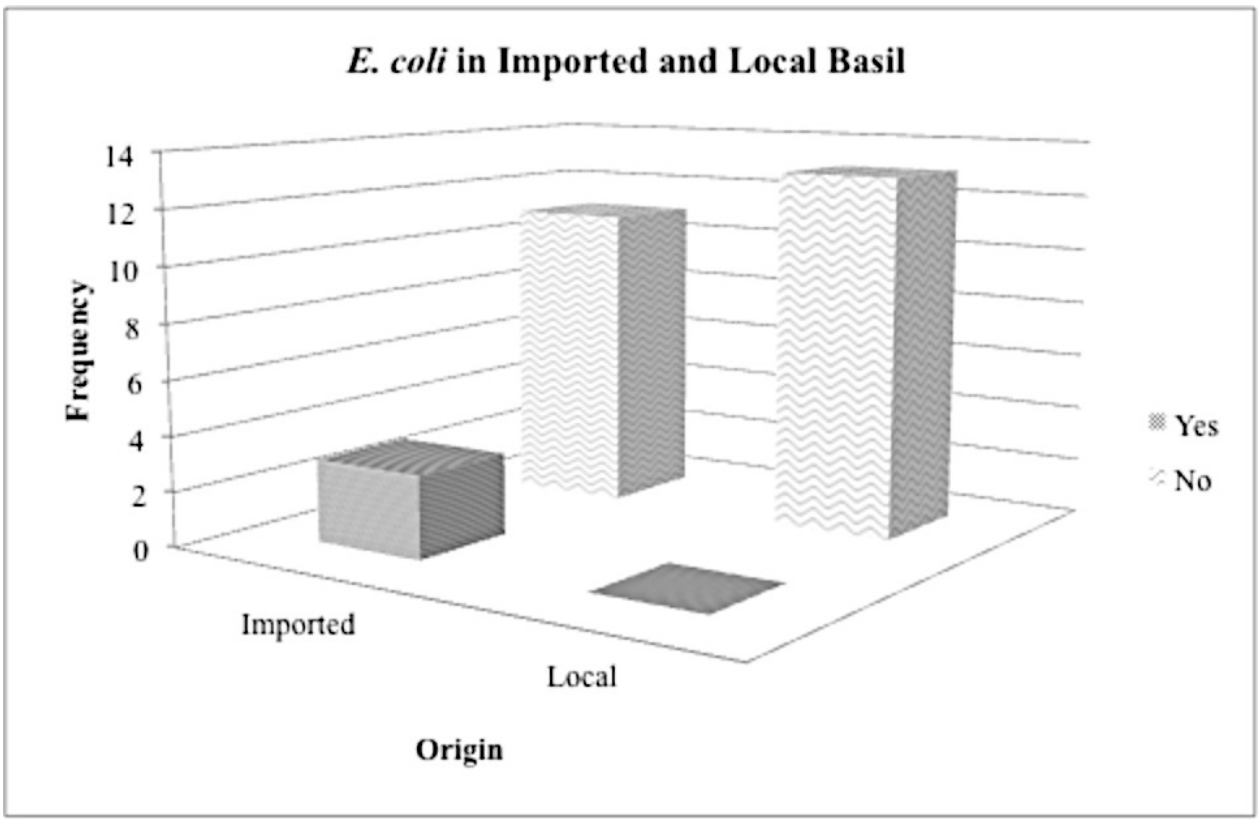

Figure 18. *E. coli* in Imported and Local Basil

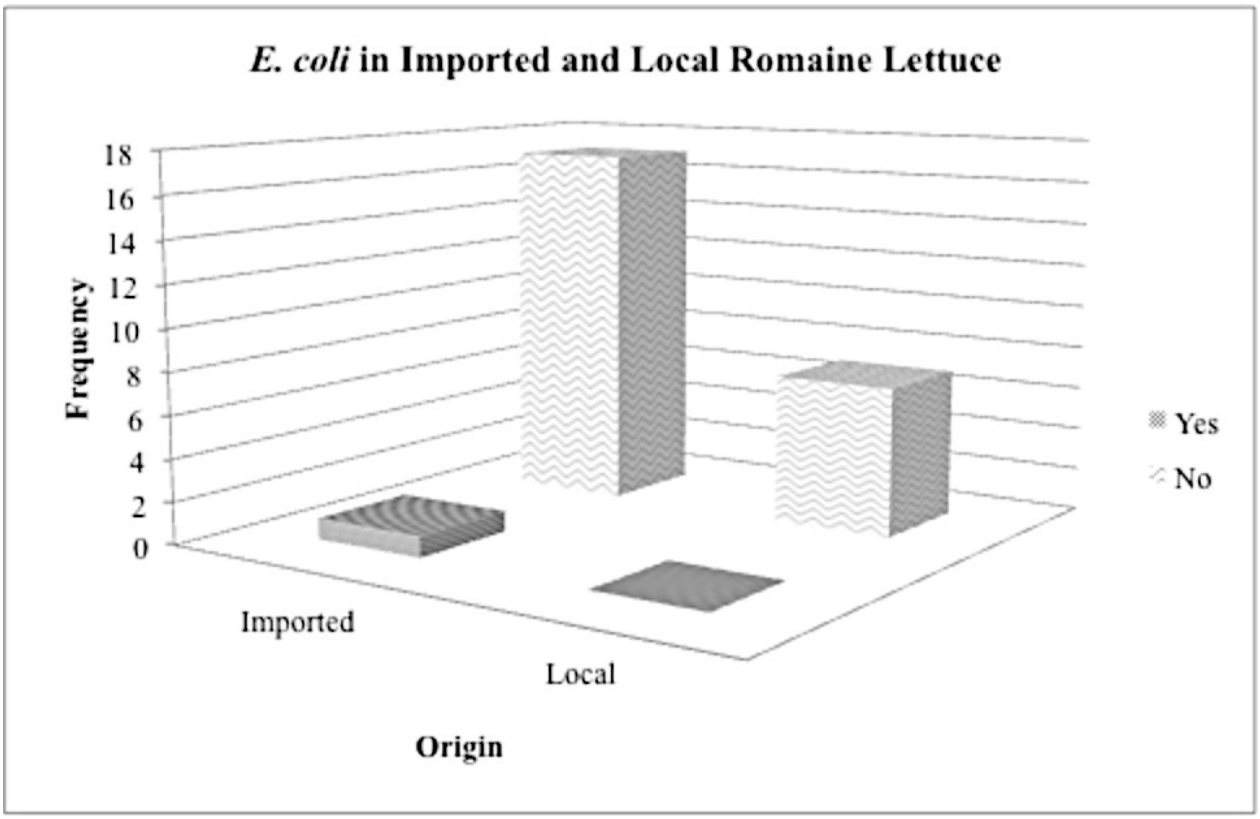

Figure 19. *E. coli* in Imported and Local Romaine Lettuce

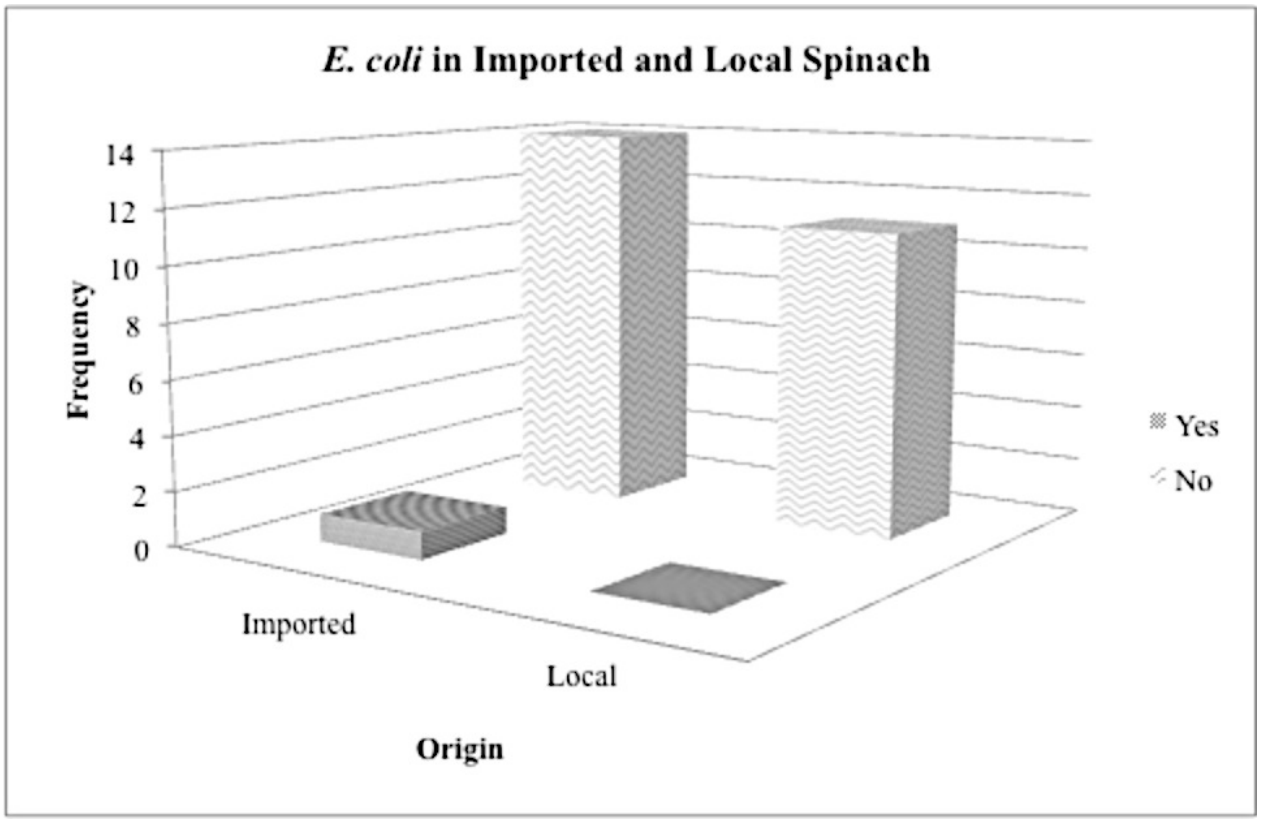

Figure 20. *E. coli* in Imported and Local Spinach

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